

# Steatorrhea and nutritional condition in cystic fibrosis children : effects of a proton-pump inhibitor

Citation for published version (APA):

Tran, T. M. (1996). *Steatorrhea and nutritional condition in cystic fibrosis children : effects of a proton-pump inhibitor*. [Doctoral Thesis, Maastricht University]. Rijksuniversiteit Limburg. <https://doi.org/10.26481/dis.19961017tt>

## Document status and date:

Published: 01/01/1996

## DOI:

[10.26481/dis.19961017tt](https://doi.org/10.26481/dis.19961017tt)

## Document Version:

Publisher's PDF, also known as Version of record

## Please check the document version of this publication:

- A submitted manuscript is the version of the article upon submission and before peer-review. There can be important differences between the submitted version and the official published version of record. People interested in the research are advised to contact the author for the final version of the publication, or visit the DOI to the publisher's website.
- The final author version and the galley proof are versions of the publication after peer review.
- The final published version features the final layout of the paper including the volume, issue and page numbers.

[Link to publication](#)

## General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal.

If the publication is distributed under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license above, please follow below link for the End User Agreement:

[www.umlib.nl/taverne-license](http://www.umlib.nl/taverne-license)

## Take down policy

If you believe that this document breaches copyright please contact us at:

[repository@maastrichtuniversity.nl](mailto:repository@maastrichtuniversity.nl)

providing details and we will investigate your claim.

**STEATORRHEA AND NUTRITIONAL CONDITION IN CYSTIC FIBROSIS CHILDREN  
EFFECTS OF A PROTON - PUMP INHIBITOR**

## CONTENTS

<b>Chapter 1</b>	<b>General introduction - literature review - Aims of the study</b>	<b>1-31</b>
	1. Genetics of cystic fibrosis	
	2. Pathogenesis	
	3. Clinical manifestations	
	4. Diagnosis	
	5. Therapy	
	6. Evaluation of steatorrhea	
	<b>Aims of the study</b>	
	References	
<b>Chapter 2</b>	<b>Methods</b>	<b>32-38</b>
	1. Methods used for fecal fat determination	
	2. Methods used for assessment of nutritional condition	
<b>Chapter 3</b>	<b>The acid steatocrit: A much improved method</b>	<b>39-49</b>
	Tran M., Forget P., Van den Neucker A., Strik J., van Kreeel B., Kuijten R.	
	J Pediatr Gastroenterol Nutr 1994; 19: 299-303	
<b>Chapter 4</b>	<b>Improved steatocrit results obtained by acidification of fecal homogenates are due to improved fat extraction</b>	<b>50-58</b>
	M. Tran, P. Forget, A. Van den Neucker, B. Van Kreeel	
	J Pediatr Gastroenterol Nutr 1996; 22: 157-160	
<b>Chapter 5</b>	<b>Clinical use of acid steatocrit</b>	<b>59-66</b>
	A. Van den Neucker, N. Pestel, T. My Dung Tran, P. Ph. Forget, H. J. Veeze, J. Bouquet, M. Sinaasappel	
	Submitted for publication	
<b>Chapter 6</b>	<b>Role of lansoprazole in children with cystic fibrosis: Evidence for improved fat malabsorption and nutritional status</b>	<b>67-83</b>
	Tran TMD, Van den Neucker A, Hendriks JJE, Forget P (junior), Forget P (senior)	
	Submitted for publication	

<b>Chapter 7</b>	<b>Anthropometry and body composition methods in children with cystic fibrosis: Effects of nutritional intervention</b>	84-107
	My-Dung T. Tran, Anita Van den Neucker, Han J. Hendriks, Bernard van Kreel, Patricia Forget, Guido Heidendal, Pierre-Philippe Forget	
	Submitted for publication	
<b>Chapter 8</b>	<b>General discussion</b>	108-112
<b>Summary</b>		113-114
<b>Samenvatting</b>		115-117
<b>Dankwoord</b>		118-120
<b>Curriculum vitae</b>		121

**STEATORRHEA AND NUTRITIONAL CONDITION IN CYSTIC FIBROSIS CHILDREN  
EFFECTS OF A PROTON-PUMP INHIBITOR**

**PROEFSCHRIFT**

Ter verkrijging van de graad van doctor  
aan de Rijksuniversiteit Limburg te Maastricht,  
op gezag van de Rector Magnificus, Prof.Mr. M.J. Cohen,  
volgens het besluit van het College van Dekanen,  
in het openbaar te verdedigen  
op donderdag 17 oktober 1996 om 16.00 uur

door

Therese Marie Pascale Thi My Dung Tran  
geboren op 27 april 1967 te Dinh Tuong, Vietnam

**Promotor:** Prof. Dr. C. Blanco

**Co-promotores:** Dr. P-Ph. Forget  
Dr. B. van Kreel

**Beoordelingscommissie:** Prof. Dr. P.B. Soeters, ( voorzitter )  
Prof. Dr. H.S.A. Heymans, ( Universiteit van Amsterdam )  
Prof. Dr. R.W. Stockbrugger  
Prof. Dr. J.M. Wit, ( Rijksuniversiteit Leiden )  
Prof. Dr. E.F.M. Wouters

Steatorrhea and nutritional condition in cystic fibrosis children:  
Effects of a proton - pump inhibitor /  
Therese Marie Pascale Thi My Dung Tran.  
Proefschrift Maastricht - Met lit. Opg. - Met samenvatting in het Nederlands.  
ISBN 90-5681-011-1

Trefw.: Steatocriet / steatorrhoe / cystic fibrosis / voedingstoestand / proton -  
pomp remmer / lichaamsamenstelling.

Vormgeving: My Dung Tran  
Omslagillustratie: Vetbollen in een microscopische faeces preparaat,  
met Soudan kleuring.

STEVENS-REISNER-SYNDROOM

*Aan mijn lieve moeder  
Voor alle cystic fibrosis kinderen . . .*

## CHAPTER 1

### GENERAL INTRODUCTION

## **1. GENETICS OF CYSTIC FIBROSIS (CF)**

Cystic fibrosis was first described in 1928 by Fanconi (1). It is an autosomal recessive disease and is reported in all racial groups with varying prevalence. In caucasians, CF occurs in 1 out of 2000 live births. Males and females are equally affected. The basic defect is a mutation of the Cystic Fibrosis Transmembrane Regulator (CFTR), a protein responsible for chloride ion transport in response to cAMP mediated signals. The most frequent CF mutation in the caucasian population is a deletion of 3 nucleotides, encoding for phenylalanine at position 508 in the CFTR protein amino acid sequence. Its overall frequency reported by the CF Genetic Analysis Consortium is 68% (2). Until now, over 200 mutations have been characterized and account for the remaining mutations.

## **2. PATHOGENESIS**

It is generally accepted that cAMP stimulated chloride conductance is a function of the CFTR (3). This function is deficient in epithelial cells of CF patients. The inability to secrete chloride and secondarily secrete water results in viscous secretions. Poor clearance of these viscid secretions from the epithelium often results in obstruction of excretory ducts. CFTR has been found in epithelial cells of several organs such as the airways, the sweat glands, the genitourinary system and the gastrointestinal tract including the pancreas and the biliary tract (4). Dysfunction of these organ systems are therefore possibly related to the same underlying defect in the CFTR-gene product.

## **3. CLINICAL MANIFESTATIONS**

CF is a multisystem disease with lungs and pancreas mostly affected in young patients.

### **3.1 Respiratory tract**

Lung disease accounts for more than 95% of the morbidity and mortality in CF (5,6). The desiccated mucus in the respiratory tract causes stasis and bronchiolar obstructions, resulting

in bacterial overgrowth and chronic lung infection. This gives rise to the production of proteases by bacteria and neutrophils. These enzymes hydrolyze important structural proteins of the lung and airways such as elastin, proteoglycans and collagen, leading to instability of bronchial walls and bronchiectasis. Furthermore, these enzymes also alter the mucosal function by increasing the secretion of macromolecular glycoconjugates contributing to a high viscosity of the mucus (7). Bronchiolitis with wheezing is frequent during the first year of life. Some patients remain however, asymptomatic for long periods. When pulmonary disease progresses, exercise intolerance occurs and finally, progressive pulmonary deterioration is the main cause of death in these patients (6,8). As a consequence of improved supportive therapy, survival has increased from 6 months at the end of the fifties (9) to nearly 30 years currently (10,11). Sinusitis and nasal polyposis sometimes occur in CF (12,13).

### **3. 2 Pancreas**

In the pancreas, obstruction of the ductules is the cause of acinar / ductular distention, followed by disruption with release of proteolytic enzymes and autodigestion of the pancreas resulting in pancreatic insufficiency with steatorrhea. The changes in the pancreas can occur early during gestation, compromising the normal maturation of the pancreas. Approximately, 85% of CF patients have steatorrhea (11). In 85 - 90% of these cases, exocrine pancreatic insufficiency develops during the first year of life. Decreased secretion of bicarbonate and water first occurs before a decrease of pancreatic enzyme concentration in duodenal fluid can be detected (14-17). Recurrent acute pancreatitis occurs in approximately 10% of CF patients (18).

Because of fat malabsorption, serum concentrations of fat soluble vitamins are often lowered. Since Vit A consists of esters of long chain fatty acids, it cannot be absorbed in the absence of pancreatic esterases. Due to its short half life, low serum levels of Vit A are often found in early untreated CF patients (19,20). Vit D deficiency resulting in decreased bone mineralization has also been reported in CF patients (21-23). Due to frequent antibiotic therapy, suppression of endogenous vit K synthesis by anaerobic intestinal bacteria often contribute to a low vit K serum level in CF patients with steatorrhea. Although Vit B12 is water soluble, serum levels may also be low in CF patients. Binding to intrinsic factor, necessary for absorption,

can only take place after cobalamin has been released from the R-protein binding by pancreatic enzymes. Decreased pancreatic bicarbonate secretion may play a role herein since the binding affinity of cobalamin for R-protein decreases at neutral or slightly alkaline pH (24). However, pancreatic enzymes supplements will normalize the Vit B12 serum level.

Abnormal glucose tolerance occurs in 30 - 75% of CF patients while diabetes mellitus develops in 10% (11). Several diabetogenic factors including increased passive sugar transport (25), increased mucosal absorption of D-glucose (26), decreased beta cell mass (27) and delayed insulin secretion (28) are present in CF. On the contrary, several antidiabetogenic factors such as an increased tissue insulin sensitivity (29) and an increased number of insulin receptors on monocytes (30) have been reported in cystic fibrosis. Moran et al. reported a decreased alpha-, beta- and pancreatic polypeptide- cell function in CF patients with exocrine disease compared to those without this disorder. Due to this finding, they suggest that either exocrine disease causes endocrine dysfunction or that a common pathogenic process simultaneously and independently impairs exocrine and endocrine function in CF patients (31). However, the exact etiology of diabetes in CF is still unknown.

### **5. 5 Malnutrition**

CF children are malnourished when compared to normal controls (32,33). Both, malabsorption accompanying pancreatic insufficiency (34) and high energy expenditure due to chronic lung infection (35,36) are thought to be responsible for the poor nutritional condition in these patients. Moreover, in CF, several intraluminal factors other than pancreatic insufficiency are also considered responsible for fat malabsorption:

1) Increased gastric acid secretion after stimulation with pentagastrin (37):

A high postprandial acid secretion could, by lowering the duodenal pH, contribute to fat malabsorption.

2) Decrease pancreatic bicarbonate secretion (13-16):

Higher gastric acid secretion after meals together with a decrease in pancreatic bicarbonate secretion, has been shown to result in a prolonged postprandial lowering of duodenal pH with

inactivation of the remaining pancreatic lipase. Moreover, low duodenal pH also results in bile acid precipitation,, fecal loss of bile acids and a decrease in the bile acid pool, contributing to fat malabsorption

### 3) Increased glycine to taurine conjugated bile acid ratios:

Due to a relatively deficient supply of taurine compared to glycine and to continuous fecal loss of bile acids, newly formed bile acids are mainly conjugated with glycine, leading to high glycine-/taurine- bile acid conjugation ratios (38). The glycine conjugated bile acids precipitate in an acidic environment contributing to the luminal bile acid deficiency in these patients.

## 3. 4 Intestinal tract

Gastroesophageal reflux and esophagitis are frequent causes of epigastric pain in CF patients (39,40) and can be responsible for decreased pulmonary functions (41). Peptic ulcers are found in up to 13% of CF patients (42) and are thought to be related to the low duodenal pH (43). Meconium ileus occurs in 15% of newborn infant with CF (44); 10% of CF patients have meconium ileus "equivalents" at a later age with a peak incidence at 20-25 years of age (45). Protein precipitation as a result of decreased duodenal pH and high secretion viscosity all probably contribute to these obstructive events (11). Up to 25% of the CF patients have rectal prolapse occurring mostly in children aged 6 - 36 months (46) while intussusception has been shown to occur in 1% (47).

## 3. 5 Biliary and Hepatic tracts

An increased incidence of cholelithiasis has been reported in CF patients (48) and is thought to be related to hypokinesia and increased fasting gallbladder volumes (49). Biliary cirrhosis with hepatosplenomegaly leading to portal hypertension occurs in 25% of CF patients. Liver steatosis has been reported in 30% of patients with CF.

## 3. 6 Genitourinary tract

More than 95% of males are infertile due to obstruction of the reproductive tracts (50). Active spermatogenesis does occur but produced spermatozoa are abnormal or immature (51). In CF women, the reproductive tracts are anatomically normal but fertility is decreased (52). Increased viscosity of the cervical mucus is thought to interfere with sperm penetration (53).

### 3.7 Sweat gland

Decreased sodium and chloride reabsorption due to dysregulation of sweat gland duct cells results in susceptibility of CF patients to salt depletion during warm weather and during gastroenteritis.

### 4. Diagnosis

The standard diagnostic procedure is the sweat test based on increased concentration of electrolytes in the sweat of the patients (54). The sweat test was developed by Gibson and Cooke (55), whereby the sweat production is stimulated by pilocarpine iontophoresis. The then collected sweat is analysed for its chloride and sodium content. However, chloride content has a better diagnostic value than sodium content, since abnormal sodium secretion can also be found in other endocrine diseases. Sweat chloride concentration higher than 60 mM or sodium above 70 mM measured minimal on two conditions is considered as abnormal, whereas chloride values under 50mM and sodium value under 30mM are found in normal persons. Chloride concentrations between 50 and 70 mM are inconclusive. For reliable results, at least 50mg of sweat should be collected. Low sweat production in the first few weeks of life is the reason for unreliable test results in this age group. In cases of doubt, identification of CFTR-mutation or measurement of intestinal current in a rectal biopsiate have been reported to be conclusive (56).

### 5. Therapy

The ideal treatment of CF should be the correction of the underlying defect by introduction of a normal copy of the defective gene into these patients genetic material. Gene therapy is

presently under intensive scrutiny. Adenovirus and recently also the retrovirus seem promising as an effective vector for normal gene transport into the target cells (57,58). Recently, transfer of the CFTR gene to the rat airways epithelia has been successfully performed (59). However, the role of gene therapy in the management of CF patients is not yet settled. Until then, treatment of CF patients has to focus on improving the nutritional condition, since malnutrition can adversely affect survival (60). The nutritional status of CF patients can be improved by firstly ameliorating the respiratory function, thereby minimizing energy expenditure and secondly, by increasing energy supply either by increasing nutrient intake or by improving nutrient digestion and absorption.

### **5.1 Respiratory function support**

Since viscous mucus in the lung is the cause of chronic lung infection, efforts should be made to improve mucus clearance. Although most patients on mucolytics such as acetylcysteine have the feeling of decreased sputum viscosity, studies with acetylcysteine either orally or as aerosol have failed to support this finding (61-63). Alternative methods such as chest percussion combined with postural drainage (64), positive expiratory pressure mask (65,66) and forced expiratory pressure (67) have been suggested to improve mucus clearance. Moreover,  $\beta_2$ -agonists as aerosol can increase sputum clearance (68) and some bronchodilating effect has been experienced in CF patients on this regimen (69,70). Corticosteroids, have been found to delay disease progression and to improve lung function in CF patients (71-74) but, short-term adverse effects such as hyperglycemia and long-term adverse effects such as development of cataract and growth retardation preclude the routine use of corticosteroids in these patients (73).

Treatment with antibiotics can reduce the progression of lung infection. Colonisation with *Ps. aeruginosa* often occur in CF patients and various regimes have failed to eradicate the bacteria (75). *Ps. aeruginosa* vaccines are presently being evaluated (76). In the end stage, lung transplantation can offer an outcome. The one and two year survival rates approach 70% and 54% respectively (77). Amiloride inhalation by blocking sodium reabsorption in the respiratory epithelium, has been shown to increase sputum clearance in a placebo-controlled cross-

ver study (78,79). Although improvement in pulmonary function was not found in one study (78), a delay in the deterioration of forced vital capacity (FVC) was reported by an other author (79). Dornase (Pulmozyme), a recombinant human desoxyribonuclease which breaks off the sputum DNA, has been reported to increase the forced expiratory volume (FEV1) and FVC safely in CF patients (80-82). Inhalation of  $\alpha$ 1-antitrypsine inhibits neutrophil elastase (83), which is released from the neutrophils and causes lung damage. Chloride channel facilitators, which directly stimulate a CFTR protein independent anion channel, are presently being evaluated (84).

## 5.2 Increase energy supply

In the past, restricted diets with **low fat content** were often prescribed for CF patients in order to minimize steatorrhea, abdominal cramps and stool bulk (85-87). Due to both unpalatability and low caloric density, these diets often resulted in **malnutrition and growth failure** in these patients (87-89). In the early 1970s, Crozier introduced the use of **high fat diets** in combination with pancreatic enzymes in order to increase the energy intake of CF patients. This regimen resulted in **better growth** with evident steatorrhea (90). Moreover, CF children from clinics using low fat diets were reported to show poorer growth (87-89) than those from clinics, encouraging the use of high fat diets (91). In order to further improve the nutritional status and growth of CF patients, feeding intervention studies have been done with different kinds of nutrients such as hypercaloric polymeric, semielemental or elemental diets. It has been shown that interventions making use of very high caloric intakes of polymeric diets (150 - 180% Recommended Daily Allowance) by overnight nasogastric tube resulted in improved nutritional status in CF adults. In children with CF, the effects of interventions with hypercaloric polymeric diets up to 130% of RDA are however unconvincing. Luder and coworkers, studying the effects of a 4 year period of nonrestricted fat diet in CF children, found improved Z-scores for weight, height and BMI for their CF patients when compared to the national population of CF patients on fat restricted diets, while no changes were seen when compared to normal children without CF (92). More recently, studies with hypercaloric polymeric diets with high fat content did not result in significant improvements of Z-scores for weight, height and skinfolds in CF patients (93), whereas **parenteral nutrition** and either oral or enteral

**elemental and semielemental** nutrition have been shown to **significantly improve the nutritional condition** of these patients (94-106). The results of short-term and long-term studies of feeding interventions on the nutritional status in CF children are summarized in table 1 and 2. The fact that predigested food can improve the nutritional status better than standard diets, strongly suggests that nutrient maldigestion plays a crucial role in the poor response to oral hypercaloric polymeric diets. The latter hypothesis is further supported by the known inactivation of pancreatic enzymes and bile acids precipitation accompanying the low duodenal pH due to low bicarbonate secretion in CF patients (107-110) . **Enteric-coated pancreatic enzyme** preparations have therefore been introduced but the low duodenal pH interferes with the release of enzymes through the acid resistant coating (111). **High doses of pancreatic enzymes** did not solve the problems of malabsorption (112) and recently, colon strictures have been observed in CF children on high doses of pancreatic enzymes (113-115). Attempts have been made to inhibit gastric acid production in the hope to improve the digestion and absorption of nutrients. However, the reported effects of **H2-receptor antagonists and prostaglandine E2** on steatorrhea have been variable and unconvincing (116-125). Results of short-term studies of cimetidine and misoprostol on fat excretion have not been consistent (table 3). This may be partly due to the lack of control of dietary fat intake. In long-term studies cimetidine showed no significant changes in fat excretion and nutritional status in CF children. on the contrary, **famotidine**, a more potent inhibitor of gastric acid secretion, showed both a significant improved fat absorption coefficient and improved growth parameters (table 4). However, interpretation of growth effects in the latter study is rather difficult because Z-score methods have not been used to evaluate growth. Further, in a double blind study, a significant improvement in steatorrhea was found when a **proton pump inhibitor** was added as adjuvant therapy in pancreatic enzyme treated CF adults (112). In children with CF, the effects of proton pump inhibitors on fat absorption and on the nutritional condition have not been reported.

**Table 1 short-term feeding intervention studies in cystic fibrosis.**

Authors	<sup>100</sup> Shepherd et al., '80	<sup>101</sup> Shepherd et al., '83	<sup>102</sup> Bertrand et al., '84	<sup>103</sup> Mansell et al., '84	<sup>104</sup> Ciociani et al., '85	<sup>105</sup> Loughlin et al., '86	<sup>106</sup> Remmel et al., '95
Number of cases	12	7	10	11	21	10	15
Age range	0.5 - 11 y	5 - 13 y	3 - 12 y	10 - 17 y	1 - 14 m	7 - 28 y	5 - 27 y
Nutritional status	malnutrition	malnutrition	malnutrition	malnutrition	normal	malnutrition	malnutrition
Study duration	1 month	6 months	1 month	1 month	5 days	6 months	3 months
Type of study	prospective own control	prospective own control	prospective own control	prospective own control	prospective CF control	prospective CF elemental CF polymeric	prospective own control
Feeding intervention	TPN 90 - 100 % RDA	elemental 20 - 40 % RDA (extra)	elemental 110 - 150 % RDA	TPN 120 % RDA	semielemental 142 kcal/kg	elemental 35% RDA (extra) versus hypercaloric	hypercaloric polymeric 130 % RDA
Route	parenteral	enteral	nasogastric	parenteral	oral	enteral	oral
Effect	SDS weight ↓ SDS height ↓ MUAC% std ↓ clinical score ↓ FVC, FeV1 ↓ PEF ↓	SDS weight ↓ SDS height ↓ MUAC % std. ↓ FM ,LBM (kg) ↓ muscle mass (kg) ↓ clinical score ↓ 3-meHis excre ↓	weight/height ↓ skinfold % std. ↓ MUAC % std. ↓ fat excretion (ns) MAMC % std. = FeV1, FEF = final work load =	weight (kg) ↓ height (cm) ↓ skinfold (mm) ↓ MAMC (cm) ↓ MIP, MEP ↓ FeV1, FEF =	Weight (kg) ↓ N-excretion ↓ fat absorption coefficient ↓	Weight/height % std. ↓ SDS height ↓ FMLBM (kg) ↓ fat excretion ↓ clinical score ↓ FeV1, FEF, FVC ↓	SDS weight (ns) SDS height (ns) growth velocity (ns) skinfold (ns) weight (kg) ↓
Follow up (duration)	all parameters improve further (after months)	NR	all parameters ↓ (after 2 months)	MIP, MEP = FeV1, FEF = (after 2 months) all other parameters ↓ (after 1-6months)	NR	NR	NR

MIP : Maximal Inspiratory Pressure

MEP : Maximal Expiratory Pressure

FeV1 : Forced expiratory volume in 1 sec.

SDS : Standard deviation score

N-excretion : Fecal Nitrogen excretion

MUAC : Mid Upper Arm Circumference

MAMC : Mid Arm Muscle Circumference

FVC : Forced Vital Capacity

FEF : Forced Expiratory Flow

PEF : Peak Expiratory Flow

LBM : Lean Body Mass

FM : Fatmass

RDA: Recommended daily allowance

3-meHis excre : 3 methylhistidine excretion in urine

↑ : Improved

↓ : Decreased

= : unchanged

(ns) : not significant

NR: not reported

**Table 2 Long-term feeding intervention studies in cystic fibrosis.**

Authors	<sup>10</sup> Allan et al, '73	<sup>11</sup> Berry et al, '75	<sup>12</sup> Yassa et al, '78	<sup>13</sup> Levy et al, '85	<sup>14</sup> Boland et al, '86	<sup>15</sup> Sheperd et al, '86	<sup>16</sup> Farrell et al, '87	<sup>17</sup> Luder et al, '89
Number of cases	17	15	43	14	10	10	36	37
Age range	2 - 21 y	10m -18y	3 - 16 y	5 - 22 y	5 - 20 y	3 - 13 y	3 - 4 m	2 - 27 y
Nutrition status	malnutrit	malnutrit	malnutrit	malnutrit	malnutrit	malnutrit	normal	malnutrit
Study duration	3 months to 3 years	1 year	1 year	1,1 year	10 - 36 months	1 year	8 months	4 years
Type of study	prospect own control	prospect CF control	prospect CF control	prospect CF control	prospect own control	prospect CF control	prospect CF control	prospect own control
Feeding intervent	elemental 50-100% RDA	elemental 100 % RDA	elemental 100 % RDA	(semi) elemental 30 % RDA (extra)	predigest non-elemental 1000 to 2000Kcal	semielemental 120-140 % RDA	pregestimil versus standard 120Kcal / kg	hypercal polymeric 120 % RDA
Route	oral	oral	oral	gastrostomy	jejunostomy	enteral	oral	oral
Effect	SDSwei ↓ SDShei ↓ clinical score ↓	SDSwei ↓ clinical score ↓ SDS hei (ns)	SDSwei ↓ SDShei ↓ SDSskf ↓ boneage ↓	weight (kg) ↓ height (cm) ↓ wei/hei % std. growth velocity ↓ BF % ↓ FFM ↓ TBK ↓ TBN ↓ ‡FVC = ‡FeV1 =	SDSwei ↓ MAMC ↓ FVC =	SDSwei ↓ SDS hei ↓ protein synthes ↓ protein catabol ↓ FeV1 ↓ FVC ↓ FEF ↓	weight (kg) ↓ height (cm) ↓ growth velocity ↓	SDSwei (ns) SDS hei (ns) FEF = BMI ↓
Follow up (duration)	NR	NR	all parameters ↓ except bone age (1 year)	NR	NR	NR	NR	NR

↓ : Improved

↓ : Decreased

= : unchanged

RDA : Recommended Daily Allowance

SDSwei : SDS weight

SDShei : SDS height

SDSskf : SDS skinfolds

LBM : Lean Body Mass

FFM : Fat Free Mass

TBN : Total body nitrogen

TBK : Total Body Kalium

FVC : Forced Vital Capacity

FeV1 : Forced expiratory volume ‡ FVC and FeV1 decrease in CF control

FEF : Forced Expiratory Flow

BMI : Body Mass Index

BF : Body fat

N-excret : Nitrogen excretion

(ns) non significant

Abs. coeff : absorption coefficient

‡ FVC and FeV1 decrease in CF control

MAMC : Mid Arm Muscle Circumference

**Table 3 Effect of short-term use of gastric acid inhibitors on steatorrhea and nutritional status in CF children.**

Authors	<sup>117</sup> Cox et al., '79	<sup>118</sup> Boyle et al., '80	<sup>119</sup> Durie et al., '80	<sup>120</sup> Gow et al., '81	<sup>121</sup> Schöni et al., '81	<sup>122</sup> Cleghorn et al., '83	<sup>123</sup> Robinson et al., '90
number of cases	10	8	15	10	10	11	15
age range	6 - 27 y	12 - 25 y	10 - 17 y	6 - 13 y	11 - 17 y	2 - 17 y	0.5 - 13.8 y
type of study	prospective crossover open	prospective randomized crossover	prospective randomized crossover	prospective randomized crossover	prospective open	prospective open	double-blind placebo controlled crossover
pancreatic enzyme	Cotazym or Viokase	Viokase	Cotazym	Pancrease	Eurobiol	Pancrease	Pancrease
intervention	cimetidine 150-200mg / day	cimetidine 300 mg / day	cimetidine 20 mg / kg / day	cimetidine 20 mg / kg / day	cimetidine 600 mg / m <sup>2</sup> / day	misoprostol 400 µg / day	misoprostol 400 µg / day
duration	1 week ?	5 days	7 days	14 days	6 days	1 week	3 weeks
effect on steatorrhea	fat excretion ↓ N-excretion ↓ fat abs coeff ↓	fat excretion ↓ fat abs coeff ↓ fecal weight ↓	fat excretion ↓ N-excretion ↓ fecal weight ↓	fat excretion (ns) N-excretion (ns) fecal weight(ns)	fat abs. coeff. (ns) N abs coeff (ns)	fat excretion normalized fat abs coeff (ns)	fat excretion ↓
effect on nutritional status	ND	ND	ND	ND	ND	ND	ND
comments	no diet evaluation per treatment period	results of diet evaluation not given	no effect on steatorrhea in patients with fat intake > 120 g / day	-	-	fat intake not controlled	fat absorption not improved in patients with < 10% fat malabsorption

abs coeff : fat absorption coefficient

N-excretion : fecal Nitrogen excretion

(ns) : not significant

**Table 4 Effect of long-term use of gastric acid inhibitors on steatorrhea and nutritional status in CF children.**

Authors	<sup>123</sup> Schöni et al, '84	<sup>124</sup> Chalmers et al, '85	<sup>125</sup> Carroccio et al, '92
number of cases	38	16	10
age range	mean 13 y	5 - 19 y	7 - 18 y
type of study	prospective randomized doubleblind	double-blind crossover	double-blind crossover
pancreatic enzyme	Pancrease	Cotazym	Pancrease
intervention	cimetidine 600mg/m <sup>2</sup> /day	cimetidine 25mg/kg/ day	famotidine 1mg/kg/day
duration	4 months	6 months	6 months
effect on steatorrhea	plasma lipid and lipoprotein (ns)	fat excretion ↓ N-excretion (ns) fecal weight (ns)	fat absorption coeff. ↓ fecal weight ↓
effect on nutritional status	weight, height (ns) skinfolds (ns) TLC, TGV (ns) Raw, sGaw ↓	SDSweight (ns) SDSheight (ns) skinfolds (ns) bone age (ns) clinical score (ns)	weight (kg) ↓ height (cm) ↓ clinical score (ns)
comments	no diet evaluation	results of diet evaluation was not given	results of diet evaluation was not given

SDS : Standard Deviation Score for age and sex

TLC : Total Lung Capacity

TGV : Thoracic Gas volume

Raw : airway resistance

sGaw : specific conductance

(ns) : not significant

## 1.6 EVALUATION OF STEATORRHEA

### 1.6.1 Fecal fat balance

The 3 days fecal fat excretion while patients are on a standard fat diet is the most reliable method for quantitative determination of fecal fat loss. The fat absorption coefficient is calculated by the following formula:

$$(\text{Fat ingested} - \text{fat excreted}) / \text{fat ingested} \times 100$$

Normal fat absorption coefficient at different ages have been reported as follows:

Age > 1 year :  $\geq 95\%$  (126-128)

Age < 1 year :  $> 83\%$  if formula fed and  $> 93\%$  if breast fed (126)

Premature infants : 38 - 73 % depending on the formula used (129)

Fecal fat can be determined by either Gravimetric or Titrimetric methods. For both methods, fecal fat is extracted with an organic solvent, the fat content is subsequently measured either by weighing (Gravimetric method) or by titration (Titrimetric method). The Gravimetric method determine all fecal lipid components, resulting in erroneously high results. On the contrary, the titrimetric method only measures fatty acids. Fecal lipids are first saponified and subsequently acidified to liberate fatty acids which are then extracted. Since its first description in 1949 (130), the **titrimetric procedure of van de Kamer** has been used as a reference method for the evaluation of malabsorption. The fat balance method is **reliable** for the quantification of fecal fat loss with a coefficient of variation of 4,6 % (131). However, the determination procedure is **time consuming, expensive and necessitates sophisticated apparatus**. Further, since the fat excretion is dependent on fat intake, patients have to keep up a **strictly fat constant diet** of more than 80 gram per day. Moreover, **fecal collection** have to be done very accurately. The balance method consequently is poorly reliable in outpatients, especially in children and infants. When fat balance is not possible, measuring **fecal fat concentration** in a fecal sample can be used for the screening of fat malabsorption. Results are then expressed as percent of wet fecal weight (fecal fat concentration). Using the  $^{14}\text{C}$ -triolein/ $^3\text{H}$ -oleic acid test as a reference method, Pedersen et al. have studied the diagnostic

value of fecal fat concentration as measured by the titrimetric method of van de Kamer in a 72 hours fecal collection without controlling for dietary fat (132). In this study, a **similar diagnostic value** was found for both **fecal fat concentration (FFC)** and **fecal fat excretion (FFE)**: The sensitivity, specificity, positive predictive value and negative predictive value of FFC versus FFE were respectively 93,1% versus 90%; 92,4% versus 89,4%; 90% versus 93% and 89% versus 90% with a **day to day coefficient of variation of 29% for FFC and 64% for FFE**. In only 6% of the patients studied, the FFC when measured in a single day sample differed from the mean 3-day fecal fat concentration value whereas the FFE differed from the mean 3-day fecal fat excretion in 37% of the patients. FFC correlated weakly but significantly with FFE ( $r = 0,55$ ;  $p < 0,01$ ) (133). FFC results in pancreatic steatorrhea being higher than in nonpancreatic steatorrhea, Bolinn et al. have suggested that FFC could be used for the differentiation of both types of steatorrhea (134). This has however not been confirmed by other investigators; who found much overlap in FFC results between pancreatic and nonpancreatic steatorrhea (132,133,135,136). Results of these studies are shown in table 5.

The utility of FFC as an screening method for fat malabsorption has been limited because of the high interday variation (29%). This interday variation might be due to the **varying fecal water content** as reported by Weijers et al. (137). This suggests that if the effect of varying water content could be eliminated, the interday variation of FFC would be much lower. A new method for the semiquantitative determination of FFC, which eliminates the influence of varying fecal water content is the **steatocrit**.

### 1. 6. 2 Steatocrit

This procedure is based on the fact that fecal fat is extracted by centrifugation of diluted stool in a hematocrit capillary at 13000 rpm for 15 minutes (138). After centrifugation, three layers are distinguished in the capillary; the upper fatty layer (FL), the middle fluid layer and the bottom solid layer (SL). The fecal fat measured by steatocrit is expressed as fecal fat concentration and is calculated as  $FL / (FL + SL)$ . Reported normal values are  $< 2\%$  (139). The steatocrit method is very suitable for use in children and infants since it is **simple, noninvasive** and can be performed on **small fecal samples** (0,5 gram). Moreover, it is inexpensive and the whole test takes only 20 minutes. Although several authors have reported this method to

be satisfactory for the evaluation of steatorrhea (138-142), some have reported the steatocrit to be **quite unreliable** (143). Sugai reported a specificity of 97% for steatocrit but a sensitivity of 98%, 79% and 29% for samples with respectively high, moderate and low fat content (144). This low sensitivity observed for samples of low fat content may be due to difficulties with either fat extraction or with obtaining a clear separation between the fatty, aqueous and solid layer, resulting in erroneous results.

### 1. 6. 3 Sudan staining method

The presence of fecal fat can be screened for by microscopic examination of stools. The fecal preparation is first acidified and stained with Sudan staining. After heating, fecal fatty acids and triglycerides are seen as fatty globules under the microscope. Dependent on the number and the size of the globules, the fatty globules are classified as normal, slightly increased or definitely increased (145). Weijers et al. studied the agreement between results of the Sudan staining method and chemically measured fecal fat (137). Satisfactory **agreement** between both methods was found in **(60 - 70 % cases) for fecal samples with very low or very high fat content ( < 3 % or > 9 % )** but the agreement dropped to **40% for samples of moderate fat content (3 - 9%)**.

### 1. 6. 4 C- Triolein absorption test

After ingestion,  $^{14}\text{C}$ -Triolein is digested by pancreatic lipase in the duodenum liberating fatty acids, which on further oxidation yield  $^{14}\text{CO}_2$  which can be detected in expired air. Although this method is **simple, rapid** to perform and gives a **direct evaluation of pancreatic function**, it is not appropriate for use in children because of the **radioactivity**. Recently, a new non radioactive substrate  $^{13}\text{C}$ -Triolein has been introduced but this is however **expensive** and a **mass spectrometer** is needed in order to use this test (131).

### 1. 6. 5 Near Infrared Reflectance Analysis

This method is based on the analysis of the infrared spectrum radiation, reflected by the

surface of the material under study. Specific peaks for the component to be investigated can be identified and their heights can be related to the concentration of the component studied by using computerised multilinear regression analysis. Besides **measuring fecal fat**, this apparatus can also be used for the determination of **fecal nitrogen and water content**. The analysis lasts less than 1 minute and can be performed on **small samples** ( 2 - 3 gram ). The variation coefficient is 2,1 % and the correlation coefficient with the van de Kamer method is 0,92 (146). However this high correlation is possibly due to the fact that this method is calibrated by the titrimetric method described by van de Kamer. The **calibration procedure is difficult** and this sophisticated instrument is **expensive** (147). Further studies are necessary in order to better evaluate the usefulness of near infrared reflectance analysis in clinical practice.

**Table 5 Diagnostic value of fecal fat concentration (FFC) and fecal fat excretion (FFE) in studies of fat malassimilation.**

Authors	<sup>122</sup> Pedersen '84	<sup>123</sup> Bolinn '84	<sup>124</sup> Roberts '86	<sup>125</sup> Lembcke '87	<sup>126</sup> Bai et al. '89
Number of cases	87	50	125	369	538
Aims of study	diagnostic value of FFC versus FFE				
Method used	†titrimetric method (72h fecal collection)	†titrimetric method (72h fecal collection)	†titrimetric method (72h fecal collection)	†titrimetric method (72h fecal collection)	†titrimetric method (72h fecal collection)
	- <sup>14</sup> C-triolein/ <sup>3</sup> H-oleic acid test as reference				
Population (n)	I. pancreatic steatorrhea (21) II. non pancreatic steatorrhea (12) III. no steatorrhea (54)	I. pancreatic steatorrhea (19) II. nonpancreatic steatorrhea (31)	I. pancreatic steatorrhea (24) II. nonpancreatic steatorrhea (70) III. no steatorrhea (31)	I. pancreatic steatorrhea (59) II. nonpancreatic steatorrhea (53) III. no steatorrhea: sick and normal controls (257)	I. pancreatic steatorrhea (88) II. nonpancreatic steatorrhea (525)
Results	I + II versus III: - pos. pred. value: FFE 0.93 FFC 0.90 - neg. pred. value: FFE 0.90 FFC 0.89 - if based on single day sampling, FFC more reliable (6% errors) than FFE (37% errors) - overlap of FFC between I and II	- FFC-pancreatic $\geq 9.5\%$ - FFC-nonpancreat $< 9.5\%$ - no overlap of FFC between I and II	- FFC-pancreatic $>$ nonpancreatic $>$ control - correlation between FFC and FFE ( $r=0.55$ ; $p<0.01$ ) - overlap of FFC between I and II	- FFC-pancreatic $>$ non-pancreatic steatorrhea $>$ pancreat. control - Overlap of FFC between I and II	- FFCsens 58% - FFCspec 70% - overlap of FFC between I and II
Comments	- free fat intake - <sup>14</sup> C excretion $\geq 10\%$ for steatorrhea - <sup>14</sup> C/ <sup>3</sup> H $> 1.3$ for pancreatic steatorrhea	- fat diet 90-100g/day	- fat diet 100g/day - 10% as cutoff for pancreatic steatorrhea	- fat diet $\geq 80$ g/day	- 10% as cutoff for pancreatic steatorrhea. - fat diet 100g/day

FFC: Fecal fat concentration

FFE: Fecal fat excretion

† Method as described by van de Kamer (111)

pos. pred. value: positive predictive value

neg. pred. value: negative predictive value

sens: sensitivity

spec: specificity

## **AIMS OF THE STUDY**

With age, children with CF show progressing malnutrition mainly attributed to either persisting malabsorption notwithstanding the use of high oral doses of pancreatic enzymes or increased energy consumption secondary to respiratory disease. Prospective studies in young children have shown malnutrition to occur only in patients with pancreatic insufficiency (34). Efforts to either maintain or restore the nutritional condition have shown that, notwithstanding the use of pancreatic enzymes, high nutrient intakes only seems to be effective when administered "digested" either as total parenteral nutrition or as (semi)elemental feedings orally or by tube feeding. The apparent insufficient effect of pancreatic enzymes does not seem to be due to too low administered doses and recently very high doses have been used with the hope of correcting malabsorption. Suggestions have been made that these high doses might be responsible for the recently reported occurrence of colitis in these patients (113-115).

Our hypothesis was that persisting malabsorption in these patients is likely to be linked to a low duodenal pH which interferes with several digestive and absorptive processes such as impeding transport of split fatty acids from the luminal lipid globules to the absorptive area through the mediation of bile salt micelles. If this was correct, antacid treatment should improve fat malabsorption in these patients. The fact that most studies hereover have been inconclusive might be due to the short and inefficient control of duodenal pH with the drugs used. A recent double-blind control study in adults patients has shown malabsorption to normalize in several patients treated with a proton pump inhibitor (omeprazol) (112). Until now, no studies with proton pump inhibitor have been reported in children.

### **The aims of the present work were:**

1. Develop an easy, noninvasive, cheap and reliable test for the monitoring of fecal fat loss in pancreatic malabsorption.
2. Evaluate the nutritional condition, the body composition and the presence or persistence of fat malabsorption in our patients with exocrine pancreatic insufficiency accompanying cystic fibrosis.

3. Evaluate whether or not the use of a proton pump inhibitor (lansoprazole) in our patients with persisting malabsorption improves both the fat malabsorption and the nutritional condition.

## REFERENCES

- (1) G. Fanconi, E. Uehlinger, C. Knauer. Das Coeliaksyndrom bei Angeborener Zystischer Pankreas fibromatose und Bronchiektasien. *Wein Med Wschr* 1936; 86: 753-756.
- (2) CF Genetic analysis consortium. Worldwide survey of the  $\Delta F$  508 mutation. *Am J Hum Genet* 1990; 47: 354-359.
- (3) M. Welsh, A. Smith. Molecular mechanisms of CFTR chloride channel dysfunction in cystic fibrosis. *Cell* 1993; 73: 1251-1254.
- (4) C. Marino, L. Matovecik, F. Gorelick, J. Cohn. Localization of the cystic fibrosis transmembrane conductance regulator in pancreas. *J Clin Invest* 1991; 88: 712-716.
- (5) T. Boat, M. Welsh, A. Beaudet. (1989). In *The Metabolic Basis of inherited Disease*, C. Scriver, A. Beaudet, W. Sly, D. Valle, eds. (New York: McGraw-Hill,inc.), pp. 2649-2680.
- (6) E. Kerem, J. Reisman, M. Corey, G. Canny, H. Levison. Prediction of mortality in patients with cystic fibrosis. *N Engl J Med* 1992; 326: 1187-91.
- (7) C. Sommerhoff, J. Nadel, C. Basbaum et al. Neutrophil elastase and cathepsin G stimulate secretion from culture bovine airway gland serous cells. *J Clin Invest* 1990;85: 682-689.
- (8) L. Sharples, T. Hathaway, C. Dennis, N. Caine, T. Higenbottam, J. Wallwork. Prognosis of patients with cystic fibrosis awaiting heart and lung transplantation. *J Heart-Lung-Transplant* 1993; 12: 669-74.
- (9) J. Britton. Effects of social class, sex, and region of residence on age at death from cystic fibrosis. *Br Med J* 1989; 298: 483-487.
- (10) M. Corey, F. McLaughlin, M. Williams, H. Levison. Comparison of survival, growth and pulmonary function in patients with cystic fibrosis in Boston and Toronto. *J Clin Epidemiol* 1988; 41: 583-591.
- (11) M. Aitken, S. Fiel. Cystic fibrosis. *Dis Mon* 1993; 39: 1-52.
- (12) P. Brihaye, P. Clement, I. Dab, B. Desprechin. Pathological changes of the lateral nasal wall in patients with cystic fibrosis. *Int J Pediatr Otorhinolaryngol* 1994; 28: 141-7.
- (13) I. Mackay, B. Djazaeri. Chronic sinusitis in cystic fibrosis. *J Roy Soc Med* 1994; 87 (Suppl 21): 17-19.
- (14) B. Hadorn, P. Johansen, C. Anderson. Pancreozymin secretin test of exocrine pancreatic function in cystic fibrosis and the significance of the result for the pathogenesis of the disease.

Can Med Assoc J 1968; 98: 377-385.

(15) B. Hadorn, G. Zoppi, D. Shmerling, A. Prader, I. McIntyre, C. Anderson. Quantitative assessment of exocrine pancreatic function in infants and children. *J Pediatr* 1968; 73: 39-50.

(16) H. Schachman, E. Lebenthal, K. Khat. Recurrent acute pancreatitis in patients with normal pancreatic enzymes. *Pediatrics* 1975; 55: 86-95.

(17) K. Gaskin, P. Durie, M. Corey, P. Wei, G. Forstner. Evidence for a primary defect of pancreatic HCO<sub>3</sub><sup>-</sup> secretion in cystic fibrosis. *Pediatr Res* 1982; 16: 554-557.

(18) A. Atlas, S. Orenstein, D. Orenstein. Pancreatitis in young children with cystic fibrosis. *J Pediatr* 1992; 120: 756-9.

(19) F. Ahmed, J. Ellis, J. Murphy, S. Wooton, A. Jackson. Excessive faecal loss of vitamin A (retinol) in cystic fibrosis. *Arch Dis Child* 1990; 65: 589-593.

(20) R. Sokol, M. Reardon, F. Accurso et al. Fat-soluble-vitamin status during the first year of life in infants with cystic fibrosis identified by screening of newborns. *Am J Clin Nutr* 1989; 50: 1064-71.

(21) V. Hubbard, P. Farrell, P. di Sant 'Agnese. 25-hydroxylcholecalciferol levels in patients with cystic fibrosis. *J Pediatr* 1979; 94: 84-86.

(22) E. Mischler, PJ Chesney, PW Chesney, R. Mazess. Demineralization in cystic fibrosis detected by direct protein absorptiometry. *Am J Dis Child* 1979; 133: 632-635.

(23) N. Solomons, J. Wagonfeld, C. Rieger et al. Some biochemical indices of nutrition in treated cystic fibrosis patients. *Am J Clin Nutr* 1981; 34: 462-474.

(24) R. Allen, B. Seetharam, E. Podell, D. Alpers. Effect of proteolytic enzymes on the binding of cobalamin to R protein and intrinsic factor. *J Clin Invest* 1978; 61: 47-54.

(25) M. Murphy, W. Sheldon, A. Brunetto et al. Active and passive sugar absorption in pancreatic insufficiency. *J Pediatr Gastroenterol Nutr* 1989; 8: 189-194.

(26) L. Frase, A. Strickland, G. Kachel, G. Krejs. Enhanced glucose absorption in the jejunum of patients with cystic fibrosis. *Gastroenterology* 1985; 88: 478-484.

(27) M. Lohr, P. Goertchen, H. Nizze et al. Cystic fibrosis associated islet changes may provide a basis for diabetes. An immunocytochemical and morphometrical study. *Virchows Arch [A]* 1989; 414: 179-185.

(28) L. Krueger, A. Lerner, S. Katz, R. Mack, D. Holsclaw, E. Lebenthal. Cystic fibrosis and diabetes mellitus: interactive or idiopathic. *J Pediatr Gastroenterol Nutr* 1991; 13: 209-219.

- (29) E. Wilmshurst, J. Soeldner, D. Holsclaw. Endogeneous and exogeneous insulin responses in patients with cystic fibrosis. *Pediatrics* 1975; 55: 75-82.
- (30) O. Andersen, S. Garner, C. Heilmann, K. Petersen, W. Petersen, C. Koch. Glucose tolerance and insulin receptor binding to monocytes and erythrocytes in patients with cystic fibrosis. *Acta Paediatr Scand* 1988; 77: 67-71.
- (31) A. Moran, P. Diem, D. Klein, M. Levitt, R. Robertson. pancreatic endocrine function in cystic fibrosis. *J Pediatr* 1991; 118: 715-723.
- (32) H. Berry, F. Kellogg, M. Hunt, R. Ingberg, L. Richter, C. Gutjahr. Dietary supplement and nutrition in children with cystic fibrosis. *Am J Dis Child* 1975; 129: 165-171.
- (33) J. Dodge, J. Yassa. Food intake and supplementary feeding programs. In: J. Sturgess, ed. *perspectives in cystic fibrosis*. Toronto: Canadian Cystic Fibrosis Foundation; 1980: 125-136.
- (34) M. Bronstein, R. Sokol, S. Abman et al. Pancreatic insufficiency, growth, and nutrition in infants identified by newborn screening as having cystic fibrosis. *J Pediatr* 1992; 120: 533-40.
- (35) J. Tomezsko, V. Stallings, D. Kawchak, J. Goin, G. Diamond, T. Scanlin. Energy expenditure and genotype of children with cystic fibrosis. *Pediatr Res* 1994; 35: 451-460.
- (36) M. Bronstein, P. Davies, K. Hambidge, F. Accurso. Normal energy expenditure in the infant with presymptomatic cystic fibrosis. *J Pediatr* 1995; 126: 28-33.
- (37) K. Cox, J. Isenberg, M. Ament. Gastric acid hypersecretion in cystic fibrosis. *J Pediatr Gastroenterol Nutr* 1982; 1: 559-565.
- (38) C. Roy, A. Weber, C. Morin et al. Abnormal biliary lipid composition in cystic fibrosis. *N Engl J Med* 1977; 297: 1301-1305.
- (39) A. Malfroot, I. Dab. New insights on gastro-oesophageal reflux in cystic fibrosis by longitudinal follow up. *Arch Dis Child* 1991; 66: 1339-1345.
- (40) S. Cucchiara, F. Santamaria, M. Andreotti et al. Mechanisms of gastro-oesophageal reflux in cystic fibrosis. *Arch Dis Child* 1991; 66: 617-622.
- (41) P. Gustafsson, S. Fransson, N. Kjellman, L. Tibbling. Gastro-oesophageal reflux and severity of pulmonary disease in cystic fibrosis. *Scand J Gastroenterol* 1991; 26: 449-456.
- (42) S. Fiedorek, R. Shulman, W. Klish. Endoscopic detection of peptic ulcer disease in cystic fibrosis. *Clin Pediatr (Phila)* 1986; 25: 243-246.
- (43) P. Robinson, A. Smith, P. Sly. Duodenal pH in cystic fibrosis and its relationship to fat malabsorption. *Dig Dis Sci* 1990; 35: 1299-1304.

- (44) J. McPartin, J. Dickson, V. Swain. Meconium ileus, immediate and longterm survival. *Arch Dis Child* 1972; 47: 207-210.
- (45) H. Andersen, K. Hjelt, E. Waever, K. Overgaard. The age related incidence of meconium ileus equivalent in a cystic fibrosis population: the impact of high energy intake. *J Pediatr Gastroenterol Nutr* 1990; 11: 356-360.
- (46) L. Kulczyki, H. Schwachman. Studies in cystic fibrosis of the pancreas: occurrence of rectal prolapse. *N Engl J Med* 1958;259: 409-412.
- (47) D. Holsclaw, C. Rocmans, H. Schwachman. Intussusception in patients with cystic fibrosis. *Pediatrics* 1971; 48:51-58.
- (48) H. Rovsing, K. Sloth. Microgallbladder and biliary calculi in mucoviscidosis. *Acta Radiol [Onco]* 1973; 14: 588-592.
- (49) F. Santamaria, P. Vajro, V. Oggero et al. Volume and emptying of the gallbladder in patients with cystic fibrosis. *J Pediatr Gastroenterol Nutr* 1990; 10: 303-306.
- (50) L. Taussig, C. Lobeck, P. Ackerman, J. Kattwinkel: Fertility in males with cystic fibrosis. *N Engl J Med* 1972; 287: 586-589.
- (51) E. Kaplan, H. Shwachman, A. Perlmutter et al.: Reproductive failures in males with cystic fibrosis. *N Engl J Med* 1968; 279: 65-69.
- (52) S. Fitzsimmons: Cystic Fibrosis Foundation Patient Registry 1990 Annual Report. Bethesda, Cystic Fibrosis Foundation, 1991.
- (53) P. Tam, P. Verdugo: Control of mucus hydration as a Donnan equilibrium process. *Nature* 1981; 292: 340-342.
- (54) P. Di Sant 'Agnese, R. Darling, G. Perera, E. Shea. Abnormal electrolyte composition of sweat in cystic fibrosis of the pancreas. Clinical significance and relationship to the disease. *Pediatrics* 1953; 12: 549-563.
- (55) L. Gibson, R. Cooke. A test for concentration of electrolytes in sweat in cystic fibrosis of the pancreas utilizing in pilocarpine by iontophoresis. *Pediatrics* 1959; 23: 545-549.
- (56) H. Veeze, A. Van den Ouweland, D. Halley et al. The diagnosis of cystic fibrosis: intestinal current measurements, a highly accurate method in case of a borderline phenotype. Submitted.
- (57) M. Rosenfeld, W. Siegfried, K. Yoshimura et al. Adenovirus-mediated transfer of a recombinant alpha 1-antitrypsin gene to the lung epithelium in vivo. *Science*.1991; 252: 431-4

- (58) B. Pitt, M. Schwarz, J. Pilewski et al. Retrovirus-mediated gene transfer in lungs of living fetal sheep. *Gene Ther* 1995; 2: 344-50.
- (59) M. Rosenfeld, K. Yoshimura, B. Trapnell et al. In vivo transfer of the human cystic fibrosis transmembrane conductance regulator gene to the airway epithelium. *Cell*. 1992; 68: 143-55.
- (60) R. Kraemer, A. Rudeberg, B. Hadorn, E. Rossi. Relative underweight in cystic fibrosis and its prognostic value. *Acta Paediatr Scand* 1978; 67: 33-37.
- (61) S. Rao, D. Wilson, R. Brooks et al. Acute effects of nebulization of n-acetylcysteine on pulmonary mechanics and gas exchange. *Am Rev Respir Dis*. 1970; 102: 17-22.
- (62) W. Waring. Current management of cystic fibrosis. *Adv Pediatr*. 1976; 23: 401-38.
- (63) M. Gotz, R. Kraemer, K. Kerrebijn et al. Oral acatylsysteine in cystic fibrosis. A cooperative study. *Eur J Respir Dis*. 1980;61: (Suppl 111): 122-6.
- (64) J. Reisman, B. Rivington-Law, M. Corey et al. Role of conventional physiotherapy in cystic fibrosis. *J Pediatr* 1988; 113: 632-6.
- (65) H. Steen, A. Redmond, D. O'Neil et al. Evaluation of the PEP mask in cystic fibrosis. *Acta Paediatr Scand*. 1991; 80: 51-6.
- (66) C. Braggion, L. Cappelletti, M. Cornacchia, L. Zanolla, G. Mastella. Short-term effects of three chest physiotherapy regimens in patients hospitalized for pulmonary exacerbations of cystic fibrosis: A cross-over randomized study. *Pediatr Pulmonol* 1995; 19: 16-22.
- (67) J. Pryor, B. Webber, M. Hobson et al. Evaluation of the forced expiratory technique as an adjunct to postural drainage in the treatment of cystic fibrosis. *J Pediatr*. 1983; 103: 538-42.
- (68) P. Sutton, H. Gemmell, N. Innes et al. Use of nebulized saline and nebulized terbutaline as an adjunct to chest physiotherapy. *Thorax*. 1988; 43: 57-60.
- (69) E. Pattishall. Longitudinal response of pulmonary function to bronchodilators in cystic fibrosis. *Pediatr Pulmonol*. 1990; 9: 80-5.
- (70) P. Konig, D. Gayer, J. Shaffer et al. Bronchodilator responsiveness and spontaneous diurnal variation of PEFr in patients with cystic fibrosis. Poster presented at the North American Cystic Fibrosis Conference. Washington, DC: 1992 Oct. Abstract.
- (71) M. Konstan, P. Byrard, C. Hoppel, P. Davis: Effect of high dose ibuprofen in patients with cystic fibrosis. *N Engl J Med* 1995; 332: 848.
- (72) H. Auerbach, M. Williams, J. Kirkpatrick et al. Alternate-day prednisone reduces morbi-

- dity and improves pulmonary function in cystic fibrosis. *Lancet*. 1985; 2: 686-8.
- (73) B. Rosenstein, H. Eigen. Risk of alternate-day prednisone in patients with cystic fibrosis. *Pediatrics*. 1991; 87: 245-6.
- (74) C. Pantin, R. Stead, M. Hodson et al. Prednisolone in the treatment of airflow obstruction in adults with cystic fibrosis. *Thorax*. 1986; 41: 34-38.
- (75) M. Zach. Pathogenesis and management of lung disease in cystic fibrosis. *J R Soc Med*. 1991; 84 (Suppl 18): 10-7.
- (76) McNeil Pharmaceutical. Pseudomonas vaccine tests start. *Cystic Fibrosis Currents*. 1991; 6(4).
- (77) A. Khaghani, B. Madden, M. Hodson et al. Heart-lung transplantation for cystic fibrosis. Paper presented at the North American Cystic Fibrosis Conference. Dallas, TX: 1991 Oct 4.
- (78) E. App, M. King, R. Helfesrieder et al. Acute and longterm amiloride inhalation in cystic fibrosis lung disease. *Am Rev Respir Dis*. 1990; 141: 605-12.
- (79) M. Knowles, N. Church, W. Waltner et al. A pilot study of aerosolized amiloride for the treatment of lung disease in cystic fibrosis. *N Engl J Med*. 1990; 322: 1189-94.
- (80) M. Hodson. Clinical studies of rhDNase in moderately and severely affected patients with cystic fibrosis - An Overview. *Respiration* 1995; 62 (suppl 1); 29-32.
- (81) M. Aitken, W. Burke, G. McDonald et al. Recombinant human Dnase inhalation in normal subjects and patients with cystic fibrosis. A phase I study. *JAMA*. 1992; 267: 1947-51.
- (82) R. Hubbard, N. McElvaney, P. Steven et al. A preliminary study of aerosolized recombinant human deoxyribonuclease I in the treatment of cystic fibrosis. *N Engl J Med*. 1992; 326:812-5.
- (83) N. McElvaney, R. Hubbard, P. Birrer et al. Aerosol alpha-1-antitrypsin treatment for cystic fibrosis. *Lancet*. 1991; 337: 392-4.
- (84) R. Boucher, E. Cheng, A. Paradiso et al. Chloride secretory response of cystic fibrosis airway epithelia: presentation of calcium but not protein kinase C and A-dependent mechanism. *J Clin Invest*. 1989;84: 1424-31.
- (85) H. Chase, M. Long, M. Lavin. Cystic fibrosis and malnutrition. *J Pediatr* 1979; 95: 337-47.
- (86) C. Roy, A. Silverman, F. Cozzetto. *Pediatric Clinical Gastroenterology*. 2nd ed. St.

Louis: CV Mosby, 1975: 615-35.

(87) J. Dodge, J. Yassa. Food intake and supplementary feeding programs. In: Sturgess JM, ed. Perspectives in cystic fibrosis. Proceedings of the 8th International Cystic Fibrosis Congress. Toronto: Canadian Cystic Fibrosis Foundation, 1980: 125-36.

(88) H. Parsons, P. Beaudry, A. Dumas, P. Pencharz. Energy needs and growth in children with cystic fibrosis. *J Pediatr Gastroenterol Nutr* 1983; 2: 44-9.

(89) H. Schwachman, R. Dooley, F. Guilmette, P. Patterson, C. Weil, H. Leubner. Cystic fibrosis of the pancreas with varying degrees of pancreatic insufficiency. *Am J Dis Child* 1956; 92: 347-68.

(90) D. Crozier. Cystic fibrosis a not so fatal disease. *Pediatr Clin North Am* 1974; 21: 935-950.

(91) D. Gurwitz, M. Corey, P. Francis, D. Crozier, H. Levison. Perspectives in cystic fibrosis. *Pediatr Clin North Am*. 1979; 26: 603-615.

(92) E. Luder, M. Kattan, J. Thornton, K. Koehler, R. Bonforte. Efficacy of a nonrestricted fat diet in patients with cystic fibrosis. *AJDC*. 1989; 143: 458-464.

(93) A. Rettammel, M. Marcus, P. Farrell, S. Sondel, R. Kosciak, E. Mischler. Oral supplementation with a high-fat, high-energy product improves nutritional status and alters serum lipids in patients with cystic fibrosis. *J Am Diet Assoc*. 1995; 95: 454-459.

(94) K. Gaskin, D. Waters, L. Baur, V. Soutter, M. Gruca. Nutritional status, growth and development in children undergoing intensive treatment for cystic fibrosis. *Acta Paediatr Scand [Suppl]*. 1990; 366: 106-110.

(95) E. O' Loughlin, D. Forbes, H. Parsons, B. Scott, D. Cooper, G. Gall. Nutritional rehabilitation of malnourished patients with cystic fibrosis. *Am J Clin Nutr*. 1986; 43: 732-737.

(96) R. Shepherd, T. Holt, B. Thomas et al. Nutritional rehabilitation in cystic fibrosis: Controlled studies of effects on nutritional growth retardation, body protein turnover, and course of pulmonary disease. *J Pediatr*. 1986; 109: 788-94.

(97) R. Shepherd, B. Thomas, D. Bennett, W. Cooksley, L. Ward. Changes in body composition and muscle protein degradation during nutritional supplementation in nutritionally growth-retarded children with cystic fibrosis. *J Pediatr Gastroenterol Nutr*. 1983; 2: 439-446.

(98) L. Levy, P. Durie, P. Pencharz, M. Corey. Effects of long-term nutritional rehabilitation on body composition and clinical status in malnourished children and adolescents with cystic

fibrosis. *J Pediatr* 1985; 107: 225-230.

(99) J. Bertrand, C. Morin, R. Lasalle, J. Patrick, A. Coates. Short-term clinical, nutritional, and functional effects of continuous elemental enteral alimentation in children with cystic fibrosis. *J Pediatr*. 1984; 104: 41-46.

(100) R. Shepherd, W. Cooksley, and W. Domville. Improved growth and clinical, nutritional, and respiratory changes in response to nutritional therapy in cystic fibrosis. *J Pediatr*. 1980; 97: 351-357.

(101) J. Yassa, R. Prosser, J. Dodge. Effects of an artificial diet on growth of patients with cystic fibrosis. *Arch Dis Child*. 1978; 53: 777-783.

(102) J. Allan, A. Mason, A. Moss. Nutritional supplementation in treatment of cystic fibrosis of the pancreas. *Am J Dis Child*. 1973; 126: 22-26.

(103) A. Mansell, J. Andersen, C. Muttart et al. Short-term pulmonary effects of total parenteral nutrition in children with cystic fibrosis. *J Pediatr* 1984; 104: 700-705.

(104) M. Canciani, G. Mastella. Absorption of a new semielemental diet in infants with cystic fibrosis. *J Pediatr Gastroenterol Nutr*. 1985; 4: 735-740.

(105) M. Boland, D. Stoski, N. Macdonald, P. Soucy, J. Patrick. Chronic jejunostomy feeding with a non-elemental formula in undernourished patients with cystic fibrosis. *Lancet* 1986; 1: 232-234.

(106) P. Farrell, E. Mischler, S. Sondel, M. Palta. Predigested formula for infants with cystic fibrosis. *Research*. 1987; 87: 1353-1356.

(107) P. Regan, J. Malagelada, E. Dimagno, and V. Go. Reduced intraluminal bile acid concentrations and fat maldigestion in pancreatic insufficiency: Correction by treatment. *Gastroenterology*. 1979; 77: 285-289.

(108) P. Zentler-Munro, W. Fitzpatrick, J. Batten, and T. Northfield. Effect of intrajejunal acidity on aqueous phase bile acid and lipid concentrations in pancreatic steatorrhea due to cystic fibrosis. *Gut*. 1984; 25: 500-507.

(109) P. Zentler-Munro, D. Fine, J. Batten, and T. Northfield. Effect of cimetidine on enzyme inactivation, bile acid precipitation, and lipid solubilisation in pancreatic steatorrhea due to cystic fibrosis. *Gut*. 1985; 26: 892-901.

(110) A. Weber, C. Roy. Intraduodenal events in cystic fibrosis. *J Pediatr Gastroenterol Nutr*. 1984; 3 (Suppl. 1): S113-S119.

- (111) S. Dutta, V. Hubbard, M. Appler. Critical examination of therapeutic efficacy of a pH-sensitive enteric-coated pancreatic enzyme preparation in treatment of exocrine pancreatic insufficiency secondary to cystic fibrosis. *Dig Dis Sci* 1988; 33: 1237-44.
- (112) H. Heijerman, C. Lamers, W. Bakker. Omeprazole enhances the efficacy of pancreatin (pancrease) in cystic fibrosis. *Ann Intern Med.* 1991; 114: 200-201.
- (113) S. Schwarzenberg, C. Wielinski, I. Shamieh et al. Cystic fibrosis-associated colitis and fibrosing colonopathy. *J Pediatr* 1995; 127: 565-70.
- (114) R. Smyth, D. van Velzen, A. Smyth, D. Lloyd, D. Heaf. Strictures of ascending colon in cystic fibrosis and high strength pancreatic enzymes. *The Lancet.* 1994; 343: 85-86.
- (115) M. Pettei, J. Leonidas, J. Levine, J. Gorvoy. Pancolon disease in cystic fibrosis and high-dose pancreatic enzyme therapy. *J Pediatr* 1994; 125: 587-9.
- (116) G. Cleghorn, R. Shepherd, T. Holt. The use of a synthetic prostaglandin E<sub>1</sub> analogue (Misoprostol) as an adjunct to pancreatic enzyme replacement in cystic fibrosis. *Scand J Gastroenterol.* 1988; 23(Suppl 143): 142-147.
- (117) K. Cox, J. Isenberg, A. Osher, R. Dooley. The effect of cimetidine on maldigestion in cystic fibrosis. 1979; 94: 488-492.
- (118) M. Schöni, R. Kraemer, E. Rossi. Cimetidine and fat malabsorption in children with cystic fibrosis. *Helv Paediat Acta.* 1981; 36: 359-369.
- (119) B. Boyle, W. Long, W. Balistreri, S. Widzer, and N. Huang. Effect of cimetidine and pancreatic enzymes on serum and fecal bile acids and fat absorption in cystic fibrosis. *Gastroenterology.* 1980; 78: 950-953.
- (120) R. Gow, R. Bradbear, P. Francis, R. Shepherd. Comparative study of varying regimens to improve steatorrhoea and creatorrhoea in cystic fibrosis: effectiveness of an enteric-coated preparation with and without antacids and cimetidine. *Lancet* 1981; 14: 1071-1074.
- (121) P. Robinson and P. Sly. Placebo-controlled trial of misoprostol in cystic fibrosis. *J Pediatr Gastroenterol Nutr.* 1990; 11: 37-40.
- (122) P. Durie, L. Bell, W. Linton, M. Corey, G. Forstner. Effect of cimetidine and sodium bicarbonate on pancreatic replacement therapy in cystic fibrosis. *Gut* 1980; 21: 778-786.
- (123) A. Carroccio, F. Pardo, G. Montalto et al. Use of famotidine in severe exocrine pancreatic insufficiency with persistent maldigestion on enzymatic replacement therapy: A long-term study in cystic fibrosis. *Dig Dis Sci* 1992; 37: 1441-1446.

- (124) D. Chalmers, R. Brown, M. Miller et al. The influence of longterm cimetidine as an adjuvant to pancreatic enzyme therapy in cystic fibrosis. *Acta Paediatr Scand.* 1985; 74: 114-117.
- (125) M. Schöni, R. Kraemer, A. Ruedeberg et al. Long-term cimetidine in children with cystic fibrosis: a randomized double blind study.
- (126) J. van de Kamer. Standard methods of clinical chemistry, edited by Seligson D. New York, Academic Press, 1958, Vol 2, p 34.
- (127) E. Wollaeger, M. Comfort, A. Osterberg. Total solids, fat and nitrogen in feces: Study of normal persons taking test diets containing moderate amount of fat; comparison with results obtained with normal persons taking test diet containing large amount of fat. *Gastroenterology* 1947; 9: 272-283.
- (128) D. Woodman, W. Yeoman. A simplified method of investigating steatorrhoea. *J Clin Pathol* 1955; 8:79-80.
- (129) M. Davidson, C. Bauer. Patterns of fat excretion in feces of premature infants fed various preparations of milk. *Pediatrics* 1960; 25: 375-84.
- (130) J. van de Kamer, H. Huinink, A. Weyers. Rapid method for the determination of fat in feces. *J Biol Chem* 1949; 177: 349-55.
- (131) B. Lembeke, B. Braden, J. Stein. Diagnostik der steatorrhoe. *Z Gastroenterol* 1994; 32: 256-261.
- (132) N. Thorsgaard Pedersen, H. Halgreen, H. Worning. Estimation of the 3-day faecal fat excretion and fat concentration as a differential test of malabsorption and maldigestion. *J Gastroenterol* 1987; 22: 91-96.
- (133) I. Roberts, C. Poturich, A. Wald. Utility of fecal fat concentrations as screening test in pancreatic insufficiency. *Dig Dis Sci* 1986; 31: 1021-4.
- (134) G. Bo-Linn, J. Fordtran. Fecal fat concentration on patients with steatorrhea. *Gastroenterology* 1984; 87: 319-322.
- (135) J. Bai, A. Andriush, G. Matelo et al. Fecal fat concentration in the differential diagnosis of steatorrhea. *Am J Gastroenterol* 1989; 84: 27-30.
- (136) B. Lembeke, K. Grimm, P. Lankisch. Raised fecal fat concentration is not a valid indicator of pancreatic steatorrhea. *Am J Gastroenterol* 1987; 82: 526-531.
- (137) H. Weijers. Fat absorption in normal and abnormal infants and children with special

reference to coeliac disease (proefscrift) 1950: 19-23.

(138) P. Phuapradit, A. Narang, P. Mendonca, D. Harris, J. Baum. The steatocrit: a simple method for estimating stool fat content in newborn infants. *Arch Dis Child* 1981; 56: 725-727.

(139) G. Iacono, A. Carroccio, G. Montalto et al. Steatocrit: normal range and physiological variations in preterm and low-birth-weight full-term newborns. *Acta Paediatr* 1992; 81: 933-4.

(140) A. Guarino, L. Tarallo, L. Greco, L. Cesarano, S. Guandalini, A. Rubino. Reference values of the steatocrit and its modifications in diarrheal diseases. *J Pediatr Gastroenterol Nutr* 1992; 14: 268-274.

(141) C. Colombo, R. Maiavacca, M. Ronchi, E. Consalvo, M. Amoretti, A. Giunta. The steatocrit: a simple method for monitoring fat malabsorption in patients with cystic fibrosis. *J Pediatr Gastroenterol Nutr* 1987; 6: 926-930.

(142) G. Iacono, A. Carroccio, F. Cavataio et al. Steatocrit test: normal range and physiological variations in infants. *J Pediatr Gastroenterol Nutr* 1990; 11: 53-57.

(143) M. Walters, J. Kelleher, J. Gilbert, J. Littlewood. Clinical monitoring of steatorrhoea in cystic fibrosis. *Arch Dis Child* 1990; 65: 99-102.

(144) E. Sugai, G. Srur, H. Vazquez et al. Steatocrit: a reliable semiquantitative method for detection of steatorrhea. *J Clin Gastroenterol* 1994; 19: 206-9.

(145) G. Drummey, J. Benson, C. Jones. Microscopical examination of the stool for steatorrhea. *N Engl J Med* 1961; 264: 85-7.

(146) L. Benini, S. Caliarì, G. Guodi. Near infrared spectrometry for faecal fat measurement: comparison with conventional gravimetric and titrimetric methods. *Gut* 1989; 30: 1344-1347.

(147) O. Bekers, C. Postma, A. Lombarts. Determination of fecal fat by Near-Infrared Spectroscopy. *Eur J Chem Clin Biochem* 1995; 33: 83-86.

## **CHAPTER 2**

### **METHODS**

The following methods were used in our studies:

## **1. Methods used for fecal fat determination:**

### **1.1 Steatocrit and Acid Steatocrit**

About 0,5 g solid stool was weighed and diluted with a volume of deionized water, equal to two times the weight of stool. The stool and water were premixed using a Vortex mixer. Subsequently, the mixture was homogenized using a 5 ml Potter Elvehjem tissue homogenizer. The fecal homogenate was aspirated into a 75  $\mu$ l plain haematocrit capillary. This capillary was sealed with wax at one end and centrifuged horizontally (13000 rpm, 15 min) in a standard haematocrit centrifuge. After centrifugation, the upper fatty layers (FL) and the bottom solid layers (SL) were measured with a graduated magnifying lens. The steatocrit was calculated as  $(FL / (FL + SL)) \times 100\%$ . Since the fat extraction was not optimal in the steatocrit procedure, we have try to increase this step by adding the perchloric acid (5N) to the fecal homogenate in a volume equal to 1/5 of the homogenate after homogenization. This acid homogenate was then mixed for 30 seconds using a Vortex mixer and the following steps were the same as the classical steatocrit. This "Acid Steatocrit" is used in our further study.

### **1.2 Titrimetric method**

The 72 hours fecal collection was first homogenized and about 5 gram of feces was weighed. The feces was saponified with concentrated potassium hydroxide (33% KOH) in ethanol, giving a solution which contains the soaps derived from neutral fats, fatty acids and also soaps originally present in the stool. By adding HCl (2N), fatty acids were obtained. After adding 125 ml toluene, the mixture was shaken vigorously for 2 - 3 minutes. 25ml of the toluene layer containing the fatty acids was then transferred to an erlenmeyer for titration with 0,1N tetrabutylammoniumhydroxide solution in propanol/methanol and thymol blue as indicator. The titration was done three times and the mean of this was used for the calculation of fecal fat excretion, which was calculated as followed:

$$\frac{125\text{ml} + 4.5\text{ml}}{25\text{ml}} (X - X_0) \times 0,1\text{N} \times 1/3 \times \frac{\text{total feces weight(g)}}{\text{sample weight (5g)}} \times 891\text{g} = \text{Total fecal fat (g/72h)}$$

with 125ml representing the toluene volume used for extraction of the fatty acids, 25ml representing the titration volume, 4,5ml is the correction for the volume interaction, X representing the number of meq of fatty acids titrated,  $X_0$  the correction for the acids present in toluene, 0,1N the concentration of tetrabutylammoniumhydroxide solution, 1/3 is the conversion factor from fatty acid to fat molecule (3 molecule fatty acids derived from 1 molecule fat) and 891 is the molecular weight of stearic acid (C-18-fat).

### 1. 3 Sudan staining method

We used the split fat stain, which identifies both triglyceride and fatty acid (1). Several drops of 100% acetic acid and several drops of Sudan III solution were added. The preparation was subsequently mixed with the coverslip, which was then applied. The slide was gently heated on a lighter until bubbling. All preparations were examined while still warm under high magnification (magnification of 400). For quantification of the amount of fat detected microscopically, we used the criteria established by Drummey et al.(2). They are as follows: normal (+): up to 100 fat globules per high power field, varying in a diameter between 1 and 4  $\mu\text{m}$ , as noted on the micrometer scale always using a magnification of 400; Increased (2+): up to 100 fat globules per high power field, the diameter of fat globules varying between 1 and 8  $\mu\text{m}$ ; Markedly increased (3+): more than 100 fat globules per high power field, varying in size from 6 to 75  $\mu\text{m}$  in diameter.

## 2. Methods used for assessment of nutritional condition:

### 2.1 Anthropometry

Weight, height and 4 skinfolds (biceps, triceps, subscapular and suprailiac) were expressed as standard deviation scores of the normal population for age and sex by using the growth charts from Gerver and de Bruin (3).

It has been found that subcutaneous fat as measured by skinfolds is related to the body density (4). This latter is again related to the body fatmass. From these theoretical principles, Gerber and de Bruin have constructed a chart, expressing the relationship between the 4 skinfolds (biceps, triceps, subscapular and suprailiac) and the percent fat free mass. In our study, the fatmass and fat free mass determined with the anthropometric method were derived from these charts.

## **2.2 Dual energy X-ray absorptiometry (DXA)**

This method first developed by Mazess et al., measures simultaneously bone mineral, fat and nonbone lean tissue. For a DXA scan, subjects lied supine on a padded table while the scintillation counter moved in a raster pattern across the body from head to foot. The Lunar DPX uses a constant x-ray source and a filter that converts the polychromatic x-ray beam into one that has two main energy peaks (40 kV and 70 kV). The ratio of soft tissue attenuation ( $R_{ST}$ ) at the two energies is measured. The attenuation of pure fat ( $R_f$ ) and of bone free lean tissue ( $R_L$ ) are known from both theoretical calculations and calibration. From this, the fatmass and lean tissue mass were calculated. The bone mineral content was calculated after correction of the overlying soft tissue (5). Body composition measurements in our study were made by a DPX with a pediatric software programme, Lunar version 1.5e. Daily quality assurance test were performed according to the manufacturer's directions. Total body analysis was performed in all children using a fast scan mode with a sample size of  $4,8 \times 9,6$ mm, sample interval of 0,03s and source collimation of 1,68mm.

## **2.3 Total body water (TBW) and extra cellular volume (ECV)**

TBW and ECV were measured by deuterium oxide (6) and bromide dilution respectively (7). Each subject received orally 20 ml of a mixture of  $D_2O$  (99,9% purity) and Bromide salt (150mMol/L) solution in a volume ratio of 1:1. Saliva and plasma samples were taken before intake of  $D_2O$  - NaBr solution and 4 hours thereafter when "an equilibrium" has been reached. To prevent saliva dilution by fluid intake which can result in a higher TBW content, patients were told not to take any fluid orally half an hour before saliva samples were taken. Urine and

fecal loss of bromide and D<sub>2</sub>O were negligible during the test since 1/2 of D<sub>2</sub>O and Bromide is 8 days (7). Saliva samples were obtained making use of dental cotton-wool, that was dried overnight at 100 °C and kept in a gas-tight tube until use. The cotton-wools and the blood samples were centrifuged and the saliva and serum thus obtained were kept in a stoppered glass vial and stored in a freezer until analysis.

### 2. 3. 1 Total body water

D<sub>2</sub>O concentrations of saliva samples were determined as follows: Calcium carbide (CaC<sub>2</sub>) was placed in the siliconized vacutainer tube and evacuated for 30 sec. with a rotatory vane pump to a total pressure of 0,01 atm. Thereafter, 25µl of salivary sample was injected in the vacutainer tube. This was done in duplicate. CaC<sub>2</sub> react with D<sub>2</sub>O forming acetylene gas. A 25µl of this gas was subsequently injected in duplicate into the GC/CF - IRMS system (gas chromatography/continous flow isotope ratio mass spectrometry) at 2 min. intervals. The mass 27/26 ratio (R<sub>27/26</sub>) was measured on a Isotope Ratio Mass Spectrometer configured for Acetylene (Finnigan MAT 252 for CF-IRMS) (6). The mean value of 4 determinations was calculated for each sample. By inserting of the tracer/tracee ratio, defined as R<sub>27/26</sub> (T<sub>4</sub>) - R<sub>27/26</sub> (T<sub>0</sub>), into the regression equation obtained from the standards, we get the dilution factor of D<sub>2</sub>O. TBW is calculated as ingested D<sub>2</sub>O volume/ dilution factor. From the TBW, LBM and FM can calculated by the following formulés:

$$\text{LBM (kg)} = \text{TBW} / ( 1,04 \times d )$$

$$\text{BF (kg)} = \text{Weight} - \text{LBM}$$

The 1,04 factor is a correction for the estimated 4% nonaqueous hydrogen exchange and d is the hydration factor of LBM which varies with age. Because our CF population was young, we used the age dependent hydration factors described by Fomon (8) for children younger than 10 year and by Boileau and Lohman (9) for older children.

### 2. 3. 2 Bromide space

Because Bromide resides mainly in the extracellular space, measured of Bromide dilution in serum give us an estimation of the extracellular volume. Bromide was determined by using a Gas Chromatograph type CP 9000 (Chrompack) equipped with an ECD detector after it was converted into a bromoacetone gas. First, perchloric acid was added to the serum sample and centrifugated for deproteinisation. An aliquot of the supernatant was then added to silver nitrate ( $\text{AgNO}_3$ ) for precipitating of silver bromide and chloride. After centrifugation, the precipitate was taken up in  $\text{NH}_3$  after that  $\text{Na}_2\text{S}$  and  $\text{NaOH}$  were added to precipitate the silver as  $\text{Ag}_2\text{S}$ . After agitation and centrifugation, the supernatant was heated until dry,  $\text{H}_2\text{O}$  was added followed by  $\text{H}_2\text{O}_2$  to oxidize sulfide. After drying,  $\text{H}_2\text{O}$  was then added and dried again. This was repeated several times. Thereafter, perchloric acid and acetone were added and the reaction is started by addition of  $\text{KmnO}_4$  with Bromoacetone formed. The solution is then extracted with benzene. The organic phase was separated from the water phase by shaking and centrifugation. The water phase was then removed. An aliquot of the organic solution is then applied to the gas chromatograph for measuring of bromoacetone/internal standard ratio. The bromide concentration was then derived from the bromoacetone standard curves. Because the distribution of Bromide depend on the potential difference between in- and extra-cellular and on the quantity of total body volume, corrected bromide space was calculated as follow:

$$\text{ECV (L)} = \frac{\text{Bromide administered (mmol)}}{\text{Bromide change T4 - T0 (mmol/L)}} - 0,036\text{TBW}$$

Where 0,036TBW is the correction factor for the cell potential and for the total body volume (7).

## REFERENCES

- (1) M. Khouri, G. Huang, Y. Shiau. Sudan stain of fecal fat : New insight into an old test. *Gastroenterology* 1989; 96: 421-7.
- (2) G. Drummey, J. Benson, C. Jones. Microscopical examination of the stool for steatorrhea. *N Engl J Med* 1961; 264: 85-7.
- (3) W. Gerver, R. de Bruin. *Paediatric Morphometrics: a reference manual*. 1th ed. Utrecht: Bunge, 1996.
- (4) J. Weststrate, P. Deurenberg. Body composition in children: proposal for a method for calculating body fat percentage from total body density or skinfold-thickness measurements. *Am J Clin Nutr* 1989; 50: 1104-15.
- (5) S. Heymsfield, J. Wang, S. Heshka, J. Kehayias, R. Pierson. Dual-photon absorptiometry: comparison of bone mineral and soft tissue mass measurements in vivo with established methods. *Am J Clin Nutr* 1989; 49: 1283-9.
- (6) B. Van Kreei, F. Van der Vegt, M. Meers, T. Wagenmakers, K. Westerterp, A. Coward. Determination of total body water by a simple and rapid mass spectrometric method. *J Mass Spectrom* 1996; 31: 108-111.
- (7) B. Van Kreei. An Improved bromide assay for the estimation of extracellular water volume by capillary gas chromatography. *Clinica Chimica Acta* 1994; 231: 117-128.
- (8) S. Fomon, F. Haschke, E. Ziegler, S. Nelson. Body composition of reference children from birth to age 10 years. *Am J Clin Nutr* 1982; 35: 1169-1175.
- (9) Boileau, R. Lohman, M. Slaughter, T. Ball, S. Going, M. Hendrikx. Hydration of the fat-free body in children during maturation. *Hum Biol.* 56: 651-666.

## CHAPTER 3

### THE ACID STEATOCRIT : A MUCH IMPROVED METHOD

Tran M, Forget P, Van den Neucker A, Strik J, van Kreel B, Kuijten R.

Departments of Pediatrics and Clinical Chemistry, University Hospital Maastricht,  
Maastricht, The netherlands.

---

J Pediatr Gastroenterol Nutr 1994; 19: 299-303

#### *Abstract*

The steatocrit method has recently been introduced as a simple screening test for steatorrhea. As it seemed likely that separation of fecal homogenate by centrifugation into a lipid phase, a watery phase and a solid phase would be pH-dependent, we evaluated the effect of fecal acidification on steatocrit results in healthy children and in patients with cystic fibrosis and studied the relationship between two steatocrit methods and fecal fat content as measured by a reference chemical method. Steatocrit results increased with the degree of fecal acidification, and maximal results were obtained at the lowest fecal pH values. Means and SEM for classical and acid steatocrit values were  $1.1 \pm 0.4\%$  (classical) versus  $3.8 \pm 1\%$  (acid) in controls ( $n = 6$ ) and  $5.4 \pm 1.9\%$  (classical) versus  $26.9 \pm 4.3\%$  (acid) in cystic fibrosis ( $n = 9$ ). The correlations between fecal fat content measured chemically and steatocrit results were 0.18 ( $p = 0.35$ ) and 0.81 ( $p < 0.0001$ ) for classical and acid steatocrit, respectively. We conclude that acidification of fecal homogenates leads to a marked improvement in the steatocrit method.

## INTRODUCTION

The diagnosis of fat malabsorption still mainly relies on the 72-hour faecal fat quantitation in which daily stool fat loss is evaluated by collecting stools for 3 days and determining stool fat content by chemical methods. The most widely used chemical method is the titrimetric method as described by van de Kamer in 1949 (1).

Work by Kouri et al has suggested that the titrimetric method largely overestimates nutritional faecal fat losses because it measures not only malabsorbed exogenous fat but also endogenous fat of various origins such as biliary lipids and lipids derived from the turnover of intestinal epithelial cells and gut bacteria (2).

Making use of the staining properties of purified lipids in an artificial matrix, Khouri et al. have suggested the fat absorption coefficient in normal adults is much higher than usually believed (2). Although the microscopic evaluation of steatorrhea by the Sudan stain provides a satisfactory screening method for steatorrhea, it is at best semiquantitative.

The steatocrit has been introduced in recent years as a simple test for the evaluation of fat malabsorption (3-6). Although several authors have reported the method to be satisfactory for the evaluation of steatorrhea, some have reported the steatocrit to be quite unreliable (6).

As it has been shown that faecal acidification results in an enhanced sensitivity of the Sudan faecal staining method (2), we wondered whether the same modification could improve fat extraction by centrifugation as performed in the steatocrit determination.

Consequently, we evaluated the effects of stool sample acidification on steatocrit determinations and to compare results from previously reported methods with acid steatocrit results in healthy children and in children with cystic fibrosis. We also determined the correlation between steatocrit results and faecal fat concentrations as measured by the reference chemical method of van de Kamer et to evaluate which of the two steatocrit methods gave the best estimate of faecal fat content.

## METHODS

### "classic" steatocrit method

Stool ( 0.5 gr ) was diluted (1/3) with deionized water and thoroughly homogenized in a 5 ml Potter Elvehjem tissue homogenizer (Heidolph Elektro KG Kelheim, no. 170-1700/20-200) stamper, tissue grind pestle (size 20 from Kontes Scientific Glassware Instruments, no. 885451-0020). The homogenate was aspirated into a 75 µl plain glass haematocrit tube. The capillary tube was subsequently centrifuged horizontally (13,000 rpm for 15 min) in a standard haematocrit centrifuge.

After centrifugation, the upper (fat) and bottom (solid) layers were measured with a graduated magnifying lens. Steatocrit was calculated as  $FL/(FL + SL)$ , where FL is the fatty-layer length and SL is the solid-layer length.

#### "Acid" steatocrit method

The method used was exactly the same as the classic steatocrit method except that, before aspirating the homogenate in the capillary tube, perchloric acid in various concentrations (5N for maximal acidification) was added to the homogenate in a volume equal to 1/5 of the homogenate volume. The resulting acid homogenate was mixed for 30 seconds with a standard Vortex mixer.

#### Chemical determination of stool fat concentration.

The method of van de Kamer et al. was used to determine stool fat content (1).

## EXPERIMENTAL DESIGN

#### Effect of stool homogenate acidification on fat extraction

To evaluate the effect of stool acidification and thus stool pH on the length of the fat column obtained by centrifugation, several stool samples from patients with and without steatorrhea were centrifuged after addition of perchloric acid solutions of various concentrations.

### Classic and acid steatocrit

To compare classic and acid steatocrit results in children with and without steatorrhea, we measured fecal steatocrit by both methods in 6 control children (mean age: 5.8 years, range 3 to 12 years; five boys and one girl) and in 9 children with cystic fibrosis (mean age: 6.9 years; range 0.5 to 20; nine boys). The control children were patients with chronic aspecific respiratory disease without gastrointestinal symptoms and with a normal sweat test. The cystic fibrosis patients all had abnormal sweat tests on several occasions and were being treated with pancreatic enzymes when steatocrit determinations were performed. As our purpose was to compare classical and acid steatocrit results in the same fecal samples, no attempt was made to quantify the fat content of the diet which was "normal" in all patients.

### Correlation between steatocrit results and fecal fat content

To further compare both steatocrit methods we looked at the relationship between results obtained by each method and fecal fat content results as measured by the method of van de Kamer et al. (1). Steatocrit measurements (classic and acid) and fecal fat content determinations (chemical method) were performed on 27 consecutive stool samples (from adults and children) sent to our laboratory for evaluation of malabsorption. No attempt was made to classify patients in disease categories as our only goal was to study the relationship between steatocrit results and fecal fat content independent of the presence of disease (clinical results will be published separately).

### Statistical methods

The coefficient of variation of each steatocrit method was determined with duplicate results of each sample for both methods. Pearson correlation coefficient was used to evaluate the relationship between steatocrit results and chemically measured fecal fat content.

## **RESULTS**

Effect of stool homogenate acidification on fat extraction

Several steatorrheal stool samples were analysed after acidification with various concentrations of perchloric acid.

A typical finding is shown in figure 1; The upper fat column was seen to increase in length with the degree of homogenate acidification. A typical normal stool sample result (no steatorrhea) is shown in figure 2. The acid steatocrit remained completely negative in normal samples.

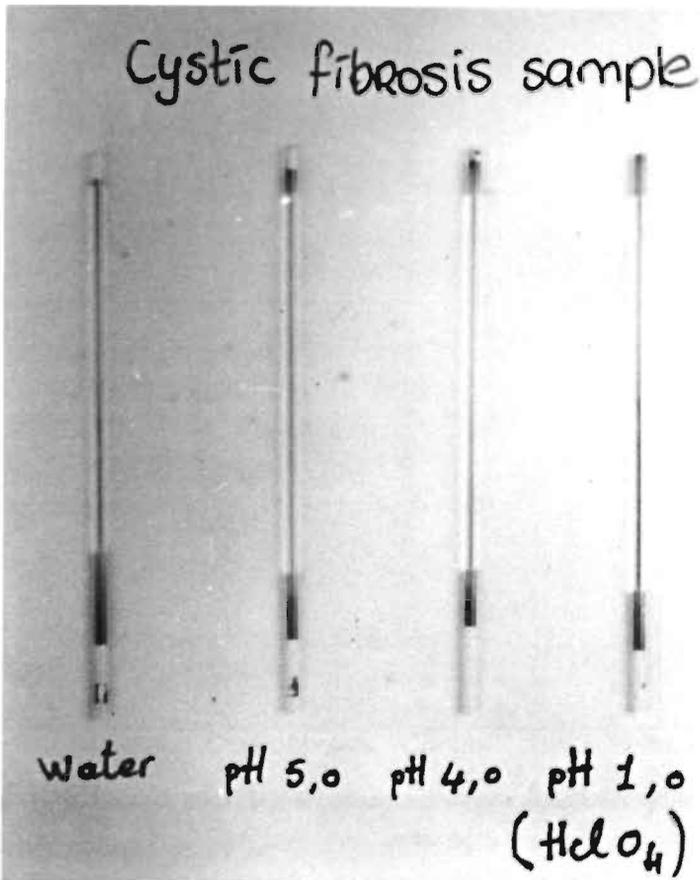
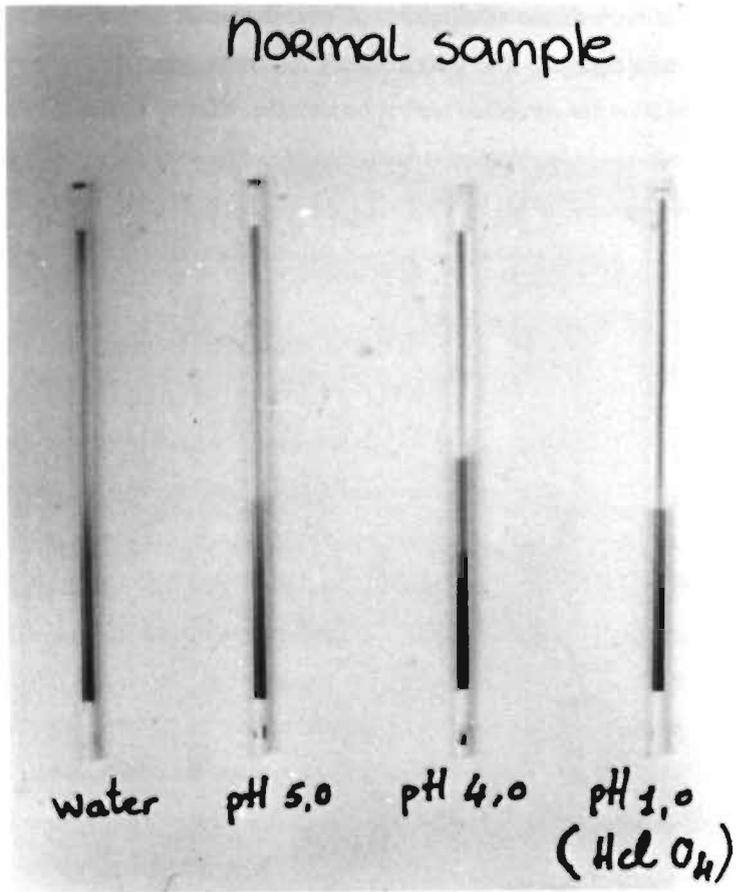


Figure 1 Effect of acidification with various concentrations of perchloric acid on the fat column length (upper part of picture) of a stool sample from a patient with cystic fibrosis.



**Figure 2** Effect of acidification with various concentrations of perchloric acid on fat extraction from a normal stool sample. Fat layer is absent at all pH values.

### Classic and acid steatocrit

Results of classic and acid steatocrit in 6 control and 9 cystic fibrosis patients (figure 3) were as follows : Steatocrit means and SEM in control patients were  $1.1 \pm 0.4$  and  $3.8 \pm 1\%$  for classic and acid steatocrit, respectively. This difference was not statistically significant. Steatocrit means and SEM in cystic fibrosis patients were  $5.4 \pm 1.9$  and  $26.9 \pm 4.3 \%$  for classic and acid steatocrit, respectively. This difference is significant ( $p < 0.01$ )

The precision of the methods was evaluated by comparing the variation coefficients; variation coefficients were 6.9 and 5.1 % for the classic and acid steatocrit methods, respectively.

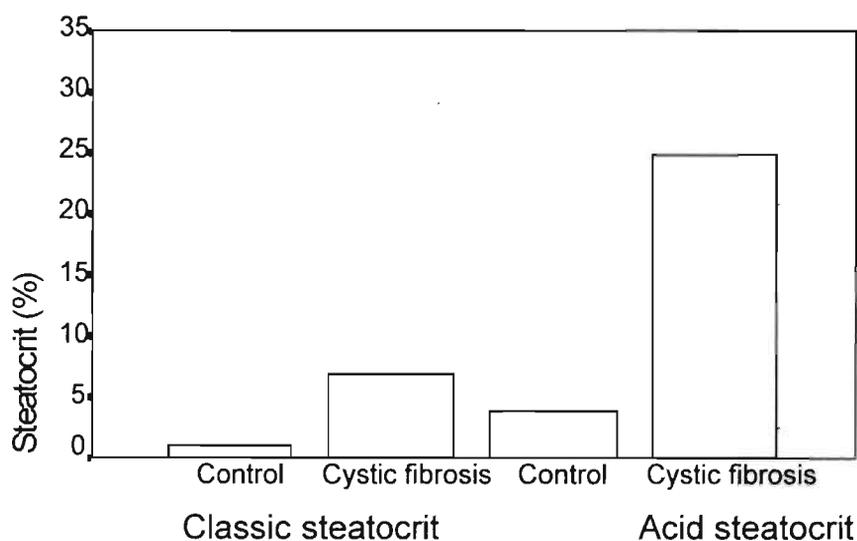
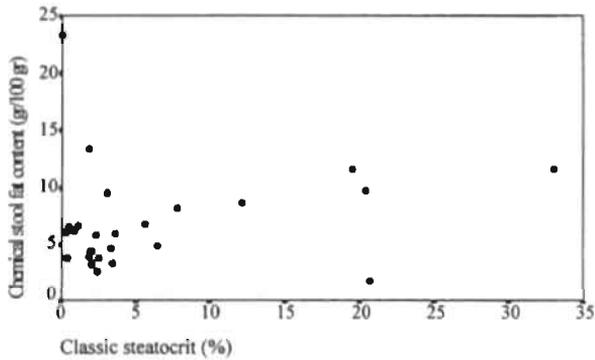


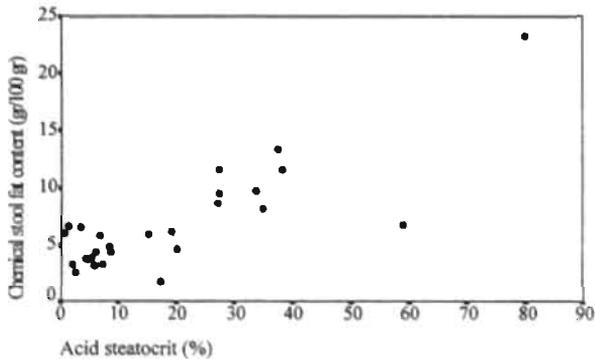
Figure 3 Classic and acid steatocrit results in six controls and nine patients with cystic fibrosis.

Correlation between steatocrit results and faecal fat content

The relationship between classic fecal steatocrit and fecal fat content as measured by the reference method of van de Kamer et al. (1) is shown in figure 4. The correlation coefficient of 0.18 is statistically non-significant ( $p = 0.35$ ). The relationship between acid fecal steatocrit and fecal fat content is shown in figure 5. The correlation coefficient of 0.81 is highly significant ( $p < 0.0001$ ).



**Figure 4** Relationship between classic fecal steatocrit and fecal fat content as measured by the method of van de Kamer et al. In 27 fecal samples ( $r = 0.18$ ;  $p = 0.35$ ).



**Figure 5** Relationship between acid fecal steatocrit and fat content as measured by the method of van de Kamer et al. In 27 fecal samples ( $r = 0.81$ ;  $p < 0.0001$ ).

## DISCUSSION

Although several authors have reported the steatocrit method to be reliable for the screening of steatorrhea ( 3-5 ), Walters et al reported the method to be completely unreliable (6). Methodological inadequacies probably underlie these discrepancies. We have been using the "classic" steatocrit in our department for a few years and have found completely negative results in some patients with proven steatorrhea. We hypothesized that in some patients fat detection might be poor and that a possible solution to the problem would be an improved method of liberating fat during the centrifugation step. It has been shown in a recent study that fecal fat in patients with pancreatic insufficiency mainly consists of fatty acids and that the fecal triglyceride content does not differ from that of normal controls (7).

Fecal fatty acid molecules exist in the form of soaps (8). Further, since the pKa of most fatty acids is lower (about 4.8) than fecal pH, most fatty acids in stool would be present as ionized species or soaps. We speculated that fecal acidification would result in the conversion of ionized fatty acid species and soaps into the protonated species leading to easier separation into lipid and water phases during the centrifugation step of the steatocrit method.

Our results show that the effect of stool homogenate acidification on the length of the upper fatty layer very nicely confirms our predictions. Although we have not checked this point in detail, it can be expected that at the low PH values obtained after maximal acidification as performed in the present study, all fatty acids will be present in the protonated form.

Further, the fact that acidification of fecal samples from patients without steatorrhea and with completely negative steatocrit results did not result in the appearance of a fatty layer, probably indicating that the improved fat extraction is not a spurious artifactual finding but the result of better extraction of lost exogenous fat.

Khouri et al have suggested that ionized fatty acids are not readily stainable with Sudan stain, although staining does occur after acidification (2). By alkalization with sodium hydroxide, the same authors showed that fatty acids lost their ability to form fat droplets and to stain with Sudan red III (2). We suppose that similar mechanisms underlie the improvement of both the fat staining method and fat extraction by fecal acidification as shown in the present study.

A further advantage of acidification is that it enhances the visible boundaries between the

various layers, resulting in improved accuracy in the reading of layers lengths. Improved fecal fat extraction by acidification should therefore result in higher diagnostic sensitivity of the steatocrit method.

Our results show classic steatocrit in control children and in children with cystic fibrosis are similar to results published by other authors (4); However, acidified steatocrit results in both control children and cystic fibrosis patients were much higher than those obtained by classic steatocrit. Ongoing work in our laboratory aims at establishing normal population values for acid steatocrit in infants and children.

In order to better interpret the differences found between the steatocrit methods, we compared steatocrit results with fecal fat concentrations measured by the most accepted reference method. Our findings show that only acid steatocrit results correlate very significantly with fecal fat content as measured by the van de Kamer method. The literature is quite varied on this point. Several studies have looked for a correlation between steatocrit results and either the fat absorption coefficient or 3-day fecal fat excretion. A good correlation was reported by two studies (4,9) while a total lack of correlation was reported by a third author (6). As steatocrit is supposed to reflect fecal fat concentration we preferred to relate steatocrit results to fecal fat concentrations rather than daily excretion or fat absorption coefficients. To our knowledge only one study reporting results in a similar way found a significant relationship between steatocrit results and fecal fat content (3). We think our finding of a lack of correlation between classic steatocrit and fecal fat content results can best be explained by the small number of observations or by the lack of homogeneity in our patient material.

This lack of homogeneity was, however, purposely chosen as we were only interested in the correlation between steatocrit results and fecal fat content. We think a positive correlation between the two steatocrit methods and fecal fat content could have been found but the acid steatocrit method would always better correlate with fecal fat content.

We conclude that acidification of fecal homogenates led to a much better fat extraction by centrifugation, increased sensitivity of the steatocrit method and to a better prediction of fecal fat content as measured by chemical methods.

*Acknowledgment:* The authors thank the clinical laboratory staff for their kind and expert technical assistance. We are very grateful to Nutricia Netherlands for financial support.

## REFERENCES

- (1) van de Kamer JH, Huinink HTB, Weyers HA. Rapid method for determination of fat in feces. *J Biol Chem* 1949 ; 177 :349-55.
- (2) Khouri MR, Huang G, Shiau YF. Sudan stain of fecal fat : new insight into an old test. *Gastroenterology* 1989 ; 96 : 421-427.
- (3) Phuapradit P, Narang A, Mendonca P, Harris DA, Baum JD. The steatocrit : a simple method for estimating stool fat content in newborn infants. *Arch Dis Child* 1981 ; 56 : 725-727.
- (4) Colombo C, Maiavacca R, Ronchi M, Consalvo E, Amoretti M, Giunta A. The steatocrit : a simple method for monitoring fat malabsorption in patients with cystic fibrosis. *J Pediatr Gastroenterol Nutr* 1987 ; 6 : 926-930.
- (5) Iacono G, Carroccio A, Cavataio F et al. Steatocrit test : normal range and physiological variation in infants. *J Pediatr Gastroenterol Nutr* 1990 ; 11 : 53-57.
- (6) Walters MP, Kelleher J, Gilbert J, Littlewood JM. Clinical monitoring of steatorrhea in cystic fibrosis. *Arch Dis Child* 1990; 65 : 99-102.
- (7) Khouri MR, Huang G, Shiau YF. Fecal triglyceride excretion is not excessive in pancreatic insufficiency. *Gastroenterology* 1989; 96 : 848-852.
- (8) Shiau YF, Popper DA, Reed M, Umstetter C, Capuzzi D, Levine GM. Intestinal triglycerides are derived from both endogenous and exogenous sources. *Am J Physiol* 1985; 248 : G164-169.
- (9) Guarino A, Tarallo L, Greco L, Cesarano L, Guandalini S, Rubino A. Reference values of the steatocrit and its modifications in diarrheal diseases. *J Pediatr Gastroenterol Nutr* 1992; 14: 268-274.

## CHAPTER 4

### IMPROVED STEATOCRIT RESULTS OBTAINED BY ACIDIFICATION OF FECAL HOMOGENATES ARE DUE TO IMPROVED FAT EXTRACTION

M. Tran, P. Forget, A. Van den Neucker and B. Van Kreel

Department of Pediatrics and Clinical Chemistry, University Hospital Maastricht,  
Maastricht, The Netherlands

---

*J Pediatr Gastroenterol Nutr* 1996; 22: 157-160

#### *Abstract*

Conflicting results have been reported on the value of the steatocrit as a screening test for steatorrhea. We recently modified the test procedure by fecal acidification with the hope of improving fat extraction and consequently the sensitivity of the test. The aim of the present study was to ascertain, whether or not fecal acidification led to improved fat extraction, by comparing the fat content of both fatty and solid layers obtained by centrifugation of 12 acidified (acid steatocrit) and unacidified (classical steatocrit) steatorrheal stool samples.

The fat content of fatty and solid layers was evaluated using of the semiquantitative (+ = 1, ++ = 2, +++ = 3) scoring system described by Drummey, for the interpretation of the sudan microscopic method for fecal fat.

The fatty layers sum of scores for the 12 samples examined, was 31 and 16, for the acid and classical steatocrit respectively. The solid layers sum of scores for the 12 samples, was 13 and 24, for the acid and classical steatocrit respectively. Fat extraction from stool samples was significantly improved after fecal sample acidification ( $p < 0.005$ ). Acid steatocrit results agreed better with chemically measured fecal fat than classical steatocrit results.

We conclude that fecal acidification, by improving fat extraction, increases the reliability of the steatocrit method for the detection of steatorrhea.

## INTRODUCTION

Several methods are in use for the diagnosis of fat malabsorption. One of these is the 72 hour fecal fat quantitation method, which is regarded as the most accurate method to evaluate steatorrhea (1). However, there are several problems. First, it is a laborious method for laboratory technicians, and second, fecal collection for 3-5 days makes the method unpleasant for the patient and sometimes poorly reliable in non collaborating children.

Another well accepted test to screen for fat malabsorption is the Sudan staining method for fecal fat (2). Unfortunately this method is only semiquantitative.

In 1981 Phuapradit introduced the steatocrit method as a new, simple and easily repeatable method for measuring fecal fat content (3).

Although several authors have reported this method to be satisfactory for the evaluation of steatorrhea (3-5), some described it as quite unreliable (6). We have been using this method for years and have often found normal steatocrit values in patients, who, when measured chemically had steatorrhea with an increased fecal fat content.

As it has been shown that fecal acidification results in an enhanced sensitivity of the Sudan fecal staining method (7), we wondered whether fecal acidification could also be used to improve the sensitivity of the steatocrit method.

We consequently modified the reported (8) steatocrit method by adding perchloric acid to the fecal homogenate. Fat extraction was evaluated for classical and acid steatocrit methods, making use of the Sudan microscopic method for fecal fat.

We further compared calculated steatocrit results from acidified and unacidified samples, and related the results to fecal fat content of the same samples, measured by the reference chemical method of van de Kamer et al. (1).

## MATERIALS AND METHODS

### *Population studied*

Twelve stool samples from 4 premature babies, 3 boys and 1 girl, with a mean gestational age of 35,3 weeks (ranged from 27 5/7 to 35 5/7 weeks), were analysed by means of both

the classical and the acid steatocrit method.

Their postnatal age varied between 11 and 18 days. They received full oral formula feedings. Their weight ranged from 1810 g to 2360 g.

### Steatocrit methods

0,5 g solid stool was weighed and diluted with a volume of deionized water, equal to two times the weight of stool. The stool and water were premixed using a Vortex mixer. Subsequently, the mixture was homogenized using a 5 ml Potter Elvehjem tissue homogenizer. Then, the homogenate was aspirated into a 75  $\mu$ l plain haematocrit capillary. This capillary was sealed with wax at one end and centrifuged horizontally (13,000 rpm, 15 min) in a standard haematocrit centrifuge.

The method used for the acid steatocrit was exactly the same as that of the classical steatocrit, the only exception being, that after homogenizing, 5N perchloric acid was added to the homogenate in a volume equal to 1/5 of the homogenate. This acid homogenate was then mixed for 30 seconds using a Vortex mixer.

After centrifugation, three layers were distinguished: a basal solid layer, an intermediate liquid layer, and an upper fatty layer. After calculating the steatocrit results for both methods as usual, the capillaries were cut in the middle of the fatty, and of the solid layers using a special glass knife. Subsequently, the layers were removed from the capillaries, using a syringe. A standard amount of each of these fatty and solid layers was then transferred to different glass slides for staining with Sudan III dye. In this way, we acquired a total of 48 slides (24 from each steatocrit method, 12 fatty and 12 solid layers) for microscopic evaluation.

### Sudan stain method for fecal fat

For this purpose we used the split fat stain, which identifies both triglyceride and fatty acid (7). Several drops of 100% acetic acid and several drops of Sudan III solution were added. The preparation was subsequently mixed with the coverslip, which was then applied. The slide was gently heated on a lighter until bubbling. All preparations were examined while

still warm under high magnification (magnification of 400), by the same person who was blind to the steatocrit method used (classical or acid).

For quantification of the amount of fat detected microscopically, we used the criteria established by Drummey et al.(2). They are as follows: normal (+): up to 100 fat globules per high power field, varying in a diameter between 1 and 4  $\mu\text{m}$ , as noted on the micrometer scale always using a magnification of 400; Increased (2+): up to 100 fat globules per high power field, the diameter of fat globules varying between 1 and 8  $\mu\text{m}$ ; and markedly increased (3+): more than 100 fat globules per high power field, varying in size from 6 to 75  $\mu\text{m}$  in diameter.

The sum of the fatty and solid layer scores of all our samples, was calculated for both steatocrit methods, and results were compared. Fisher's exact probability test was used to test whether or not the solid layers microscopic fat content was dependent on sample acidification. Finally, calculated acidified and unacidified steatocrit results were compared and related to the chemically measured fecal fat content.

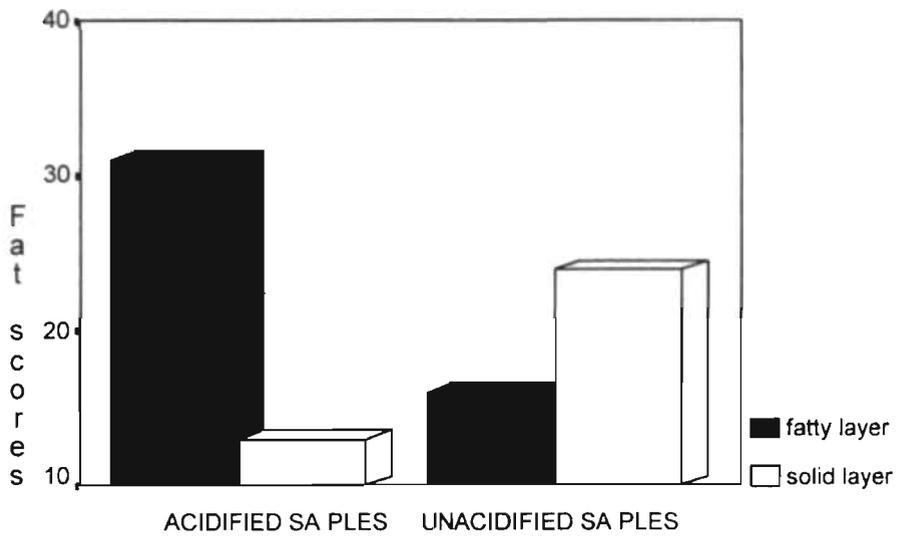
## RESULTS

Table 1 shows that acidification of the fecal homogenates before centrifugation (acid steatocrit method) results in a higher Drummey score in the fatty layers and a lower score in the solid layers. In four specimens (sample 6, 7, 8 and 10), the fatty layers obtained by the classical steatocrit method were so small that we did not succeed in making microscopical slides. Equal results were obtained by both the classical and the acid steatocrit method, in only one sample (sample 11).

**Table 1 Fatty and solid layer microscopical fat scores for both acid and classic steatocrit methods.**

SAMPLES	SCORES OF FAT GLOBULES (+, ++, +++)			
	FATTY LAYERS		SOLID LAYERS	
	ACID	CLASSIC	ACID	CLASSIC
2	+++	++	+	+
3	++	++	+	+++
4	+++	+	+	+++
5	+++	++	+	+++
6	+	-	+	++
7	++	-	+	++
8	+++	-	+	+
9	+++	+++	+	++
10	++	-	+	+
11	+++	+++	+	+
12	+++	++	+	+++
<b>SUM</b>	<b>31</b>	<b>16</b>	<b>13</b>	<b>24</b>

The sum of the fat scores for fatty and solid layers, and for both steatocrit methods is summarized in figure 1. The number of solid layer samples with low microscopic ( $\leq +$ ) fat content, was 11 of 12, and 4 of 12, for the acidified and unacidified samples respectively ( $p < 0.005$ , Fisher's exact probability test).



**Figure 1** Sum of 12 microscopical Sudan fat globule scores (1, 2, 3) performed on fatty and solid layers of acidified and unacidified fecal samples.

The calculated steatocrit results for both steatocrit methods, and the chemically measured fecal fat content for the 12 samples, are shown in table 2. Chemical fat measurements of two samples (1 and 2) were not performed. The chemically measured fecal fat concentration was very high in all samples and corresponded with high acid steatocrit results, while only 5 classical steatocrit results were elevated.

**Table 2 Classic and acid steatocrit results compared to chemically measured fecal fat in 12 steatorrheal fecal samples.**

SAMPLES	CLASSIC STEATOCRIT (%)	ACID STEATOCRIT (%)	FECAL FAT (GRAM %)
1	5.3	81.7	-
2	2.5	72.4	-
3	5.3	71.1	16.6
4	28.8	93.3	28.5
5	26	90.9	26.7
6	6.2	92.6	28.3
7	3.1	92.5	18.7
8	2.8	94.2	26.5
9	59.8	93.7	24.3
10	6.2	96.4	10.3
11	63.9	96	27.3
12	48.7	94.4	20.6

## DISCUSSION

There has always been a need for a simple, rapid and easy to perform screening test for fat malabsorption. Such a test would not only be useful for the detection of steatorrhea but also for the therapeutic monitoring of children treated for pancreatic insufficiency.

The steatocrit is a simple and rapid micromethod that can be repeated at short time intervals

(3). It is inexpensive and not invasive (5). Some authors have reported it as a very satisfactory screening test (3-5), but others have found it quite unreliable (6). Our experience with the method has shown the steatocrit to often be normal, in fecal samples with a very high chemically measured fecal fat content. This could be due to inefficient fecal fat extraction at the centrifugation step. Therefore we recently improved the steatocrit method by acidifying the fecal homogenate before centrifugation (8).

The present study was set up to study the effect of fecal homogenate acidification on fat extraction at the centrifugation step. If fat extraction improves by acidification, we would expect to find more fat globules in the fatty layer and less in the solid layer, after centrifugation of acidified fecal samples, when compared to unacidified samples. Our results are in agreement with our expectations and support the hypothesis that fecal acidification improves fat extraction, and should consequently improve the sensitivity of the steatocrit. Due to various reasons, the fat content of premature infants' stool, is known to be very high. Confirming the latter, chemical fat measurements of all our samples from 4 premature babies showed very high values. The acid steatocrit seems to reflect these very high fat contents, while classical steatocrit results were high in only 5 of 12 samples. The correlation between chemical fat measurement and acid steatocrit has been reported previously (8). Such a correlation cannot be shown in the present study where only high-fat-content stools were evaluated.

Results of the present study do support our previous findings, confirming, that fecal acidification improves fat extraction at the centrifugation step, and consequently increases the reliability of steatocrit results for the detection of fat malabsorption. Because the Sudan staining method for fecal fat is only semiquantitative, we suggest using the acid steatocrit as a good alternative to chemical fat measurement.

*Acknowledgement:* The authors wish to thank the clinical laboratory staff for their kind and expert technical assistance. We are very grateful to Nutricia Netherlands for financial support.

## REFERENCES

- (1) Van de Kamer JH, Huinink HTB, Weyers HA. Rapid method for determination of fat in feces. *J Biol Chem* 1949; 177: 349-55.
- (2) Drummey GD, Benson JA, Jones CM. Microscopical examination of the stool for steatorrhea. *N.Engl J Med* 1961; 264: 85-7.
- (3) Phuapradit P, Narang A, Mendonca P, Harris DA, Baum JD. The steatocrit: a simple method for estimating stool fat content in newborn infants. *Arch Dis Child* 1981; 56: 725-727.
- (4) Iacono G, Carroccio A, Cavataio F, Montalto G, Mancusco C, Balsamo V et al. Steatocrit test: normal range and physiological variation in infants. *J Pediatr Gastroenterol Nutr* 1990; 11: 53-57.
- (5) Columbo C, Maiavacca R, Ronchi M, Consalvo E, Amoretti M, Giunta A. The steatocrit: a simple method for monitoring fat malabsorption in patients with cystic fibrosis. *J Pediatr Gastroenterol Nutr* 1987; 6 : 926-930.
- (6) Walters MP, Kelleher J, Gilbert J, Littlewood JM. Clinical monitoring of steatorrhea in cystic fibrosis. *Arch Dis Child* 1990; 65: 99-102.
- (7) Khouri MR, Huang G, Shiao YF. Sudan stain of fecal fat: new insight into an old test. *Gastroenterology* 1989; 96: 421-427.
- (8) Tran M, Forget P, Van den Neucker A, Strik J, van Kreel B, Kuijten R. The acid steatocrit: a much improved method. *J. Pediatr. Gastroenterol Nutr.* 1994; 19: 299-303

## CHAPTER 5

### CLINICAL USE OF ACID STEATOCRIT

A. Van den Neucker<sup>1</sup>, N. Pestel<sup>1</sup>, T. My Dung Tran<sup>1</sup>, P.Ph. Forget<sup>1</sup>, H.J. Veeze<sup>2</sup>,  
J. Bouquet<sup>2</sup>, M. Sinaasappel<sup>2</sup>.

<sup>1</sup>Department of Pediatrics, University Hospital Maastricht and <sup>2</sup>Sophia Children's Hospital  
Rotterdam, The Netherlands

---

Submitted for publication

#### *Abstract*

Malabsorption of fat is an important gastrointestinal cause of malnutrition and growth retardation in childhood. The golden standard for the evaluation of fat malabsorption is the fecal fat balance method. The acid steatocrit method has recently been introduced as a simple method to evaluate fecal fat. The present study aimed at evaluating the acid steatocrit in clinical practice. Fecal fat excretion and acid steatocrit results were determined in 42 children, half with and half without fat malabsorption. Acid steatocrit results correlated significantly with both fecal fat excretion ( $p < 0.01$ ) and fecal fat concentration ( $p < 0.001$ ). Sensitivity and specificity of the acid steatocrit for the diagnosis of malabsorption was 90% and 100% respectively. We consider the acid steatocrit method useful for the screening and monitoring of patients with steatorrhea. Acid steatocrit, Steatorrhoea, Cystic Fibrosis.

## INTRODUCTION

Malabsorption of fat is the most important gastrointestinal cause of malnutrition and growth retardation in childhood. The detection of steatorrhea is useful for the diagnosis of intestinal and pancreatic disease. The gold standard for the evaluation of patients suspected of fat malabsorption is the fat balance method whereby fecal fat is chemically measured according to the method of van de Kamer (1). This method is cumbersome, laborious, expensive and often difficult to manage in children. In 1981 Phuapradit et al. introduced a simple test to evaluate fecal fat content (2). Although some authors found this test quite reliable (3), others did not (4). As previously reported, substantial improvement of the method was obtained by acidification of fecal samples, acid steatocrit (AS) (5).

The present study was designed to compare the fecal fat excretion with the acid steatocrit for the diagnosis of fat malabsorption in children.

## PATIENTS

Forty two children, 23 boys and 19 girls, aged between 6.5 months and 18 years (mean: 6.6 years) were selected for the study. All these children were suspected of fat malabsorption, on the basis of anamnestic and clinical parameters. The different diagnoses of our patients are shown in table 1.

## METHODS AND MATERIAL

Three days stool collections from each patient were collected in separate containers, one container for each day. The stools were frozen at  $-18^{\circ}\text{C}$  Celsius. Fat content in each collection was determined by the titrimetric method described by van de Kamer et al. (1). Acid steatocrit from a single stool sample on day 1 and from a sample out of the homogenized 72 hours collection were determined by the method of Tran et al. (5) Feces (0.5 gr.) was diluted (1/4) with deionized water and thoroughly homogenized making use of a 5ml. Potter Elvehjem tissue homogenizer. Perchloric acid 5N was added to the homogenate in a volume equal to 1/5 of the homogenate volume. The resulting acid homogenate was mixed for 30 seconds making

**Table 1 List of diagnosis (n = 42).**

DIAGNOSIS	NUMBER OF CASES
CYSTIC FIBROSIS	20
MENTAL RETARDATION	2
RECURRENT DIARRHEA	5
FAILURE TO THRIVE	5
COELIAC DISEASE	2
INFLAMMATORY BOWEL DISEASE	1
SHORT BOWEL	1
CHOLEDOCHAL CYSTE	1
SUCRASE-ISOMALTASE DEFICIENCE	1
RECURRENT ABDOMINAL PAIN	1
UNKNOWN	3

use of a standard Vortex mixer. The homogenate was aspirated into a 75  $\mu$ l plain glass haematocrit capillary. The capillary was subsequently centrifuged horizontally (13000 rpm. for 15 min.) in a standard centrifuge. After centrifugation, the lengths of the upper (fat) and the bottom (solid) layers were measured by means of a graduated magnifying lens. Steatocrit was calculated as follows: percentage of (the fatty layer length / (fatty layer length + solid layer length)).

In order to validate the diagnostic value of the acid steatocrit we studied two patients groups, one with and one without steatorrhea. We divided the patients according to previous clinical data and fat excretion results, whereby a fecal fat excretion  $\geq$  3gr./day was considered as abnormal (6).

## RESULTS

Correlation coefficients between acid steatocrit results from either a single stool sample or from the sample taken from the 72 hours homogenized collection, and both the fecal fat excretion and the fecal fat concentration are shown in table 2.

**Table 2** Correlation between the results of the acid steatocrit from either a single stool sample and a sample from the homogenised stool collection and the results of both fat excretion and fecal fat concentration in 42 children suspected of malabsorption.

ACID STEATOCRIT	FAT EXCRETION	FAT CONCENTRATION
SINGLE STOOL	$r = 0.4 (p \leq 0.01)$	$r = 0.82 (p \leq 0.001)$
COLLECTION	$r = 0.68 (p \leq 0.001)$	$r = 0.82 (p \leq 0.001)$

The sensitivity and the specificity of the acid steatocrit determination from either a single stool sample or a sample taken from the 72 hours homogenized collection, and of the fecal fat concentration, for the diagnosis of steatorrhea are shown in table 3.

**Table 3** Sensitivity and specificity of the acid steatocrit determination from a single stool sample and from a homogenised stool collection sample and of the fecal concentration, for the diagnosis of steatorrhea.

	SENSITIVITY	SPECIFICITY
AS SINGLE STOOL (%)	75%	84%
AS COLLECTION (%)	90%	100%
FAT CONCENTRATION (%)	100%	76%

AS: Acid steatocrit

Fig.1 shows our AS results from the homogenized fecal collection sample related to the fecal fat excretion (g/day). The reference line for AS was set at the level of 10% (5), and the cut off reference line for the daily fat excretion was set at the level of 3 gram per day (6). As can be seen from the figure, one false positive and three false negative acid steatocrit results were found in our study population. Regarding these results one should notice that they are very close to the reference lines: the false positive steatocrit result had a value of 16% and the results of the fecal fat excretion corresponding to the false negative steatocrit results were 4.9; 6.4 and 7.7g/day, and concern children aged 12. 6 and 13 years respectively.

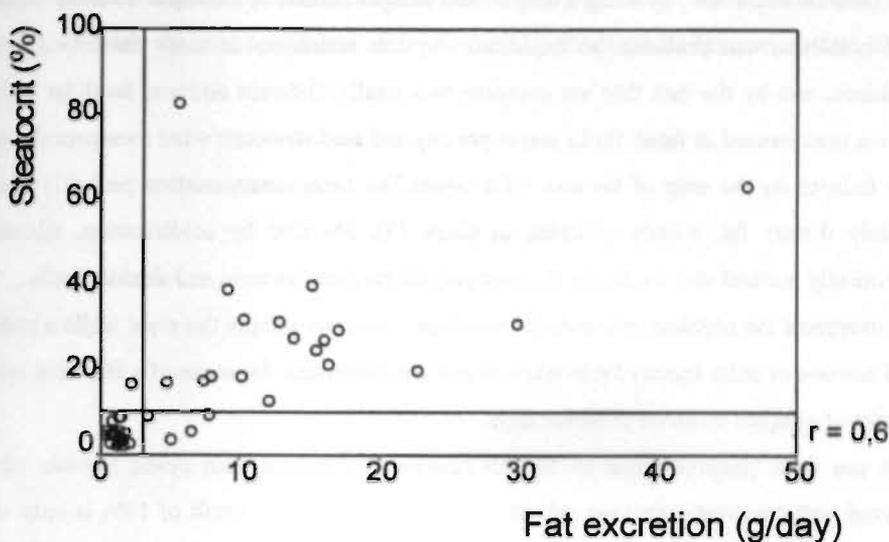


Figure 1 Relationship between acid steatocrit and fat excretion. Reference lines for acid steatocrit at 10 % and for fat excretion at 3 gram / day.

## DISCUSSION

Since the fecal fat balance excretion as described by van de Kamer is cumbersome, expensive and unpleasant for all involved, there is a need for a simple test. Some authors reported the steatocrit micromethod described by Phuapradit as a simple method for monitoring fat malabsorption (3), and reported a good correlation ( $r=0.93$ ) with the fecal fat excretion. Although others considered the steatocrit method of Phuapradit unreliable and mentioned the difficulty to delineate the fatty layer (4) and the impression that fat remains in the solid layer, as a problem of this method. This problem was solved by acidification of the fecal sample, whereby fat extraction is improved, and steatocrit results correlate much better with chemically measured fecal fat (5).

Our AS results correlate satisfactorily with chemically measured fecal fat concentrations and somewhat less, but still significantly, with fecal fat excretion. However, our correlation coefficient is lower than the correlation coefficient of the steatocrit without acidification as published in a previous study (3). We have no explanation for this discrepancy, and other

authors also failed to reproduce these results (4). The lesser correlation of the AS results with the fecal fat excretion, by using a single stool sample instead of a sample from the homogenized collection can probably be explained by the variability of daily fat consumption in children, and by the fact that we compare two totally different entities: fecal fat excretion, which is expressed as fecal fat in grams per day and acid steatocrit what measures the ratio of the fatlayer on the sum of fat and solid layers. The latter determination probably measures mainly dietary fat, mostly occurring as soaps (7), liberated by acidification, whereas the chemically method also measures the endogenous fat from bacteria and shedded cells.

To overcome the problem of the daily variability, one can sample the stool while a standardized amount of daily dietary fat is taken or one can determine the mean of a few acid steatocrit results of samples taken on different days.

The one false positive result of the AS concerned a patient with cystic fibrosis who was treated with pancreatic enzyme substitution therapy. This AS result of 16% is only slightly elevated considering the values obtained in cystic fibrosis patients on substitution therapy, which are mostly between 20 and 30%.

The three false negative results of the acid steatocrit can probably be explained by the choice of the reference line for the normal fecal fat excretion. Fecal fat excretion higher than 4.5g/24hours is considered pathologic (6,8) for children and adolescents, whereas other authors consider 7g/day the upper limit of normal fecal fat excretion in adults (9). The reference line for normal daily fecal fat excretion varies clearly with age and dietary fat intake as previously suggested by Williams (8). Taking account of these remarks, the fat excretion studies of 2 of our 3 patients with false negative steatocrit results could, due to their ages (12 and 13 years), still be considered "normal" and in agreement with AS results. This would reduce the disagreement between the methods to only a few ones.

## CONCLUSION

Acid steatocrit results are highly correlated with the chemically measured fecal fat concentration and significantly correlated with the fecal fat excretion. Although single sample acid steatocrit results are slightly less sensitive and specific than other measured parameters for the diagnosis of steatorrhoea, acid steatocrit measured in the stool samples taken from the homo-

genized collection compare favourably with the fecal fat concentration. We consider the acid steatocrit method useful for the screening and monitoring of patients with steatorrhea. If it is necessary to know the real coefficient of fat absorption, the fecal fat balance method is needed.

## REFERENCES

- (1) van de Kamer JH, Huinink HTB, Weyers HA. Rapid method for determination of fat in feces. *J Biol Chem* 1949; 177:349-55.
- (2) Phuapradit P, Narang A, Mendonca P, Harris DA, Baum JD. The steatocrit: a simple method for estimating stool fat content in newborn infants. *Arch Dis Child* 1981; 56:725-7.
- (3) Colombo C, Maiavacca R, Ronchi M, Consalvo E, Amoretti M, Giunta A. The steatocrit: a simple method for monitoring fat malabsorption in patients with cystic fibrosis. *J Pediatr Gastroenterol Nutr* 1987;6:926-30.
- (4) Walters MP, Kelleher J, Gilbert J, Littelwood JM. Clinical monitoring of steatorrhea in cystic fibrosis. *J Pediatr Gastroenterol Nutr* 1990; 65:99-102.
- (5) Tran M, Forget P, Van den Neucker A, Strik J, Kreel van B, Kuijten R. The acid steatocrit: a much improved method. *J Pediatr Gastroenterol Nutr* 1994; 19: 299-303.
- (6) Navarro J, Schmitz J. *Gastroenterologie pédiatrique*, Flammarion Médecine Sciences, Paris 1986.
- (7) Quinlan PT, Lockton S, Irwin J, Lucas AL. The relationship between stool hardness and stool composition in breast- and formula-fed infants. *J Pediatr Gastroenterol Nutr* 1995; 20:81-90.
- (8) Williams HH, Endicott EN, Shepherd ML, Galbraith H, Macy IG. Fat excretion by normal children. *J. of Nutrition* 1943; 25, 379.
- (9) Bai JC, Andrúsh A, Matelo G, Martinez C, Vazquez H, Boerr L, Sambuelli A. Fecal fat concentration in the differential diagnosis of steatorrhea. *Am. J. Gastroenterol.* 1989; 27-30.

## CHAPTER 6

### ROLE OF LANSOPRAZOLE IN CHILDREN WITH CYSTIC FIBROSIS: EVIDENCE FOR IMPROVED FAT ABSORPTION AND NUTRITIONAL STATUS

Tran TMD, Van den Neucker A, Hendriks JJE, Forget P ( junior ), Forget P ( senior )

Department of Pediatrics, University Hospital Maastricht, Maastricht, The Netherlands

---

Submitted for publication

#### *Abstract*

Stearrhea and nutritional parameters were investigated in 15 cystic fibrosis children before starting lansoprazole, after 3 months on lansoprazole (15mg/day) and 3 months after stopping lansoprazole. There were 5 girls and 10 boys with a mean age of 9.5 years (range: 3.1 - 22.6y). Patients were their own controls. Acid steatocrit, anthropometric methods and DXA were used to evaluate steatorrhea and the nutritional status respectively. On lansoprazole, mean  $\pm$  SD acid steatocrit values decreased from  $37.1 \pm 8.8$  % to  $28.5 \pm 10.6$  % ( $p = 0.02$ ). During lansoprazole therapy, significant mean Z-score changes were found for weight ( $+0.14$  /  $p = 0.02$ ), length ( $+0.15$  /  $p = 0.03$ ), subscapular ( $+ 0.61$  /  $p = 0.003$ ), suprailiaca ( $+0.8$  /  $p = 0.002$ ) and the sum of 4 skinfolds ( $+0.61$  /  $p = 0.002$ ) . Z-scores deteriorated again after stopping lansoprazole. Fatmass and bone mineral content increased significantly on lansoprazole ( $p = 0.008$  and  $p = 0.005$  resp.). Improvement of subscapular Z-score was related to improvement of acid steatocrit values ( $p = 0.01$ ) during treatment. We conclude that lansoprazole as adjuvant therapy significantly improves fat absorption and the nutritional status in CF children.

## INTRODUCTION

Cystic fibrosis (CF) is an autosomal recessive inherited disease. Defect in the chloride transepithelial transport system results in viscous mucus in various organs with lung and pancreas mostly affected (1). Both, pancreatic insufficiency resulting in malabsorption and high energy expenditure due to increased respiratory work (2-3), are thought to be responsible for the poor nutritional condition in CF patients. Since malnutrition can compromise absorptive and immune function resulting in a shortened survival (4), all efforts should be made in order to improve the nutritional status of these patients. Unfortunately, high doses of pancreatic enzymes did not solve the problems of malabsorption (5) and colon stricture has been observed in CF children on this regimen (6,7). Further, the use of hypercaloric diets did not result in significant improvements of Z-scores for weight, length and skinfolds in CF children (8). Only parenteral nutrition and either oral or enteral elemental and semielemental nutrition have been shown to significantly improve the nutritional condition of these children (9-15). The latter strongly suggests that nutrient maldigestion plays a crucial role in the poor response to oral hypercaloric diets. As cystic fibrosis patients have a low duodenal pH probably linked to fat maldigestion (16), inhibition of gastric acid production could improve absorption. The reported effects of H<sub>2</sub>-receptor antagonists and prostaglandine E<sub>2</sub> on steatorrhea have been variable (17-22). Insufficient inhibition of gastric acid could be responsible for these unconvincing results. Recently, in a double blind study, a significant improvement in steatorrhea was found when a proton-pump inhibitor was added as adjuvant therapy in pancreatic enzyme treated cystic fibrosis patients (23). The effect of proton pump inhibitors on the nutritional condition of children with CF have not yet been reported. The aims of our study were to evaluate the effects of lansoprazole (proton-pump inhibitor) on both steatorrhea and the nutritional condition of CF patients while on enzyme therapy.

## SUBJECTS AND METHODS

### *Study design*

As the effect of a proton pump inhibitor on fat balance has been convincingly proven in a

double blind study (23), we adapted a prospective open study design wherein patients were their own controls. In the month preceding the study, all patients were screened for steatorrhea by measuring fecal acid steatocrit once every 10 days. Patients with a mean acid steatocrit value higher than 25% (normal values in our laboratory < 20%) were invited to take part in the study. After evaluation of nutritional parameters by DXA and anthropometric methods, lansoprazole was added to their standard treatment in a dose of 15 mg day before breakfast for 3 months. When fat malabsorption did not change after 2 months, the dose was doubled in children older than 10 years and weighing more than 30 kg. During the lansoprazole treatment period 9 fecal samples were taken with an interval of 10 days for acid steatocrit measurements. The mean of these 9 measurements was used as a measure of steatorrhea during the treatment period. After 3 months on treatment, the nutritional condition assessment was repeated. All measurements of nutritional condition were performed on a single day. Three fecal samples for acid steatocrit determinations and anthropometric parameters were again measured respectively 1 month and 3 months after stopping lansoprazole therapy. Dietary evaluations were performed at the start, at the end and one month after stopping lansoprazole.

#### Patients population

23 CF out-clinic patients from the academic Hospital Maastricht were recruited. All patients were treated with pancreatic enzymes. Of these, 2 patients were too ill to participate in the study. 21 patients were screened for steatorrhea while on pancrease enzyme. 15 of them who had steatorrhea were included. In most children, the CF diagnosis had been made during the first year of life by repeated positive sweat tests, all 15 children were considered to have pancreatic insufficiency on the basis of abnormal fecal chymotrypsin, 72 hours fat balance (24) and increased acid steatocrit results (25). Mean energy intake was 113 % RDA (recommended daily allowance). The mean number of pancreas enzyme capsules (Pancrease) taken by 13 of these patients was 20 (range: 11 - 33), one patient took 3 Pancrease capsules (5000E lipase, 2900E amylase, 330E protease) and 6 Panzytrat tablets (25000E lipase, 22500E amylase, 1250E protease) and another one took 10 Creon capsules (8000E lipase, 9000E amylase, 450E protease) per day. Mean age, weight and length of those 15 children were 9.5 y (range: 3.1 - 22.6 y); 29.3 kg (range: 13.6 - 67.6 kg) and 131 cm (range: 97.7 - 184.9 cm)

respectively. Their nutritional status was moderately altered with a mean Body Mass Index (BMI) of 15.6 (range : 13.2 - 18.3). Mean predicted values of FEV1 and FVC were respectively 81.3% (range: 39 - 114%) and 85.5% (range: 44 - 108%). Informed patient and parental consent were obtained.

#### Evaluation of fat malabsorption by acid steatocrit

The acid steatocrit was determined as previously reported (25). In short, 0.5g solid stool was weighed and diluted with a volume of deionized water, equal to two times the weight of stool. The stool and water were premixed using a Vortex mixer. Subsequently, the mixture was homogenized using a 5 ml Potter Elvehjem tissue homogenizer. After then 5N perchloric acid was added to the homogenate in a volume equal to 1/5 of the homogenate. After mixing with the Vortex, the acidified homogenate was aspirated into a 75  $\mu$ l plain haematocrit capillary. This capillary was sealed with wax at one end and centrifuged horizontally (13000 rpm, 15 min). After centrifugation, 3 layers were distinguished: a basal solid layer (SL), an intermediate liquid layer and an upper fatty layer (FL). Acid steatocrit was calculated as  $(FL / (FL + SL)) \times 100\%$

### EVALUATION OF NUTRITIONAL STATUS

#### Anthropometry

The arm circumference, biceps, triceps, subscapular and suprailiac skinfolds were measured 3 times on the left side of the body using the Harpenden caliper. Average values were taken. Weight and length were also measured. BMI was calculated as  $\text{weight} / (\text{length}^2)$ . The Z-score

(Z-score is defined as  $(X - x) / S$  where X is the patients 's measurement, x is the mean value for age and sex and S is the standard deviation of x) of all these anthropometric parameters were calculated based on the reference data described by Gerver and de Bruin (26). A negative value indicates values under the mean reference value and a positive or negative change in Z-score means catch up or slowing down of growth respectively. All measurements were done

by the same investigator (TT).

#### Dual - energy x-ray absorptiometry (DXA)

DXA measurement is based on the differential tissue attenuation of photons of two energy levels from an X-ray source (27). All patients underwent total body scan performed with a DPX (Lunar Radiation Corp, Madison, WI) total body scanner. The results were analysed with a paediatric software programme, version 1.5e. Daily quality assurance test was performed according to the manufacturer's directions. Total non bone LBM (lean body mass), total BMC (bone mineral content), total body FM (fatmass) and BMD (bone mineral density) Z-score were measured by DXA procedure. These results were compared to those of the reference population, recently described by Ogle et al., who studied the body composition by DXA in 265 normal individuals aged 4 - 26 year (28).

#### Diet evaluation

At the beginning, at the end and one month after stopping lansoprazole nutrient intake was assessed by a specially trained clinical CF dietitian from consecutive 3 day food diaries including one weekend day. Intakes were expressed as kilocalorie per kg bodyweight for the energy intake and gram per kg bodyweight for fat-, carbohydrate- and protein-intakes, using the netherlands nutrients table "NEVO" 1993.

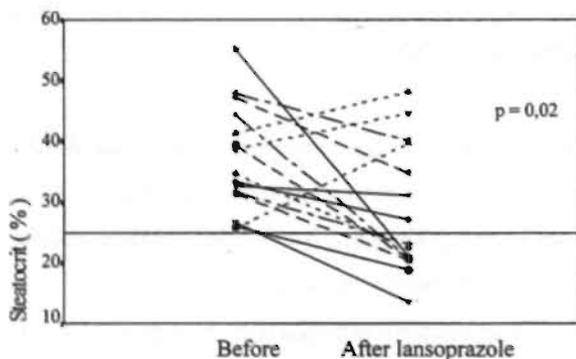
#### Statistic

All data were analysed by using SPSS statistic program. Anthropometric parameters and body composition results measured before the start and at the end of the trial were compared making use of Wilcoxon one sample test. The sign test was used to compare LBM, FM and BMC assessed by DXA, with the reference population described by Ogle.

## RESULTS

### Fat malabsorption

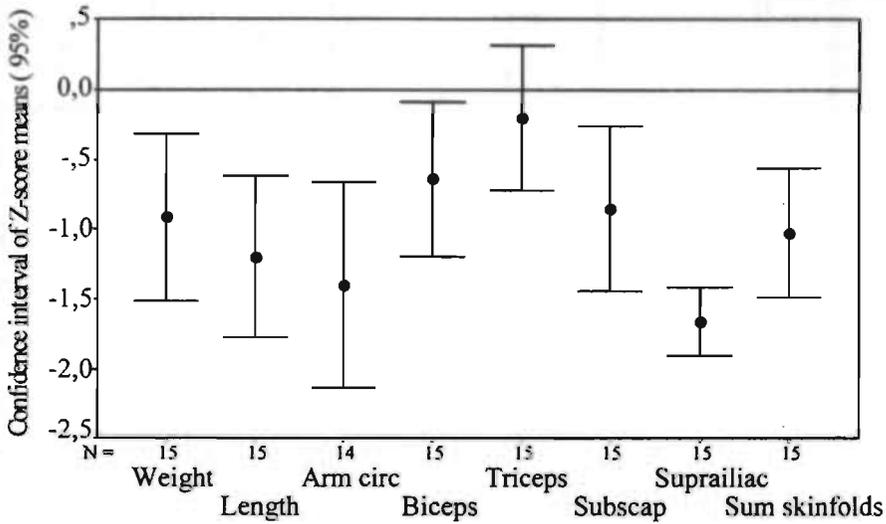
Despite standard pancreas enzyme, 15 of 21 children had steatorrhea with an average  $\pm$  SD pretreatment acid steatocrit value (mean of 3 determinations in each patient) of  $37.1\% \pm 8.8\%$ . After 3 months of treatment with lansoprazole, there was a significant ( $p = 0.02$ ) improvement in steatorrhea with a mean  $\pm$  SD acid steatocrit value of  $28.5\% \pm 10.6\%$ . Eight patients on lansoprazole had a mean acid steatocrit value lower than 25% (fig 1). In this group the mean decrease was 16% (44.2% of start value). In 3 children the acid steatocrit value decreased with 9% (20.6% of start value) but was not completely corrected. In 4 children fat malabsorption did not improve at all. Four children received a double dose of lansoprazole for 1 month, resulting in a decreased acid steatocrit results in 2 (decrease of 8.6% and 19%). Due to social problems one child was dropped out of the study after the lansoprazole period. Mean  $\pm$  SD acid steatocrit value for the remaining 14 children in the first month after stopping lansoprazole was  $29.7\% \pm 13.9\%$ , which was not significantly different from the values on lansoprazole. Of 4 children whose acid steatocrit was not changed on lansoprazole, 2 had higher acid steatocrit values after stopping.



**Figure 1** Acid steatocrit before and after 3 months treatment with lansoprazole in 15 CF children. A line through the 25 % value is drawn showing the study inclusion limit. Acid steatocrit values on lansoprazole are significantly decreased ( $p = 0.02$ ).

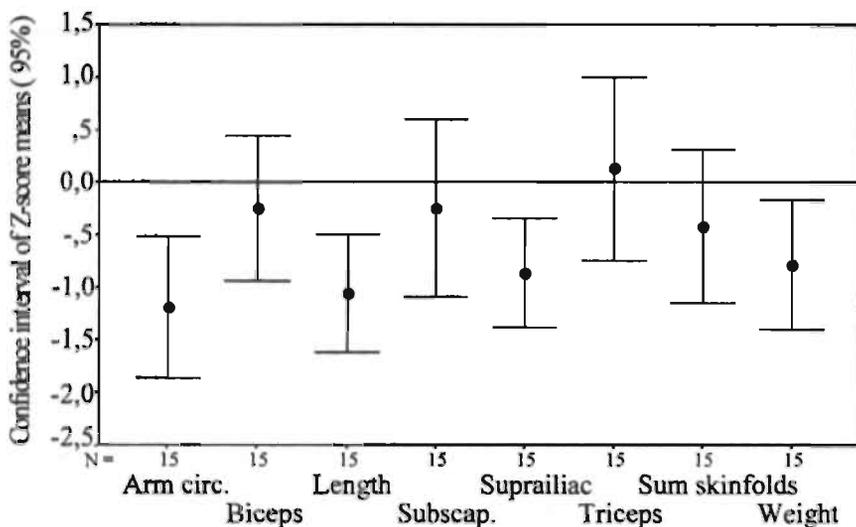
### Anthropometric parameters

Mean and 95% CI (confidence interval) for weight, length, arm circumference, 4 skinfolds and the sum of these 4 skinfolds expressed as Z-score are shown in fig.2. For all parameters except the triceps, the CI do not include the reference 50th centile line (Z-score 0), underscoring the fact that except for the triceps skinfold, all other anthropometric parameters mean Z-scores were significantly decreased in our CF children when compared to those of the normal population. The suprailiac skinfold was most abnormal and showed the smallest interindividual variation.



**Figure 2** Mean Z-scores and 95% confidence interval of anthropometric parameters in 15 CF children before lansoprazole therapy. The line through 0 represents the 50th centile of the reference population. The differences between the study group and the reference population are significant when the CI do not include the Z-score 0 line. All anthropometric parameters were significantly decreased in our CF children except for the triceps skinfold.

After treatment with lansoprazole, Z-scores of anthropometric parameters improved significantly. All parameters moved toward the Z-score 0 line (50th centile for reference population). The Z-scores of biceps, subscapular and sum of the 4 skinfolds did not significantly differ from the reference population any more (fig.3).



**Figure 3** Mean Z-scores and 95% confidence interval of anthropometric parameters in 15 CF children on lansoprazole for 3 months. The differences between the study group and the reference population are significant when the CI do not include the Z-score 0 line. Several parameters (biceps, subscapular and sum of 4 skinfolds) normalized during lansoprazole treatment (see figure 2).

Z-score changes for all anthropometric parameters studied are shown in table 1. Except for arm circumference, biceps and triceps skinfolds, Z-scores of all parameters improved significantly. Subscapular, suprailiac and the sum of the 4 skinfolds showed the most significant changes. The acid steatocrit results during lansoprazole treatment were significantly lower ( $p = 0.01$ ) in our patient subgroup with subscapular Z-score improvement  $\geq 0.5$  when compared to the subgroup showing lower Z-score changes. Three months after lansoprazole was stopped, 5 children were dropped out of the study; 2 because of the far distances from home, 1 had

taken lansoprazole again because of increased symptoms of steatorrhea and abdominal pain and 2 because of social problems. Nutritional parameters were therefore evaluated in only 10 children 3 months after stopping lansoprazole. Z-scores of all anthropometric parameters deteriorated, with weight and subscapular Z-score changes reaching statistical significance (table 1).

**Table 1** Mean Z-scores of anthropometric parameters in 15 CF children before (T0), after 3 months on lansoprazole (T3) and 3 months after stopping lansoprazole (T6).

Anthrop. Parameters	T0 Mean Z-score (n = 15)	T3 Mean Z-score (n = 15)	T6 Mean Z-score (n = 10)	T3 - T0 Mean Z-score (n = 15)	T6 - T3 Mean Z-score (n = 10)
Weight	-0.91	-0.78	-1.38	0.14 (p = 0.02)	-0.6 (p = 0.01)
Length	-1.2	-1.05	-1.23	0.15 (p = 0.03)	-0.18 (p = 0.16)
Armcircum	-1.4	-1.19	-1.74	0.22 (p = 0.05)	-0.55 (p = 0.52)
Biceps	-0.63	-0.24	-1	0.39 (p = 0.06)	-0.76 (p = 0.21)
Triceps	-0.2	0.14	-0.72	0.34 (p = 0.2)	-0.86 (p = 0.26)
Subscapular	-0.85	-0.24	-1.14	0.61 (p = 0.003)	-0.9 (p = 0.03)
Suprailiaca	-1.66	-0.86	-1.13	0.8 (p = 0.002)	-0.27 (p = 0.68)
Sum skinfolds	-1.02	-0.41	-1.16	0.61 (p = 0.002)	-0.75 (p = 0.31)

### Body composition

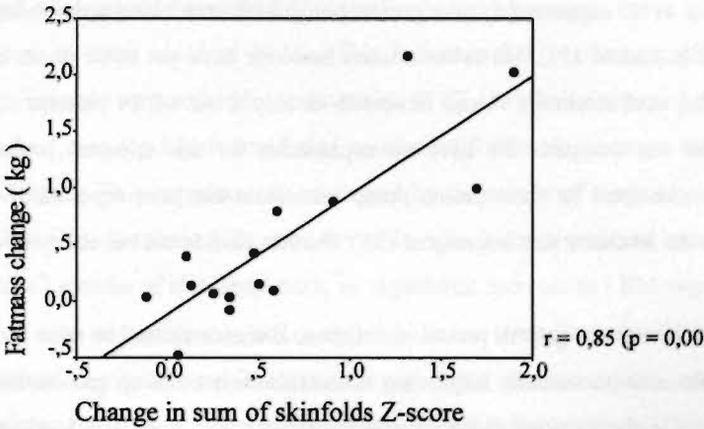
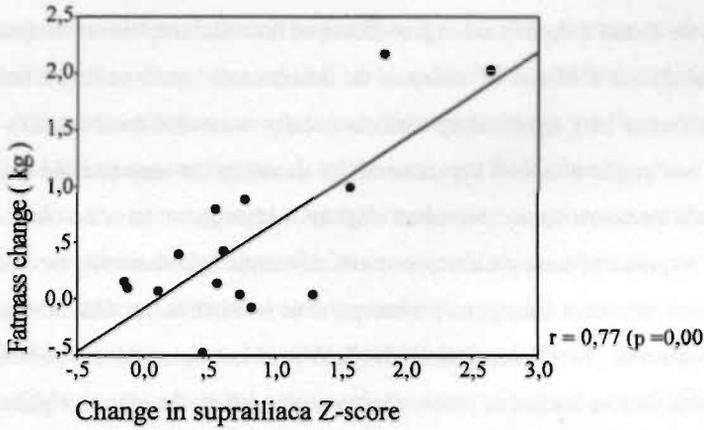
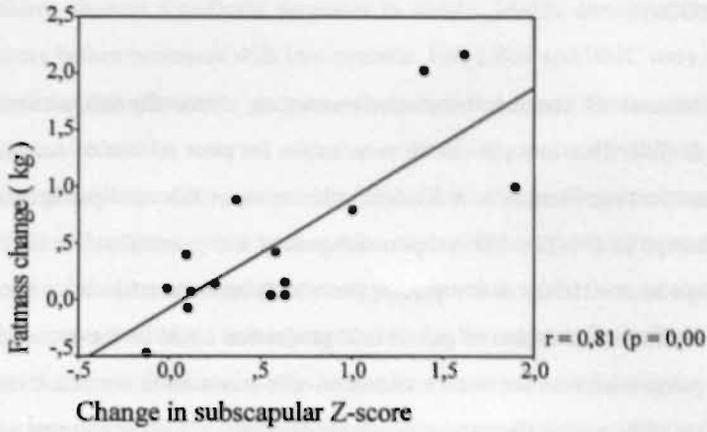
Body composition data before and after lansoprazole are given in table 2. All 3 components FM, LBM and BMC were significantly decreased in our 15 CF children when compared to the reference population described by Ogle et al. (p = 0.01; p = 0.02 and p = 0.005 respectively). Bone mineral density Z-score was significantly decreased (p ≤ 0.05). Significant increases of FM and BMC occurred after 3 months of treatment with lansoprazole. Changes in the subscapular, suprailiaca and sum of the 4 skinfolds Z-scores were highly correlated with changes in FM by DXA (r = 0.81 / r = 0.77 / r = 0.85 resp. with p = 0.001) (fig 4).

Diet evaluation

Mean fat, protein, carbohydrate and energy intakes were 3.1 - 2.8 - 3.0 g / kg ; 3.0 - 2.7 - 2.8 g / kg ; 10.4 - 9.7 - 9.8 g / kg and 2095 - 1986 - 1977 kcal / kg bodyweight at the start, at the end and one month after lansoprazole trial respectively. None of these changes were significant.

**Table 2 Body composition before (T0) and after (T3) lansoprazole by DXA.**

<b>BODY COMPOSITION</b>	<b>T0 ( n = 15 )</b>	<b>T3 ( n = 15 )</b>	<b>SIGNIFICANCY ( p )</b>
<b>MEAN FATMASS ( Kg )</b>	<b>3.97</b>	<b>4.76</b>	<b>0.008</b>
<b>MEAN LBM ( Kg )</b>	<b>22.83</b>	<b>24.03</b>	<b>0.06</b>
<b>MEAN BMC ( Kg )</b>	<b>1.02</b>	<b>1.08</b>	<b>0.005</b>
<b>MEAN BMD ( Z-SCORE )</b>	<b>-0.55</b>	<b>-0.58</b>	<b>0.65</b>



**Figure 4** Relation between changes in subscapular skinfold Z-score, suprailiaca skinfold Z-score, sum of 4 skinfolds Z-score and fatmass as measured by DXA (dual energy X-ray absorptiometry) in 15 CF children.

## DISCUSSION

Due to a decrease of pancreas bicarbonate secretion, cystic fibrosis patients have a low duodenal pH (29). This low pH can be responsible for poor release of enzymes through the acid resistant coating. Further, low duodenal pH can cause bile acid precipitation resulting in lipid malabsorption (30-32). H<sub>2</sub>-receptor antagonists and prostaglandine E<sub>2</sub> have been used with the hope to reverse the above proces, but results on steatorrhea have been controversial (17-22). Insufficient inhibition of gastric acid production could be the cause of these failures. As proton pump inhibitors are known to control acid secretion in a much more effective way (33), they could be more effective in increasing duodenal pH. In agreement with Heijermans et al. (23), we found a significant improvement of fat malabsorption as measured by the acid steatocrit in all but 4 of our CF children on lansoprazole. Acid steatocrit results have been shown to correlate very significantly with chemically measured fecal fat (25). Acid steatocrit values did not improve in 4 of our patients. By doubling the lansoprazole dose in 2 of these patients, acid steatocrit values decreased slightly. Although we have no clear explanation for the lack of response of these patients, poor efficacy could hypothetically be due to host factors including poor intestinal lansoprazole absorption or interindividual differences in bioavailability of lansoprazole (34). Further, since the halflife of lansoprazole is between 1 and 2 hours and inhibition will be limited to proton pumps active during the effective plasma levels of the drugs, Sachs et al. suggested to give proton pump inhibitors twice a day whenever effective pH control is desired (33). However, studies hereover have yet to be done. Contrary to our expectations, acid steatocrit values increased in only 8 out of 14 patients one month after lansoprazole was stopped. We have no explanation for this apparent prolonged effect of lansoprazole on fecal fat since proton pump restoration has been reported to occur 50 to 72 hours after the inhibitor was interrupted (33). Further studies are necessary in order to clarify this point.

Because of the normal growth proces in children, Z-scores should be used for the evaluation of anthropometric parameters. Improving Z-scores reflect catch-up growth and consequently improvement in the nutritional status while the reverse is true for deteriorating Z-scores. The recent introduction of DXA methodology makes it possible to evaluate FM, LBM and BMC rapidly and non invasively in children (27). In agreement with previous studies, all except 2

of our CF children showed significant decreases in weight, length, arm circumference and 4 skinfolds Z-scores before treatment with lansoprazole. FM, LBM and BMC were also significantly decreased despite pancreatic enzyme substitution and hypercaloric supplementation. The catabolic process could be reversed after 3 months of lansoprazole resulting in Z-score improvement of all anthropometric parameters, FM, BMC and to a lesser extent LBM. The improved nutritional condition could be due either to higher energy intakes or to improved absorption. As we found somewhat lower energy intakes during the lansoprazole treatment period, a higher intake probably is not responsible for the improved nutritional condition. Further, the fact that lower fecal acid steatocrit results were found in our patients with the best nutritional response as assessed by subscapular Z-score improvements, supports the idea that improved absorption is the main factor responsible for the improvement of the nutritional status in our patients. However increased FM, LBM and BMC are difficult to interpret because results for reference populations expressed as Z-scores have not yet been reported. As Z-score of BMD did not change on lansoprazole, the increased BMC found in our CF children, is probably linked to the growth process. Our results showing deterioration of the nutritional condition after interruption of lansoprazole "intervention", are in agreement with those of Bertrand et al., who reported nutritional deterioration after stopping elemental enteral alimentation (14). Oral hypercaloric diets have not been shown to improve Z-scores of weight, length and skinfolds as parameters for nutritional status and growth process of CF children (8). Only parenteral or elemental "predigested" enteral nutrition have been shown to reverse the catabolic process in these children (9-15). This indicates that persisting maldigestion or malabsorption is mainly responsible for malnutrition in CF. As alkalinization of duodenal pH improves malabsorption (16,22), it could also, as elemental diets do, improve the nutritional status of CF children. This hypothesis is confirmed by the results of our study. In contrast with O' Loughlin (10), Shepherd (12) and Levy (13), who found significant improvement in LBM after 6 to 12 months of elemental diets, no significant increase in LBM was seen in our study. The 3 months of treatment in our study could be too short for a significant change in LBM to be noticed. Our results are in agreement with those of other authors, who evaluated the effect of gluten free diet on body compartments by DXA. Only FM and BMC improved after a 6 months gluten free diet while LBM did not (35). As H<sub>2</sub> - receptor antagonists have been shown to significantly decrease nitrogen malabsorption in CF patients (36), we do not

think that the poor improvement of LBM in our patients can be ascribed to a selective improvement of fat and not of protein malabsorption.

In conclusion, most of our CF children maintained steatorrhea and were malnourished despite optimal treatment with hypercaloric diets and pancreatic enzymes. Lansoprazole as adjuvant therapy resulted in decreased fat malabsorption and improved nutritional status in these CF children after 3 months of treatment. Longterm evaluation of the effect of lansoprazole on both the nutritional status and lung function parameters have yet to be performed.

#### *Acknowledgment*

we are gratefull to Liesbeth van der Ploeg, Lianne Schoorlemmer dieticians and Piet Willems, Sandra Zimny from the department of nuclear medicine for their invaluable assistance. We also thank all nurses of the pediatric polyclinic for their welwilling support.

## REFERENCES

- (1) M. Welsh, A. Smith. Molecular mechanisms of CFTR chloride channel dysfunction in cystic fibrosis. *Cell* 1993; 73:1251-1254.
- (2) J. Tomezsko, V. Stallings, D. Kawchak, J. Goin, G. Diamond, T. Scanlin. Energy expenditure and genotype of children with cystic fibrosis. *Pediatr Res* 1994; 35: 451- 460.
- (3) M. Bronstein, P. Davies, K. Hambidge, F. Accurso. Normal energy expenditure in the infant with presymptomatic cystic fibrosis. *J Pediatr* 1995; 126: 28-33.
- (4) R.Kraemer, A. Rudeberg, B. Hadorn, E. Rossi. Relative underweight in cystic fibrosis and its prognostic value. *Acta Paediatr Scand* 1978; 67: 33-37.
- (5) P. Robinson, P. Sly. High dose pancreatic enzymes in cystic fibrosis. *Arch Dis Child*. 1990; 65: 311-312.
- (6) E. Lebenthal. High strength pancreatic exocrine enzyme capsules associated with colonic strictures in patients with cystic fibrosis: "more is not necessarily better". *J Pediatr Gastroenterol Nutr*. 1994; 18: 423-425.
- (7) R. Smyth, D. van Velzen, A. Smyth, D. Lloyd, D. Heaf. Strictures of ascending colon in cystic fibrosis and high strength pancreatic enzymes. *The Lancet*. 1994; 343: 85-86.
- (8) A. Rettammel, M. Marcus, P. Farrell, S. Sondel, R. Kosciak, E. Mischler. *J Am Diet Assoc*. 1995; 95: 454-459.
- (9) K. Gaskin, D. Waters, L. Baur, V. Soutter, M. Gruca. *Acta Paediatr Scand [Suppl]*. 1990; 366: 106-110.
- (10) E. O' Loughlin, D. Forbes, H. Parsons, B. Scott, D. Cooper, G. Gall. Nutritional rehabilitation of malnourished patients with cystic fibrosis. *Am J Clin Nutr*. 1986: 43: 732-737.
- (11) R. Shepherd, T. Holt, B. Thomas et al. Nutritional rehabilitation in cystic fibrosis: Controlled studies of effects on nutritional growth retardation, body protein turnover, and course of pulmonary disease. *J Pediatr*. 1986; 109: 788-94.
- (12) R. Shepherd, B. Thomas, D. Bennett, W. Cooksley, L. Ward. Changes in body composition and muscle protein degradation during nutritional supplementation in nutritionally growth-retarded children with cystic fibrosis. *J Pediatr Gastroenterol Nutr*. 1983; 2: 439-446.
- (13) L. Levy, P. Durie, P. Pencharz, M. Corey. Effects of long-term nutritional rehabilitation on body composition and clinical status in malnourished children and adolescents with cystic

fibrosis. *J Pediatr* 1985; 107: 225-230.

(14) J. Bertrand, C. Morin, R. Lasalle, J. Patrick, A. Coates. Short-term clinical, nutritional, and functional effects of continuous elemental enteral alimentation in children with cystic fibrosis. *J Pediatr*. 1984; 104: 41-46.

(15) R. Shepherd, W. Cooksley, and W. Domville. Improved growth and clinical, nutritional, and respiratory changes in response to nutritional therapy in cystic fibrosis. *J Pediatr*. 1980; 97: 351-357.

(16) P. Robinson, A. Smith, and P. Sly. Duodenal pH in Cystic fibrosis and its relationship to fat malabsorption. *Dig Dis Sci*. 1990; 35: 1299-1304.

(17) A. Carroccio, F. Pardo, G. Montalto et al. Use of famotidine in severe exocrine pancreatic insufficiency with persistent maldigestion on enzymatic replacement therapy: A long-term study in cystic fibrosis. *Dig Dis Sci* 1992; 37: 1441-1446.

(18) D. Chalmers, R. Brown, M. Miller et al. The influence of longterm cimetidine as an adjuvant to pancreatic enzyme therapy in cystic fibrosis. *Acta Paediatr Scand*. 1985; 74: 114-117.

(19) P. Robinson and P. Sly. Placebo-controlled trial of misoprostol in cystic fibrosis. *J Pediatr Gastroenterol Nutr*. 1990; 11: 37-40.

(20) H. Heijerman, C. Lamers, J. Dijkman, and W. Bakker. Ranitidine compared with the dimethylprostaglandin E2 analogue enprostil as adjunct to pancreatic enzyme replacement in adult cystic fibrosis. *Scand J Gastroenterol*. 1990; 25 (Suppl 178): 26-31.

(21) M. Schöni, R. Kraemer, E. Rossi. Cimetidine and fat malabsorption in children with cystic fibrosis. *Helv Paediat Acta*. 1981; 36: 359-369.

(22) B. Boyle, W. Long, W. Balistreri, S. Widzer, and N. Huang. Effect of cimetidine and pancreatic enzymes on serum and fecal bile acids and fat absorption in cystic fibrosis. *Gastroenterology*. 1980; 78: 950-953.

(23) H. Heijerman, C. Lamers, W. Bakker. Omeprazole enhances the efficacy of pancreatic (pancreas) in cystic fibrosis. *Ann Intern Med*. 1991; 114: 200-201.

(24) J. van de Kamer, H. Huinink, H. Weyers. Rapid method for determination of fat in feces. *J Biol Chem*. 1949; 177: 349-55.

(25) M. Tran, P. Forget, A. Van den Neucker, J. Strik, B. van Kreel, and R. Kuijten. The acid steatocrit: A much improved method. *J Pediatr Gastroenterol Nutr*. 1994; 19: 299-303.

- (26) W. Gerver, R. de Bruin. *Paediatric Morphometrics: A reference manual*. 1th ed. Utrecht: Bunge, 1995.
- (27) R. Mazess, H. Barden, J. Bisek, and J. Hanson. Dual-energy x-ray absorptiometry for total-body and regional bone-mineral and soft-tissue composition. *Am J Clin Nutr*. 1990; 51:1106-12.
- (28) G. Ogle, J. Allen, I. Humphries et al. Body-composition assessment by dual-energy x-ray absorptiometry in subjects aged 4-26 y. *Am J Clin Nutr*. 1995; 61:746-53.
- (29) A. Weber, C. Roy. Intraduodenal events in cystic fibrosis. *J Pediatr Gastroenterol Nutr*. 1984; 3 (Suppl. 1): S113-S119.
- (30) P. Regan, J. Malagelada, E. Dimagno, and V. Go. Reduced intraluminal bile acid concentrations and fat maldigestion in pancreatic insufficiency: Correction by treatment. *Gastroenterology*. 1979; 77: 285-289.
- (31) P. Zentler-Munro, W. Fitzpatrick, J. Batten, and T. Northfield. Effect of intrajejunal acidity on aqueous phase bile acid and lipid concentrations in pancreatic steatorrhoea due to cystic fibrosis. *Gut*. 1984; 25: 500-507.
- (32) P. Zentler-Munro, D. Fine, J. Batten, and T. Northfield. Effect of cimetidine on enzyme inactivation, bile acid precipitation, and lipid solubilisation in pancreatic steatorrhoea due to cystic fibrosis. *Gut*. 1985; 26: 892-901.
- (33) G. Sachs, J. Shin, C. Briving, B. Wallmark, S. Hersey. The pharmacology of the gastric acid pump: The H<sup>+</sup>, K<sup>+</sup> ATPase. *Annu Rev Pharmacol Toxicol*. 1995; 35: 277-305.
- (34) C. Spencer and D. Faulds. *Drugs: Focus on Lansoprazole*. 1994; 48: 404-430.
- (35) G. Barera, P. Brambilla, P. Manzoni, S. Acciuffi, G. Caccia, C. Bianchi. Changes in body composition evaluated by DXA during gluten free diet in celiac children. *J Pediatr Gastroenterol Nutr*. 1995; 20: 476 "abstr".
- (36) K. Cox, J. Isenberg, A. Osher, R. Dooley. The effect of cimetidine on maldigestion in cystic fibrosis. *J. Pediatr*. 1979; 94: 488-492.

## CHAPTER 7

### ANTHROPOMETRY AND BODY COMPOSITION IN CHILDREN WITH CYSTIC FIBROSIS: EFFECTS OF A PROTON - PUMP INHIBITOR

<sup>(1)</sup>My-Dung T. Tran, <sup>(1)</sup>Anita Van den Neucker, <sup>(1)</sup>Han J. Hendriks, <sup>(2)</sup>Bernard van Kreel,  
<sup>(1)</sup>Patricia Forget, <sup>(3)</sup>Guido Heidendal, <sup>(1)</sup>Pierre-Philippe Forget

<sup>(1)</sup>Department of Pediatrics, <sup>(2)</sup> Clinical Chemistry and <sup>(3)</sup>Nuclear Medicine, University Hospital  
Maastricht, Maastricht, the Netherlands.

---

Submitted for publication

#### *Abstract*

We studied the body composition of 18 CF children making use of dual-energy X-ray absorptiometry (DEXA), deuterium-bromide and skinfold methods and evaluated the efficacy of these body composition methods for the detection of body composition changes during 3 months therapeutic intervention with lansoprazole. Our CF patients were malnourished with decreased mean Z-scores for armcircumference (-1.62), biceps (-0.77), subscapular (-0.92), suprailliac skinfolds (-1.66), weight (-1.03) and height (-1.31). Their fatmass was significantly depleted as shown by DEXA, skinfold and total body water (TBW) methods. Extracellular volume (%) was increased, while intracellular volume (%) was normal. Only the lean body mass (LBM) as measured by DEXA was decreased ( $p = 0.02$ ). Decreased bone mineral content and bone mineral density Z-scores were also found ( $p = 0.005$  and  $p = 0.03$  respectively). After treatment with lansoprazole, significant increases in fatmass was found by DEXA and skinfold methods (53% and 97% of weight changes respectively) whereas weight increase was exclusively ascribed to an increase in LBM with the TBW method. Changes in body-weight however, were not correlated with either fatmass and fat free mass changes as measured by any of these methods. We conclude that results of DEXA, TBW and skinfold methods are not interchangeable and that the methods used are not accurate enough for the differential detection of small changes in fatmass and fat free mass as found in the present study.

## INTRODUCTION

Due to malabsorption (1), chronic lung infections with increased energy expenditure (2,3) and poor appetite, most cystic fibrosis patients show signs of malnutrition. As malnutrition can affect pulmonary function and shorten survival (4), feeding interventions are sometimes necessary to restore normal growth and body composition. Assessment of body composition changes is necessary for the precise evaluation of nutritional interventions. While different body composition methods have been described, only few studies have, to our knowledge, compared different measurement techniques in pediatric subjects and there are no reports on the efficacy of these methods for the detection of body composition changes during therapeutic interventions. In children, Dual-energy X-ray absorptiometry (DEXA), Total Body Water (TBW) and Skinfold methods are frequently used for the determination of body composition since they are all noninvasive. In this age group, methods used for measuring body composition have to be very precise in order to detect small changes in body composition. The precision for repeated measurements has been reported to be 1-2 % for DEXA, 1.6% for TBW and either 5% (intraobserver) or 15% (interobserver) for the skinfold method (5). In the present study we first evaluated the body composition of our 18 CF children making use of DEXA, skinfolds and TBW (deuterium-bromide) methods and subsequently evaluated the agreement between these results. Secondly, we investigated the sensitivity of these 3 methods for the detection of small changes in body composition of 15 CF children whose nutritional condition improved significantly after intervention with lansoprazole for 3 months. For the purpose of the present study we defined fat free mass by DEXA (FFM-DEXA) as the sum of lean body mass (LBM) and bone mineral content (BMC) and total mass (TM) as DEXA constructed weight (LBM + FM + BMC).

## SUBJECTS

### *Population studied for the comparison of body composition methods*

23 CF children were recruited from the Academic Hospital Maastricht, The Netherlands. Of these 2 patients were too ill to take part in the study and 3 children refused to participate. 18

children who had no exacerbation 4 weeks before the study were included. Thirteen of them were prepubertal and younger than ten years, 3 children were postpubertal and were between 11.6 - 14.1 years. Two subjects were adolescents of 16.1 and 22.6 years. Fourteen of these 18 children were diagnosed during the first year of life while 16 of them had pancreatic insufficiency (abnormal fecal chymotrypsin and 72h fecal fat balance). Their nutritional status was moderate with a mean BMI (body mass index) of 15.6 (range: 13.2 - 23.2). Mean age, weight and height were respectively 9.0 y (range: 2.9 - 22.6 y); 27.4 kg (range: 13.6 - 67.6 kg) and 127.5 cm (range: 96.2 - 184.9 cm). Mean FEV1 (forced expiratory volume in 1 second) and FVC (forced vital capacity) were respectively 84% (range: 39 - 117%) and 86% (range: 44 - 109%) of predicted values. Mean energy intake was 113% RDA (recommended daily allowance). All patients were on conventional physiotherapy, pancreatic enzymes and some of them received antibiotics regularly for pulmonary exacerbations. Weight, height, armcircumferences, TBW, DEXA and skinfolds were measured on the same day. All usual CF medications were continued during the study.

#### *Population studied for the evaluation of changes in body composition*

We included 15 out of 16 children with pancreatic insufficiency as described above who maintained steatorrhea despite pancreatic enzymes and were treated with lansoprazole for 3 months with significant improvement of anthropometric parameters (results will be published separately). Mean age, weight and height of these 15 children were 9.5 y (range: 3.1 - 22.6 y); 29.3 kg (range: 13.6 - 67.6 kg) and 131 cm (range: 97.7 - 184.9 cm) respectively. Their nutritional status was moderately altered with a mean Body Mass Index (BMI) of 15,6 (range : 13.2 - 18.3). Mean FEV1 and FVC were respectively 81.3% (range: 39 - 114%) and 85.5% (range: 44 - 108%) of predicted values.

Anthropometry, DEXA and TBW were measured on the same day just before starting and 3 months after treatment with lansoprazole (15mg / day). Other usual CF medications were continued throughout the study. Informed patient and parental consent were obtained from all study subjects.

## METHODS

### Growth parameters

Weight, height, upper armcircumferences and 4 skinfold thicknesses (biceps, triceps, subscapular and suprailiac) were measured on the left side of the body in triplicate, using the Harpenden caliper. Average of three measures was taken and was expressed as standard deviation scores of the normal population for age and sex using the growth charts from Gerver and de Bruin (6). BMI was calculated as  $\text{weight}/\text{height}^2$ . Results of BMI were compared to the reference population described by Westrate and Deurenberg et al. (7). Mid upper arm muscle area was calculated from the mid upper armcircumference and the sum of biceps and triceps skinfolds (6).

### Body composition

Body composition results obtained from all three methods were compared to those of a recently reported pediatric reference population (8). The percentage of fatmass and fat free mass measured by the skinfold method were also compared to those of the reference population described by Gerver and de Bruin (6).

### Body composition by anthropometry

It has been found that subcutaneous fat as measured by skinfolds is related to the body density (9). This latter is itself related to the body fatmass. From these theoretical principles, Gerver and de Bruin have constructed a chart, expressing the relationship between the 4 skinfolds (biceps, triceps, subscapular and suprailiac) and the percent fat free mass (6). In our study, fat free mass determined by this method was derived from these charts and fatmass was then calculated by subtracting FFM from bodyweight.

### Body composition by dual-energy x-ray absorptiometry

The theoretical principles for DEXA measurement of body composition and the precision of this method have been described previously (10-12). All DEXA measurements were performed with a Dual Photon X-ray ( Lunar Radiation Corp, Madison, WI ) total body scanner. These results were analysed with a pediatric software programme, version 1.5e. Daily quality assurance tests were performed according to the manufacturer 's directions. Total body analysis was performed in all children using a fast scan mode with a sample size of 4.8 x 9.6mm, sample interval of 0.03s and source collimation of 1.68mm. The following body compartments were assessed: total non bone lean body mass, total bone mineral content, total bone mineral density (BMD), total body fatness and Z-score of BMD.

#### Body composition by total body water and bromide space

TBW and ECV were measured by deuterium oxide (13) and bromide dilution respectively (14). Each subject received orally 20 ml (40 ml was given to the 2 adolescent patients) of a mixture of D<sub>2</sub>O (99.9% purity) and Bromide salt (150mMol/L) solution in a volume ratio of 1:1. Saliva and plasma samples were taken before intake of D<sub>2</sub>O - NaBr solution and 4 hours thereafter when an "plateau" has been reached. To prevent saliva dilution by fluid intake which can result in a higher TBW content, patients were told not to take any fluid orally half an hour before saliva samples were taken. Urine and fecal loss of bromide and D<sub>2</sub>O during the equilibration period were considered negligible as the D<sub>2</sub>O and bromide T<sub>1/2</sub> are about 8 days (14). Saliva samples were obtained making use of dental cotton-wool, that was dried overnight at 100 °C and kept in a gas-tight tube until use. The cotton-wools and the blood samples were centrifuged and the saliva and serum thus obtained were kept in a stoppered glass vial and stored in a freezer at -20 °C until analysis. Results of TBW, ECV and ICV were compared to the reference values described by Friis-Hansen (15).

#### 1. TBW ANALYSIS

D<sub>2</sub>O concentrations of saliva samples were determined as described by van Kreel (14): Calcium carbide (CaC<sub>2</sub>) was placed in the siliconized vacutainer tube and evacuated for 30 sec with a rotatory vane pump to a total pressure of 0,01 atm. Thereafter, 25µl of salivary sample

was injected in the vacutainer tube. This was done in duplicate.  $\text{CaC}_2$  react with  $\text{D}_2\text{O}$  forming acetylene gas. A  $25\mu\text{l}$  sample of this gas was subsequently injected in duplicate into the GC/CF - IRMS system (gas chromatography/continuous flow isotope ratio mass spectrometry) at 2 min. intervals. The mass 27/26 ratio ( $R_{27/26}$ ) was measured on a Isotope Ratio Mass Spectrometer configured for Acetylene (Finnigan MAT 252 for CF-IRMS). The mean value of 4 determinations was calculated for each sample. By inserting the tracer/tracee ratio, defined as  $R_{27/26}(\text{T4}) - R_{27/26}(\text{T0})$ , into the regression equation obtained from the standards, we get the dilution factor of  $\text{D}_2\text{O}$ . TBW is calculated as ingested  $\text{D}_2\text{O}$  volume/dilution factor. FFM and FM are then calculated by the following formulae:

$$\text{FFM (kg)} = \text{TBW} / (1,04 \times d)$$

$$\text{FM (kg)} = \text{Weight} - \text{FFM}$$

The 1,04 factor is a correction for the estimated 4% nonaqueous hydrogen exchange and  $d$  is the hydration factor of LBM which varies with age and sex. Because our CF population was young, we used the age dependent hydration factors described by Fomon (16) for children younger than 10 year and by Boileau and Lohman (17) for older children.

## 2. BROMIDE DILUTION ANALYSIS

Because bromide resides mainly in the extracellular space, the measurement of bromide dilution gives an estimate of the extracellular volume. Bromide was determined by using a Gas Chromatograph type CP 9000 (Chrompack) equipped with an ECD detector after it was converted into bromoacetone gas (14). First, perchloric acid was added to the serum sample and centrifuged for deproteinisation. An aliquot of the supernatant was then added to silver nitrate ( $\text{AgNO}_3$ ) for precipitation of silver bromide and chloride. After centrifugation, the precipitate was taken up in  $\text{NH}_3$  after adding  $\text{Na}_2\text{S}$  and  $\text{NaOH}$  in order to eliminate the silver. After agitation and centrifugation, the supernatant was heated until dry.  $\text{H}_2\text{O}$  was added followed by  $\text{H}_2\text{O}_2$  in order to oxidize sulfide. After drying,  $2\text{H}_2\text{O}$  was then added and dried again. This was repeated several times. Thereafter, perchloric acid and acetone were added and the reaction was started by addition of  $\text{KmnO}_4$  with Bromoacetone formed. The solution

is then extracted with benzene. The organic phase was separated from the water phase by shaking and centrifugation. The water phase was then removed. An aliquot of the organic solution is then applied to the gas chromatograph for measuring of the bromoacetone/internal standard ratio. The bromide concentration was then derived from the bromoacetone standard curves. Because the distribution of bromide depends on the potential difference between the in- and extra-cellular compartments and on the total body volume, the corrected bromide space was calculated as follows:

$$\text{ECV (L)} = \frac{\text{Bromide administered (mmol)}}{\text{Bromide change T4 - T0 (mmol/L)}} - 0.036\text{TBW}$$

Where 0.036TBW is the correction factor for the cell potential and for the total body volume (14). Body cell mass (BCM) was then calculated by subtracting ECV from TBW.

### Statistics

All data were analysed with SPSS statistic program version 6.0. The sign test was used to compare the growth parameters and body composition results with those of the reference population. The Pearson correlation coefficient was used to determine the relationship between measurements obtained by the various body composition methods. Partial correlation coefficients, controlling for age, were used for the evaluation of the relationships between measured ICV, ECV, mid-upper-arm muscle area and the various body composition results. The between method differences were compared, using the Wilcoxon rank test. The agreement between methods were evaluated by the Statistical method of Bland and Altman (18).

## RESULTS

### *Body composition of 18 cf children*

Mean age, nutritional parameters expressed as standard deviation scores (Z-scores) and results of body composition measured by skinfold, DEXA and TBW from 18 CF children are shown in table 1. Compared to the reference population, mean Z-scores for weight, height, BMI, arm circumference and skinfolds (except for the triceps) were significantly decreased (fig 1). The mid-upper-arm muscle area was significantly decreased in our CF population ( $p = 0.005$ ). In absolute terms, all body composition components measured by DEXA such as FM, LBM, BMC and BMD Z-scores were significantly decreased ( $p = 0.01$ ;  $p = 0.02$ ;  $p = 0.005$  and  $p = 0.03$  respectively) compared to the control population described by Oggle (8). When compared to the normal DEXA body composition data reported by Oggle (8), results obtained with either the TBW or the skinfold methods showed only fat mass to be significantly decreased in our patients ( $p = 0.03$  and  $p = 0.05$  respectively). In relative terms, FM-DEXA (compared to normal DEXA data reported by Oggle) and FM-skinfolds (compared to normal skinfolds data reported by Gerber) were also significantly decreased. The BMI was correlated with both FM and FFM measured by all three methods (FM-DEXA  $r = 0.90$ ; FM-skinfold  $r = 0.87$ ; FM-TBW  $r = 0.75$ ; FFM-DEXA  $r = 0.79$ ; FFM-skinfold  $r = 0.81$ ; FFM-TBW  $r = 0.8$  with  $p = 0.001$  for all correlations). As expected strong correlations were found between age on the one hand and FM-DEXA ( $r = 0.67$   $p = 0.003$ ), FFM-DEXA ( $r = 0.95$   $p = 0.001$ ), FM-skinfolds ( $r = 0.59$   $p = 0.01$ ), FFM-skinfolds ( $r = 0.95$   $p = 0.001$ ), FM-TBW ( $r = 0.47$   $p = 0.047$ ), FFM-TBW ( $r = 0.94$   $p = 0.001$ ) and BMC-DEXA ( $r = 0.94$   $p = 0.001$ ). No sex differences in body composition data were found. When compared to the reference values described by Fris-Hansen (15), the ECV and TBW as a percentage of bodyweight were significantly increased ( $p < 0.005$  both) while the ICV as percent of bodyweight was normal. The partial correlations (controlling for age) between ECV, ICV, the mid-upper-arm muscle area LBM and FM are shown in table 2.

**Table 1 Characteristics of 18 CF children.**

	MEAN	SEM	RANGE
AGE (Yr)	8.97	1.16	2.9 - 22.6
BW (Kg)	27.43	3.66	13.6 - 67.6
BW (SDS)	-1.03	0.25	-2.33 - 1.36
TM (Kg)	27.82	3.84	12.96 - 67.33
HEIGHT (cm)	128	6.0	96.2 - 184.9
HEIGHT (SDS)	-1.31	0.25	-2.89 - 0.40
BMI (Kg / m <sup>2</sup> )	15.6	0.57	13.21 - 23.17
ARMCIRCUMFER. (SDS)	-1.62	0.31	-3.14 - 1.43
BICEPS (SDS)	-0.77	0.23	-1.86 - 1.33
TRICEPS (SDS)	-0.33	0.23	-2 - 2.33
SUBSCAPULAR (SDS)	-0.92	0.23	-2 - 1.6
SUPRAILIACA (SDS)	-1.66	0.09	2.43 - -0.57
SUM 4 SKINFOLDS (SDS)	-1.17	0.20	-2.2 - 1.2
FM-DXA (%)	12.11	1.48	6.1 - 28.8
FM-TBW (%)	10.39	1.61	0.0 - 22.2
FM-SKINFOLD (%)	14.44	0.94	8 - 23
FFM-DXA (%)	87.9	1.5	71.2 - 93.9
FFM-TBW (%)	89.8	1.6	77.8 - 101
FFM-SKINFOLD (%)	85.6	0.9	77 - 92
BMC-DXA (Kg)	1.02	0.17	0.37 - 2.68
TBW (L)	19.06	2.38	9.9 - 47.4
TBW (%)	70.82	1.32	59.62 - 79.70
ICV (L)	10.96	1.78	3.71 - 31.93
ICV (%)	38.41	1.42	27.28 - 51.01
ECV (L)	8.10	0.65	4.45 - 15.47
ECV (%)	32.44	1.75	19.01 - 47.69

BW: Body weight

FM: Fat mass

TBW: Total body water

TM: DEXA constructed weight

FFM: Fat free mass

ECV: Extracellular volume

BMI: Body mass index

BMC: Bone mineral content

ICV: Intracellular volume

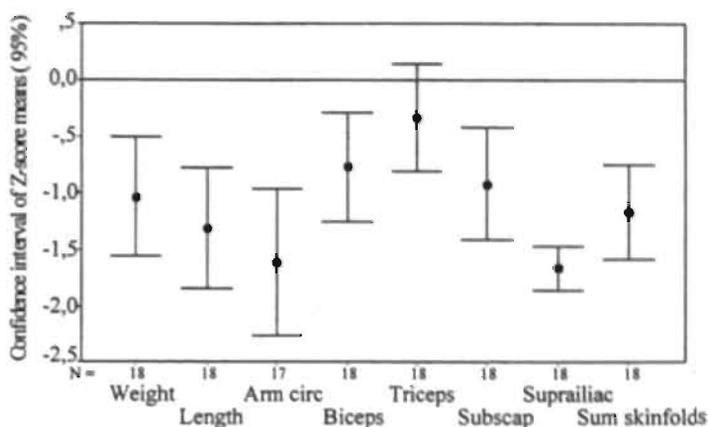


Figure 1 Confidence interval of Z-score means for various anthropometric parameters in cystic fibrosis children showing significantly lower values for all parameters except for the triceps skinfold.

Table 2 Correlation coefficients between muscle area, ECV, ICV and body composition results

	MUSCLE AREA (cm <sup>2</sup> )	ECV (L)	ICV (L)
LBM-DEXA (Kg)	0.84 ( p = 0.001 )	0.67 ( p = 0.004 )	0.91 ( p = 0.001 )
LBM-SKINF (Kg)	0.90 ( p = 0.001 )	0.58 ( p = 0.02 )	0.92 ( p = 0.001 )
LBM-TBW (Kg)	0.86 ( p = 0.001 )	0.63 ( p = 0.007 )	0.95 ( p = 0.001 )
FM-DEXA (Kg)	0.62 ( p = 0.02 )	0.15 ( p = 0.58 )	0.53 ( p = 0.04 )
FM-SKINF (Kg)	0.61 ( p = 0.02 )	0.23 ( p = 0.38 )	0.53 ( p = 0.03 )
FM-TBW (Kg)	0.33 ( p = 0.21 )	-0.004 ( p = 0.99 )	0.20 ( p = 0.43 )
ECV (L)	0.45 ( p = 0.08 )		
ICV (L)	0.86 ( p = 0.001 )		

LBM-DEXA: Lean body mass by DEXA method

LBM-SKINF: Lean body mass by skinfolds method

LBM-TBW: Lean body mass by TBW method

ECV: Extracellular volume

FM-DEXA: Fatmass by DEXA

FM-SKINF: Fatmass by skinfolds method

FM-TBW: Fatmass by TBW method

ICV: Intracellular volume

**Table 3 Changes in bodyweight and body composition after 3 months on lansoprazole in 15 CF children.**

	T3 - T0		
	MEAN ± SEM	MINIMUM	MAXIMUM
BW (Kg)	0.97 ± 0.13	0.4	2.1
TM (Kg)	0.97 ± 0.15	-0.05	1.9
FM-DXA (Kg)	0.52 ± 0.19	-0.46	2.18
FM-TBW (Kg)	-0.23 ± 0.52	-4.85	3.44
FM-SKINFOLD (Kg)	0.94 ± 0.26	0.05	3.52
LBM-DXA (Kg)	0.43 ± 0.18	-0.96	1.46
BMC-DXA (Kg)	0.03 ± 0.01	-0.03	0.09
FFM-DXA (Kg)	0.41 ± 0.17	-0.98	1.03
FFM-TBW (Kg)	1.27 ± 0.49	-1.84	5.35
FFM-SKINFOLD (Kg)	0.04 ± 0.26	-3.02	0.98
TBW (L)	0.99 ± 0.39	-1.4	4.1
ICV (L)	0.09 ± 0.41	-3.49	3.17
ECV (L)	0.92 ± 0.46	-2.38	4.91

T0: Before start lansoprazole

T3: 3 months after lansoprazole

TM: Total mass (DEXA)

BW: Body weight

FFM: Fat free mass

FM: Fatmass

BMC: Bone mineral content

LBM: Lean body mass

TBW: Total body water

ECV: Extracellular water

ICV: Intracellular water

### Limits of agreement between methods

As only 5 of our patients 3 girls and 2 boys were postpubertal, all results of both boys and girls were analysed together. There was a high correlation between the 3 body composition methods for measuring of FM and FFM (fig 2 and 3). The best correlation for FM determination was between DEXA and the skinfold method ( $r = 0.98$ ). As DEXA is a 3 compartments model, BMC was not included in the lean body mass. After correction for BMC, the correlation coefficient was unchanged. Plots of the paired differences for FM and FFM measured in kilogram versus their mean, with indication of the limits of agreement are shown in figure 4 and figure 5 respectively. Since our population was small, we preferred to use the 10th and 90th centile values instead of  $\pm 2$  SD for defining the limits of agreement. No intermethod correlations were found between means and differences as shown in figures 4 and 5. The 50th centile of the differences between FM-TBW versus FM-skinfolds was -1.68kg (-2.64 - -0.46kg); FM-skinfolds versus FM-DEXA was 0.75kg (-3.01 - 1.21kg); FM-TBW versus FM-DEXA was -0,96kg (-5.05 - -0.03kg). The 50th centile of the differences between FFM-TBW versus FFM-DEXA was 0.45kg (-0.70 - 4.85kg); FFM-skinfolds versus FFM-DEXA was -0.17kg (-0.77 - 3.46); FFM-TBW versus FFM-skinfolds was 1.68kg (0.46 - 2.96kg). The DEXA constructed weight was highly correlated with scale weight ( $r = 0.999$   $p = 0.001$ ). However, the DEXA weight was significantly lower than scale weight ( $p = 0.003$ ); the 50th centile of the differences between bodyweight constructed from DEXA (TM) and scale weight was -0.52kg (-0.79 - 0.12). Significant differences were found between the means of FFM measured by the TBW and the skinfold methods ( $p = 0.02$ ), the skinfolds and DEXA methods ( $p = 0.001$ ) as well as the TBW and DEXA methods ( $p = 0.001$ ). Only FM results from TBW and skinfolds were significantly different ( $p = 0.01$ ).

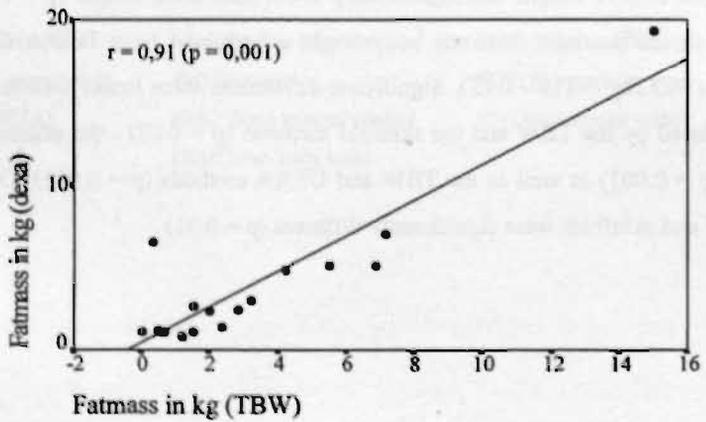
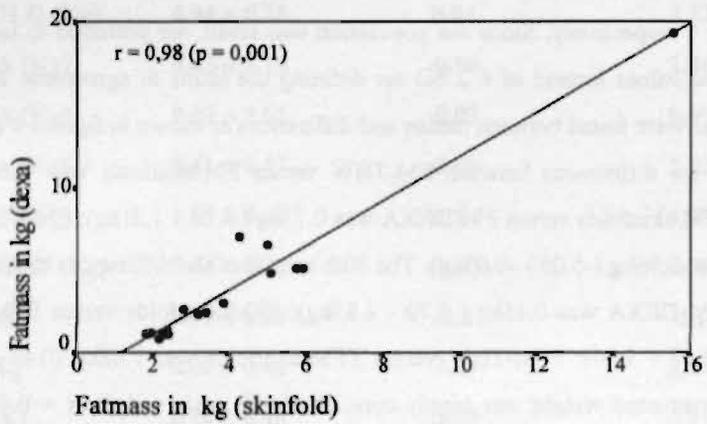
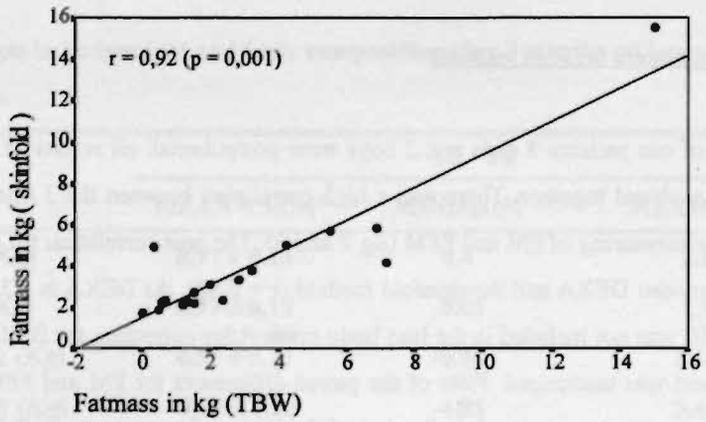


Figure 2 Intermethod fatmasses correlation coefficients.

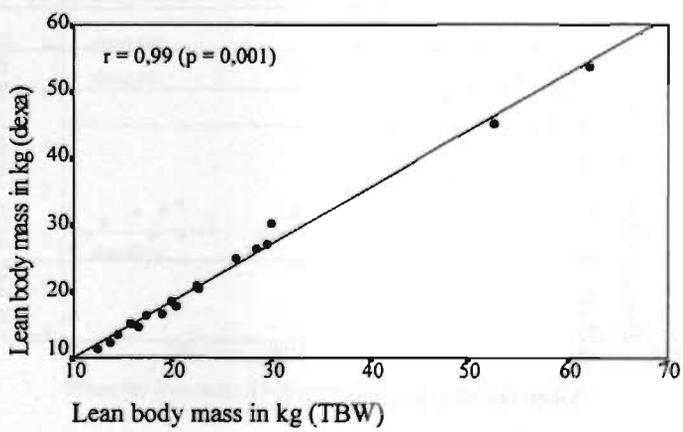
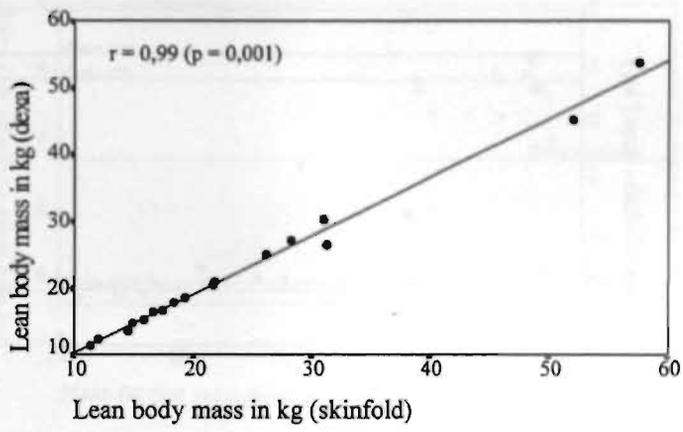
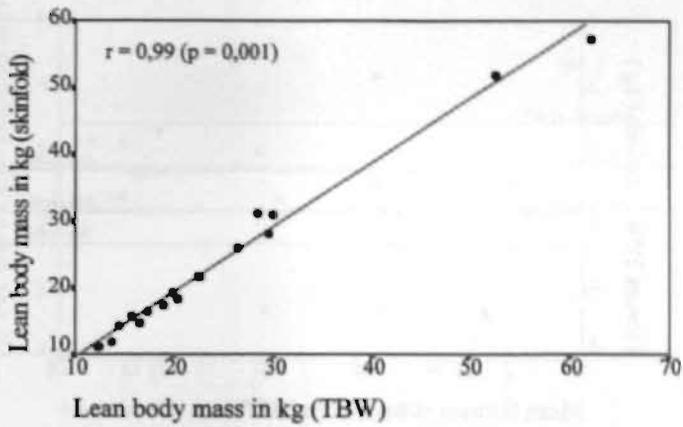


Figure 3 Intermethod lean body mass correlation coefficients.

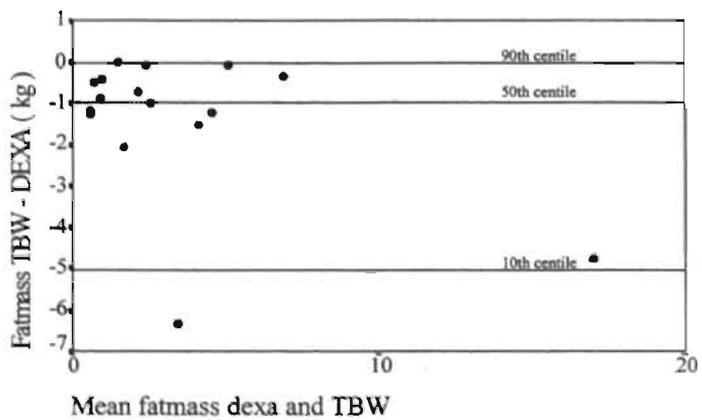
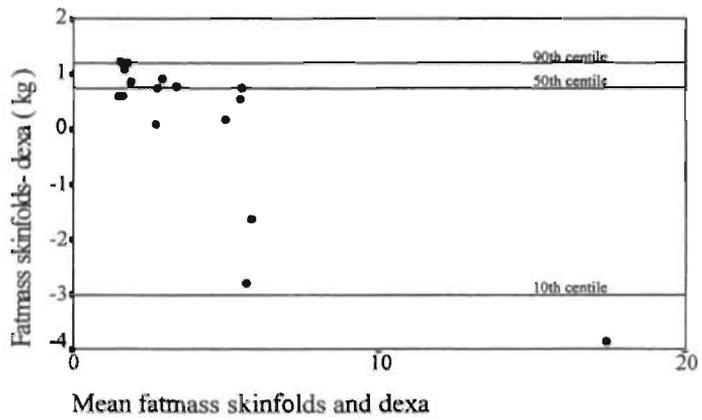
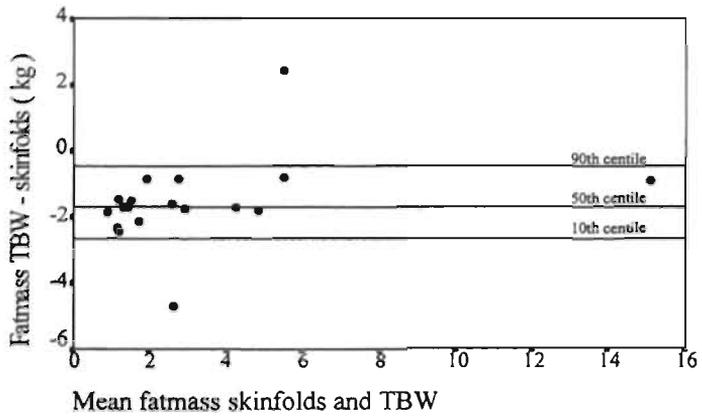


Figure 4 Limits of agreement for fatmass measured by the various methods.

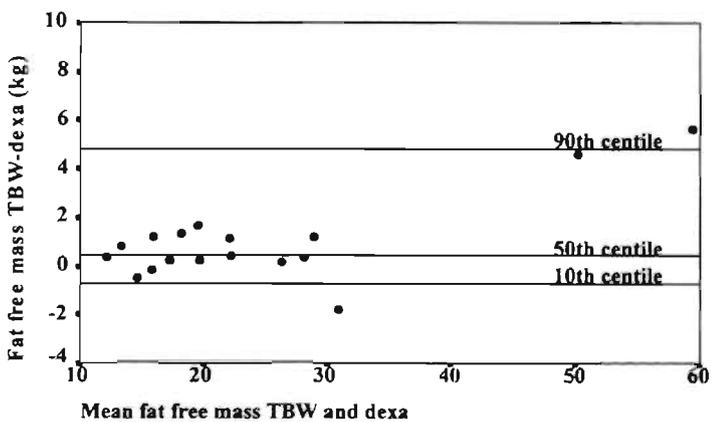
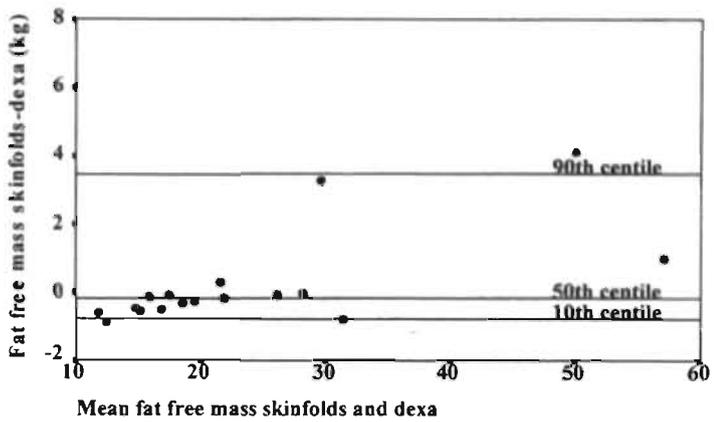
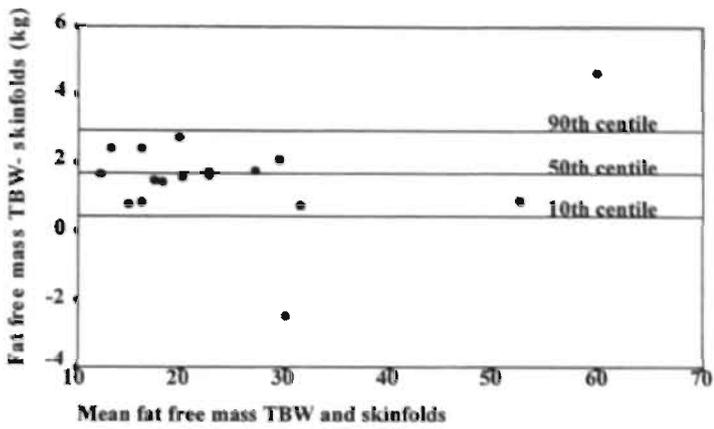


Figure 5 Limits of agreement for fat free mass measured by the various methods.

### Body composition changes

The changes in bodyweight, FM and FFM measured by skinfold, DEXA and TBW are shown in table 3. The increase in bodyweight was the same for both DEXA constructed weight and scale weight. However changes both in FM and FFM were different between methods. Both changes in FM and FFM measured by skinfold were highly correlated with those measured by DEXA ( $r=0.91$   $p = 0.001$  and  $r = 0.84$   $p = 0.001$  for FM and FFM respectively). Changes in FM measured by the TBW method were not correlated with changes of the same parameter measured by either the DEXA or the skinfold method, whereas changes in FFM-TBW were negatively correlated with those measured by skinfold and DEXA (FFM-TBW versus FFM-skinfold:  $r = -0.56$   $p = 0.03$  and FFM-TBW versus FFM-DEXA:  $r = -0.52$   $p = 0.05$ ). No correlation was found between changes in bodyweight and changes in FM or FFM measured by any method. Changes in ECV and ICV did not correlate with bodyweight changes. No correlation was found between changes in ICV and changes in LBM by any method.

## DISCUSSION

In children, effective evaluation of deterioration or catch-up growth can only be achieved by using the Z-score method. Despite high caloric polymeric intake, treatment of steatorrhea and support of pulmonary function, significantly lowered Z-scores for arm circumference, biceps, subscapular, suprailliac, sum of the 4 skinfolds weight and height were found in our patients. As our patients showed decreased weight, height Z-scores and mid upper arm muscle area, we expected both FM and FFM to be decreased. Since all body composition methods are based on assumptions, we used 3 noninvasive methods (DEXA, TBW and skinfolds) to evaluate the body composition of our patients. However, interpretation of the body composition results is difficult due to the lack of reference values for several measuring methods. The results of various methods used were strongly correlated with each other but still showed differences. In absolute terms, only DEXA results were as expected; showing a decrease in all 3 body components measured. Results of the TBW and skinfolds method could not be assessed accurately due to the lack of reference data expressed in absolute terms. When compared to the DEXA reference values, TBW and skinfolds methods only showed a decrease in FM. In relative terms, our CF populations showed an increase in TBW and ECV while the ICV (body cell water mass) appeared well maintained. These results imply a decrease of fatmass, associated with a relative increase in TBW, ECV and consequently FFM (19). In agreement with these data, our results showing an increased percentage of FFM as evaluated by the skinfold method also implies a decrease in fatmass percentage in our CF patients. According to these results, we believe our CF patients mainly have a depletion of fatmass and bone mineral content with a slight decreased in lean body mass in absolute terms. Comparison of our results with other body composition studies in CF children is difficult: First, the general condition of the studied populations differed between studies and second, methods used for the assessment of the nutritional condition were different. Tomezsko et al. found no significant decrease in body FM and FFM in their CF children with only significantly decreased suprailliac skinfold thicknesses and subscapular Z-scores. However their CF population was very young and showed only mild symptoms (2). In another study concerning older CF children with abnormal pulmonary function, Johnston et al. did find a significantly lower percentage of body fat (FFM not reported) compared to matched control children similar to our findings with all 3

methods (20). In agreement with our study, Miller et al. who studied the body composition and muscle protein metabolism in a group undernourished CF children with Z-scores for weight and height similar to those of our CF population, found a significant decrease in FM, FFM and muscle mass (21). The strong correlation we found between components of body composition and age are well known (8,22,23). The lack of sex differences can probably be explained by the prepubertal age of most patients (22). A high correlation was found between mid-upper-arm muscle area, ECV, ICV and LBM and FM. As expected, correlation coefficients between mid-upper-arm muscle area, ECV, ICV and LBM remained high while only weak correlations were found with FM. Correlations were also evaluated after "homogenizing" our patient group by excluding the 2 adolescent patients. Highly significant correlations were again found between the above parameters and LBM while none were found with FM. As expected the between methods results differed significantly. Despite a high correlation between DEXA constructed weight and scale weight, mean DEXA weight was significantly and about 520 gram lower than scale weight. This is in agreement with results from Oggle et al. (8). FM measured by TBW was lower than that measured by either skinfolds or DEXA methods whereas FM-skinfold was often higher than FM-DEXA. The low values of FM when measured by the TBW method might be due to overestimation of FFM by this method. The FFM calculated by TBW is based on the assumption that a fraction of FFM is water. As the water content of FFM decreases with age (17), we used the age dependent FFM hydration fraction to calculate the FFM (17). The mean FFM hydration fraction used in our study was 76.27% (range: 73.7 - 77.5%). In a study of body composition of CF prepubertal children, making use of skinfolds and TBW methods, Tomezco et al. also found a significantly lower body fat percentage with the TBW when compared to the skinfold method, which showed normal results (2). In two compartment models such as the TBW and the skinfolds methods, the densities of FFM is assumed to be constant in the range 18 - 67 years but the density does vary depending on the concentrations of water and mineral in FFM (24,25). Although in our study the variation in water and mineral content was taken into account in the regression equations of skinfolds and TBW methods for the calculation of FFM and FM in the age range below 18 years, the water and mineral content are still population specific depending on the presence of illnesses. The percentage of TBW in CF children has been reported to be increased compared to control children (19,26). Theoretically, the DEXA method has the advantage

of being independent of biological assumptions about the densities and level of tissue hydration but the accuracy of the method still depends on the internal calibration (27,28). It has been reported that when compared to chemical analysis, DEXA overestimate fat measured in meat blocks with lower fat content and underestimate the content in those with high fat content (29). Moreover, studies comparing DEXA results with those obtained from chemical analysis, using piglets, showed slight inadequacies in the estimation of fatmass and lean body mass (27,30). We think that the between methods differences are most likely related to the various body compartments measured by these 3 methods rather than to inherent inaccuracies in the techniques themselves. This means that results obtained from each of these methods are not interchangeable. An important question to answer is whether or not any of the used methods is capable of detecting body composition changes occurring during nutritional interventions. DEXA has been introduced as direct method with very good reproducibility (12,31). In this study we compared the sensitivity of DEXA, TBW and skinfolds for detecting small body composition changes in children. For this purpose, we assessed the body composition of 15 CF children before and 3 months after they were treated with lansoprazole as an adjunct therapy for pancreatic enzymes in order to decrease steatorrhea. All 15 CF children showed significant increases in Z-scores for weight, height and skinfolds (unpublished observations). The evaluation of body composition changes differed depending on the method used. With the DEXA method, 53% of the weight increase was ascribed to FM, 44% to FFM and 3% to BMC. Both DEXA and skinfolds methods showed significant increases in fatmass but the increased FFM was not significant. In contrast, weight increase was exclusively ascribed to an increase in FFM with the TBW method. However, no significant correlations were found between weight changes and either FM, LBM or BMC changes by any method. The correlation coefficient of 0.40 found between weight changes and changes in FFM by DEXA just failed to reach statistical significance ( $p = 0.07$ ). There were also no significant changes in ECV and ICV after intervention. This is in contrast to results reported by Going et al., who studied the changes in body compartments induced by dehydration - rehydration with oral fluid using DEXA method for assessment of body composition changes. They found a correlation between bodyweight changes and changes in TM, soft tissue mass (LBM + FM) and LBM. However, the total weight changes induced in their study was higher than in our study (approximately 1.2 kg versus 0.97 kg in our study) and as the changes in bodyweight were

induced by water content, the total bodyweight changes were exclusively ascribed to changes in the water content of STM, reflected by the exclusive increase in LBM (32). Since fatmass was mostly depleted in our patients, it is likely that this body compartment will normalize first as a result of an effective intervention.

From the results of this study, we conclude that results measured by different methods are not interchangeable. It is consequently important to use the same method for longitudinal evaluation of body composition. However, the use of DEXA, TBW and skinfolds methods is limited in children in whom only slight changes in bodyweight after intervention are expected (3% in this study) since the sensitivity is apparently not high enough for the detection of small differential changes in FM and FFM.

*Acknowledgment:* The authors wish to thank Mia Meers from the department of clinical laboratory, Sandra Zimny and Piet Willems from the department of nuclear medicine for their kind and expert technical assistance.

## REFERENCES

- (1) M. Aitken, S. Fiel. Cystic Fibrosis. *Dis Mon* 1993;39: 1-52.
- (2) J. Tomezsko, T. Scanlin, V. Stallings. Body composition of children with cystic fibrosis with mild clinical manifestations compared with normal children. *Am J Clin Nutr* 1994; 59: 123-8.
- (3) M. Bronstein, P. Davies, K. Hambidge, F. Accurso. Normal energy expenditure in the infant with presymptomatic cystic fibrosis. *J Pediatr* 1995; 126: 28-33.
- (4) R. Kraemer, A. R udeberg, B. Hadorn, E. Rossi. Relative underweight in cystic fibrosis and its prognostic value. *Acta Paediatr Scand* 1978; 67: 33-37.
- (5) R. Branson, Y. Vaucher, G. Harrison, M. Vargas, C. Thies. Inter- and intra-observer reliability of skinfold thickness measurements in newborn infants. *Hum Biol* 1982; 54: 137-143.
- (6) W. Gerver, R. de Bruin. *Paediatric Morphometrics: A reference manual*. 1th ed. Utrecht: Bunge, 1996.
- (7) J. Westrate, P. Deurenberg, H. Van Tinteren. *Int J Obesity*. 1989; 13: 465-477.
- (8) G. Ogle, J. Allen, I. Humphries et al. Body-composition assessment by dual-energy x-ray absorptiometry in subjects aged 4-26 y. *Am J Clin Nutr*. 1995; 61:746-53.
- (9) J. Westrate, P. Deurenberg. Body composition in children: proposal for a method for calculating body fat percentage from total body density or skinfold-thickness measurements. *Am J Clin Nutr* 1989; 50: 1104-15.
- (10) R. Mazess, B. Collick, J. Trempe, H. Barden, J. Hanson. Performance evaluation of a dual-energy x-ray bone densitometer. *Calcif Tissue Int* 1989; 44: 228-232.
- (11) W. Peppler, R. Mazess. Total body bone mineral and lean body mass by dual-photon absorptiometry. I. Theory and measurement procedure. *Calcif Tissue Int* 1981; 33: 353-359.
- (12) R. Mazess, H. Barden, J. Bisek, J. Hanson. Dual-energy x-ray absorptiometry for total-body regional bone-mineral and soft-tissue composition. *Am J Clin Nutr* 1990; 51: 1106-12.
- (13) B. Van Kreel, F. Van der Vegt, M. Meers, T. Wagenmakers, K. Westerterp, A. Coward. Determination of total body water by a simple and rapid mass spectrometric method. *J Mass Spectrom* 1996; 31: 108-111.
- (14) B. Van Kreel. An improved bromide assay for the estimation of extracellular water

- volume by capillary gas chromatography. *Clinica Chimica Acta* 1994; 231: 117-128.
- (15) B. Friis-Hansen. Body water compartments in children: Changes during growth and related changes in body composition. *Pediatrics* 1961; 28: 169-181.
- (16) S. Fomon, F. Haschke, E. Ziegler, S. Nelson. Body composition of reference children from birth to age 10 years. *Am J Clin Nutr* 1982; 35: 1169-1175.
- (17) R. Boileau, T. Lohman, M. Slaughter, T. Ball, S. Going and M. Hendrix. Hydration of the fat-free body in children during maturation. *Hum Biol* 1984; 56: 651-666.
- (18) J. Bland, D. Altman. Statistical methods for assessing agreement between two methods of clinical measurement. *The Lancet* 1986; 8: 307-310.
- (19) M. Miller, D. Kornhauser. Bromide pharmacokinetics in cystic fibrosis. *Arch Pediatr Adolesc Med* 1994; 148:266-271.
- (20) J. Johnston, M. Leong, E. Checkland, P. Zuberbuhler, P. Conger, A. Quinney. Body fat assessed from body density and estimated from skinfold thickness in normal children and children with cystic fibrosis. *Am J Clin Nutr* 1988; 48: 1362-6.
- (21) M. Miller, L. Ward, B. Thomas, W. Cooksley, R. Shepherd. Altered body composition and muscle protein degradation in nutritionally growth-retarded children with cystic fibrosis. *Am J Clin Nutr* 1982; 36: 492-499.
- (22) H. Rico, M. Revilla, L.F. Villa, E. Hernández, M. Alvarez de Buergo and M. Villa. Body composition in children and Tanner's stages: A study with Dual-energy X-ray absorptiometry. *Metabolism* 1993; 42: 967-970.
- (23) R. Faulkner, D. Bailey, D. Drinkwater, A. Wilkinson, C. Houston and H. McKay. Regional and total body bone mineral content, bone mineral density and total body tissue composition in children 8 - 16 years of age. *Calcif Tissue Int* 1993; 53: 7-12.
- (24) G. Forbes. *Human body composition*. New York: Springer-Verlag, 1987.
- (25) T. Lohman. *Advances in body composition assessment*. Champaign, IL: Human Kinetics, 1992.
- (26) M. Newby, N. Keim, D. Brown. Body composition of adult cystic fibrosis patients and control subjects as determined by densitometry, bioelectrical impedance, total body electrical conductivity, skinfold measurements, and deuterium oxide dilution. *Am J Clin Nutr* 1990; 52: 209-13.
- (27) K. Ellis, R. Shypailo, J. Pratt, W. Pond. Accuracy of dual-energy x-ray absorptiometry

for body composition measurements in children. *Am J Clin Nutr* 1994; 60: 660-5.

(28) R. Wellens, W. Chumlea, S. Guo, A. Roche, N. Reo, R. Siervogel. Body composition in white adults by dual-energy x-ray absorptiometry, densitometry, and total body water. *Am J Clin Nutr* 1994; 59: 547-55.

(29) M. Jensen, J. Kanaley, L. Roust et al. Assessment of body composition with use of dual-energy x-ray absorptiometry: Evaluation and comparison with other methods. *Mayo Clin Proc* 1993; 68: 867-873.

(30) J. Brunton, H. Bayley, S. Atkinson. Validation and application of dual-energy x-ray absorptiometry to measure bone mass and body composition in small infants. *Am J Clin Nutr* 1993; 58: 839-45.

(31) P. Chilibeck, A. Calder, D. Sale, C. Webber. Reproducibility of dual-energy x-ray absorptiometry. *Can Assoc Radiol J* 1994; 45: 297-302.

(32) S. Going, M. Massett, M. Hall et al. Detection of small changes in body composition by dual-energy x-ray absorptiometry. *Am J Clin Nutr* 1993; 57: 845-50.

## CHAPTER 8

### GENERAL DISCUSSION

Chronic pulmonary infections and poor appetite together with fat malabsorption are the main causes of malnutrition and growth retardation in CF children (1-3). The ideal treatment of CF should be the correction of the underlying defect by introduction of a normal copy of the defective gene into these patients genetic material. Although gene therapy is presently under intensive scrutiny (4-6), the role of this treatment in CF patients is not yet settled. Until then, treatment of these patients has to focus on improving the nutritional condition, since malnutrition can adversely affect survival (7). As 85% of CF patients have pancreatic insufficiency (8), improved absorption by pancreatic enzymes substitution is one of the main goals. Diagnosis and regular monitoring of fecal fat loss along with close evaluation of growth and the nutritional condition are consequently necessary in the follow up of these patients. Although the fat balance method is considered to be the golden standard for the evaluation of steatorrhea, it is too cumbersome to be used for the frequent monitoring of fat losses in these children. Several studies have shown the measurement of fecal fat concentration to be a valuable alternative to fat excretion studies for the diagnosis of fat malabsorption (9). These studies also shown that the differences in fat excretion between either 3 or 1 day collections are mainly due to day to day variation in stool volume, the stool fat concentration being much more constant. These studies led us to suppose that the repeated measurement of stool fat concentration in stool samples would be a valuable aid to the monitoring of steatorrhea. As chemical measurement of stool fat is time consuming, we looked for an alternative easy measure of fat content. Although the steatocrit looked quite attractive (10) our first results and also results reported by others (11,12) disappointingly often showed low steatocrit results in stools of high fat content.

By acidification of stool homogenates, we could show fat extraction to be much improved and to result in a satisfactory correlation coefficient between chemically measured fecal fat and "acid steatocrit" results. We consequently decided to use the acid steatocrit in an intervention study (proton pump inhibitor) aiming at improving both steatorrhea and the nutritional condi-

tion in children with CF.

Both anthropometric parameters and body composition methods were used for the evaluation of the nutritional condition. Difficulties arise due to the fact that weight, height and skinfolds are age and sex specific. Although several authors have overcome this problem by expressing results of these parameters as a percentage of the predicted values for age and sex, the use of Z-scores is the preferred method for most authors. Z-scores measure deviations from the median value expressed in standard deviation units. Improving Z-scores reflect catch-up growth while the reverse is true for deteriorating Z-scores. Recently, Gerver and de Bruin have constructed growth charts with standard deviation for weight, height armcircumferences and the 4 skinfolds (biceps, triceps, subscapular and suprailiac) (13). Anthropometric parameters can be easily converted into Z-scores through the use of these reference data for normal children. As weight changes could be due to either changes in fatmass, fat free mass or both, we measured body composition by several methods in order to evaluate body composition before and after our intervention (proton pump inhibitor) study.

Our study results show significant decreases of most measured anthropometric parameters in children with cystic fibrosis. Decrease in skinfold thicknesses were most significant and contrary to a commonly held belief triceps skinfolds were often normal while subscapular and suprailiac skinfolds were very sensitive indicators of chronic malnutrition in these patients. Our findings support the use of these simple anthropometric measurements for the evaluation of the nutritional condition in children. As far as body composition results are concerned, interpretation of results is uneasy due to the lack of reference values for several measuring methods. Notwithstanding these drawbacks, results of the various methods used were strongly correlated with each other but, still showed differences which preclude the use of these various methods interchangeably. Results should be looked at both in relative and in absolute terms.

In absolute terms, the DEXA method showed a severe decrease of fatmass and a slight decrease of fat free mass and of bone mineral content. Results of the total body water and skinfold method agreed with the DEXA results but could not be accurately assessed due to the lack of reliable reference data.

In relative terms, the deuterium - bromide results showed a relatively increased total body water and extracellular water compartment while the relative body cell water mass appeared

well maintained. These results imply a decrease of fatmass as percent of bodyweight. Likewise, the fat free mass (%) measured by the skinfold method was increased in our CF children. All these results agree with each other rather well and show that children with CF have a lowered bodyweight accompanied by a decreased fatmass (%), an increased fat free mass (%) and an increased extracellular water compartment (%) while the intracellular water compartment (%) appears to be well maintained (table 1).

**Table 1** Body composition in children with cystic fibrosis.

	<b>FM</b>	<b>FFM</b>	<b>TBW</b>	<b>ECV</b>	<b>ICV</b>
	<b>kg (%)</b>	<b>kg (%)</b>	<b>(%)</b>	<b>(%)</b>	<b>(%)</b>
<b>DEXA</b>	↓ (↓)	↓ (↑)			
<b>Skinfold</b>	? (↓)	? (↑)			
<b>Deuterium</b>					
<b>Bromide</b>	? (↓↓)	? (↑)	(↑)	(↑)	(n)

DEXA: Dual energy X - Ray Absorptiometry

FM: Fatmass

FFM: Fat free mass

TBW: Total Body Water

ECV: Extracellular volume

ICV: Intracellular volume

A positive effect of omeprazole on fat absorption has been found in adults with CF (14). However the role of proton pump inhibitors on steatorrhea and its effects on the nutritional condition has not been evaluated in children. We have studied the effect of 3 months treatment of lansoprazole on fat malabsorption and body composition in 15 CF children, maintaining steatorrhea while on pancreatic enzymes. These children showed significant improvements of both fat absorption (as measured by the acid steatocrit) and Z-scores for all parameters except for the biceps and triceps skinfolds and deteriorated again 3 months after lansoprazole was stopped. The increase in skinfold thicknesses Z-scores were accompanied by signifi-

cant increases in fatmass as measured by the skinfold and the DEXA methods.

Different body composition methods have been described but, only few studies have compared different measurement techniques in pediatric subjects. An important question to answer is whether or not any of these methods is capable of detecting body composition changes occurring during nutritional interventions. Our study comparing the changes in body composition measured by DEXA, TBW and skinfolds methods in 15 CF children, whose nutritional condition improved significantly after intervention with lansoprazole for 3 months, showed different results for each method. Both DEXA and skinfolds methods showed significant increases in fatmass but not in lean body mass in absolute terms. Likewise, the percentage of body cell water mass did not increase significantly after nutritional intervention. On the other hand, the increases in bodyweight were completely ascribed to increases in lean body mass but not in fatmass when evaluated by the TBW method. Since fatmass was mostly depleted in our CF children (as shown by DEXA, skinfolds and total body water methods), it is likely that this body compartment will normalize first as a result of an effective intervention. Our results do not allow firm conclusions as to the effect of lansoprazole on FFM while a significant increase in bone mineral content was found. The bodyweight changes occurring during lansoprazole intervention were unrelated to either fatmass or FFM changes measured by any of the three methods used. We think the weight changes in the various body compartments were too small to be accurately measured by body composition methods.

In conclusion, the acid steatocrit is a reliable, cheap and noninvasive alternative method for the monitoring of fat malabsorption. Most cystic fibrosis patients are malnourished even when lung functions are stable and a hypercaloric diet is used. Body composition studies in these patients mainly show a loss of fat mass and bone mineral content with a relative increase in extracellular water and a normal intracellular water mass (%). Inhibition of gastric acid secretion by a proton pump inhibitor improved both fat absorption and the nutritional condition of our patients. Methods for the assessment of body composition are not interchangeable and not accurate enough for detecting small changes in fatmass and fat free mass such as measured in our 3 months study. A longterm study is needed in order to better evaluate the effects of lansoprazole on body composition in children with cystic fibrosis.

## REFERENCES

- (1) J. Dodge, J. Yassa. Food intake and supplementary feeding programs. In: J. Sturgess, ed. perspectives in cystic fibrosis. Toronto: Canadian Cystic Fibrosis Foundation; 1980: 125-136.
- (2) M. Bronstein, R. Sokol, S. Abman et al. Pancreatic insufficiency, growth, and nutrition in infants identified by newborn screening as having cystic fibrosis. *J Pediatr* 1992; 120: 533-40.
- (3) J. Tomezsco, V. Stallings, D. Kawchak, J. Goin, G. Diamond, T. Scanlin. Energy expenditure and genotype of children with cystic fibrosis. *Pediatr Res* 1994; 35: 451-460.
- (4) M. Rosenfeld, W. Siegfried, K. Yoshimura et al. Adenovirus-mediated transfer of a recombinant alpha 1-antitrypsin gene to the lung epithelium in vivo. *Science*. 1991;252: 431-4
- (5) B. Pitt, M. Schwarz, J. Pilewski et al. Retrovirus-mediated gene transfer in lungs of living fetal sheep. *Gene Ther* 1995; 2: 344-50.
- (6) M. Rosenfeld, K. Yoshimura, B. Trapnell et al. In vivo transfer of the human cystic fibrosis transmembrane conductance regulator gene to the airway epithelium. *Cell*. 1992; 68: 143-55.
- (7) R. Kraemer, A. Rudeberg, B. Hadorn, E. Rossi. Relative underweight in cystic fibrosis and its prognostic value. *Acta Paediatr Scand* 1978; 67: 33-37.
- (8) M. Aitken, S. Fiel. Cystic fibrosis. *Dis Mon* 1993; 39: 1-52.
- (9) N. Thorsgaard Pedersen, H. Halgreen, H. Worning. Estimation of the 3-day faecal fat excretion and fat concentration as a differential test of malabsorption and maldigestion. *J Gastroenterol* 1987; 22: 91-96.
- (10) P. Phuapradit, A. Narang, P. Mendonca, D. Harris, J. Baum. The steatocrit: a simple method for estimating stool fat content in newborn infants. *Arch Dis Child* 1981; 56: 725-727.
- (11) M. Walters, J. Kelleher, J. Gilbert, J. Littlewood. Clinical monitoring of steatorrhea in cystic fibrosis. *Arch Dis Child* 1990; 65: 99-102.
- (12) E. Sugai, G. Srur, H. Vazquez et al. Steatocrit: a reliable semiquantitative method for detection of steatorrhea. *J Clin Gastroenterol* 1994; 19: 206-9.
- (13) W. Gerver, R. de Bruin. *Paediatric Morphometrics: A reference manual*. 1th ed. Utrecht: Bunge, 1996.
- (14) H. Heijerman, C. Lamers, W. Bakker. Omeprazole enhances the efficacy of pancreatin (pancrease) in cystic fibrosis. *Ann Intern Med*. 1991; 114: 200-201.

## SUMMARY

**In chapter one**, the pathogenesis, clinical manifestations and treatment modalities of cystic fibrosis are briefly summarized. CF is a multisystem disease, the basic defect is a mutation of the CFTR gene. Until now, more than 200 mutations have been characterized. CFTR has been found in epithelial cells of several organs with the lung and pancreas being mostly affected. The role of gene therapy in the management of CF patients is not yet settled. Until then treatment of these patients has to focus on support of lung function and improved fat absorption in order to maintain a normal nutritional status. From our literature review, only predigested foods such as (semi)elemental diets and very high-energy polymeric diets, have been reported to improve the nutritional condition in CF patients. Low duodenal pH is thought to be at least partly responsible for the persisting maldigestion. Inhibition of gastric acid secretion by a proton pump inhibitor has been shown to improve steatorrhea in CF adults. The effect of proton pump inhibitors on fat absorption and on the nutritional status of children with CF has not been reported. The effect of treatment on steatorrhea can only be evaluated by regular monitoring of fecal fat loss. The fat balance method being too cumbersome for the repeated evaluation of steatorrhea, we first aimed at developing an alternative test suitable for our purpose. This test (acid steatocrit) was subsequently used to evaluate the effect of lansoprazole (proton pump inhibitor) on steatorrhea in CF patients showing persisting malabsorption while on pancreatic enzymes. The effects of therapy on the nutritional condition of our patients was evaluated simultaneously.

**In chapter two**, the methods used in this study are described. For the determination of fecal fat, the titrimetric method described by van de Kamer and the Sudan staining method were used for the comparison of steatocrit and acid steatocrit methods. Anthropometry, dual-energy X-ray absorptiometry, total body water and bromide dilution techniques were used to assess body composition.

**In chapter three, four and five**, we describe the steatocrit test as an alternative method for the 3 days fecal fat balance method for the monitoring of steatorrhea. Although the steatocrit

test has been reported to be cheap, simple and noninvasive test, its reliability has been questioned. As this might be due to inadequate fat extraction during the centrifugation step of the steatocrit procedure, we aimed at improving fat extraction by acidification of the fecal homogenate. Results obtained by our modified steatocrit method, called the "acid steatocrit", were highly correlated with those obtained by chemical analysis. We found a high sensitivity and specificity for the acid steatocrit.

Results of the evaluation of the nutritional condition of our patients as well as results concerning the presence of persisting steatorrhea in patients on pancreatic enzymes are described in **chapter six**. Despite hypercaloric intake and the use of pancreatic enzymes, our CF patients maintained steatorrhea and showed signs of malnutrition with significantly decreased Z-scores for weight, height, arm circumference, biceps, subscapular and suprailiac skinfolds. Moreover, their fatmass, lean body mass and bone mineral content were significantly decreased when compared to the reference population described by Oggle et al. Treatment of these CF children with lansoprazole as an adjunct therapy of pancreatic enzymes, resulted in a significant decrease in steatorrhea accompanied by a significant improvement in their nutritional condition.

In **chapter seven**, we describe results of our body composition studies in our patients before and after treatment with lansoprazole. Although highly correlated, results from these various methods were shown not to be interchangeable. In absolute terms, the DEXA, the TBW and the skinfold methods showed children with CF to have a severe depletion of fatmass and a slight decrease of FFM. In relative terms, the above results point to lower body fat percentage accompanied by a higher percentage of LBM. Our results with deuterium - bromide do confirm the above results by showing a high relative TBW content and consequently a low relative fat content. Bromide results further show the relative increase of water percentage to be due to a relatively increased extracellular water compartment with a maintained relative body cell water mass. Although small changes in bodyweight were correctly detected by DEXA examination, the latter method was not accurate enough for the differential detection of small changes in FM and FFM. The usefulness of DEXA, TBW and skinfold methods for the assessment of small body composition changes in children is therefore limited.

## SAMENVATTING

**In hoofdstuk een**, zijn de pathogenese, de klinische manifestaties en de therapeutische mogelijkheden voor cystic fibrosis (CF) kort samengevat. Cystic fibrosis is een multisysteem ziekte, waarvan mutatie van de CFTR (cystic fibrosis transmembrane regulator) gene is het basis defect.

Tot dus ver, zijn er meer dan 200 mutaties beschreven. CFTR werd in de epitheel cellen van verschillende organen gevonden. De longen en de pancreas zijn het meest betrokken by deze erfelijke aandoening. De rol van de gen therapie is nog niet bevestigd in de behandeling van CF patienten. De behandeling van deze patienten is er dan ook gericht op de long functies te ondersteunen en een normale voedingstoestand te behouden door het verbeteren van de vet malabsorptie.

Uit het litteratuur overzicht blijkt dat de voedingsstatus van CF patienten alleen effectief te verbeteren is met voorverteerd voedsel zoals (semi)elementaire voeding, of met een zeer hoge energie inname. Een lage duodenale pH is mede verantwoordelijk voor het slechte verteringsproces. Het is bij volwassen CF patienten bekend dat de vet absorptie significant te verbeteren is door remming van de maagzuur secretie met een proton pomp remmer. Er is nog geen studie gedaan naar het effect van dit middel op de vet vertering en de voedings status bij CF kinderen.

Regelmatig monitoring van vet in de ontlasting is noodzakelijk voor de behandeling van vet malabsorptie. De gebruikelijke vet balans methode is hiervoor te omslachtig. Ons eerste doel was het ontwikkelen van een alternatieve test die snel en makkelijk uitvoerbaar is. Deze test (zure steatocriet) werd dan gebruikt om het effect van een proton pomp remmer (lansoprazol) op steatorrhoea in CF patienten met persisterende malabsorptie onder pancreas enzymen, te evalueren. Daarnaast, werd het effect van lansoprazol op de voedingstoestand van onze patienten geevalueerd.

**In hoofdstuk twee**, beschrijven we de methoden die we gebruikt hebben in deze studie. Voor de bepaling van vet in de ontlasting, werden de titrimetrische methode, beschreven door van de Kamer, en de Sudan kleurings techniek gebruikt om de klassieke steatocriet te vergelijken

met de zure steatocriet. De anthropometrische methode, de dual-energy X-ray absorptiometry (DEXA), het totale lichaamswater (TBW) en de bromide dilutie technieken werden toegepast om de lichaamsamenstelling te beoordelen.

In hoofdstuk drie, vier en vijf, beschrijven we de steatocriet test als een alternatieve methode voor de 3 dagen vet balans ter monitoring van vet in de ontlasting. Hoewel de steatocriet test werd gezien als een goedkope, simpele en noninvasieve test, de betrouwbaarheid van deze test wordt betwist. Dit is mogelijk toe te schrijven aan de inadequate vet extractie tijdens het centrifugeren van de steatocriet procedure. Ons doel was de vet extractie te verbeteren door het aanzuren van het faeces homogenaat. De resultaten verkregen met deze gemodificeerde steatocriet genaamd "zure steatocriet", correleerden goed met de resultaten van de chemische vet analyse. We vonden een hoge sensitiviteit en specificiteit voor de zure steatocriet test.

In hoofdstuk zes, bestuderen we de mate van vet malabsorptie en de voedingstoestand van onze CF kinderen behandeld met pancreas enzymen. Ondanks de hypercalorische voeding en de behandeling met pancreas enzymen, hadden onze patiënten aanhoudende steatorrhoea en toonden tekenen van malnutritie met significante verslechtering van de gemiddelde Z-scores voor gewicht, lengte, armomtrek, biceps, subscapulaire en supriliacale huidplooiën. Bovendien, hun vetmassa, spiermassa en botmineral is significant lager dan die van de normale kinderen, beschreven door Oggle. Na de behandeling van deze kinderen met een proton pomp remmer (lansoprazol) als supplementaire therapie by pancreas enzymen, vonden we een significante vermindering van steatorrhoea met verbetering van de voedingstoestand.

In hoofdstuk zeven, beschrijven we de resultaten van de lichaamsamenstelling van onze patiënten voor en na de behandeling met lansoprazol. Ondanks de hoge correlatie tussen de resultaten van de gebruikte lichaamsamenstelling methodes, zijn deze technieken niet uitwisselbaar. In absolute zin, toonden de DEXA, de TBW en de huidplooi methode een ernstige depletie van de vetmassa en een lichte afname van de vet-vrije massa by CF kinderen. In relatieve zin, wijzen deze resultaten in de richting van een afname van het vet percentage gepaard aan een hoger percentage van lean body mass (LBM). Dit komt overeen met de resultaten van deuterium-bromide, waarbij een hoog TBW percentage en dus een laag vet

percentage gevonden werd. De toename in het TBW percentage is toe te schrijven aan het verhoogde percentage extracellulair water terwijl intracellulair water normaal blijft. Alhoewel de verandering in lichaamsgewicht door het DEXA onderzoek correct werd geschat, was geen van de gebruikte lichaamssamenstelling methodes nauwkeurig genoeg voor het schatten van kleine veranderingen in de vetmassa en vet-vrije massa. De bruikbaarheid van DEXA, TBW en huidplooi methoden voor het schatten van kleine veranderingen in de lichaamssamenstelling bij kinderen is daarom beperkt.

## DANKWOORD

Woorden schieten tekort om mijn dank uit te drukken. Ik ben niet zo goed in taal expressie, toch hoop ik met enkele eenvoudige zinnen iedereen te kunnen bedanken, die het mij mogelijk hebben gemaakt dit proefschrift vorm te geven.

Zonder iemand tekort te willen doen, richt ik een speciaal dankwoord tot de volgende personen:

**Prof. Dr. C. Blanco**, promotor, beste Carlos, ondanks je drukke taak, heb je toch heel snel en kritisch mijn werkstukken doorgenomen. Hiervoor dank ik je extra.

**Dr. P. Ph. Forget**, copromotor, beste Philippe, het lukte mij nooit je te tutoyeren, niet vanwege onze persoonlijke contacten, maar vanwege mijn respect voor jou. De manier waarop je het onderzoek stuurde waarbij je mij geheel in mijn waarde en vrijheid liet, was van buitengewoon hoog niveau. Je leerde mij wetenschappelijk denken. Waar nodig was bood je hulp aan, soms ook met het verwerken van de resultaten. De correctie van het manuscript was binnen korte tijd klaar. Zelfs in je vakantie, nam je mijn werkstukken mee en was je bereid hiervoor terug te komen. Ik heb genoten van je onuitputtelijke bron van nieuwe ideeën.

Ook in het persoonlijk contact was je aangenaam. Je heb altijd in mij geloofd en stond altijd achter mij. Beste Philippe, zonder jouw inzet en je vertrouwen als begeleider, zou dit proefschrift nooit deze vorm hebben gekregen.

**Dr. B. van Kreel**, copromotor, de helft van mijn tijd als onderzoeker heb ik in uw laboratorium doorgebracht. Uw deur stond altijd voor mij open. Als het niet lukte met de steatocrit-bepaling, heb u altijd nieuwe suggesties. Uw deskundigheid en eerlijkheid was onmisbaar voor het slagen van dit onderzoek.

**Prof. Dr. R. H. Kuijten**, bedankt voor de mogelijkheden die u hebt gecreeërd voor dit onderzoek.

**Drs. A. Van den Neucker**, beste Anita, al die jaren ben je voor mij een goede vriendin geweest. Ook als het mij tegen zat, wist je met je nuchtere kijk en eerlijkheid mijn problemen te relativeren. Ik heb genoten van onze discussies en van je gezelschap op verscheidene congressen. Je interesse in anderen en je brede algemene kennis maakte het zeer boeiend. Anita, je hebt mijn "gat" in de Westerse cultuur opgevuld.

**Hooggeleerde leden van de beoordelingscommissie**, bedankt voor uw vlotte en kritische beoordeling van dit manuscript.

**Alle kinderartsen, neonatologen en arts-assistenten** kindergeneeskunde in het AZM dank ik hierbij voor de aanspraak in de afgelopen jaren.

**Dr. W. J. M. Gerver en Dr. R. De Bruin**, jullie groeicurven hebben grote waarde toegevoegd aan dit onderzoek. Bedankt voor jullie voortreffelijke bijdrage.

**Jolanda van Golde en Rony Neefjes**, beste Rony en Jolanda, bedankt voor het meelevende en de gezellige uren in het AIO-hok, in het restaurant, in het theater aan het Vrijthof, in de bioscoop, bij een van ons thuis of in het zwembad. Bedankt voor het aanhoren van mijn "gezeurd". We hebben goede en slechte tijden met elkaar doorgemaakt. Ik hoop dat onze vriendschap hierdoor alleen maar sterker is geworden.

**Alle medewerkers van het klinisch chemisch laboratorium** van het AZM, met name **Serva, Lou, Michel, Mia, Theo, Peter en Marian**, bedankt voor jullie inzet en betrokkenheid tijdens het onderzoek. Jullie wetenschappelijke interesse was van niveau. Als ik hulp nodig had waren jullie bereid, soms ook ongevroegd, het eigen werk neer te leggen en mij bij te staan. Bedankt voor de aangename sfeer en de gezellige samenwerking.

**Liesbeth van der Ploeg en Lianne Schoorlemmer**, dietisten, wil ik danken voor het uitrekenen van de calorieën bij mijn patiënten populatie.

**Dr. G. A. K. Heidendal, Piet Willems en Sandra Zimny** van de nucleaire afdeling, bedankt voor jullie fijnzinnige instructies over de DEXA scan.

**Alle poli-assistenten en de secretaresses** van de kindergeneeskunde, wil ik danken voor de samenwerking in de afgelopen jaren.

**Oom Wim en tante Margriet van der Avoort**, bedankt voor jullie steun en betrokkenheid in de afgelopen 15 jaren van mijn leven in Nederland.

Ik ben de firma's **Hoechst Marion Roussel B.V.** (Hoevelaken) en **Janssen-Cilag B.V.** (Tilburg) dankbaar voor hun financiële ondersteuning in de drukkosten van dit proefschrift.

Tenslotte, zou dit boek niet volledig zijn zonder hulp en meelevende van mijn familie. Lieve mama, oom Kiet, Manh Hung en Manh Cong, terwijl ik rustig aan mijn proefschrift werkte, hebben jullie voor mijn verhuizing gezorgd.

**Manh Cong**, bedankt voor het ter beschikbaar stellen van je computer en **Manh Hung** voor je

deskundige steun. Als ik met de computer problemen had, kon ik altijd op jullie terugvalen. Oom Kiet, bedankt voor je inzet en betrokkenheid. Nooit hoefde ik je om hulp te vragen, je was er gewoon.

Lieve Mama, zonder jou zou dit boek er nooit zijn gekomen. Heel je leven lang heb je voor ons klaar gestaan. Jouw droom is een goede toekomst voor je kinderen. Daarvoor heb je 15 jaar geleden je leven op het spel gezet. Je stimuleerde ons om te studeren. Rijkdom is niet belangrijk, maar kennis, dat is de beste bagage die je op onze weg aan ons hebt kunnen meegeven. Mama, bedankt voor je betrokkenheid en het aanhoren van mijn frustraties. Met een glimlach en een schouderklop wist je al mijn problemen op te lossen. Mama, ik hou van jou en ik ben trots dat jij mijn moeder ben.

## CURRICULUM VITAE

Thi My Dung Tran werd op 27 april 1967 te Dinh Tuong in Vietnam geboren.

Na het doorlopen van het basisonderwijs volgde zij, eveneens in Vietnam, drie jaren vervolgonderwijs op voorbereidend wetenschappelijk niveau.

In 1981 kwam zij met haar familie in Nederland.

Op 2 juni 1986 verwierf zij aan de Rijksscholengemeenschap "Den Hulster" te Venlo het diploma Atheneum B. met als eindexamenpakket de vakken: Nederlands, Engels, Wiskunde I en II, Natuurkunde, Scheikunde en Biologie.

Vanaf het najaar 1986 studeerde zij geneeskunde aan de Rijksuniversiteit te Maastricht. Zij behaalde op 13 augustus 1990 haar doctoraal getuigschrift. Op 1 februari 1993 werd het diploma basisarts aldaar aan haar uitgereikt.

Tijdens haar studie verrichte zij wetenschappelijk onderzoek onder leiding van Dr. P. PH. Forget op de afdeling kindergeneeskunde van het Academisch Ziekenhuis Maastricht: "Singel Stool analysis for fat, alfa-animo nitrogen and electrolyt".

Vanaf 1 maart tot 3 november 1993 werkte zij als arts-onderzoeker bij de vakgroep kindergeneeskunde van het A.Z.M. aan het project "Effect of ranitidine in children with chronic abdominal pain".

Van 1 december 1993 tot 1 december 1994 was zij werkzaam als AGNIO kindergeneeskunde in het A.Z.M.

In de periode 1 december 1994 tot 1 april 1996 werkte zij onder leiding van Dr. P. PH. Forget, kinder-gastroenteroloog in het A.Z.M., aan haar promotie-onderzoek.



**Steatorrhea and nutritional condition  
in cystic fibrosis children effects  
of a proton-pump inhibitor**



T.M.D. Tran

**STEATORRHEA AND NUTRITIONAL CONDITION IN CYSTIC FIBROSIS CHILDREN  
EFFECTS OF A PROTON - PUMP INHIBITOR**

## CONTENTS

<b>Chapter 1</b>	<b>General introduction - literature review - Aims of the study</b>	<b>1-31</b>
	1. Genetics of cystic fibrosis	
	2. Pathogenesis	
	3. Clinical manifestations	
	4. Diagnosis	
	5. Therapy	
	6. Evaluation of steatorrhea	
	<b>Aims of the study</b>	
	References	
<b>Chapter 2</b>	<b>Methods</b>	<b>32-38</b>
	1. Methods used for fecal fat determination	
	2. Methods used for assessment of nutritional condition	
<b>Chapter 3</b>	<b>The acid steatocrit: A much improved method</b>	<b>39-49</b>
	Tran M., Forget P., Van den Neucker A., Strik J., van Kreeel B., Kuijten R.	
	J Pediatr Gastroenterol Nutr 1994; 19: 299-303	
<b>Chapter 4</b>	<b>Improved steatocrit results obtained by acidification of fecal homogenates are due to improved fat extraction</b>	<b>50-58</b>
	M. Tran, P. Forget, A. Van den Neucker, B. Van Kreeel	
	J Pediatr Gastroenterol Nutr 1996; 22: 157-160	
<b>Chapter 5</b>	<b>Clinical use of acid steatocrit</b>	<b>59-66</b>
	A. Van den Neucker, N. Pestel, T. My Dung Tran, P. Ph. Forget, H. J. Veeze, J. Bouquet, M. Sinaasappel	
	Submitted for publication	
<b>Chapter 6</b>	<b>Role of lansoprazole in children with cystic fibrosis: Evidence for improved fat malabsorption and nutritional status</b>	<b>67-83</b>
	Tran TMD, Van den Neucker A, Hendriks JJE, Forget P (junior), Forget P (senior)	
	Submitted for publication	

<b>Chapter 7</b>	<b>Anthropometry and body composition methods in children with cystic fibrosis: Effects of nutritional intervention</b>	84-107
	My-Dung T. Tran, Anita Van den Neucker, Han J. Hendriks, Bernard van Kreel, Patricia Forget, Guido Heidendal, Pierre-Philippe Forget	
	Submitted for publication	
<b>Chapter 8</b>	<b>General discussion</b>	108-112
<b>Summary</b>		113-114
<b>Samenvatting</b>		115-117
<b>Dankwoord</b>		118-120
<b>Curriculum vitae</b>		121

**STEATORRHEA AND NUTRITIONAL CONDITION IN CYSTIC FIBROSIS CHILDREN  
EFFECTS OF A PROTON-PUMP INHIBITOR**

**PROEFSCHRIFT**

Ter verkrijging van de graad van doctor  
aan de Rijksuniversiteit Limburg te Maastricht,  
op gezag van de Rector Magnificus, Prof.Mr. M.J. Cohen,  
volgens het besluit van het College van Dekanen,  
in het openbaar te verdedigen  
op donderdag 17 oktober 1996 om 16.00 uur

door

Therese Marie Pascale Thi My Dung Tran  
geboren op 27 april 1967 te Dinh Tuong, Vietnam

**Promotor:** Prof. Dr. C. Blanco

**Co-promotores:** Dr. P-Ph. Forget  
Dr. B. van Kreel

**Beoordelingscommissie:** Prof. Dr. P.B. Soeters, ( voorzitter )  
Prof. Dr. H.S.A. Heymans, ( Universiteit van Amsterdam )  
Prof. Dr. R.W. Stockbrugger  
Prof. Dr. J.M. Wit, ( Rijksuniversiteit Leiden )  
Prof. Dr. E.F.M. Wouters

Steatorrhea and nutritional condition in cystic fibrosis children:  
Effects of a proton - pump inhibitor /  
Therese Marie Pascale Thi My Dung Tran.  
Proefschrift Maastricht - Met lit. Opg. - Met samenvatting in het Nederlands.  
ISBN 90-5681-011-1

Trefw.: Steatocriet / steatorrhoe / cystic fibrosis / voedingstoestand / proton -  
pomp remmer / lichaamsamenstelling.

Vormgeving: My Dung Tran  
Omslagillustratie: Vetbollen in een microscopische faeces preparaat,  
met Soudan kleuring.

STEVENS-REISSNER SYNDROOM

*Aan mijn lieve moeder  
Voor alle cystic fibrosis kinderen . . .*

## CHAPTER 1

### GENERAL INTRODUCTION

## **1. GENETICS OF CYSTIC FIBROSIS (CF)**

Cystic fibrosis was first described in 1928 by Fanconi (1). It is an autosomal recessive disease and is reported in all racial groups with varying prevalence. In caucasians, CF occurs in 1 out of 2000 live births. Males and females are equally affected. The basic defect is a mutation of the Cystic Fibrosis Transmembrane Regulator (CFTR), a protein responsible for chloride ion transport in response to cAMP mediated signals. The most frequent CF mutation in the caucasian population is a deletion of 3 nucleotides, encoding for phenylalanine at position 508 in the CFTR protein amino acid sequence. Its overall frequency reported by the CF Genetic Analysis Consortium is 68% (2). Until now, over 200 mutations have been characterized and account for the remaining mutations.

## **2. PATHOGENESIS**

It is generally accepted that cAMP stimulated chloride conductance is a function of the CFTR (3). This function is deficient in epithelial cells of CF patients. The inability to secrete chloride and secondarily secrete water results in viscous secretions. Poor clearance of these viscid secretions from the epithelium often results in obstruction of excretory ducts. CFTR has been found in epithelial cells of several organs such as the airways, the sweat glands, the genitourinary system and the gastrointestinal tract including the pancreas and the biliary tract (4). Dysfunction of these organ systems are therefore possibly related to the same underlying defect in the CFTR-gene product.

## **3. CLINICAL MANIFESTATIONS**

CF is a multisystem disease with lungs and pancreas mostly affected in young patients.

### **3.1 Respiratory tract**

Lung disease accounts for more than 95% of the morbidity and mortality in CF (5,6). The desiccated mucus in the respiratory tract causes stasis and bronchiolar obstructions, resulting

in bacterial overgrowth and chronic lung infection. This gives rise to the production of proteases by bacteria and neutrophils. These enzymes hydrolyze important structural proteins of the lung and airways such as elastin, proteoglycans and collagen, leading to instability of bronchial walls and bronchiectasis. Furthermore, these enzymes also alter the mucosal function by increasing the secretion of macromolecular glycoconjugates contributing to a high viscosity of the mucus (7). Bronchiolitis with wheezing is frequent during the first year of life. Some patients remain however, asymptomatic for long periods. When pulmonary disease progresses, exercise intolerance occurs and finally, progressive pulmonary deterioration is the main cause of death in these patients (6,8). As a consequence of improved supportive therapy, survival has increased from 6 months at the end of the fifties (9) to nearly 30 years currently (10,11). Sinusitis and nasal polyposis sometimes occur in CF (12,13).

### **3. 2 Pancreas**

In the pancreas, obstruction of the ductules is the cause of acinar / ductular distention, followed by disruption with release of proteolytic enzymes and autodigestion of the pancreas resulting in pancreatic insufficiency with steatorrhea. The changes in the pancreas can occur early during gestation, compromising the normal maturation of the pancreas. Approximately, 85% of CF patients have steatorrhea (11). In 85 - 90% of these cases, exocrine pancreatic insufficiency develops during the first year of life. Decreased secretion of bicarbonate and water first occurs before a decrease of pancreatic enzyme concentration in duodenal fluid can be detected (14-17). Recurrent acute pancreatitis occurs in approximately 10% of CF patients (18).

Because of fat malabsorption, serum concentrations of fat soluble vitamins are often lowered. Since Vit A consists of esters of long chain fatty acids, it cannot be absorbed in the absence of pancreatic esterases. Due to its short half life, low serum levels of Vit A are often found in early untreated CF patients (19,20). Vit D deficiency resulting in decreased bone mineralization has also been reported in CF patients (21-23). Due to frequent antibiotic therapy, suppression of endogenous vit K synthesis by anaerobic intestinal bacteria often contribute to a low vit K serum level in CF patients with steatorrhea. Although Vit B12 is water soluble, serum levels may also be low in CF patients. Binding to intrinsic factor, necessary for absorption,

can only take place after cobalamin has been released from the R-protein binding by pancreatic enzymes. Decreased pancreatic bicarbonate secretion may play a role herein since the binding affinity of cobalamin for R-protein decreases at neutral or slightly alkaline pH (24). However, pancreatic enzymes supplements will normalize the Vit B12 serum level.

Abnormal glucose tolerance occurs in 30 - 75% of CF patients while diabetes mellitus develops in 10% (11). Several diabetogenic factors including increased passive sugar transport (25), increased mucosal absorption of D-glucose (26), decreased beta cell mass (27) and delayed insulin secretion (28) are present in CF. On the contrary, several antidiabetogenic factors such as an increased tissue insulin sensitivity (29) and an increased number of insulin receptors on monocytes (30) have been reported in cystic fibrosis. Moran et al. reported a decreased alpha-, beta- and pancreatic polypeptide- cell function in CF patients with exocrine disease compared to those without this disorder. Due to this finding, they suggest that either exocrine disease causes endocrine dysfunction or that a common pathogenic process simultaneously and independently impairs exocrine and endocrine function in CF patients (31). However, the exact etiology of diabetes in CF is still unknown.

### **5. 5 Malnutrition**

CF children are malnourished when compared to normal controls (32,33). Both, malabsorption accompanying pancreatic insufficiency (34) and high energy expenditure due to chronic lung infection (35,36) are thought to be responsible for the poor nutritional condition in these patients. Moreover, in CF, several intraluminal factors other than pancreatic insufficiency are also considered responsible for fat malabsorption:

1) Increased gastric acid secretion after stimulation with pentagastrin (37):

A high postprandial acid secretion could, by lowering the duodenal pH, contribute to fat malabsorption.

2) Decrease pancreatic bicarbonate secretion (13-16):

Higher gastric acid secretion after meals together with a decrease in pancreatic bicarbonate secretion, has been shown to result in a prolonged postprandial lowering of duodenal pH with

inactivation of the remaining pancreatic lipase. Moreover, low duodenal pH also results in bile acid precipitation,, fecal loss of bile acids and a decrease in the bile acid pool, contributing to fat malabsorption

### 3) Increased glycine to taurine conjugated bile acid ratios:

Due to a relatively deficient supply of taurine compared to glycine and to continuous fecal loss of bile acids, newly formed bile acids are mainly conjugated with glycine, leading to high glycine-/taurine- bile acid conjugation ratios (38). The glycine conjugated bile acids precipitate in an acidic environment contributing to the luminal bile acid deficiency in these patients.

## 3. 4 Intestinal tract

Gastroesophageal reflux and esophagitis are frequent causes of epigastric pain in CF patients (39,40) and can be responsible for decreased pulmonary functions (41). Peptic ulcers are found in up to 13% of CF patients (42) and are thought to be related to the low duodenal pH (43). Meconium ileus occurs in 15% of newborn infant with CF (44); 10% of CF patients have meconium ileus "equivalents" at a later age with a peak incidence at 20-25 years of age (45). Protein precipitation as a result of decreased duodenal pH and high secretion viscosity all probably contribute to these obstructive events (11). Up to 25% of the CF patients have rectal prolapse occurring mostly in children aged 6 - 36 months (46) while intussusception has been shown to occur in 1% (47).

## 3. 5 Biliary and Hepatic tracts

An increased incidence of cholelithiasis has been reported in CF patients (48) and is thought to be related to hypokinesia and increased fasting gallbladder volumes (49). Biliary cirrhosis with hepatosplenomegaly leading to portal hypertension occurs in 25% of CF patients. Liver steatosis has been reported in 30% of patients with CF.

## 3. 6 Genitourinary tract

More than 95% of males are infertile due to obstruction of the reproductive tracts (50). Active spermatogenesis does occur but produced spermatozoa are abnormal or immature (51). In CF women, the reproductive tracts are anatomically normal but fertility is decreased (52). Increased viscosity of the cervical mucus is thought to interfere with sperm penetration (53).

### 3.7 Sweat gland

Decreased sodium and chloride reabsorption due to dysregulation of sweat gland duct cells results in susceptibility of CF patients to salt depletion during warm weather and during gastroenteritis.

### 4. Diagnosis

The standard diagnostic procedure is the sweat test based on increased concentration of electrolytes in the sweat of the patients (54). The sweat test was developed by Gibson and Cooke (55), whereby the sweat production is stimulated by pilocarpine iontophoresis. The then collected sweat is analysed for its chloride and sodium content. However, chloride content has a better diagnostic value than sodium content, since abnormal sodium secretion can also be found in other endocrine diseases. Sweat chloride concentration higher than 60 mM or sodium above 70 mM measured minimal on two conditions is considered as abnormal, whereas chloride values under 50mM and sodium value under 30mM are found in normal persons. Chloride concentrations between 50 and 70 mM are inconclusive. For reliable results, at least 50mg of sweat should be collected. Low sweat production in the first few weeks of life is the reason for unreliable test results in this age group. In cases of doubt, identification of CFTR-mutation or measurement of intestinal current in a rectal biopsiate have been reported to be conclusive (56).

### 5. Therapy

The ideal treatment of CF should be the correction of the underlying defect by introduction of a normal copy of the defective gene into these patients genetic material. Gene therapy is

presently under intensive scrutiny. Adenovirus and recently also the retrovirus seem promising as an effective vector for normal gene transport into the target cells (57,58). Recently, transfer of the CFTR gene to the rat airways epithelia has been successfully performed (59). However, the role of gene therapy in the management of CF patients is not yet settled. Until then, treatment of CF patients has to focus on improving the nutritional condition, since malnutrition can adversely affect survival (60). The nutritional status of CF patients can be improved by firstly ameliorating the respiratory function, thereby minimizing energy expenditure and secondly, by increasing energy supply either by increasing nutrient intake or by improving nutrient digestion and absorption.

### **5.1 Respiratory function support**

Since viscous mucus in the lung is the cause of chronic lung infection, efforts should be made to improve mucus clearance. Although most patients on mucolytics such as acetylcysteine have the feeling of decreased sputum viscosity, studies with acetylcysteine either orally or as aerosol have failed to support this finding (61-63). Alternative methods such as chest percussion combined with postural drainage (64), positive expiratory pressure mask (65,66) and forced expiratory pressure (67) have been suggested to improve mucus clearance. Moreover,  $\beta_2$ -agonists as aerosol can increase sputum clearance (68) and some bronchodilating effect has been experienced in CF patients on this regimen (69,70). Corticosteroids, have been found to delay disease progression and to improve lung function in CF patients (71-74) but, short-term adverse effects such as hyperglycemia and long-term adverse effects such as development of cataract and growth retardation preclude the routine use of corticosteroids in these patients (73).

Treatment with antibiotics can reduce the progression of lung infection. Colonisation with *Ps. aeruginosa* often occur in CF patients and various regimes have failed to eradicate the bacteria (75). *Ps. aeruginosa* vaccines are presently being evaluated (76). In the end stage, lung transplantation can offer an outcome. The one and two year survival rates approach 70% and 54% respectively (77). Amiloride inhalation by blocking sodium reabsorption in the respiratory epithelium, has been shown to increase sputum clearance in a placebo-controlled cross-

ver study (78,79). Although improvement in pulmonary function was not found in one study (78), a delay in the deterioration of forced vital capacity (FVC) was reported by an other author (79). Dornase (Pulmozyme), a recombinant human desoxyribonuclease which breaks off the sputum DNA, has been reported to increase the forced expiratory volume (FEV1) and FVC safely in CF patients (80-82). Inhalation of  $\alpha$ 1-antitrypsine inhibits neutrophil elastase (83), which is released from the neutrophils and causes lung damage. Chloride channel facilitators, which directly stimulate a CFTR protein independent anion channel, are presently being evaluated (84).

## 5.2 Increase energy supply

In the past, restricted diets with **low fat content** were often prescribed for CF patients in order to minimize steatorrhea, abdominal cramps and stool bulk (85-87). Due to both unpalatability and low caloric density, these diets often resulted in **malnutrition and growth failure** in these patients (87-89). In the early 1970s, Crozier introduced the use of **high fat diets** in combination with pancreatic enzymes in order to increase the energy intake of CF patients. This regimen resulted in **better growth** with evident steatorrhea (90). Moreover, CF children from clinics using low fat diets were reported to show poorer growth (87-89) than those from clinics, encouraging the use of high fat diets (91). In order to further improve the nutritional status and growth of CF patients, feeding intervention studies have been done with different kinds of nutrients such as hypercaloric polymeric, semielemental or elemental diets. It has been shown that interventions making use of very high caloric intakes of polymeric diets (150 - 180% Recommended Daily Allowance) by overnight nasogastric tube resulted in improved nutritional status in CF adults. In children with CF, the effects of interventions with hypercaloric polymeric diets up to 130% of RDA are however unconvincing. Luder and coworkers, studying the effects of a 4 year period of nonrestricted fat diet in CF children, found improved Z-scores for weight, height and BMI for their CF patients when compared to the national population of CF patients on fat restricted diets, while no changes were seen when compared to normal children without CF (92). More recently, studies with hypercaloric polymeric diets with high fat content did not result in significant improvements of Z-scores for weight, height and skinfolds in CF patients (93), whereas **parenteral nutrition** and either oral or enteral

**elemental and semielemental** nutrition have been shown to **significantly improve the nutritional condition** of these patients (94-106). The results of short-term and long-term studies of feeding interventions on the nutritional status in CF children are summarized in table 1 and 2. The fact that predigested food can improve the nutritional status better than standard diets, strongly suggests that nutrient maldigestion plays a crucial role in the poor response to oral hypercaloric polymeric diets. The latter hypothesis is further supported by the known inactivation of pancreatic enzymes and bile acids precipitation accompanying the low duodenal pH due to low bicarbonate secretion in CF patients (107-110) . **Enteric-coated pancreatic enzyme** preparations have therefore been introduced but the low duodenal pH interferes with the release of enzymes through the acid resistant coating (111). **High doses of pancreatic enzymes** did not solve the problems of malabsorption (112) and recently, colon strictures have been observed in CF children on high doses of pancreatic enzymes (113-115). Attempts have been made to inhibit gastric acid production in the hope to improve the digestion and absorption of nutrients. However, the reported effects of **H2-receptor antagonists and prostaglandine E2** on steatorrhea have been variable and unconvincing (116-125). Results of short-term studies of cimetidine and misoprostol on fat excretion have not been consistent (table 3). This may be partly due to the lack of control of dietary fat intake. In long-term studies cimetidine showed no significant changes in fat excretion and nutritional status in CF children. on the contrary, **famotidine**, a more potent inhibitor of gastric acid secretion, showed both a significant improved fat absorption coefficient and improved growth parameters (table 4). However, interpretation of growth effects in the latter study is rather difficult because Z-score methods have not been used to evaluate growth. Further, in a double blind study, a significant improvement in steatorrhea was found when a **proton pump inhibitor** was added as adjuvant therapy in pancreatic enzyme treated CF adults (112). In children with CF, the effects of proton pump inhibitors on fat absorption and on the nutritional condition have not been reported.

**Table 1 short-term feeding intervention studies in cystic fibrosis.**

Authors	<sup>10</sup> Shepherd et al., '80	<sup>11</sup> Shepherd et al., '83	<sup>12</sup> Bertrand et al., '84	<sup>13</sup> Mansell et al., '84	<sup>14</sup> Ciociani et al., '85	<sup>15</sup> Loughlin et al., '86	<sup>16</sup> Remmel et al., '95
Number of cases	12	7	10	11	21	10	15
Age range	0.5 - 11 y	5 - 13 y	3 - 12 y	10 - 17 y	1 - 14 m	7 - 28 y	5 - 27 y
Nutritional status	malnutrition	malnutrition	malnutrition	malnutrition	normal	malnutrition	malnutrition
Study duration	1 month	6 months	1 month	1 month	5 days	6 months	3 months
Type of study	prospective own control	prospective own control	prospective own control	prospective own control	prospective CF control	prospective CF elemental CF polymeric	prospective own control
Feeding intervention	TPN 90 - 100 % RDA	elemental 20 - 40 % RDA (extra)	elemental 110 - 150 % RDA	TPN 120 % RDA	semielemental 142 kcal/kg	elemental 35% RDA (extra) versus hypercaloric	hypercaloric polymeric 130 % RDA
Route	parenteral	enteral	nasogastric	parenteral	oral	enteral	oral
Effect	SDS weight ↑ SDS height ↑ MUAC% std ↓ clinical score ↑ FVC, FeV1 ↑ PEF ↑	SDS weight ↑ SDS height ↑ MUAC % std. ↓ FM ,LBM (kg) ↑ muscle mass (kg) ↑ clinical score ↓ 3-meHis excre ↓	weight/height ↑ skinfold % std. ↓ MUAC % std. ↓ fat excretion (ns) MAMC % std. = FeV1, FEF = final work load =	weight (kg) ↑ height (cm) ↑ skinfold (mm) ↓ MAMC (cm) ↑ MIP, MEP ↑ FeV1, FEF =	Weight (kg) ↓ N-excretion ↓ fat absorption coefficient ↓	Weight/height % std. ↓ SDS height ↑ FMLBM (kg) ↓ fat excretion ↓ clinical score ↑ FeV1, FEF, FVC ↓	SDS weight (ns) SDS height (ns) growth velocity (ns) skinfold (ns) weight (kg) ↓
Follow up (duration)	all parameters improve further (after months)	NR	all parameters ↓ (after 2 months)	MIP, MEP = FeV1, FEF = (after 2 months) all other parameters ↓ (after 1-6months)	NR	NR	NR

MIP : Maximal Inspiratory Pressure

MEP : Maximal Expiratory Pressure

FeV1 : Forced expiratory volume in 1 sec.

SDS : Standard deviation score

N-excretion : Fecal Nitrogen excretion

MUAC : Mid Upper Arm Circumference

MAMC : Mid Arm Muscle Circumference

FVC : Forced Vital Capacity

FEF : Forced Expiratory Flow

PEF : Peak Expiratory Flow

LBM : Lean Body Mass

FM : Fatmass

RDA: Recommended daily allowance

3-meHis excre : 3 methylhistidine excretion in urine

↑ : Improved

↓ : Decreased

= : unchanged

(ns) : not significant

NR: not reported

**Table 2 Long-term feeding intervention studies in cystic fibrosis.**

Authors	<sup>10</sup> Allan et al., '73	<sup>11</sup> Berry et al., '75	<sup>12</sup> Yassa et al., '78	<sup>13</sup> Levy et al., '85	<sup>14</sup> Boland et al., '86	<sup>15</sup> Sheperd et al., '86	<sup>16</sup> Farrell et al., '87	<sup>17</sup> Luder et al., '89
Number of cases	17	15	43	14	10	10	36	37
Age range	2 - 21 y	10m -18y	3 - 16 y	5 - 22 y	5 - 20 y	3 - 13 y	3 - 4 m	2 - 27 y
Nutrition status	malnutrit	malnutrit	malnutrit	malnutrit	malnutrit	malnutrit	normal	malnutrit
Study duration	3 months to 3 years	1 year	1 year	1,1 year	10 - 36 months	1 year	8 months	4 years
Type of study	prospect own control	prospect CF control	prospect CF control	prospect CF control	prospect own control	prospect CF control	prospect CF control	prospect own control
Feeding intervent	elemental 50-100% RDA	elemental 100 % RDA	elemental 100 % RDA	(semi) elemental 30 % RDA (extra)	predigest non-elemental 1000 to 2000Kcal	semielemental 120-140 % RDA	pregestimil versus standard 120Kcal / kg	hypercal polymeric 120 % RDA
Route	oral	oral	oral	gastrostomy	jejunostomy	enteral	oral	oral
Effect	SDSwei ↓ SDShei ↓ clinical score ↓	SDSwei ↓ clinical score ↓ SDS hei (ns)	SDSwei ↓ SDShei ↓ SDSskf ↓ boneage ↓	weight (kg) ↓ height (cm) ↓ wei/hei % std. growth velocity ↓ BF % ↓ FFM ↓ TBK ↓ TBN ↓ ‡FVC = ‡FeV1 =	SDSwei ↓ MAMC ↓ FVC =	SDSwei ↓ SDS hei ↓ protein synthes ↓ protein catabol ↓ FeV1 ↓ FVC ↓ FEF ↓	weight (kg) ↓ height (cm) ↓ growth velocity ↓	SDSwei (ns) SDS hei (ns) FEF = BMI ↓
Follow up (duration)	NR	NR	all parameters ↓ except bone age (1 year)	NR	NR	NR	NR	NR

↓ : Improved

↓ : Decreased

= : unchanged

RDA : Recommended Daily Allowance

SDSwei : SDS weight

SDShei : SDS height

SDSskf : SDS skinfolds

LBM : Lean Body Mass

FFM : Fat Free Mass

TBN : Total body nitrogen

TBK : Total Body Kalium

FVC : Forced Vital Capacity

FeV1 : Forced expiratory volume ‡ FVC and FeV1 decrease in CF control

FEF : Forced Expiratory Flow

BMI : Body Mass Index

BF : Body fat

N-excret : Nitrogen excretion

(ns) non significant

Abs. coeff : absorption coefficient

‡ FVC and FeV1 decrease in CF control

MAMC : Mid Arm Muscle Circumference

**Table 3 Effect of short-term use of gastric acid inhibitors on steatorrhea and nutritional status in CF children.**

Authors	<sup>117</sup> Cox et al., '79	<sup>118</sup> Boyle et al., '80	<sup>119</sup> Durie et al., '80	<sup>120</sup> Gow et al., '81	<sup>121</sup> Schöni et al., '81	<sup>122</sup> Cleghorn et al., '83	<sup>123</sup> Robinson et al., '90
number of cases	10	8	15	10	10	11	15
age range	6 - 27 y	12 - 25 y	10 - 17 y	6 - 13 y	11 - 17 y	2 - 17 y	0.5 - 13.8 y
type of study	prospective crossover open	prospective randomized crossover	prospective randomized crossover	prospective randomized crossover	prospective open	prospective open	double-blind placebo controlled crossover
pancreatic enzyme	Cotazym or Viokase	Viokase	Cotazym	Pancrease	Eurobiol	Pancrease	Pancrease
intervention	cimetidine 150-200mg / day	cimetidine 300 mg / day	cimetidine 20 mg / kg / day	cimetidine 20 mg / kg / day	cimetidine 600 mg / m <sup>2</sup> / day	misoprostol 400 µg / day	misoprostol 400 µg / day
duration	1 week ?	5 days	7 days	14 days	6 days	1 week	3 weeks
effect on steatorrhea	fat excretion ↓ N-excretion ↓ fat abs coeff ↓	fat excretion ↓ fat abs coeff ↓ fecal weight ↓	fat excretion ↓ N-excretion ↓ fecal weight ↓	fat excretion (ns) N-excretion (ns) fecal weight(ns)	fat abs. coeff. (ns) N abs coeff (ns)	fat excretion normalized fat abs coeff (ns)	fat excretion ↓
effect on nutritional status	ND	ND	ND	ND	ND	ND	ND
comments	no diet evaluation per treatment period	results of diet evaluation not given	no effect on steatorrhea in patients with fat intake > 120 g / day	-	-	fat intake not controlled	fat absorption not improved in patients with < 10% fat malabsorption

abs coeff : fat absorption coefficient

N-excretion : fecal Nitrogen excretion

(ns) : not significant

**Table 4 Effect of long-term use of gastric acid inhibitors on steatorrhea and nutritional status in CF children.**

Authors	<sup>123</sup> Schöni et al, '84	<sup>124</sup> Chalmers et al, '85	<sup>125</sup> Carroccio et al, '92
number of cases	38	16	10
age range	mean 13 y	5 - 19 y	7 - 18 y
type of study	prospective randomized doubleblind	double-blind crossover	double-blind crossover
pancreatic enzyme	Pancrease	Cotazym	Pancrease
intervention	cimetidine 600mg/m <sup>2</sup> /day	cimetidine 25mg/kg/ day	famotidine 1mg/kg/day
duration	4 months	6 months	6 months
effect on steatorrhea	plasma lipid and lipoprotein (ns)	fat excretion ↓ N-excretion (ns) fecal weight (ns)	fat absorption coeff. ↓ fecal weight ↓
effect on nutritional status	weight, height (ns) skinfolds (ns) TLC, TGV (ns) Raw, sGaw ↓	SDSweight (ns) SDSheight (ns) skinfolds (ns) bone age (ns) clinical score (ns)	weight (kg) ↓ height (cm) ↓ clinical score (ns)
comments	no diet evaluation	results of diet evaluation was not given	results of diet evaluation was not given

SDS : Standard Deviation Score for age and sex

TLC : Total Lung Capacity

TGV : Thoracic Gas volume

Raw : airway resistance

sGaw : specific conductance

(ns) : not significant

## 1.6 EVALUATION OF STEATORRHEA

### 1.6.1 Fecal fat balance

The 3 days fecal fat excretion while patients are on a standard fat diet is the most reliable method for quantitative determination of fecal fat loss. The fat absorption coefficient is calculated by the following formula:

$$(\text{Fat ingested} - \text{fat excreted}) / \text{fat ingested} \times 100$$

Normal fat absorption coefficient at different ages have been reported as follows:

Age > 1 year :  $\geq 95\%$  (126-128)

Age < 1 year :  $> 83\%$  if formula fed and  $> 93\%$  if breast fed (126)

Premature infants : 38 - 73 % depending on the formula used (129)

Fecal fat can be determined by either Gravimetric or Titrimetric methods. For both methods, fecal fat is extracted with an organic solvent, the fat content is subsequently measured either by weighing (Gravimetric method) or by titration (Titrimetric method). The Gravimetric method determine all fecal lipid components, resulting in erroneously high results. On the contrary, the titrimetric method only measures fatty acids. Fecal lipids are first saponified and subsequently acidified to liberate fatty acids which are then extracted. Since its first description in 1949 (130), the **titrimetric procedure of van de Kamer** has been used as a reference method for the evaluation of malabsorption. The fat balance method is **reliable** for the quantification of fecal fat loss with a coefficient of variation of 4,6 % (131). However, the determination procedure is **time consuming, expensive and necessitates sophisticated apparatus**. Further, since the fat excretion is dependent on fat intake, patients have to keep up a **strictly fat constant diet** of more than 80 gram per day. Moreover, **fecal collection** have to be done very accurately. The balance method consequently is poorly reliable in outpatients, especially in children and infants. When fat balance is not possible, measuring **fecal fat concentration** in a fecal sample can be used for the screening of fat malabsorption. Results are then expressed as percent of wet fecal weight (fecal fat concentration). Using the  $^{14}\text{C}$ -triolein/ $^3\text{H}$ -oleic acid test as a reference method, Pedersen et al. have studied the diagnostic

value of fecal fat concentration as measured by the titrimetric method of van de Kamer in a 72 hours fecal collection without controlling for dietary fat (132). In this study, a **similar diagnostic value** was found for both **fecal fat concentration (FFC)** and **fecal fat excretion (FFE)**: The sensitivity, specificity, positive predictive value and negative predictive value of FFC versus FFE were respectively 93,1% versus 90%; 92,4% versus 89,4%; 90% versus 93% and 89% versus 90% with a **day to day coefficient of variation of 29% for FFC and 64% for FFE**. In only 6% of the patients studied, the FFC when measured in a single day sample differed from the mean 3-day fecal fat concentration value whereas the FFE differed from the mean 3-day fecal fat excretion in 37% of the patients. FFC correlated weakly but significantly with FFE ( $r = 0,55$ ;  $p < 0,01$ ) (133). FFC results in pancreatic steatorrhea being higher than in nonpancreatic steatorrhea, Bolinn et al. have suggested that FFC could be used for the differentiation of both types of steatorrhea (134). This has however not been confirmed by other investigators; who found much overlap in FFC results between pancreatic and nonpancreatic steatorrhea (132,133,135,136). Results of these studies are shown in table 5.

The utility of FFC as an screening method for fat malabsorption has been limited because of the high interday variation (29%). This interday variation might be due to the **varying fecal water content** as reported by Weijers et al. (137). This suggests that if the effect of varying water content could be eliminated, the interday variation of FFC would be much lower. A new method for the semiquantitative determination of FFC, which eliminates the influence of varying fecal water content is the **steatocrit**.

### 1. 6. 2 Steatocrit

This procedure is based on the fact that fecal fat is extracted by centrifugation of diluted stool in a hematocrit capillary at 13000 rpm for 15 minutes (138). After centrifugation, three layers are distinguished in the capillary; the upper fatty layer (FL), the middle fluid layer and the bottom solid layer (SL). The fecal fat measured by steatocrit is expressed as fecal fat concentration and is calculated as  $FL / (FL + SL)$ . Reported normal values are  $< 2\%$  (139). The steatocrit method is very suitable for use in children and infants since it is **simple, noninvasive** and can be performed on **small fecal samples** (0,5 gram). Moreover, it is inexpensive and the whole test takes only 20 minutes. Although several authors have reported this method to

be satisfactory for the evaluation of steatorrhea (138-142), some have reported the steatocrit to be **quite unreliable** (143). Sugai reported a specificity of 97% for steatocrit but a sensitivity of 98%, 79% and 29% for samples with respectively high, moderate and low fat content (144). This low sensitivity observed for samples of low fat content may be due to difficulties with either fat extraction or with obtaining a clear separation between the fatty, aqueous and solid layer, resulting in erroneous results.

### 1. 6. 3 Sudan staining method

The presence of fecal fat can be screened for by microscopic examination of stools. The fecal preparation is first acidified and stained with Sudan staining. After heating, fecal fatty acids and triglycerides are seen as fatty globules under the microscope. Dependent on the number and the size of the globules, the fatty globules are classified as normal, slightly increased or definitely increased (145). Weijers et al. studied the agreement between results of the Sudan staining method and chemically measured fecal fat (137). Satisfactory **agreement** between both methods was found in **(60 - 70 % cases) for fecal samples with very low or very high fat content ( < 3 % or > 9 % )** but the agreement dropped to **40% for samples of moderate fat content (3 - 9%)**.

### 1. 6. 4 C- Triolein absorption test

After ingestion,  $^{14}\text{C}$ -Triolein is digested by pancreatic lipase in the duodenum liberating fatty acids, which on further oxidation yield  $^{14}\text{CO}_2$  which can be detected in expired air. Although this method is **simple, rapid** to perform and gives a **direct evaluation of pancreatic function**, it is not appropriate for use in children because of the **radioactivity**. Recently, a new non radioactive substrate  $^{13}\text{C}$ -Triolein has been introduced but this is however **expensive** and a **mass spectrometer** is needed in order to use this test (131).

### 1. 6. 5 Near Infrared Reflectance Analysis

This method is based on the analysis of the infrared spectrum radiation, reflected by the

surface of the material under study. Specific peaks for the component to be investigated can be identified and their heights can be related to the concentration of the component studied by using computerised multilinear regression analysis. Besides **measuring fecal fat**, this apparatus can also be used for the determination of **fecal nitrogen and water content**. The analysis lasts less than 1 minute and can be performed on **small samples** ( 2 - 3 gram ). The variation coefficient is 2,1 % and the correlation coefficient with the van de Kamer method is 0,92 (146). However this high correlation is possibly due to the fact that this method is calibrated by the titrimetric method described by van de Kamer. The **calibration procedure is difficult** and this sophisticated instrument is **expensive** (147). Further studies are necessary in order to better evaluate the usefulness of near infrared reflectance analysis in clinical practice.

**Table 5 Diagnostic value of fecal fat concentration (FFC) and fecal fat excretion (FFE) in studies of fat malassimilation.**

Authors	<sup>122</sup> Pedersen '84	<sup>123</sup> Bolinn '84	<sup>124</sup> Roberts '86	<sup>125</sup> Lembcke '87	<sup>126</sup> Bai et al. '89
Number of cases	87	50	125	369	538
Aims of study	diagnostic value of FFC versus FFE				
Method used	†titrimetric method (72h fecal collection)	†titrimetric method (72h fecal collection)	†titrimetric method (72h fecal collection)	†titrimetric method (72h fecal collection)	†titrimetric method (72h fecal collection)
	- <sup>14</sup> C-triolein/ <sup>3</sup> H-olein acid test as reference				
Population (n)	I. pancreatic steatorrhea (21) II. non pancreatic steatorrhea (12) III. no steatorrhea (54)	I. pancreatic steatorrhea (19) II. nonpancreatic steatorrhea (31)	I. pancreatic steatorrhea (24) II. nonpancreatic steatorrhea (70) III. no steatorrhea (31)	I. pancreatic steatorrhea (59) II. nonpancreatic steatorrhea (53) III. no steatorrhea: sick and normal controls (257)	I. pancreatic steatorrhea (88) II. nonpancreatic steatorrhea (525)
Results	I + II versus III: - pos. pred. value: FFE 0.93 - FFC 0.90 - neg. pred. value: FFE 0.90 - FFC 0.89 - if based on single day sampling, FFC more reliable (6% errors) than FFE (37% errors) - overlap of FFC between I and II	- FFC-pancreatic $\geq 9.5\%$ - FFC-nonpancreat $< 9.5\%$ - no overlap of FFC between I and II	- FFC-pancreatic $>$ nonpancreatic $>$ control - correlation between FFC and FFE ( $r=0.55$ ; $p<0.01$ ) - overlap of FFC between I and II	- FFC-pancreatic $>$ non-pancreatic steatorrhea $>$ pancreat. control - Overlap of FFC between I and II	- FFCsens 58% - FFCspec 70% - overlap of FFC between I and II
Comments	- free fat intake - <sup>14</sup> C excretion $\geq 10\%$ for steatorrhea - <sup>14</sup> C/ <sup>3</sup> H $> 1.3$ for pancreatic steatorrhea	- fat diet - 90-100g/day	- fat diet 100g/day - 10% as cutoff for pancreatic steatorrhea	- fat diet $\geq 80$ g/day	- 10% as cutoff for pancreatic steatorrhea. - fat diet 100g/day

FFC: Fecal fat concentration

FFE: Fecal fat excretion

† Method as described by van de Kamer (111)

pos. pred. value: positive predictive value

neg. pred. value: negative predictive value

sens: sensitivity

spec: specificity

## **AIMS OF THE STUDY**

With age, children with CF show progressing malnutrition mainly attributed to either persisting malabsorption notwithstanding the use of high oral doses of pancreatic enzymes or increased energy consumption secondary to respiratory disease. Prospective studies in young children have shown malnutrition to occur only in patients with pancreatic insufficiency (34). Efforts to either maintain or restore the nutritional condition have shown that, notwithstanding the use of pancreatic enzymes, high nutrient intakes only seems to be effective when administered "digested" either as total parenteral nutrition or as (semi)elemental feedings orally or by tube feeding. The apparent insufficient effect of pancreatic enzymes does not seem to be due to too low administered doses and recently very high doses have been used with the hope of correcting malabsorption. Suggestions have been made that these high doses might be responsible for the recently reported occurrence of colitis in these patients (113-115).

Our hypothesis was that persisting malabsorption in these patients is likely to be linked to a low duodenal pH which interferes with several digestive and absorptive processes such as impeding transport of split fatty acids from the luminal lipid globules to the absorptive area through the mediation of bile salt micelles. If this was correct, antacid treatment should improve fat malabsorption in these patients. The fact that most studies hereover have been inconclusive might be due to the short and inefficient control of duodenal pH with the drugs used. A recent double-blind control study in adults patients has shown malabsorption to normalize in several patients treated with a proton pump inhibitor (omeprazol) (112). Until now, no studies with proton pump inhibitor have been reported in children.

### **The aims of the present work were:**

1. Develop an easy, noninvasive, cheap and reliable test for the monitoring of fecal fat loss in pancreatic malabsorption.
2. Evaluate the nutritional condition, the body composition and the presence or persistence of fat malabsorption in our patients with exocrine pancreatic insufficiency accompanying cystic fibrosis.

3. Evaluate whether or not the use of a proton pump inhibitor (lansoprazole) in our patients with persisting malabsorption improves both the fat malabsorption and the nutritional condition.

## REFERENCES

- (1) G. Fanconi, E. Uehlinger, C. Knauer. Das Coeliaksyndrom bei Angeborener Zystischer Pankreas fibromatose und Bronchiektasien. *Wein Med Wschr* 1936; 86: 753-756.
- (2) CF Genetic analysis consortium. Worldwide survey of the  $\Delta F$  508 mutation. *Am J Hum Genet* 1990; 47: 354-359.
- (3) M. Welsh, A. Smith. Molecular mechanisms of CFTR chloride channel dysfunction in cystic fibrosis. *Cell* 1993; 73: 1251-1254.
- (4) C. Marino, L. Matovecik, F. Gorelick, J. Cohn. Localization of the cystic fibrosis transmembrane conductance regulator in pancreas. *J Clin Invest* 1991; 88: 712-716.
- (5) T. Boat, M. Welsh, A. Beaudet. (1989). In *The Metabolic Basis of inherited Disease*, C. Scriver, A. Beaudet, W. Sly, D. Valle, eds. (New York: McGraw-Hill,inc.), pp. 2649-2680.
- (6) E. Kerem, J. Reisman, M. Corey, G. Canny, H. Levison. Prediction of mortality in patients with cystic fibrosis. *N Engl J Med* 1992; 326: 1187-91.
- (7) C. Sommerhoff, J. Nadel, C. Basbaum et al. Neutrophil elastase and cathepsin G stimulate secretion from culture bovine airway gland serous cells. *J Clin Invest* 1990;85: 682-689.
- (8) L. Sharples, T. Hathaway, C. Dennis, N. Caine, T. Higenbottam, J. Wallwork. Prognosis of patients with cystic fibrosis awaiting heart and lung transplantation. *J Heart-Lung-Transplant* 1993; 12: 669-74.
- (9) J. Britton. Effects of social class, sex, and region of residence on age at death from cystic fibrosis. *Br Med J* 1989; 298: 483-487.
- (10) M. Corey, F. McLaughlin, M. Williams, H. Levison. Comparison of survival, growth and pulmonary function in patients with cystic fibrosis in Boston and Toronto. *J Clin Epidemiol* 1988; 41: 583-591.
- (11) M. Aitken, S. Fiel. Cystic fibrosis. *Dis Mon* 1993; 39: 1-52.
- (12) P. Brihaye, P. Clement, I. Dab, B. Desprechin. Pathological changes of the lateral nasal wall in patients with cystic fibrosis. *Int J Pediatr Otorhinolaryngol* 1994; 28: 141-7.
- (13) I. Mackay, B. Djazaeri. Chronic sinusitis in cystic fibrosis. *J Roy Soc Med* 1994; 87 (Suppl 21): 17-19.
- (14) B. Hadorn, P. Johansen, C. Anderson. Pancreozymin secretin test of exocrine pancreatic function in cystic fibrosis and the significance of the result for the pathogenesis of the disease.

Can Med Assoc J 1968; 98: 377-385.

(15) B. Hadorn, G. Zoppi, D. Shmerling, A. Prader, I. McIntyre, C. Anderson. Quantitative assessment of exocrine pancreatic function in infants and children. *J Pediatr* 1968; 73: 39-50.

(16) H. Schachman, E. Lebenthal, K. Khat. Recurrent acute pancreatitis in patients with normal pancreatic enzymes. *Pediatrics* 1975; 55: 86-95.

(17) K. Gaskin, P. Durie, M. Corey, P. Wei, G. Forstner. Evidence for a primary defect of pancreatic HCO<sub>3</sub><sup>-</sup> secretion in cystic fibrosis. *Pediatr Res* 1982; 16: 554-557.

(18) A. Atlas, S. Orenstein, D. Orenstein. Pancreatitis in young children with cystic fibrosis. *J Pediatr* 1992; 120: 756-9.

(19) F. Ahmed, J. Ellis, J. Murphy, S. Wooton, A. Jackson. Excessive faecal loss of vitamin A (retinol) in cystic fibrosis. *Arch Dis Child* 1990; 65: 589-593.

(20) R. Sokol, M. Reardon, F. Accurso et al. Fat-soluble-vitamin status during the first year of life in infants with cystic fibrosis identified by screening of newborns. *Am J Clin Nutr* 1989; 50: 1064-71.

(21) V. Hubbard, P. Farrell, P. di Sant 'Agnese. 25-hydroxylcholecalciferol levels in patients with cystic fibrosis. *J Pediatr* 1979; 94: 84-86.

(22) E. Mischler, PJ Chesney, PW Chesney, R. Mazess. Demineralization in cystic fibrosis detected by direct protein absorptiometry. *Am J Dis Child* 1979; 133: 632-635.

(23) N. Solomons, J. Wagonfeld, C. Rieger et al. Some biochemical indices of nutrition in treated cystic fibrosis patients. *Am J Clin Nutr* 1981; 34: 462-474.

(24) R. Allen, B. Seetharam, E. Podell, D. Alpers. Effect of proteolytic enzymes on the binding of cobalamin to R protein and intrinsic factor. *J Clin Invest* 1978; 61: 47-54.

(25) M. Murphy, W. Sheldon, A. Brunetto et al. Active and passive sugar absorption in pancreatic insufficiency. *J Pediatr Gastroenterol Nutr* 1989; 8: 189-194.

(26) L. Frase, A. Strickland, G. Kachel, G. Krejs. Enhanced glucose absorption in the jejunum of patients with cystic fibrosis. *Gastroenterology* 1985; 88: 478-484.

(27) M. Lohr, P. Goertchen, H. Nizze et al. Cystic fibrosis associated islet changes may provide a basis for diabetes. An immunocytochemical and morphometrical study. *Virchows Arch [A]* 1989; 414: 179-185.

(28) L. Krueger, A. Lerner, S. Katz, R. Mack, D. Holsclaw, E. Lebenthal. Cystic fibrosis and diabetes mellitus: interactive or idiopathic. *J Pediatr Gastroenterol Nutr* 1991; 13: 209-219.

- (29) E. Wilmshurst, J. Soeldner, D. Holsclaw. Endogeneous and exogeneous insulin responses in patients with cystic fibrosis. *Pediatrics* 1975; 55: 75-82.
- (30) O. Andersen, S. Garner, C. Heilmann, K. Petersen, W. Petersen, C. Koch. Glucose tolerance and insulin receptor binding to monocytes and erythrocytes in patients with cystic fibrosis. *Acta Paediatr Scand* 1988; 77: 67-71.
- (31) A. Moran, P. Diem, D. Klein, M. Levitt, R. Robertson. pancreatic endocrine function in cystic fibrosis. *J Pediatr* 1991; 118: 715-723.
- (32) H. Berry, F. Kellogg, M. Hunt, R. Ingberg, L. Richter, C. Gutjahr. Dietary supplement and nutrition in children with cystic fibrosis. *Am J Dis Child* 1975; 129: 165-171.
- (33) J. Dodge, J. Yassa. Food intake and supplementary feeding programs. In: J. Sturgess, ed. *perspectives in cystic fibrosis*. Toronto: Canadian Cystic Fibrosis Foundation; 1980: 125-136.
- (34) M. Bronstein, R. Sokol, S. Abman et al. Pancreatic insufficiency, growth, and nutrition in infants identified by newborn screening as having cystic fibrosis. *J Pediatr* 1992; 120: 533-40.
- (35) J. Tomezsco, V. Stallings, D. Kawchak, J. Goin, G. Diamond, T. Scanlin. Energy expenditure and genotype of children with cystic fibrosis. *Pediatr Res* 1994; 35: 451-460.
- (36) M. Bronstein, P. Davies, K. Hambidge, F. Accurso. Normal energy expenditure in the infant with presymptomatic cystic fibrosis. *J Pediatr* 1995; 126: 28-33.
- (37) K. Cox, J. Isenberg, M. Ament. Gastric acid hypersecretion in cystic fibrosis. *J Pediatr Gastroenterol Nutr* 1982; 1: 559-565.
- (38) C. Roy, A. Weber, C. Morin et al. Abnormal biliary lipid composition in cystic fibrosis. *N Engl J Med* 1977; 297: 1301-1305.
- (39) A. Malfroot, I. Dab. New insights on gastro-oesophageal reflux in cystic fibrosis by longitudinal follow up. *Arch Dis Child* 1991; 66: 1339-1345.
- (40) S. Cucchiara, F. Santamaria, M. Andreotti et al. Mechanisms of gastro-oesophageal reflux in cystic fibrosis. *Arch Dis Child* 1991; 66: 617-622.
- (41) P. Gustafsson, S. Fransson, N. Kjellman, L. Tibbling. Gastro-oesophageal reflux and severity of pulmonary disease in cystic fibrosis. *Scand J Gastroenterol* 1991; 26: 449-456.
- (42) S. Fiedorek, R. Shulman, W. Klish. Endoscopic detection of peptic ulcer disease in cystic fibrosis. *Clin Pediatr (Phila)* 1986; 25: 243-246.
- (43) P. Robinson, A. Smith, P. Sly. Duodenal pH in cystic fibrosis and its relationship to fat malabsorption. *Dig Dis Sci* 1990; 35: 1299-1304.

- (44) J. McPartin, J. Dickson, V. Swain. Meconium ileus, immediate and longterm survival. *Arch Dis Child* 1972; 47: 207-210.
- (45) H. Andersen, K. Hjelt, E. Waever, K. Overgaard. The age related incidence of meconium ileus equivalent in a cystic fibrosis population: the impact of high energy intake. *J Pediatr Gastroenterol Nutr* 1990; 11: 356-360.
- (46) L. Kulczyki, H. Schwachman. Studies in cystic fibrosis of the pancreas: occurrence of rectal prolapse. *N Engl J Med* 1958;259: 409-412.
- (47) D. Holsclaw, C. Rocmans, H. Schwachman. Intussusception in patients with cystic fibrosis. *Pediatrics* 1971; 48:51-58.
- (48) H. Rovsing, K. Sloth. Microgallbladder and biliary calculi in mucoviscidosis. *Acta Radiol [Onco]* 1973; 14: 588-592.
- (49) F. Santamaria, P. Vajro, V. Oggero et al. Volume and emptying of the gallbladder in patients with cystic fibrosis. *J Pediatr Gastroenterol Nutr* 1990; 10: 303-306.
- (50) L. Taussig, C. Lobeck, P. Ackerman, J. Kattwinkel: Fertility in males with cystic fibrosis. *N Engl J Med* 1972; 287: 586-589.
- (51) E. Kaplan, H. Shwachman, A. Perlmutter et al.: Reproductive failures in males with cystic fibrosis. *N Engl J Med* 1968; 279: 65-69.
- (52) S. Fitzsimmons: Cystic Fibrosis Foundation Patient Registry 1990 Annual Report. Bethesda, Cystic Fibrosis Foundation, 1991.
- (53) P. Tam, P. Verdugo: Control of mucus hydration as a Donnan equilibrium process. *Nature* 1981; 292: 340-342.
- (54) P. Di Sant 'Agnese, R. Darling, G. Perera, E. Shea. Abnormal electrolyte composition of sweat in cystic fibrosis of the pancreas. Clinical significance and relationship to the disease. *Pediatrics* 1953; 12: 549-563.
- (55) L. Gibson, R. Cooke. A test for concentration of electrolytes in sweat in cystic fibrosis of the pancreas utilizing in pilocarpine by iontophoresis. *Pediatrics* 1959; 23: 545-549.
- (56) H. Veeze, A. Van den Ouweland, D. Halley et al. The diagnosis of cystic fibrosis: intestinal current measurements, a highly accurate method in case of a borderline phenotype. Submitted.
- (57) M. Rosenfeld, W. Siegfried, K. Yoshimura et al. Adenovirus-mediated transfer of a recombinant alpha 1-antitrypsin gene to the lung epithelium in vivo. *Science*.1991; 252: 431-4

- (58) B. Pitt, M. Schwarz, J. Pilewski et al. Retrovirus-mediated gene transfer in lungs of living fetal sheep. *Gene Ther* 1995; 2: 344-50.
- (59) M. Rosenfeld, K. Yoshimura, B. Trapnell et al. In vivo transfer of the human cystic fibrosis transmembrane conductance regulator gene to the airway epithelium. *Cell*. 1992; 68: 143-55.
- (60) R. Kraemer, A. Rudeberg, B. Hadorn, E. Rossi. Relative underweight in cystic fibrosis and its prognostic value. *Acta Paediatr Scand* 1978; 67: 33-37.
- (61) S. Rao, D. Wilson, R. Brooks et al. Acute effects of nebulization of n-acetylcysteine on pulmonary mechanics and gas exchange. *Am Rev Respir Dis*. 1970; 102: 17-22.
- (62) W. Waring. Current management of cystic fibrosis. *Adv Pediatr*. 1976; 23: 401-38.
- (63) M. Gotz, R. Kraemer, K. Kerrebijn et al. Oral acatylsysteine in cystic fibrosis. A cooperative study. *Eur J Respir Dis*. 1980;61: (Suppl 111): 122-6.
- (64) J. Reisman, B. Rivington-Law, M. Corey et al. Role of conventional physiotherapy in cystic fibrosis. *J Pediatr* 1988; 113: 632-6.
- (65) H. Steen, A. Redmond, D. O'Neil et al. Evaluation of the PEP mask in cystic fibrosis. *Acta Paediatr Scand*. 1991; 80: 51-6.
- (66) C. Braggion, L. Cappelletti, M. Cornacchia, L. Zanolla, G. Mastella. Short-term effects of three chest physiotherapy regimens in patients hospitalized for pulmonary exacerbations of cystic fibrosis: A cross-over randomized study. *Pediatr Pulmonol* 1995; 19: 16-22.
- (67) J. Pryor, B. Webber, M. Hobson et al. Evaluation of the forced expiratory technique as an adjunct to postural drainage in the treatment of cystic fibrosis. *J Pediatr*. 1983; 103: 538-42.
- (68) P. Sutton, H. Gemmell, N. Innes et al. Use of nebulized saline and nebulized terbutaline as an adjunct to chest physiotherapy. *Thorax*. 1988; 43: 57-60.
- (69) E. Pattishall. Longitudinal response of pulmonary function to bronchodilators in cystic fibrosis. *Pediatr Pulmonol*. 1990; 9: 80-5.
- (70) P. Konig, D. Gayer, J. Shaffer et al. Bronchodilator responsiveness and spontaneous diurnal variation of PEFr in patients with cystic fibrosis. Poster presented at the North American Cystic Fibrosis Conference. Washington, DC: 1992 Oct. Abstract.
- (71) M. Konstan, P. Byrard, C. Hoppel, P. Davis: Effect of high dose ibuprofen in patients with cystic fibrosis. *N Engl J Med* 1995; 332: 848.
- (72) H. Auerbach, M. Williams, J. Kirkpatrick et al. Alternate-day prednisone reduces morbi-

- dity and improves pulmonary function in cystic fibrosis. *Lancet*. 1985; 2: 686-8.
- (73) B. Rosenstein, H. Eigen. Risk of alternate-day prednisone in patients with cystic fibrosis. *Pediatrics*. 1991; 87: 245-6.
- (74) C. Pantin, R. Stead, M. Hodson et al. Prednisolone in the treatment of airflow obstruction in adults with cystic fibrosis. *Thorax*. 1986; 41: 34-38.
- (75) M. Zach. Pathogenesis and management of lung disease in cystic fibrosis. *J R Soc Med*. 1991; 84 (Suppl 18): 10-7.
- (76) McNeil Pharmaceutical. Pseudomonas vaccine tests start. *Cystic Fibrosis Currents*. 1991; 6(4).
- (77) A. Khaghani, B. Madden, M. Hodson et al. Heart-lung transplantation for cystic fibrosis. Paper presented at the North American Cystic Fibrosis Conference. Dallas, TX: 1991 Oct 4.
- (78) E. App, M. King, R. Helfesrieder et al. Acute and longterm amiloride inhalation in cystic fibrosis lung disease. *Am Rev Respir Dis*. 1990; 141: 605-12.
- (79) M. Knowles, N. Church, W. Waltner et al. A pilot study of aerosolized amiloride for the treatment of lung disease in cystic fibrosis. *N Engl J Med*. 1990; 322: 1189-94.
- (80) M. Hodson. Clinical studies of rhDNase in moderately and severely affected patients with cystic fibrosis - An Overview. *Respiration* 1995; 62 (suppl 1); 29-32.
- (81) M. Aitken, W. Burke, G. McDonald et al. Recombinant human Dnase inhalation in normal subjects and patients with cystic fibrosis. A phase I study. *JAMA*. 1992; 267: 1947-51.
- (82) R. Hubbard, N. McElvaney, P. Steven et al. A preliminary study of aerosolized recombinant human deoxyribonuclease I in the treatment of cystic fibrosis. *N Engl J Med*. 1992; 326:812-5.
- (83) N. McElvaney, R. Hubbard, P. Birrer et al. Aerosol alpha-1-antitrypsin treatment for cystic fibrosis. *Lancet*. 1991; 337: 392-4.
- (84) R. Boucher, E. Cheng, A. Paradiso et al. Chloride secretory response of cystic fibrosis airway epithelia: presentation of calcium but not protein kinase C and A-dependent mechanism. *J Clin Invest*. 1989;84: 1424-31.
- (85) H. Chase, M. Long, M. Lavin. Cystic fibrosis and malnutrition. *J Pediatr* 1979; 95: 337-47.
- (86) C. Roy, A. Silverman, F. Cozzetto. *Pediatric Clinical Gastroenterology*. 2nd ed. St.

Louis: CV Mosby, 1975: 615-35.

(87) J. Dodge, J. Yassa. Food intake and supplementary feeding programs. In: Sturgess JM, ed. Perspectives in cystic fibrosis. Proceedings of the 8th International Cystic Fibrosis Congress. Toronto: Canadian Cystic Fibrosis Foundation, 1980: 125-36.

(88) H. Parsons, P. Beaudry, A. Dumas, P. Pencharz. Energy needs and growth in children with cystic fibrosis. *J Pediatr Gastroenterol Nutr* 1983; 2: 44-9.

(89) H. Schwachman, R. Dooley, F. Guilmette, P. Patterson, C. Weil, H. Leubner. Cystic fibrosis of the pancreas with varying degrees of pancreatic insufficiency. *Am J Dis Child* 1956; 92: 347-68.

(90) D. Crozier. Cystic fibrosis a not so fatal disease. *Pediatr Clin North Am* 1974; 21: 935-950.

(91) D. Gurwitz, M. Corey, P. Francis, D. Crozier, H. Levison. Perspectives in cystic fibrosis. *Pediatr Clin North Am*. 1979; 26: 603-615.

(92) E. Luder, M. Kattan, J. Thornton, K. Koehler, R. Bonforte. Efficacy of a nonrestricted fat diet in patients with cystic fibrosis. *AJDC*. 1989; 143: 458-464.

(93) A. Rettammel, M. Marcus, P. Farrell, S. Sondel, R. Kosciak, E. Mischler. Oral supplementation with a high-fat, high-energy product improves nutritional status and alters serum lipids in patients with cystic fibrosis. *J Am Diet Assoc*. 1995; 95: 454-459.

(94) K. Gaskin, D. Waters, L. Baur, V. Soutter, M. Gruca. Nutritional status, growth and development in children undergoing intensive treatment for cystic fibrosis. *Acta Paediatr Scand [Suppl]*. 1990; 366: 106-110.

(95) E. O' Loughlin, D. Forbes, H. Parsons, B. Scott, D. Cooper, G. Gall. Nutritional rehabilitation of malnourished patients with cystic fibrosis. *Am J Clin Nutr*. 1986; 43: 732-737.

(96) R. Shepherd, T. Holt, B. Thomas et al. Nutritional rehabilitation in cystic fibrosis: Controlled studies of effects on nutritional growth retardation, body protein turnover, and course of pulmonary disease. *J Pediatr*. 1986; 109: 788-94.

(97) R. Shepherd, B. Thomas, D. Bennett, W. Cooksley, L. Ward. Changes in body composition and muscle protein degradation during nutritional supplementation in nutritionally growth-retarded children with cystic fibrosis. *J Pediatr Gastroenterol Nutr*. 1983; 2: 439-446.

(98) L. Levy, P. Durie, P. Pencharz, M. Corey. Effects of long-term nutritional rehabilitation on body composition and clinical status in malnourished children and adolescents with cystic

fibrosis. *J Pediatr* 1985; 107: 225-230.

(99) J. Bertrand, C. Morin, R. Lasalle, J. Patrick, A. Coates. Short-term clinical, nutritional, and functional effects of continuous elemental enteral alimentation in children with cystic fibrosis. *J Pediatr*. 1984; 104: 41-46.

(100) R. Shepherd, W. Cooksley, and W. Domville. Improved growth and clinical, nutritional, and respiratory changes in response to nutritional therapy in cystic fibrosis. *J Pediatr*. 1980; 97: 351-357.

(101) J. Yassa, R. Prosser, J. Dodge. Effects of an artificial diet on growth of patients with cystic fibrosis. *Arch Dis Child*. 1978; 53: 777-783.

(102) J. Allan, A. Mason, A. Moss. Nutritional supplementation in treatment of cystic fibrosis of the pancreas. *Am J Dis Child*. 1973; 126: 22-26.

(103) A. Mansell, J. Andersen, C. Muttart et al. Short-term pulmonary effects of total parenteral nutrition in children with cystic fibrosis. *J Pediatr* 1984; 104: 700-705.

(104) M. Canciani, G. Mastella. Absorption of a new semielemental diet in infants with cystic fibrosis. *J Pediatr Gastroenterol Nutr*. 1985; 4: 735-740.

(105) M. Boland, D. Stoski, N. Macdonald, P. Soucy, J. Patrick. Chronic jejunostomy feeding with a non-elemental formula in undernourished patients with cystic fibrosis. *Lancet* 1986; 1: 232-234.

(106) P. Farrell, E. Mischler, S. Sondel, M. Palta. Predigested formula for infants with cystic fibrosis. *Research*. 1987; 87: 1353-1356.

(107) P. Regan, J. Malagelada, E. Dimagno, and V. Go. Reduced intraluminal bile acid concentrations and fat maldigestion in pancreatic insufficiency: Correction by treatment. *Gastroenterology*. 1979; 77: 285-289.

(108) P. Zentler-Munro, W. Fitzpatrick, J. Batten, and T. Northfield. Effect of intrajejunal acidity on aqueous phase bile acid and lipid concentrations in pancreatic steatorrhea due to cystic fibrosis. *Gut*. 1984; 25: 500-507.

(109) P. Zentler-Munro, D. Fine, J. Batten, and T. Northfield. Effect of cimetidine on enzyme inactivation, bile acid precipitation, and lipid solubilisation in pancreatic steatorrhea due to cystic fibrosis. *Gut*. 1985; 26: 892-901.

(110) A. Weber, C. Roy. Intraduodenal events in cystic fibrosis. *J Pediatr Gastroenterol Nutr*. 1984; 3 (Suppl. 1): S113-S119.

- (111) S. Dutta, V. Hubbard, M. Appler. Critical examination of therapeutic efficacy of a pH-sensitive enteric-coated pancreatic enzyme preparation in treatment of exocrine pancreatic insufficiency secondary to cystic fibrosis. *Dig Dis Sci* 1988; 33: 1237-44.
- (112) H. Heijerman, C. Lamers, W. Bakker. Omeprazole enhances the efficacy of pancreatin (pancrease) in cystic fibrosis. *Ann Intern Med.* 1991; 114: 200-201.
- (113) S. Schwarzenberg, C. Wielinski, I. Shamieh et al. Cystic fibrosis-associated colitis and fibrosing colonopathy. *J Pediatr* 1995; 127: 565-70.
- (114) R. Smyth, D. van Velzen, A. Smyth, D. Lloyd, D. Heaf. Strictures of ascending colon in cystic fibrosis and high strength pancreatic enzymes. *The Lancet.* 1994; 343: 85-86.
- (115) M. Pettei, J. Leonidas, J. Levine, J. Gorvoy. Pancolonic disease in cystic fibrosis and high-dose pancreatic enzyme therapy. *J Pediatr* 1994; 125: 587-9.
- (116) G. Cleghorn, R. Shepherd, T. Holt. The use of a synthetic prostaglandin E<sub>1</sub> analogue (Misoprostol) as an adjunct to pancreatic enzyme replacement in cystic fibrosis. *Scand J Gastroenterol.* 1988; 23(Suppl 143): 142-147.
- (117) K. Cox, J. Isenberg, A. Osher, R. Dooley. The effect of cimetidine on maldigestion in cystic fibrosis. 1979; 94: 488-492.
- (118) M. Schöni, R. Kraemer, E. Rossi. Cimetidine and fat malabsorption in children with cystic fibrosis. *Helv Paediat Acta.* 1981; 36: 359-369.
- (119) B. Boyle, W. Long, W. Balistreri, S. Widzer, and N. Huang. Effect of cimetidine and pancreatic enzymes on serum and fecal bile acids and fat absorption in cystic fibrosis. *Gastroenterology.* 1980; 78: 950-953.
- (120) R. Gow, R. Bradbear, P. Francis, R. Shepherd. Comparative study of varying regimens to improve steatorrhoea and creatorrhoea in cystic fibrosis: effectiveness of an enteric-coated preparation with and without antacids and cimetidine. *Lancet* 1981; 14: 1071-1074.
- (121) P. Robinson and P. Sly. Placebo-controlled trial of misoprostol in cystic fibrosis. *J Pediatr Gastroenterol Nutr.* 1990; 11: 37-40.
- (122) P. Durie, L. Bell, W. Linton, M. Corey, G. Forstner. Effect of cimetidine and sodium bicarbonate on pancreatic replacement therapy in cystic fibrosis. *Gut* 1980; 21: 778-786.
- (123) A. Carroccio, F. Pardo, G. Montalto et al. Use of famotidine in severe exocrine pancreatic insufficiency with persistent maldigestion on enzymatic replacement therapy: A long-term study in cystic fibrosis. *Dig Dis Sci* 1992; 37: 1441-1446.

- (124) D. Chalmers, R. Brown, M. Miller et al. The influence of longterm cimetidine as an adjuvant to pancreatic enzyme therapy in cystic fibrosis. *Acta Paediatr Scand.* 1985; 74: 114-117.
- (125) M. Schöni, R. Kraemer, A. Ruedeberg et al. Long-term cimetidine in children with cystic fibrosis: a randomized double blind study.
- (126) J. van de Kamer. Standard methods of clinical chemistry, edited by Seligson D. New York, Academic Press, 1958, Vol 2, p 34.
- (127) E. Wollaeger, M. Comfort, A. Osterberg. Total solids, fat and nitrogen in feces: Study of normal persons taking test diets containing moderate amount of fat; comparison with results obtained with normal persons taking test diet containing large amount of fat. *Gastroenterology* 1947; 9: 272-283.
- (128) D. Woodman, W. Yeoman. A simplified method of investigating steatorrhoea. *J Clin Pathol* 1955; 8:79-80.
- (129) M. Davidson, C. Bauer. Patterns of fat excretion in feces of premature infants fed various preparations of milk. *Pediatrics* 1960; 25: 375-84.
- (130) J. van de Kamer, H. Huinink, A. Weyers. Rapid method for the determination of fat in feces. *J Biol Chem* 1949; 177: 349-55.
- (131) B. Lembeke, B. Braden, J. Stein. Diagnostik der steatorrhoe. *Z Gastroenterol* 1994; 32: 256-261.
- (132) N. Thorsgaard Pedersen, H. Halgreen, H. Worning. Estimation of the 3-day faecal fat excretion and fat concentration as a differential test of malabsorption and maldigestion. *J Gastroenterol* 1987; 22: 91-96.
- (133) I. Roberts, C. Poturich, A. Wald. Utility of fecal fat concentrations as screening test in pancreatic insufficiency. *Dig Dis Sci* 1986; 31: 1021-4.
- (134) G. Bo-Linn, J. Fordtran. Fecal fat concentration on patients with steatorrhea. *Gastroenterology* 1984; 87: 319-322.
- (135) J. Bai, A. Andriush, G. Matelo et al. Fecal fat concentration in the differential diagnosis of steatorrhea. *Am J Gastroenterol* 1989; 84: 27-30.
- (136) B. Lembeke, K. Grimm, P. Lankisch. Raised fecal fat concentration is not a valid indicator of pancreatic steatorrhea. *Am J Gastroenterol* 1987; 82: 526-531.
- (137) H. Weijers. Fat absorption in normal and abnormal infants and children with special

reference to coeliac disease (proefscrift) 1950: 19-23.

(138) P. Phuapradit, A. Narang, P. Mendonca, D. Harris, J. Baum. The steatocrit: a simple method for estimating stool fat content in newborn infants. *Arch Dis Child* 1981; 56: 725-727.

(139) G. Iacono, A. Carroccio, G. Montalto et al. Steatocrit: normal range and physiological variations in preterm and low-birth-weight full-term newborns. *Acta Paediatr* 1992; 81: 933-4.

(140) A. Guarino, L. Tarallo, L. Greco, L. Cesarano, S. Guandalini, A. Rubino. Reference values of the steatocrit and its modifications in diarrheal diseases. *J Pediatr Gastroenterol Nutr* 1992; 14: 268-274.

(141) C. Colombo, R. Maiavacca, M. Ronchi, E. Consalvo, M. Amoretti, A. Giunta. The steatocrit: a simple method for monitoring fat malabsorption in patients with cystic fibrosis. *J Pediatr Gastroenterol Nutr* 1987; 6: 926-930.

(142) G. Iacono, A. Carroccio, F. Cavataio et al. Steatocrit test: normal range and physiological variations in infants. *J Pediatr Gastroenterol Nutr* 1990; 11: 53-57.

(143) M. Walters, J. Kelleher, J. Gilbert, J. Littlewood. Clinical monitoring of steatorrhoea in cystic fibrosis. *Arch Dis Child* 1990; 65: 99-102.

(144) E. Sugai, G. Srur, H. Vazquez et al. Steatocrit: a reliable semiquantitative method for detection of steatorrhea. *J Clin Gastroenterol* 1994; 19: 206-9.

(145) G. Drummey, J. Benson, C. Jones. Microscopical examination of the stool for steatorrhea. *N Engl J Med* 1961; 264: 85-7.

(146) L. Benini, S. Caliarì, G. Guodi. Near infrared spectrometry for faecal fat measurement: comparison with conventional gravimetric and titrimetric methods. *Gut* 1989; 30: 1344-1347.

(147) O. Bekers, C. Postma, A. Lombarts. Determination of faecal fat by Near-Infrared Spectroscopy. *Eur J Chem Clin Biochem* 1995; 33: 83-86.

## **CHAPTER 2**

### **METHODS**

The following methods were used in our studies:

## **1. Methods used for fecal fat determination:**

### **1.1 Steatocrit and Acid Steatocrit**

About 0,5 g solid stool was weighed and diluted with a volume of deionized water, equal to two times the weight of stool. The stool and water were premixed using a Vortex mixer. Subsequently, the mixture was homogenized using a 5 ml Potter Elvehjem tissue homogenizer. The fecal homogenate was aspirated into a 75  $\mu$ l plain haematocrit capillary. This capillary was sealed with wax at one end and centrifuged horizontally (13000 rpm, 15 min) in a standard haematocrit centrifuge. After centrifugation, the upper fatty layers (FL) and the bottom solid layers (SL) were measured with a graduated magnifying lens. The steatocrit was calculated as  $(FL / (FL + SL)) \times 100\%$ . Since the fat extraction was not optimal in the steatocrit procedure, we have try to increase this step by adding the perchloric acid (5N) to the fecal homogenate in a volume equal to 1/5 of the homogenate after homogenization. This acid homogenate was then mixed for 30 seconds using a Vortex mixer and the following steps were the same as the classical steatocrit. This "Acid Steatocrit" is used in our further study.

### **1.2 Titrimetric method**

The 72 hours fecal collection was first homogenized and about 5 gram of feces was weighed. The feces was saponified with concentrated potassium hydroxide (33% KOH) in ethanol, giving a solution which contains the soaps derived from neutral fats, fatty acids and also soaps originally present in the stool. By adding HCl (2N), fatty acids were obtained. After adding 125 ml toluene, the mixture was shaken vigorously for 2 - 3 minutes. 25ml of the toluene layer containing the fatty acids was then transferred to an erlenmeyer for titration with 0,1N tetrabutylammoniumhydroxide solution in propanol/methanol and thymol blue as indicator. The titration was done three times and the mean of this was used for the calculation of fecal fat excretion, which was calculated as followed:

$$\frac{125\text{ml} + 4.5\text{ml}}{25\text{ml}} (X - X_0) \times 0,1\text{N} \times 1/3 \times \frac{\text{total feces weight(g)}}{\text{sample weight (5g)}} \times 891\text{g} = \text{Total fecal fat (g/72h)}$$

with 125ml representing the toluene volume used for extraction of the fatty acids, 25ml representing the titration volume, 4,5ml is the correction for the volume interaction, X representing the number of meq of fatty acids titrated,  $X_0$  the correction for the acids present in toluene, 0,1N the concentration of tetrabutylammoniumhydroxide solution, 1/3 is the conversion factor from fatty acid to fat molecule (3 molecule fatty acids derived from 1 molecule fat) and 891 is the molecular weight of stearic acid (C-18-fat).

### 1. 3 Sudan staining method

We used the split fat stain, which identifies both triglyceride and fatty acid (1). Several drops of 100% acetic acid and several drops of Sudan III solution were added. The preparation was subsequently mixed with the coverslip, which was then applied. The slide was gently heated on a lighter until bubbling. All preparations were examined while still warm under high magnification (magnification of 400). For quantification of the amount of fat detected microscopically, we used the criteria established by Drummey et al.(2). They are as follows: normal (+): up to 100 fat globules per high power field, varying in a diameter between 1 and 4  $\mu\text{m}$ , as noted on the micrometer scale always using a magnification of 400; Increased (2+): up to 100 fat globules per high power field, the diameter of fat globules varying between 1 and 8  $\mu\text{m}$ ; Markedly increased (3+): more than 100 fat globules per high power field, varying in size from 6 to 75  $\mu\text{m}$  in diameter.

## 2. Methods used for assessment of nutritional condition:

### 2.1 Anthropometry

Weight, height and 4 skinfolds (biceps, triceps, subscapular and suprailiac) were expressed as standard deviation scores of the normal population for age and sex by using the growth charts from Gerver and de Bruin (3).

It has been found that subcutaneous fat as measured by skinfolds is related to the body density (4). This latter is again related to the body fatmass. From these theoretical principles, Gerber and de Bruin have constructed a chart, expressing the relationship between the 4 skinfolds (biceps, triceps, subscapular and suprailiac) and the percent fat free mass. In our study, the fatmass and fat free mass determined with the anthropometric method were derived from these charts.

## **2.2 Dual energy X-ray absorptiometry (DXA)**

This method first developed by Mazess et al., measures simultaneously bone mineral, fat and nonbone lean tissue. For a DXA scan, subjects lied supine on a padded table while the scintillation counter moved in a raster pattern across the body from head to foot. The Lunar DPX uses a constant x-ray source and a filter that converts the polychromatic x-ray beam into one that has two main energy peaks (40 kV and 70 kV). The ratio of soft tissue attenuation ( $R_{ST}$ ) at the two energies is measured. The attenuation of pure fat ( $R_f$ ) and of bone free lean tissue ( $R_L$ ) are known from both theoretical calculations and calibration. From this, the fatmass and lean tissue mass were calculated. The bone mineral content was calculated after correction of the overlying soft tissue (5). Body composition measurements in our study were made by a DPX with a pediatric software programme, Lunar version 1.5e. Daily quality assurance test were performed according to the manufacturer's directions. Total body analysis was performed in all children using a fast scan mode with a sample size of  $4,8 \times 9,6$ mm, sample interval of 0,03s and source collimation of 1,68mm.

## **2.3 Total body water (TBW) and extra cellular volume (ECV)**

TBW and ECV were measured by deuterium oxide (6) and bromide dilution respectively (7). Each subject received orally 20 ml of a mixture of  $D_2O$  (99,9% purity) and Bromide salt (150mMol/L) solution in a volume ratio of 1:1. Saliva and plasma samples were taken before intake of  $D_2O$  - NaBr solution and 4 hours thereafter when "an equilibrium" has been reached. To prevent saliva dilution by fluid intake which can result in a higher TBW content, patients were told not to take any fluid orally half an hour before saliva samples were taken. Urine and

fecal loss of bromide and D<sub>2</sub>O were negligible during the test since 1/2 of D<sub>2</sub>O and Bromide is 8 days (7). Saliva samples were obtained making use of dental cotton-wool, that was dried overnight at 100 °C and kept in a gas-tight tube until use. The cotton-wools and the blood samples were centrifuged and the saliva and serum thus obtained were kept in a stoppered glass vial and stored in a freezer until analysis.

### 2. 3. 1 Total body water

D<sub>2</sub>O concentrations of saliva samples were determined as follows: Calcium carbide (CaC<sub>2</sub>) was placed in the siliconized vacutainer tube and evacuated for 30 sec. with a rotatory vane pump to a total pressure of 0,01 atm. Thereafter, 25µl of salivary sample was injected in the vacutainer tube. This was done in duplicate. CaC<sub>2</sub> react with D<sub>2</sub>O forming acetylene gas. A 25µl of this gas was subsequently injected in duplicate into the GC/CF - IRMS system (gas chromatography/continous flow isotope ratio mass spectrometry) at 2 min. intervals. The mass 27/26 ratio (R<sub>27/26</sub>) was measured on a Isotope Ratio Mass Spectrometer configured for Acetylene (Finnigan MAT 252 for CF-IRMS) (6). The mean value of 4 determinations was calculated for each sample. By inserting of the tracer/tracee ratio, defined as R<sub>27/26</sub> (T<sub>4</sub>) - R<sub>27/26</sub> (T<sub>0</sub>), into the regression equation obtained from the standards, we get the dilution factor of D<sub>2</sub>O. TBW is calculated as ingested D<sub>2</sub>O volume/ dilution factor. From the TBW, LBM and FM can calculated by the following formulés:

$$\text{LBM (kg)} = \text{TBW} / ( 1,04 \times d )$$

$$\text{BF (kg)} = \text{Weight} - \text{LBM}$$

The 1,04 factor is a correction for the estimated 4% nonaqueous hydrogen exchange and d is the hydration factor of LBM which varies with age. Because our CF population was young, we used the age dependent hydration factors described by Fomon (8) for children younger than 10 year and by Boileau and Lohman (9) for older children.

### 2. 3. 2 Bromide space

Because Bromide resides mainly in the extracellular space, measured of Bromide dilution in serum give us an estimation of the extracellular volume. Bromide was determined by using a Gas Chromatograph type CP 9000 (Chrompack) equipped with an ECD detector after it was converted into a bromoacetone gas. First, perchloric acid was added to the serum sample and centrifugated for deproteinisation. An aliquot of the supernatant was then added to silver nitrate ( $\text{AgNO}_3$ ) for precipitating of silver bromide and chloride. After centrifugation, the precipitate was taken up in  $\text{NH}_3$  after that  $\text{Na}_2\text{S}$  and  $\text{NaOH}$  were added to precipitate the silver as  $\text{Ag}_2\text{S}$ . After agitation and centrifugation, the supernatant was heated until dry,  $\text{H}_2\text{O}$  was added followed by  $\text{H}_2\text{O}_2$  to oxidize sulfide. After drying,  $\text{H}_2\text{O}$  was then added and dried again. This was repeated several times. Thereafter, perchloric acid and acetone were added and the reaction is started by addition of  $\text{KmnO}_4$  with Bromoacetone formed. The solution is then extracted with benzene. The organic phase was separated from the water phase by shaking and centrifugation. The water phase was then removed. An aliquot of the organic solution is then applied to the gas chromatograph for measuring of bromoacetone/internal standard ratio. The bromide concentration was then derived from the bromoacetone standard curves. Because the distribution of Bromide depend on the potential difference between in- and extra-cellular and on the quantity of total body volume, corrected bromide space was calculated as follow:

$$\text{ECV (L)} = \frac{\text{Bromide administered (mmol)}}{\text{Bromide change T4 - T0 (mmol/L)}} - 0,036\text{TBW}$$

Where 0,036TBW is the correction factor for the cell potential and for the total body volume (7).

## REFERENCES

- (1) M. Khouri, G. Huang, Y. Shiau. Sudan stain of fecal fat : New insight into an old test. *Gastroenterology* 1989; 96: 421-7.
- (2) G. Drummey, J. Benson, C. Jones. Microscopical examination of the stool for steatorrhea. *N Engl J Med* 1961; 264: 85-7.
- (3) W. Gerver, R. de Bruin. *Paediatric Morphometrics: a reference manual*. 1th ed. Utrecht: Bunge, 1996.
- (4) J. Weststrate, P. Deurenberg. Body composition in children: proposal for a method for calculating body fat percentage from total body density or skinfold-thickness measurements. *Am J Clin Nutr* 1989; 50: 1104-15.
- (5) S. Heymsfield, J. Wang, S. Heshka, J. Kehayias, R. Pierson. Dual-photon absorptiometry: comparison of bone mineral and soft tissue mass measurements in vivo with established methods. *Am J Clin Nutr* 1989; 49: 1283-9.
- (6) B. Van Kreef, F. Van der Vegt, M. Meers, T. Wagenmakers, K. Westerterp, A. Coward. Determination of total body water by a simple and rapid mass spectrometric method. *J Mass Spectrom* 1996; 31: 108-111.
- (7) B. Van Kreef. An Improved bromide assay for the estimation of extracellular water volume by capillary gas chromatography. *Clinica Chimica Acta* 1994; 231: 117-128.
- (8) S. Fomon, F. Haschke, E. Ziegler, S. Nelson. Body composition of reference children from birth to age 10 years. *Am J Clin Nutr* 1982; 35: 1169-1175.
- (9) Boileau, R. Lohman, M. Slaughter, T. Ball, S. Going, M. Hendrikx. Hydration of the fat-free body in children during maturation. *Hum Biol.* 56: 651-666.

## CHAPTER 3

### THE ACID STEATOCRIT : A MUCH IMPROVED METHOD

Tran M, Forget P, Van den Neucker A, Strik J, van Kreel B, Kuijten R.

Departments of Pediatrics and Clinical Chemistry, University Hospital Maastricht,  
Maastricht, The netherlands.

---

J Pediatr Gastroenterol Nutr 1994; 19: 299-303

#### *Abstract*

The steatocrit method has recently been introduced as a simple screening test for steatorrhea. As it seemed likely that separation of fecal homogenate by centrifugation into a lipid phase, a watery phase and a solid phase would be pH-dependent, we evaluated the effect of fecal acidification on steatocrit results in healthy children and in patients with cystic fibrosis and studied the relationship between two steatocrit methods and fecal fat content as measured by a reference chemical method. Steatocrit results increased with the degree of fecal acidification, and maximal results were obtained at the lowest fecal pH values. Means and SEM for classical and acid steatocrit values were  $1.1 \pm 0.4\%$  (classical) versus  $3.8 \pm 1\%$  (acid) in controls ( $n = 6$ ) and  $5.4 \pm 1.9\%$  (classical) versus  $26.9 \pm 4.3\%$  (acid) in cystic fibrosis ( $n = 9$ ). The correlations between fecal fat content measured chemically and steatocrit results were 0.18 ( $p = 0.35$ ) and 0.81 ( $p < 0.0001$ ) for classical and acid steatocrit, respectively. We conclude that acidification of fecal homogenates leads to a marked improvement in the steatocrit method.

## INTRODUCTION

The diagnosis of fat malabsorption still mainly relies on the 72-hour faecal fat quantitation in which daily stool fat loss is evaluated by collecting stools for 3 days and determining stool fat content by chemical methods. The most widely used chemical method is the titrimetric method as described by van de Kamer in 1949 (1).

Work by Kouri et al has suggested that the titrimetric method largely overestimates nutritional faecal fat losses because it measures not only malabsorbed exogenous fat but also endogenous fat of various origins such as biliary lipids and lipids derived from the turnover of intestinal epithelial cells and gut bacteria (2).

Making use of the staining properties of purified lipids in an artificial matrix, Khouri et al. have suggested the fat absorption coefficient in normal adults is much higher than usually believed (2). Although the microscopic evaluation of steatorrhea by the Sudan stain provides a satisfactory screening method for steatorrhea, it is at best semiquantitative.

The steatocrit has been introduced in recent years as a simple test for the evaluation of fat malabsorption (3-6). Although several authors have reported the method to be satisfactory for the evaluation of steatorrhea, some have reported the steatocrit to be quite unreliable (6).

As it has been shown that faecal acidification results in an enhanced sensitivity of the Sudan faecal staining method (2), we wondered whether the same modification could improve fat extraction by centrifugation as performed in the steatocrit determination.

Consequently, we evaluated the effects of stool sample acidification on steatocrit determinations and to compare results from previously reported methods with acid steatocrit results in healthy children and in children with cystic fibrosis. We also determined the correlation between steatocrit results and faecal fat concentrations as measured by the reference chemical method of van de Kamer et to evaluate which of the two steatocrit methods gave the best estimate of faecal fat content.

## METHODS

### "classic" steatocrit method

Stool ( 0.5 gr ) was diluted (1/3) with deionized water and thoroughly homogenized in a 5 ml Potter Elvehjem tissue homogenizer (Heidolph Elektro KG Kelheim, no. 170-1700/20-200) stamper, tissue grind pestle (size 20 from Kontes Scientific Glassware Instruments, no. 885451-0020). The homogenate was aspirated into a 75 µl plain glass haematocrit tube. The capillary tube was subsequently centrifuged horizontally (13,000 rpm for 15 min) in a standard haematocrit centrifuge.

After centrifugation, the upper (fat) and bottom (solid) layers were measured with a graduated magnifying lens. Steatocrit was calculated as  $FL/(FL + SL)$ , where FL is the fatty-layer length and SL is the solid-layer length.

#### "Acid" steatocrit method

The method used was exactly the same as the classic steatocrit method except that, before aspirating the homogenate in the capillary tube, perchloric acid in various concentrations (5N for maximal acidification) was added to the homogenate in a volume equal to 1/5 of the homogenate volume. The resulting acid homogenate was mixed for 30 seconds with a standard Vortex mixer.

#### Chemical determination of stool fat concentration.

The method of van de Kamer et al. was used to determine stool fat content (1).

## EXPERIMENTAL DESIGN

### Effect of stool homogenate acidification on fat extraction

To evaluate the effect of stool acidification and thus stool pH on the length of the fat column obtained by centrifugation, several stool samples from patients with and without steatorrhea were centrifuged after addition of perchloric acid solutions of various concentrations.

### Classic and acid steatocrit

To compare classic and acid steatocrit results in children with and without steatorrhea, we measured fecal steatocrit by both methods in 6 control children (mean age: 5.8 years, range 3 to 12 years; five boys and one girl) and in 9 children with cystic fibrosis (mean age: 6.9 years; range 0.5 to 20; nine boys). The control children were patients with chronic aspecific respiratory disease without gastrointestinal symptoms and with a normal sweat test. The cystic fibrosis patients all had abnormal sweat tests on several occasions and were being treated with pancreatic enzymes when steatocrit determinations were performed. As our purpose was to compare classical and acid steatocrit results in the same fecal samples, no attempt was made to quantify the fat content of the diet which was "normal" in all patients.

### Correlation between steatocrit results and fecal fat content

To further compare both steatocrit methods we looked at the relationship between results obtained by each method and fecal fat content results as measured by the method of van de Kamer et al. (1). Steatocrit measurements (classic and acid) and fecal fat content determinations (chemical method) were performed on 27 consecutive stool samples (from adults and children) sent to our laboratory for evaluation of malabsorption. No attempt was made to classify patients in disease categories as our only goal was to study the relationship between steatocrit results and fecal fat content independent of the presence of disease (clinical results will be published separately).

### Statistical methods

The coefficient of variation of each steatocrit method was determined with duplicate results of each sample for both methods. Pearson correlation coefficient was used to evaluate the relationship between steatocrit results and chemically measured fecal fat content.

## **RESULTS**

Effect of stool homogenate acidification on fat extraction

Several steatorrheal stool samples were analysed after acidification with various concentrations of perchloric acid.

A typical finding is shown in figure 1; The upper fat column was seen to increase in length with the degree of homogenate acidification. A typical normal stool sample result (no steatorrhea) is shown in figure 2. The acid steatocrit remained completely negative in normal samples.

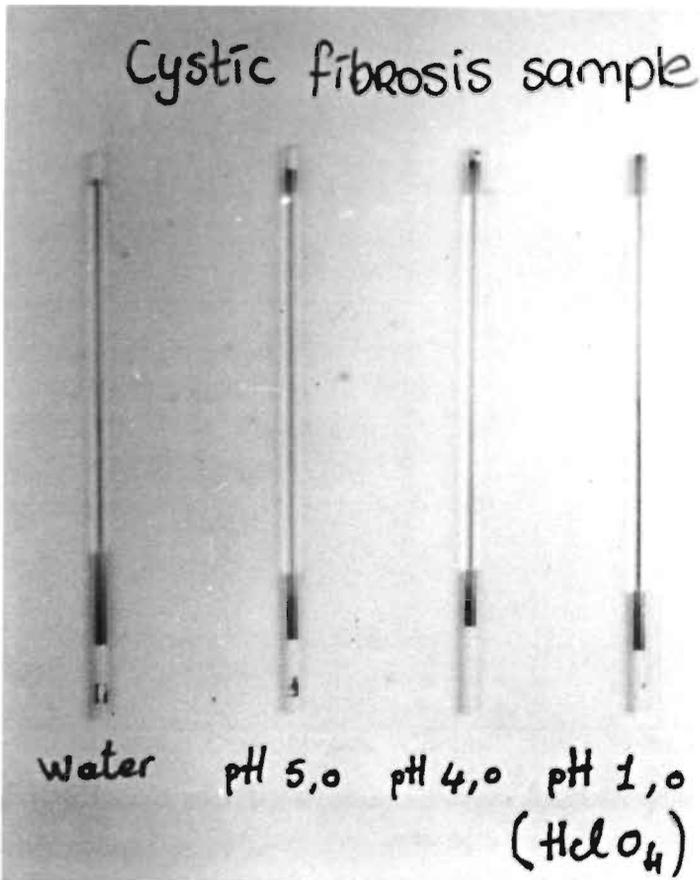
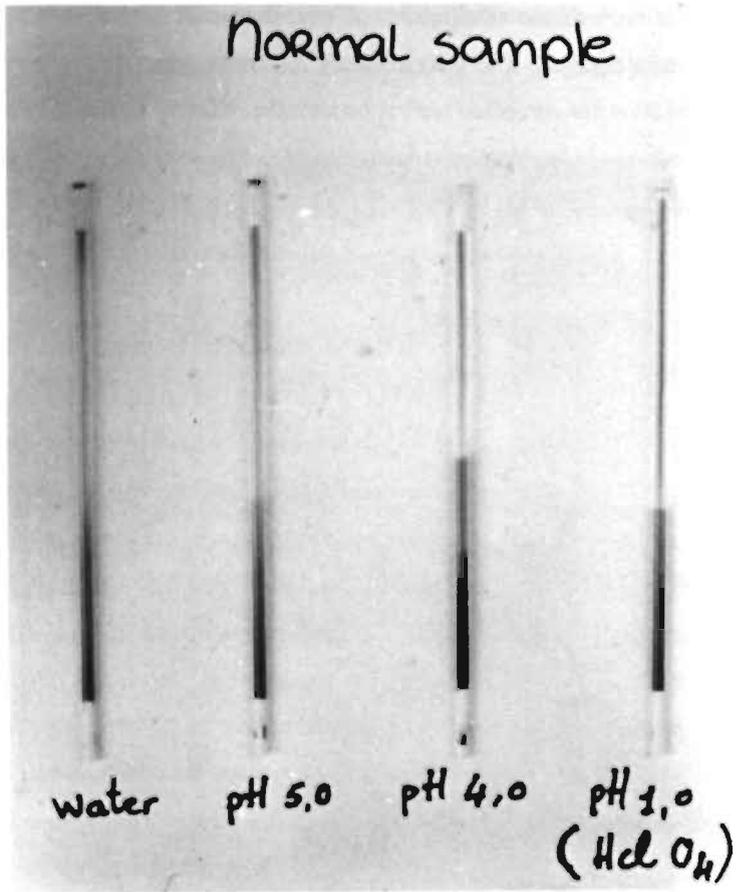


Figure 1 Effect of acidification with various concentrations of perchloric acid on the fat column length (upper part of picture) of a stool sample from a patient with cystic fibrosis.



**Figure 2** Effect of acidification with various concentrations of perchloric acid on fat extraction from a normal stool sample. Fat layer is absent at all pH values.

### Classic and acid steatocrit

Results of classic and acid steatocrit in 6 control and 9 cystic fibrosis patients (figure 3) were as follows : Steatocrit means and SEM in control patients were  $1.1 \pm 0.4$  and  $3.8 \pm 1\%$  for classic and acid steatocrit, respectively. This difference was not statistically significant. Steatocrit means and SEM in cystic fibrosis patients were  $5.4 \pm 1.9$  and  $26.9 \pm 4.3 \%$  for classic and acid steatocrit, respectively. This difference is significant ( $p < 0.01$ )

The precision of the methods was evaluated by comparing the variation coefficients; variation coefficients were 6.9 and 5.1 % for the classic and acid steatocrit methods, respectively.

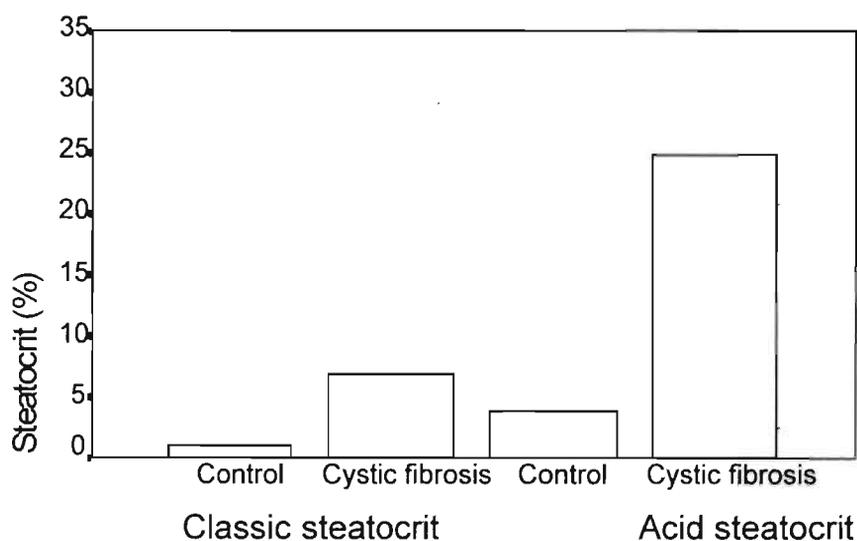
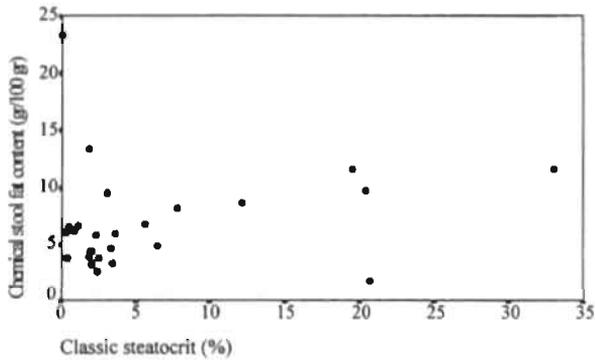


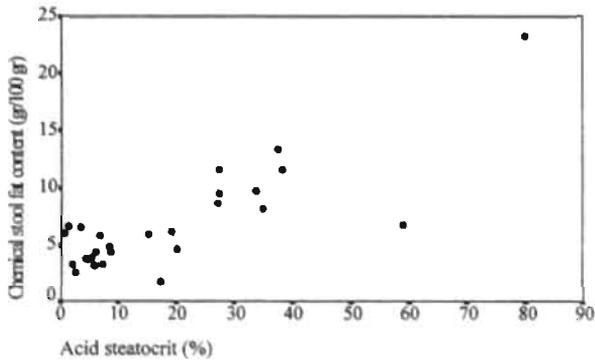
Figure 3 Classic and acid steatocrit results in six controls and nine patients with cystic fibrosis.

Correlation between steatocrit results and faecal fat content

The relationship between classic fecal steatocrit and fecal fat content as measured by the reference method of van de Kamer et al. (1) is shown in figure 4. The correlation coefficient of 0.18 is statistically non-significant ( $p = 0.35$ ). The relationship between acid fecal steatocrit and fecal fat content is shown in figure 5. The correlation coefficient of 0.81 is highly significant ( $p < 0.0001$ ).



**Figure 4** Relationship between classic fecal steatocrit and fecal fat content as measured by the method of van de Kamer et al. In 27 fecal samples ( $r = 0.18$ ;  $p = 0.35$ ).



**Figure 5** Relationship between acid fecal steatocrit and fat content as measured by the method of van de Kamer et al. In 27 fecal samples ( $r = 0.81$ ;  $p < 0.0001$ ).

## DISCUSSION

Although several authors have reported the steatocrit method to be reliable for the screening of steatorrhea ( 3-5 ), Walters et al reported the method to be completely unreliable (6). Methodological inadequacies probably underlie these discrepancies. We have been using the "classic" steatocrit in our department for a few years and have found completely negative results in some patients with proven steatorrhea. We hypothesized that in some patients fat detection might be poor and that a possible solution to the problem would be an improved method of liberating fat during the centrifugation step. It has been shown in a recent study that fecal fat in patients with pancreatic insufficiency mainly consists of fatty acids and that the fecal triglyceride content does not differ from that of normal controls (7).

Fecal fatty acid molecules exist in the form of soaps (8). Further, since the pKa of most fatty acids is lower (about 4.8) than fecal pH, most fatty acids in stool would be present as ionized species or soaps. We speculated that fecal acidification would result in the conversion of ionized fatty acid species and soaps into the protonated species leading to easier separation into lipid and water phases during the centrifugation step of the steatocrit method.

Our results show that the effect of stool homogenate acidification on the length of the upper fatty layer very nicely confirms our predictions. Although we have not checked this point in detail, it can be expected that at the low PH values obtained after maximal acidification as performed in the present study, all fatty acids will be present in the protonated form.

Further, the fact that acidification of fecal samples from patients without steatorrhea and with completely negative steatocrit results did not result in the appearance of a fatty layer, probably indicating that the improved fat extraction is not a spurious artifactual finding but the result of better extraction of lost exogenous fat.

Khouri et al have suggested that ionized fatty acids are not readily stainable with Sudan stain, although staining does occur after acidification (2). By alkalization with sodium hydroxide, the same authors showed that fatty acids lost their ability to form fat droplets and to stain with Sudan red III (2). We suppose that similar mechanisms underlie the improvement of both the fat staining method and fat extraction by fecal acidification as shown in the present study.

A further advantage of acidification is that it enhances the visible boundaries between the

various layers, resulting in improved accuracy in the reading of layers lengths. Improved fecal fat extraction by acidification should therefore result in higher diagnostic sensitivity of the steatocrit method.

Our results show classic steatocrit in control children and in children with cystic fibrosis are similar to results published by other authors (4); However, acidified steatocrit results in both control children and cystic fibrosis patients were much higher than those obtained by classic steatocrit. Ongoing work in our laboratory aims at establishing normal population values for acid steatocrit in infants and children.

In order to better interpret the differences found between the steatocrit methods, we compared steatocrit results with fecal fat concentrations measured by the most accepted reference method. Our findings show that only acid steatocrit results correlate very significantly with fecal fat content as measured by the van de Kamer method. The literature is quite varied on this point. Several studies have looked for a correlation between steatocrit results and either the fat absorption coefficient or 3-day fecal fat excretion. A good correlation was reported by two studies (4,9) while a total lack of correlation was reported by a third author (6). As steatocrit is supposed to reflect fecal fat concentration we preferred to relate steatocrit results to fecal fat concentrations rather than daily excretion or fat absorption coefficients. To our knowledge only one study reporting results in a similar way found a significant relationship between steatocrit results and fecal fat content (3). We think our finding of a lack of correlation between classic steatocrit and fecal fat content results can best be explained by the small number of observations or by the lack of homogeneity in our patient material.

This lack of homogeneity was, however, purposely chosen as we were only interested in the correlation between steatocrit results and fecal fat content. We think a positive correlation between the two steatocrit methods and fecal fat content could have been found but the acid steatocrit method would always better correlate with fecal fat content.

We conclude that acidification of fecal homogenates led to a much better fat extraction by centrifugation, increased sensitivity of the steatocrit method and to a better prediction of fecal fat content as measured by chemical methods.

*Acknowledgment:* The authors thank the clinical laboratory staff for their kind and expert technical assistance. We are very grateful to Nutricia Netherlands for financial support.

## REFERENCES

- (1) van de Kamer JH, Huinink HTB, Weyers HA. Rapid method for determination of fat in feces. *J Biol Chem* 1949 ; 177 :349-55.
- (2) Khouri MR, Huang G, Shiau YF. Sudan stain of fecal fat : new insight into an old test. *Gastroenterology* 1989 ; 96 : 421-427.
- (3) Phuapradit P, Narang A, Mendonca P, Harris DA, Baum JD. The steatocrit : a simple method for estimating stool fat content in newborn infants. *Arch Dis Child* 1981 ; 56 : 725-727.
- (4) Colombo C, Maiavacca R, Ronchi M, Consalvo E, Amoretti M, Giunta A. The steatocrit : a simple method for monitoring fat malabsorption in patients with cystic fibrosis. *J Pediatr Gastroenterol Nutr* 1987 ; 6 : 926-930.
- (5) Iacono G, Carroccio A, Cavataio F et al. Steatocrit test : normal range and physiological variation in infants. *J Pediatr Gastroenterol Nutr* 1990 ; 11 : 53-57.
- (6) Walters MP, Kelleher J, Gilbert J, Littlewood JM. Clinical monitoring of steatorrhea in cystic fibrosis. *Arch Dis Child* 1990; 65 : 99-102.
- (7) Khouri MR, Huang G, Shiau YF. Fecal triglyceride excretion is not excessive in pancreatic insufficiency. *Gastroenterology* 1989; 96 : 848-852.
- (8) Shiau YF, Popper DA, Reed M, Umstetter C, Capuzzi D, Levine GM. Intestinal triglycerides are derived from both endogenous and exogenous sources. *Am J Physiol* 1985; 248 : G164-169.
- (9) Guarino A, Tarallo L, Greco L, Cesarano L, Guandalini S, Rubino A. Reference values of the steatocrit and its modifications in diarrheal diseases. *J Pediatr Gastroenterol Nutr* 1992; 14: 268-274.

## CHAPTER 4

### IMPROVED STEATOCRIT RESULTS OBTAINED BY ACIDIFICATION OF FECAL HOMOGENATES ARE DUE TO IMPROVED FAT EXTRACTION

M. Tran, P. Forget, A. Van den Neucker and B. Van Kreel

Department of Pediatrics and Clinical Chemistry, University Hospital Maastricht,  
Maastricht, The Netherlands

---

*J Pediatr Gastroenterol Nutr* 1996; 22: 157-160

#### *Abstract*

Conflicting results have been reported on the value of the steatocrit as a screening test for steatorrhea. We recently modified the test procedure by fecal acidification with the hope of improving fat extraction and consequently the sensitivity of the test. The aim of the present study was to ascertain, whether or not fecal acidification led to improved fat extraction, by comparing the fat content of both fatty and solid layers obtained by centrifugation of 12 acidified (acid steatocrit) and unacidified (classical steatocrit) steatorrheal stool samples.

The fat content of fatty and solid layers was evaluated using of the semiquantitative (+ = 1, ++ = 2, +++ = 3) scoring system described by Drummey, for the interpretation of the sudan microscopic method for fecal fat.

The fatty layers sum of scores for the 12 samples examined, was 31 and 16, for the acid and classical steatocrit respectively. The solid layers sum of scores for the 12 samples, was 13 and 24, for the acid and classical steatocrit respectively. Fat extraction from stool samples was significantly improved after fecal sample acidification ( $p < 0.005$ ). Acid steatocrit results agreed better with chemically measured fecal fat than classical steatocrit results.

We conclude that fecal acidification, by improving fat extraction, increases the reliability of the steatocrit method for the detection of steatorrhea.

## INTRODUCTION

Several methods are in use for the diagnosis of fat malabsorption. One of these is the 72 hour fecal fat quantitation method, which is regarded as the most accurate method to evaluate steatorrhea (1). However, there are several problems. First, it is a laborious method for laboratory technicians, and second, fecal collection for 3-5 days makes the method unpleasant for the patient and sometimes poorly reliable in non collaborating children.

Another well accepted test to screen for fat malabsorption is the Sudan staining method for fecal fat (2). Unfortunately this method is only semiquantitative.

In 1981 Phuapradit introduced the steatocrit method as a new, simple and easily repeatable method for measuring fecal fat content (3).

Although several authors have reported this method to be satisfactory for the evaluation of steatorrhea (3-5), some described it as quite unreliable (6). We have been using this method for years and have often found normal steatocrit values in patients, who, when measured chemically had steatorrhea with an increased fecal fat content.

As it has been shown that fecal acidification results in an enhanced sensitivity of the Sudan fecal staining method (7), we wondered whether fecal acidification could also be used to improve the sensitivity of the steatocrit method.

We consequently modified the reported (8) steatocrit method by adding perchloric acid to the fecal homogenate. Fat extraction was evaluated for classical and acid steatocrit methods, making use of the Sudan microscopic method for fecal fat.

We further compared calculated steatocrit results from acidified and unacidified samples, and related the results to fecal fat content of the same samples, measured by the reference chemical method of van de Kamer et al. (1).

## MATERIALS AND METHODS

### *Population studied*

Twelve stool samples from 4 premature babies, 3 boys and 1 girl, with a mean gestational age of 35,3 weeks (ranged from 27 5/7 to 35 5/7 weeks), were analysed by means of both

the classical and the acid steatocrit method.

Their postnatal age varied between 11 and 18 days. They received full oral formula feedings. Their weight ranged from 1810 g to 2360 g.

### Steatocrit methods

0,5 g solid stool was weighed and diluted with a volume of deionized water, equal to two times the weight of stool. The stool and water were premixed using a Vortex mixer. Subsequently, the mixture was homogenized using a 5 ml Potter Elvehjem tissue homogenizer. Then, the homogenate was aspirated into a 75  $\mu$ l plain haematocrit capillary. This capillary was sealed with wax at one end and centrifuged horizontally (13,000 rpm, 15 min) in a standard haematocrit centrifuge.

The method used for the acid steatocrit was exactly the same as that of the classical steatocrit, the only exception being, that after homogenizing, 5N perchloric acid was added to the homogenate in a volume equal to 1/5 of the homogenate. This acid homogenate was then mixed for 30 seconds using a Vortex mixer.

After centrifugation, three layers were distinguished: a basal solid layer, an intermediate liquid layer, and an upper fatty layer. After calculating the steatocrit results for both methods as usual, the capillaries were cut in the middle of the fatty, and of the solid layers using a special glass knife. Subsequently, the layers were removed from the capillaries, using a syringe. A standard amount of each of these fatty and solid layers was then transferred to different glass slides for staining with Sudan III dye. In this way, we acquired a total of 48 slides (24 from each steatocrit method, 12 fatty and 12 solid layers) for microscopic evaluation.

### Sudan stain method for fecal fat

For this purpose we used the split fat stain, which identifies both triglyceride and fatty acid (7). Several drops of 100% acetic acid and several drops of Sudan III solution were added. The preparation was subsequently mixed with the coverslip, which was then applied. The slide was gently heated on a lighter until bubbling. All preparations were examined while

still warm under high magnification (magnification of 400), by the same person who was blind to the steatocrit method used (classical or acid).

For quantification of the amount of fat detected microscopically, we used the criteria established by Drummey et al.(2). They are as follows: normal (+): up to 100 fat globules per high power field, varying in a diameter between 1 and 4  $\mu\text{m}$ , as noted on the micrometer scale always using a magnification of 400; Increased (2+): up to 100 fat globules per high power field, the diameter of fat globules varying between 1 and 8  $\mu\text{m}$ ; and markedly increased (3+): more than 100 fat globules per high power field, varying in size from 6 to 75  $\mu\text{m}$  in diameter.

The sum of the fatty and solid layer scores of all our samples, was calculated for both steatocrit methods, and results were compared. Fisher's exact probability test was used to test whether or not the solid layers microscopic fat content was dependent on sample acidification. Finally, calculated acidified and unacidified steatocrit results were compared and related to the chemically measured fecal fat content.

## RESULTS

Table 1 shows that acidification of the fecal homogenates before centrifugation (acid steatocrit method) results in a higher Drummey score in the fatty layers and a lower score in the solid layers. In four specimens (sample 6, 7, 8 and 10), the fatty layers obtained by the classical steatocrit method were so small that we did not succeed in making microscopical slides. Equal results were obtained by both the classical and the acid steatocrit method, in only one sample (sample 11).

**Table 1 Fatty and solid layer microscopical fat scores for both acid and classic steatocrit methods.**

SAMPLES	SCORES OF FAT GLOBULES (+, ++, +++)			
	FATTY LAYERS		SOLID LAYERS	
	ACID	CLASSIC	ACID	CLASSIC
2	+++	++	+	+
3	++	++	+	+++
4	+++	+	+	+++
5	+++	++	+	+++
6	+	-	+	++
7	++	-	+	++
8	+++	-	+	+
9	+++	+++	+	++
10	++	-	+	+
11	+++	+++	+	+
12	+++	++	+	+++
<b>SUM</b>	<b>31</b>	<b>16</b>	<b>13</b>	<b>24</b>

The sum of the fat scores for fatty and solid layers, and for both steatocrit methods is summarized in figure 1. The number of solid layer samples with low microscopic ( $\leq +$ ) fat content, was 11 of 12, and 4 of 12, for the acidified and unacidified samples respectively ( $p < 0.005$ , Fisher's exact probability test).

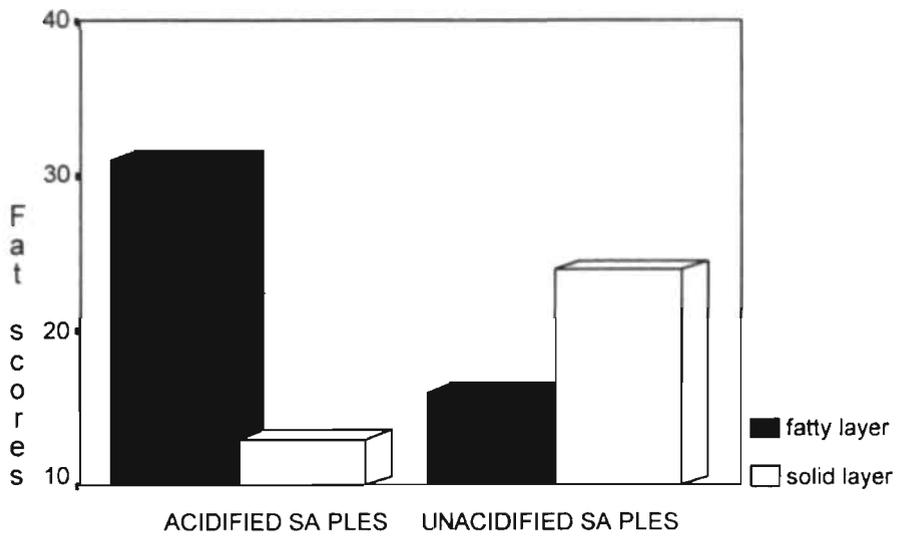


Figure 1 Sum of 12 microscopical Sudan fat globule scores (1, 2, 3) performed on fatty and solid layers of acidified and unacidified fecal samples.

The calculated steatocrit results for both steatocrit methods, and the chemically measured fecal fat content for the 12 samples, are shown in table 2. Chemical fat measurements of two samples (1 and 2) were not performed. The chemically measured fecal fat concentration was very high in all samples and corresponded with high acid steatocrit results, while only 5 classical steatocrit results were elevated.

**Table 2 Classic and acid steatocrit results compared to chemically measured fecal fat in 12 steatorrheal fecal samples.**

SAMPLES	CLASSIC STEATOCRIT (%)	ACID STEATOCRIT (%)	FECAL FAT (GRAM %)
1	5.3	81.7	-
2	2.5	72.4	-
3	5.3	71.1	16.6
4	28.8	93.3	28.5
5	26	90.9	26.7
6	6.2	92.6	28.3
7	3.1	92.5	18.7
8	2.8	94.2	26.5
9	59.8	93.7	24.3
10	6.2	96.4	10.3
11	63.9	96	27.3
12	48.7	94.4	20.6

## DISCUSSION

There has always been a need for a simple, rapid and easy to perform screening test for fat malabsorption. Such a test would not only be useful for the detection of steatorrhea but also for the therapeutic monitoring of children treated for pancreatic insufficiency.

The steatocrit is a simple and rapid micromethod that can be repeated at short time intervals

(3). It is inexpensive and not invasive (5). Some authors have reported it as a very satisfactory screening test (3-5), but others have found it quite unreliable (6). Our experience with the method has shown the steatocrit to often be normal, in fecal samples with a very high chemically measured fecal fat content. This could be due to inefficient fecal fat extraction at the centrifugation step. Therefore we recently improved the steatocrit method by acidifying the fecal homogenate before centrifugation (8).

The present study was set up to study the effect of fecal homogenate acidification on fat extraction at the centrifugation step. If fat extraction improves by acidification, we would expect to find more fat globules in the fatty layer and less in the solid layer, after centrifugation of acidified fecal samples, when compared to unacidified samples. Our results are in agreement with our expectations and support the hypothesis that fecal acidification improves fat extraction, and should consequently improve the sensitivity of the steatocrit. Due to various reasons, the fat content of premature infants' stool, is known to be very high. Confirming the latter, chemical fat measurements of all our samples from 4 premature babies showed very high values. The acid steatocrit seems to reflect these very high fat contents, while classical steatocrit results were high in only 5 of 12 samples. The correlation between chemical fat measurement and acid steatocrit has been reported previously (8). Such a correlation cannot be shown in the present study where only high-fat-content stools were evaluated.

Results of the present study do support our previous findings, confirming, that fecal acidification improves fat extraction at the centrifugation step, and consequently increases the reliability of steatocrit results for the detection of fat malabsorption. Because the Sudan staining method for fecal fat is only semiquantitative, we suggest using the acid steatocrit as a good alternative to chemical fat measurement.

*Acknowledgement:* The authors wish to thank the clinical laboratory staff for their kind and expert technical assistance. We are very grateful to Nutricia Netherlands for financial support.

## REFERENCES

- (1) Van de Kamer JH, Huinink HTB, Weyers HA. Rapid method for determination of fat in feces. *J Biol Chem* 1949; 177: 349-55.
- (2) Drummey GD, Benson JA, Jones CM. Microscopical examination of the stool for steatorrhea. *N.Engl J Med* 1961; 264: 85-7.
- (3) Phuapradit P, Narang A, Mendonca P, Harris DA, Baum JD. The steatocrit: a simple method for estimating stool fat content in newborn infants. *Arch Dis Child* 1981; 56: 725-727.
- (4) Iacono G, Carroccio A, Cavataio F, Montalto G, Mancusco C, Balsamo V et al. Steatocrit test: normal range and physiological variation in infants. *J Pediatr Gastroenterol Nutr* 1990; 11: 53-57.
- (5) Columbo C, Maiavacca R, Ronchi M, Consalvo E, Amoretti M, Giunta A. The steatocrit: a simple method for monitoring fat malabsorption in patients with cystic fibrosis. *J Pediatr Gastroenterol Nutr* 1987; 6 : 926-930.
- (6) Walters MP, Kelleher J, Gilbert J, Littlewood JM. Clinical monitoring of steatorrhea in cystic fibrosis. *Arch Dis Child* 1990; 65: 99-102.
- (7) Khouri MR, Huang G, Shiao YF. Sudan stain of fecal fat: new insight into an old test. *Gastroenterology* 1989; 96: 421-427.
- (8) Tran M, Forget P, Van den Neucker A, Strik J, van Kreel B, Kuijten R. The acid steatocrit: a much improved method. *J. Pediatr. Gastroenterol Nutr.* 1994; 19: 299-303

## CHAPTER 5

### CLINICAL USE OF ACID STEATOCRIT

A. Van den Neucker<sup>1</sup>, N. Pestel<sup>1</sup>, T. My Dung Tran<sup>1</sup>, P.Ph. Forget<sup>1</sup>, H.J. Veeze<sup>2</sup>,  
J. Bouquet<sup>2</sup>, M. Sinaasappel<sup>2</sup>.

<sup>1</sup>Department of Pediatrics, University Hospital Maastricht and <sup>2</sup>Sophia Children's Hospital  
Rotterdam, The Netherlands

---

Submitted for publication

#### *Abstract*

Malabsorption of fat is an important gastrointestinal cause of malnutrition and growth retardation in childhood. The golden standard for the evaluation of fat malabsorption is the fecal fat balance method. The acid steatocrit method has recently been introduced as a simple method to evaluate fecal fat. The present study aimed at evaluating the acid steatocrit in clinical practice. Fecal fat excretion and acid steatocrit results were determined in 42 children, half with and half without fat malabsorption. Acid steatocrit results correlated significantly with both fecal fat excretion ( $p < 0.01$ ) and fecal fat concentration ( $p < 0.001$ ). Sensitivity and specificity of the acid steatocrit for the diagnosis of malabsorption was 90% and 100% respectively. We consider the acid steatocrit method useful for the screening and monitoring of patients with steatorrhea. Acid steatocrit, Steatorrhoea, Cystic Fibrosis.

## INTRODUCTION

Malabsorption of fat is the most important gastrointestinal cause of malnutrition and growth retardation in childhood. The detection of steatorrhea is useful for the diagnosis of intestinal and pancreatic disease. The gold standard for the evaluation of patients suspected of fat malabsorption is the fat balance method whereby fecal fat is chemically measured according to the method of van de Kamer (1). This method is cumbersome, laborious, expensive and often difficult to manage in children. In 1981 Phuapradit et al. introduced a simple test to evaluate fecal fat content (2). Although some authors found this test quite reliable (3), others did not (4). As previously reported, substantial improvement of the method was obtained by acidification of fecal samples, acid steatocrit (AS) (5).

The present study was designed to compare the fecal fat excretion with the acid steatocrit for the diagnosis of fat malabsorption in children.

## PATIENTS

Forty two children, 23 boys and 19 girls, aged between 6.5 months and 18 years (mean: 6.6 years) were selected for the study. All these children were suspected of fat malabsorption, on the basis of anamnestic and clinical parameters. The different diagnoses of our patients are shown in table 1.

## METHODS AND MATERIAL

Three days stool collections from each patient were collected in separate containers, one container for each day. The stools were frozen at  $-18^{\circ}\text{C}$  Celsius. Fat content in each collection was determined by the titrimetric method described by van de Kamer et al. (1). Acid steatocrit from a single stool sample on day 1 and from a sample out of the homogenized 72 hours collection were determined by the method of Tran et al. (5) Feces (0.5 gr.) was diluted (1/4) with deionized water and thoroughly homogenized making use of a 5ml. Potter Elvehjem tissue homogenizer. Perchloric acid 5N was added to the homogenate in a volume equal to 1/5 of the homogenate volume. The resulting acid homogenate was mixed for 30 seconds making

**Table 1 List of diagnosis (n = 42).**

DIAGNOSIS	NUMBER OF CASES
CYSTIC FIBROSIS	20
MENTAL RETARDATION	2
RECURRENT DIARRHEA	5
FAILURE TO THRIVE	5
COELIAC DISEASE	2
INFLAMMATORY BOWEL DISEASE	1
SHORT BOWEL	1
CHOLEDOCHAL CYSTE	1
SUCRASE-ISOMALTASE DEFICIENCE	1
RECURRENT ABDOMINAL PAIN	1
UNKNOWN	3

use of a standard Vortex mixer. The homogenate was aspirated into a 75  $\mu$ l plain glass haematocrit capillary. The capillary was subsequently centrifuged horizontally (13000 rpm. for 15 min.) in a standard centrifuge. After centrifugation, the lengths of the upper (fat) and the bottom (solid) layers were measured by means of a graduated magnifying lens. Steatocrit was calculated as follows: percentage of (the fatty layer length / (fatty layer length + solid layer length)).

In order to validate the diagnostic value of the acid steatocrit we studied two patients groups, one with and one without steatorrhea. We divided the patients according to previous clinical data and fat excretion results, whereby a fecal fat excretion  $\geq$  3gr./day was considered as abnormal (6).

## RESULTS

Correlation coefficients between acid steatocrit results from either a single stool sample or from the sample taken from the 72 hours homogenized collection, and both the fecal fat excretion and the fecal fat concentration are shown in table 2.

**Table 2** Correlation between the results of the acid steatocrit from either a single stool sample and a sample from the homogenised stool collection and the results of both fat excretion and fecal fat concentration in 42 children suspected of malabsorption.

ACID STEATOCRIT	FAT EXCRETION	FAT CONCENTRATION
SINGLE STOOL	$r = 0.4 (p \leq 0.01)$	$r = 0.82 (p \leq 0.001)$
COLLECTION	$r = 0.68 (p \leq 0.001)$	$r = 0.82 (p \leq 0.001)$

The sensitivity and the specificity of the acid steatocrit determination from either a single stool sample or a sample taken from the 72 hours homogenized collection, and of the fecal fat concentration, for the diagnosis of steatorrhea are shown in table 3.

**Table 3** Sensitivity and specificity of the acid steatocrit determination from a single stool sample and from a homogenised stool collection sample and of the fecal concentration, for the diagnosis of steatorrhea.

	SENSITIVITY	SPECIFICITY
AS SINGLE STOOL (%)	75%	84%
AS COLLECTION (%)	90%	100%
FAT CONCENTRATION (%)	100%	76%

AS: Acid steatocrit

Fig.1 shows our AS results from the homogenized fecal collection sample related to the fecal fat excretion (g/day). The reference line for AS was set at the level of 10% (5), and the cut off reference line for the daily fat excretion was set at the level of 3 gram per day (6). As can be seen from the figure, one false positive and three false negative acid steatocrit results were found in our study population. Regarding these results one should notice that they are very close to the reference lines: the false positive steatocrit result had a value of 16% and the results of the fecal fat excretion corresponding to the false negative steatocrit results were 4.9; 6.4 and 7.7g/day, and concern children aged 12. 6 and 13 years respectively.

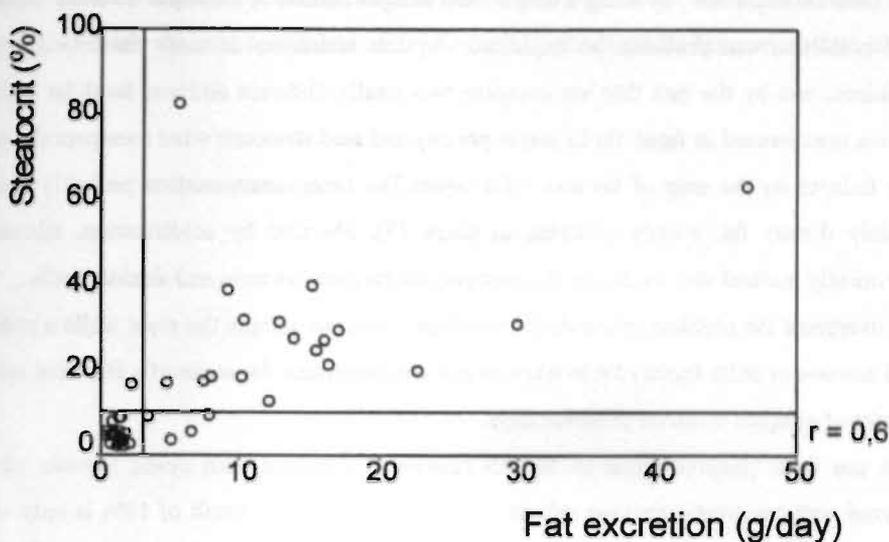


Figure 1 Relationship between acid steatocrit and fat excretion. Reference lines for acid steatocrit at 10 % and for fat excretion at 3 gram / day.

## DISCUSSION

Since the fecal fat balance excretion as described by van de Kamer is cumbersome, expensive and unpleasant for all involved, there is a need for a simple test. Some authors reported the steatocrit micromethod described by Phuapradit as a simple method for monitoring fat malabsorption (3), and reported a good correlation ( $r=0.93$ ) with the fecal fat excretion. Although others considered the steatocrit method of Phuapradit unreliable and mentioned the difficulty to delineate the fatty layer (4) and the impression that fat remains in the solid layer, as a problem of this method. This problem was solved by acidification of the fecal sample, whereby fat extraction is improved, and steatocrit results correlate much better with chemically measured fecal fat (5).

Our AS results correlate satisfactorily with chemically measured fecal fat concentrations and somewhat less, but still significantly, with fecal fat excretion. However, our correlation coefficient is lower than the correlation coefficient of the steatocrit without acidification as published in a previous study (3). We have no explanation for this discrepancy, and other

authors also failed to reproduce these results (4). The lesser correlation of the AS results with the fecal fat excretion, by using a single stool sample instead of a sample from the homogenized collection can probably be explained by the variability of daily fat consumption in children, and by the fact that we compare two totally different entities: fecal fat excretion, which is expressed as fecal fat in grams per day and acid steatocrit what measures the ratio of the fatlayer on the sum of fat and solid layers. The latter determination probably measures mainly dietary fat, mostly occurring as soaps (7), liberated by acidification, whereas the chemically method also measures the endogenous fat from bacteria and shedded cells.

To overcome the problem of the daily variability, one can sample the stool while a standardized amount of daily dietary fat is taken or one can determine the mean of a few acid steatocrit results of samples taken on different days.

The one false positive result of the AS concerned a patient with cystic fibrosis who was treated with pancreatic enzyme substitution therapy. This AS result of 16% is only slightly elevated considering the values obtained in cystic fibrosis patients on substitution therapy, which are mostly between 20 and 30%.

The three false negative results of the acid steatocrit can probably be explained by the choice of the reference line for the normal fecal fat excretion. Fecal fat excretion higher than 4.5g/24hours is considered pathologic (6,8) for children and adolescents, whereas other authors consider 7g/day the upper limit of normal fecal fat excretion in adults (9). The reference line for normal daily fecal fat excretion varies clearly with age and dietary fat intake as previously suggested by Williams (8). Taking account of these remarks, the fat excretion studies of 2 of our 3 patients with false negative steatocrit results could, due to their ages (12 and 13 years), still be considered "normal" and in agreement with AS results. This would reduce the disagreement between the methods to only a few ones.

## CONCLUSION

Acid steatocrit results are highly correlated with the chemically measured fecal fat concentration and significantly correlated with the fecal fat excretion. Although single sample acid steatocrit results are slightly less sensitive and specific than other measured parameters for the diagnosis of steatorrhoea, acid steatocrit measured in the stool samples taken from the homo-

genized collection compare favourably with the fecal fat concentration. We consider the acid steatocrit method useful for the screening and monitoring of patients with steatorrhea. If it is necessary to know the real coefficient of fat absorption, the fecal fat balance method is needed.

## REFERENCES

- (1) van de Kamer JH, Huinink HTB, Weyers HA. Rapid method for determination of fat in feces. *J Biol Chem* 1949; 177:349-55.
- (2) Phuapradit P, Narang A, Mendonca P, Harris DA, Baum JD. The steatocrit: a simple method for estimating stool fat content in newborn infants. *Arch Dis Child* 1981; 56:725-7.
- (3) Colombo C, Maiavacca R, Ronchi M, Consalvo E, Amoretti M, Giunta A. The steatocrit: a simple method for monitoring fat malabsorption in patients with cystic fibrosis. *J Pediatr Gastroenterol Nutr* 1987;6:926-30.
- (4) Walters MP, Kelleher J, Gilbert J, Littelwood JM. Clinical monitoring of steatorrhea in cystic fibrosis. *J Pediatr Gastroenterol Nutr* 1990; 65:99-102.
- (5) Tran M, Forget P, Van den Neucker A, Strik J, Kreel van B, Kuijten R. The acid steatocrit: a much improved method. *J Pediatr Gastroenterol Nutr* 1994; 19: 299-303.
- (6) Navarro J, Schmitz J. *Gastroenterologie pédiatrique*, Flammarion Médecine Sciences, Paris 1986.
- (7) Quinlan PT, Lockton S, Irwin J, Lucas AL. The relationship between stool hardness and stool composition in breast- and formula-fed infants. *J Pediatr Gastroenterol Nutr* 1995; 20:81-90.
- (8) Williams HH, Endicott EN, Shepherd ML, Galbraith H, Macy IG. Fat excretion by normal children. *J. of Nutrition* 1943; 25, 379.
- (9) Bai JC, Andrúsh A, Matelo G, Martinez C, Vazquez H, Boerr L, Sambuelli A. Fecal fat concentration in the differential diagnosis of steatorrhea. *Am. J. Gastroenterol.* 1989; 27-30.

## CHAPTER 6

### ROLE OF LANSOPRAZOLE IN CHILDREN WITH CYSTIC FIBROSIS: EVIDENCE FOR IMPROVED FAT ABSORPTION AND NUTRITIONAL STATUS

Tran TMD, Van den Neucker A, Hendriks JJE, Forget P ( junior ), Forget P ( senior )

Department of Pediatrics, University Hospital Maastricht, Maastricht, The Netherlands

---

Submitted for publication

#### *Abstract*

Statorrhea and nutritional parameters were investigated in 15 cystic fibrosis children before starting lansoprazole, after 3 months on lansoprazole (15mg/day) and 3 months after stopping lansoprazole. There were 5 girls and 10 boys with a mean age of 9.5 years (range: 3.1 - 22.6y). Patients were their own controls. Acid steatocrit, anthropometric methods and DXA were used to evaluate steatorrhea and the nutritional status respectively. On lansoprazole, mean  $\pm$  SD acid steatocrit values decreased from  $37.1 \pm 8.8$  % to  $28.5 \pm 10.6$  % ( $p = 0.02$ ). During lansoprazole therapy, significant mean Z-score changes were found for weight ( $+0.14$  /  $p = 0.02$ ), length ( $+0.15$  /  $p = 0.03$ ), subscapular ( $+ 0.61$  /  $p = 0.003$ ), suprailiaca ( $+0.8$  /  $p = 0.002$ ) and the sum of 4 skinfolds ( $+0.61$  /  $p = 0.002$ ) . Z-scores deteriorated again after stopping lansoprazole. Fatmass and bone mineral content increased significantly on lansoprazole ( $p = 0.008$  and  $p = 0.005$  resp.). Improvement of subscapular Z-score was related to improvement of acid steatocrit values ( $p = 0.01$ ) during treatment. We conclude that lansoprazole as adjuvant therapy significantly improves fat absorption and the nutritional status in CF children.

## INTRODUCTION

Cystic fibrosis (CF) is an autosomal recessive inherited disease. Defect in the chloride transepithelial transport system results in viscous mucus in various organs with lung and pancreas mostly affected (1). Both, pancreatic insufficiency resulting in malabsorption and high energy expenditure due to increased respiratory work (2-3), are thought to be responsible for the poor nutritional condition in CF patients. Since malnutrition can compromise absorptive and immune function resulting in a shortened survival (4), all efforts should be made in order to improve the nutritional status of these patients. Unfortunately, high doses of pancreatic enzymes did not solve the problems of malabsorption (5) and colon stricture has been observed in CF children on this regimen (6,7). Further, the use of hypercaloric diets did not result in significant improvements of Z-scores for weight, length and skinfolds in CF children (8). Only parenteral nutrition and either oral or enteral elemental and semielemental nutrition have been shown to significantly improve the nutritional condition of these children (9-15). The latter strongly suggests that nutrient maldigestion plays a crucial role in the poor response to oral hypercaloric diets. As cystic fibrosis patients have a low duodenal pH probably linked to fat maldigestion (16), inhibition of gastric acid production could improve absorption. The reported effects of H<sub>2</sub>-receptor antagonists and prostaglandine E<sub>2</sub> on steatorrhea have been variable (17-22). Insufficient inhibition of gastric acid could be responsible for these unconvincing results. Recently, in a double blind study, a significant improvement in steatorrhea was found when a proton-pump inhibitor was added as adjuvant therapy in pancreatic enzyme treated cystic fibrosis patients (23). The effect of proton pump inhibitors on the nutritional condition of children with CF have not yet been reported. The aims of our study were to evaluate the effects of lansoprazole (proton-pump inhibitor) on both steatorrhea and the nutritional condition of CF patients while on enzyme therapy.

## SUBJECTS AND METHODS

### *Study design*

As the effect of a proton pump inhibitor on fat balance has been convincingly proven in a

double blind study (23), we adapted a prospective open study design wherein patients were their own controls. In the month preceding the study, all patients were screened for steatorrhea by measuring fecal acid steatocrit once every 10 days. Patients with a mean acid steatocrit value higher than 25% (normal values in our laboratory < 20%) were invited to take part in the study. After evaluation of nutritional parameters by DXA and anthropometric methods, lansoprazole was added to their standard treatment in a dose of 15 mg day before breakfast for 3 months. When fat malabsorption did not change after 2 months, the dose was doubled in children older than 10 years and weighing more than 30 kg. During the lansoprazole treatment period 9 fecal samples were taken with an interval of 10 days for acid steatocrit measurements. The mean of these 9 measurements was used as a measure of steatorrhea during the treatment period. After 3 months on treatment, the nutritional condition assessment was repeated. All measurements of nutritional condition were performed on a single day. Three fecal samples for acid steatocrit determinations and anthropometric parameters were again measured respectively 1 month and 3 months after stopping lansoprazole therapy. Dietary evaluations were performed at the start, at the end and one month after stopping lansoprazole.

#### Patients population

23 CF out-clinic patients from the academic Hospital Maastricht were recruited. All patients were treated with pancreatic enzymes. Of these, 2 patients were too ill to participate in the study. 21 patients were screened for steatorrhea while on pancrease enzyme. 15 of them who had steatorrhea were included. In most children, the CF diagnosis had been made during the first year of life by repeated positive sweat tests, all 15 children were considered to have pancreatic insufficiency on the basis of abnormal fecal chymotrypsin, 72 hours fat balance (24) and increased acid steatocrit results (25). Mean energy intake was 113 % RDA (recommended daily allowance). The mean number of pancreas enzyme capsules (Pancrease) taken by 13 of these patients was 20 (range: 11 - 33), one patient took 3 Pancrease capsules (5000E lipase, 2900E amylase, 330E protease) and 6 Panzytrat tablets (25000E lipase, 22500E amylase, 1250E protease) and another one took 10 Creon capsules (8000E lipase, 9000E amylase, 450E protease) per day. Mean age, weight and length of those 15 children were 9.5 y (range: 3.1 - 22.6 y); 29.3 kg (range: 13.6 - 67.6 kg) and 131 cm (range: 97.7 - 184.9 cm)

respectively. Their nutritional status was moderately altered with a mean Body Mass Index (BMI) of 15.6 (range : 13.2 - 18.3). Mean predicted values of FEV1 and FVC were respectively 81.3% (range: 39 - 114%) and 85.5% (range: 44 - 108%). Informed patient and parental consent were obtained.

#### Evaluation of fat malabsorption by acid steatocrit

The acid steatocrit was determined as previously reported (25). In short, 0.5g solid stool was weighed and diluted with a volume of deionized water, equal to two times the weight of stool. The stool and water were premixed using a Vortex mixer. Subsequently, the mixture was homogenized using a 5 ml Potter Elvehjem tissue homogenizer. After then 5N perchloric acid was added to the homogenate in a volume equal to 1/5 of the homogenate. After mixing with the Vortex, the acidified homogenate was aspirated into a 75  $\mu$ l plain haematocrit capillary. This capillary was sealed with wax at one end and centrifuged horizontally (13000 rpm, 15 min). After centrifugation, 3 layers were distinguished: a basal solid layer (SL), an intermediate liquid layer and an upper fatty layer (FL). Acid steatocrit was calculated as  $(FL / (FL + SL)) \times 100\%$

### EVALUATION OF NUTRITIONAL STATUS

#### Anthropometry

The arm circumference, biceps, triceps, subscapular and suprailiac skinfolds were measured 3 times on the left side of the body using the Harpenden caliper. Average values were taken. Weight and length were also measured. BMI was calculated as  $\text{weight} / (\text{length}^2)$ . The Z-score

(Z-score is defined as  $(X - x) / S$  where X is the patients 's measurement, x is the mean value for age and sex and S is the standard deviation of x) of all these anthropometric parameters were calculated based on the reference data described by Gerver and de Bruin (26). A negative value indicates values under the mean reference value and a positive or negative change in Z-score means catch up or slowing down of growth respectively. All measurements were done

by the same investigator (TT).

#### Dual - energy x-ray absorptiometry (DXA)

DXA measurement is based on the differential tissue attenuation of photons of two energy levels from an X-ray source (27). All patients underwent total body scan performed with a DPX (Lunar Radiation Corp, Madison, WI) total body scanner. The results were analysed with a paediatric software programme, version 1.5e. Daily quality assurance test was performed according to the manufacturer's directions. Total non bone LBM (lean body mass), total BMC (bone mineral content), total body FM (fatmass) and BMD (bone mineral density) Z-score were measured by DXA procedure. These results were compared to those of the reference population, recently described by Ogle et al., who studied the body composition by DXA in 265 normal individuals aged 4 - 26 year (28).

#### Diet evaluation

At the beginning, at the end and one month after stopping lansoprazole nutrient intake was assessed by a specially trained clinical CF dietitian from consecutive 3 day food diaries including one weekend day. Intakes were expressed as kilocalorie per kg bodyweight for the energy intake and gram per kg bodyweight for fat-, carbohydrate- and protein-intakes, using the netherlands nutrients table "NEVO" 1993.

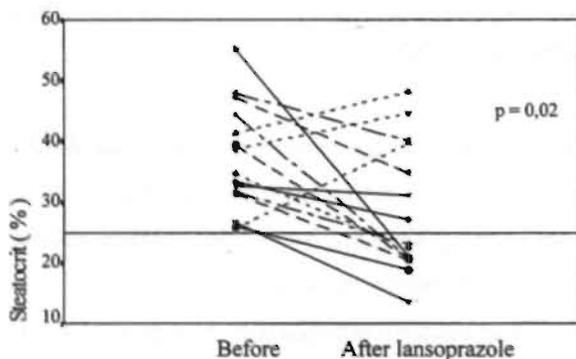
#### Statistic

All data were analysed by using SPSS statistic program. Anthropometric parameters and body composition results measured before the start and at the end of the trial were compared making use of Wilcoxon one sample test. The sign test was used to compare LBM, FM and BMC assessed by DXA, with the reference population described by Ogle.

## RESULTS

### Fat malabsorption

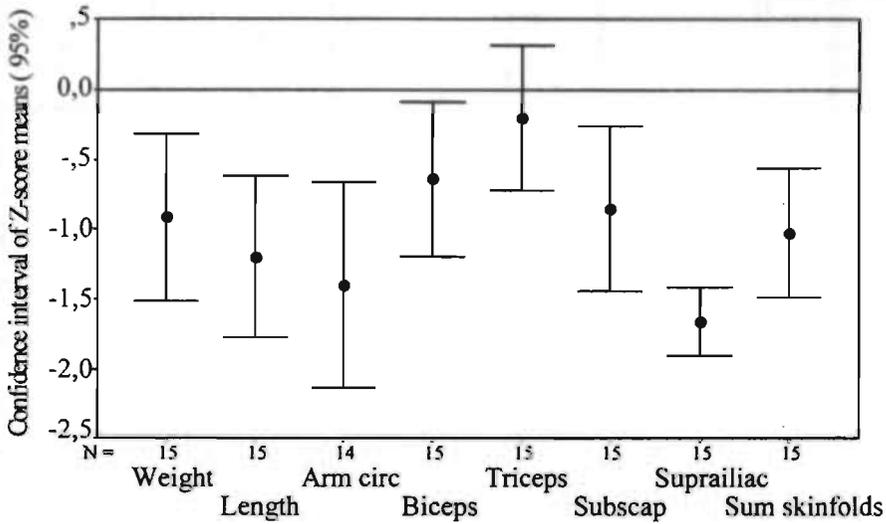
Despite standard pancreas enzyme, 15 of 21 children had steatorrhea with an average  $\pm$  SD pretreatment acid steatocrit value (mean of 3 determinations in each patient) of  $37.1\% \pm 8.8\%$ . After 3 months of treatment with lansoprazole, there was a significant ( $p = 0.02$ ) improvement in steatorrhea with a mean  $\pm$  SD acid steatocrit value of  $28.5\% \pm 10.6\%$ . Eight patients on lansoprazole had a mean acid steatocrit value lower than 25% (fig 1). In this group the mean decrease was 16% (44.2% of start value). In 3 children the acid steatocrit value decreased with 9% (20.6% of start value) but was not completely corrected. In 4 children fat malabsorption did not improve at all. Four children received a double dose of lansoprazole for 1 month, resulting in a decreased acid steatocrit results in 2 (decrease of 8.6% and 19%). Due to social problems one child was dropped out of the study after the lansoprazole period. Mean  $\pm$  SD acid steatocrit value for the remaining 14 children in the first month after stopping lansoprazole was  $29.7\% \pm 13.9\%$ , which was not significantly different from the values on lansoprazole. Of 4 children whose acid steatocrit was not changed on lansoprazole, 2 had higher acid steatocrit values after stopping.



**Figure 1** Acid steatocrit before and after 3 months treatment with lansoprazole in 15 CF children. A line through the 25 % value is drawn showing the study inclusion limit. Acid steatocrit values on lansoprazole are significantly decreased ( $p = 0.02$ ).

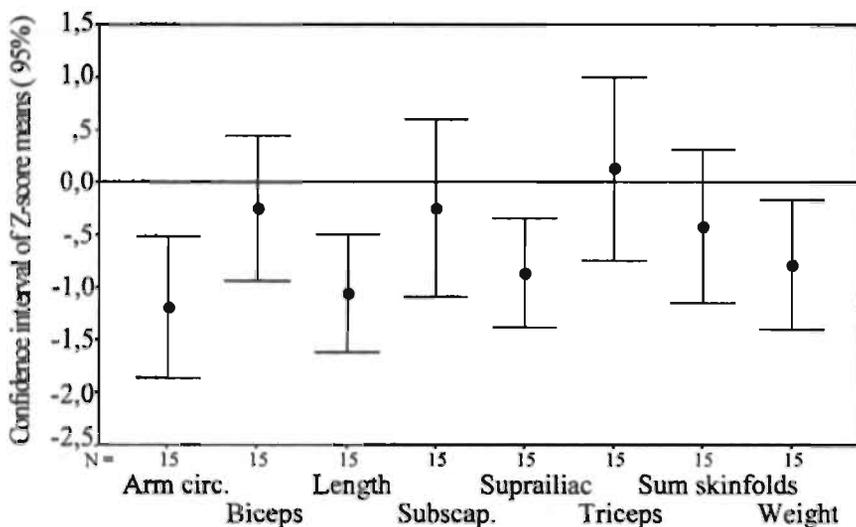
### Anthropometric parameters

Mean and 95% CI (confidence interval) for weight, length, arm circumference, 4 skinfolds and the sum of these 4 skinfolds expressed as Z-score are shown in fig.2. For all parameters except the triceps, the CI do not include the reference 50th centile line (Z-score 0), underscoring the fact that except for the triceps skinfold, all other anthropometric parameters mean Z-scores were significantly decreased in our CF children when compared to those of the normal population. The suprailiac skinfold was most abnormal and showed the smallest interindividual variation.



**Figure 2** Mean Z-scores and 95% confidence interval of anthropometric parameters in 15 CF children before lansoprazole therapy. The line through 0 represents the 50th centile of the reference population. The differences between the study group and the reference population are significant when the CI do not include the Z-score 0 line. All anthropometric parameters were significantly decreased in our CF children except for the triceps skinfold.

After treatment with lansoprazole, Z-scores of anthropometric parameters improved significantly. All parameters moved toward the Z-score 0 line (50th centile for reference population). The Z-scores of biceps, subscapular and sum of the 4 skinfolds did not significantly differ from the reference population any more (fig.3).



**Figure 3** Mean Z-scores and 95% confidence interval of anthropometric parameters in 15 CF children on lansoprazole for 3 months. The differences between the study group and the reference population are significant when the CI do not include the Z-score 0 line. Several parameters (biceps, subscapular and sum of 4 skinfolds) normalized during lansoprazole treatment (see figure 2).

Z-score changes for all anthropometric parameters studied are shown in table 1. Except for arm circumference, biceps and triceps skinfolds, Z-scores of all parameters improved significantly. Subscapular, suprailiac and the sum of the 4 skinfolds showed the most significant changes. The acid steatocrit results during lansoprazole treatment were significantly lower ( $p = 0.01$ ) in our patient subgroup with subscapular Z-score improvement  $\geq 0.5$  when compared to the subgroup showing lower Z-score changes. Three months after lansoprazole was stopped, 5 children were dropped out of the study; 2 because of the far distances from home, 1 had

taken lansoprazole again because of increased symptoms of steatorrhea and abdominal pain and 2 because of social problems. Nutritional parameters were therefore evaluated in only 10 children 3 months after stopping lansoprazole. Z-scores of all anthropometric parameters deteriorated, with weight and subscapular Z-score changes reaching statistical significance (table 1).

**Table 1** Mean Z-scores of anthropometric parameters in 15 CF children before (T0), after 3 months on lansoprazole (T3) and 3 months after stopping lansoprazole (T6).

Anthrop. Parameters	T0 Mean Z-score (n = 15)	T3 Mean Z-score (n = 15)	T6 Mean Z-score (n = 10)	T3 - T0 Mean Z-score (n = 15)	T6 - T3 Mean Z-score (n = 10)
Weight	-0.91	-0.78	-1.38	0.14 (p = 0.02)	-0.6 (p = 0.01)
Length	-1.2	-1.05	-1.23	0.15 (p = 0.03)	-0.18 (p = 0.16)
Armcircum	-1.4	-1.19	-1.74	0.22 (p = 0.05)	-0.55 (p = 0.52)
Biceps	-0.63	-0.24	-1	0.39 (p = 0.06)	-0.76 (p = 0.21)
Triceps	-0.2	0.14	-0.72	0.34 (p = 0.2)	-0.86 (p = 0.26)
Subscapular	-0.85	-0.24	-1.14	0.61 (p = 0.003)	-0.9 (p = 0.03)
Suprailiaca	-1.66	-0.86	-1.13	0.8 (p = 0.002)	-0.27 (p = 0.68)
Sum skinfolds	-1.02	-0.41	-1.16	0.61 (p = 0.002)	-0.75 (p = 0.31)

### Body composition

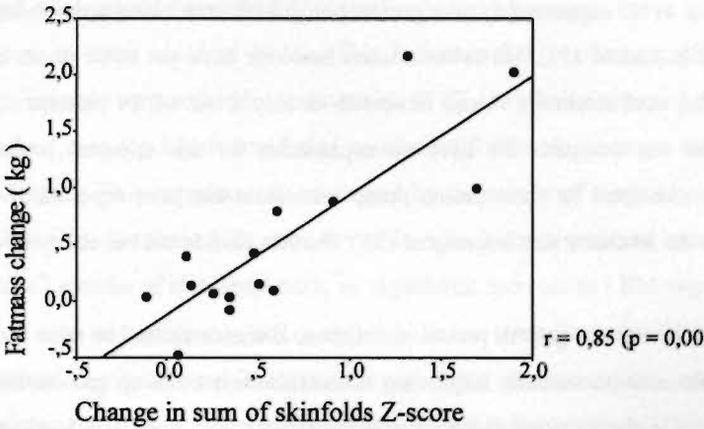
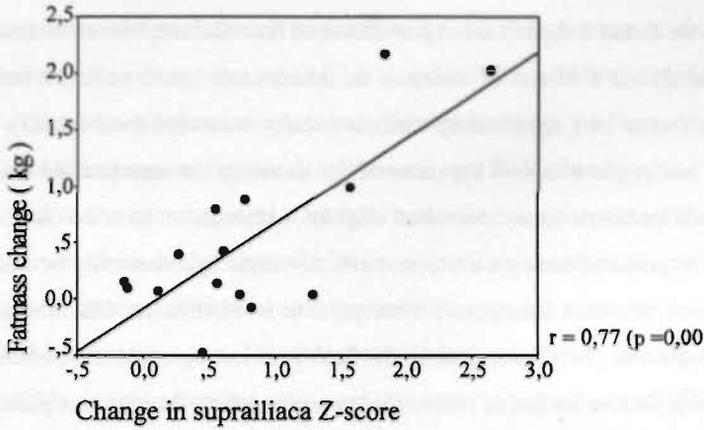
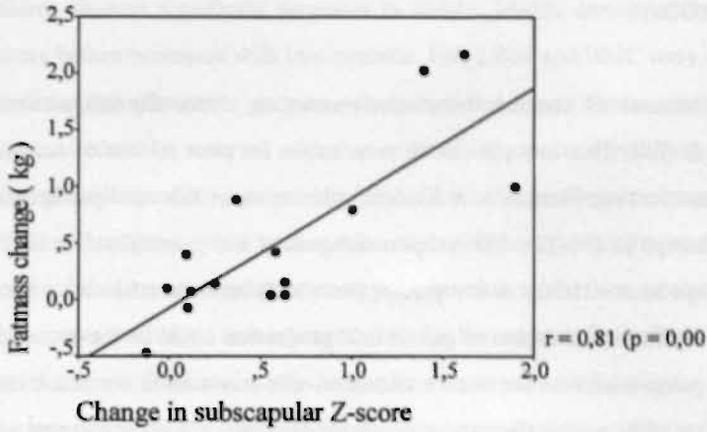
Body composition data before and after lansoprazole are given in table 2. All 3 components FM, LBM and BMC were significantly decreased in our 15 CF children when compared to the reference population described by Ogle et al. (p = 0.01; p = 0.02 and p = 0.005 respectively). Bone mineral density Z-score was significantly decreased (p ≤ 0.05). Significant increases of FM and BMC occurred after 3 months of treatment with lansoprazole. Changes in the subscapular, suprailiaca and sum of the 4 skinfolds Z-scores were highly correlated with changes in FM by DXA (r = 0.81 / r = 0.77 / r = 0.85 resp. with p = 0.001) (fig 4).

Diet evaluation

Mean fat, protein, carbohydrate and energy intakes were 3.1 - 2.8 - 3.0 g / kg ; 3.0 - 2.7 - 2.8 g / kg ; 10.4 - 9.7 - 9.8 g / kg and 2095 - 1986 - 1977 kcal / kg bodyweight at the start, at the end and one month after lansoprazole trial respectively. None of these changes were significant.

**Table 2 Body composition before (T0) and after (T3) lansoprazole by DXA.**

<b>BODY COMPOSITION</b>	<b>T0 ( n = 15 )</b>	<b>T3 ( n = 15 )</b>	<b>SIGNIFICANCY ( p )</b>
<b>MEAN FATMASS ( Kg )</b>	<b>3.97</b>	<b>4.76</b>	<b>0.008</b>
<b>MEAN LBM ( Kg )</b>	<b>22.83</b>	<b>24.03</b>	<b>0.06</b>
<b>MEAN BMC ( Kg )</b>	<b>1.02</b>	<b>1.08</b>	<b>0.005</b>
<b>MEAN BMD ( Z-SCORE )</b>	<b>-0.55</b>	<b>-0.58</b>	<b>0.65</b>



**Figure 4** Relation between changes in subscapular skinfold Z-score, suprailiac skinfold Z-score, sum of 4 skinfolds Z-score and fatmass as measured by DXA (dual energy X-ray absorptiometry) in 15 CF children.

## DISCUSSION

Due to a decrease of pancreas bicarbonate secretion, cystic fibrosis patients have a low duodenal pH (29). This low pH can be responsible for poor release of enzymes through the acid resistant coating. Further, low duodenal pH can cause bile acid precipitation resulting in lipid malabsorption (30-32). H<sub>2</sub>-receptor antagonists and prostaglandine E<sub>2</sub> have been used with the hope to reverse the above proces, but results on steatorrhea have been controversial (17-22). Insufficient inhibition of gastric acid production could be the cause of these failures. As proton pump inhibitors are known to control acid secretion in a much more effective way (33), they could be more effective in increasing duodenal pH. In agreement with Heijermans et al. (23), we found a significant improvement of fat malabsorption as measured by the acid steatocrit in all but 4 of our CF children on lansoprazole. Acid steatocrit results have been shown to correlate very significantly with chemically measured fecal fat (25). Acid steatocrit values did not improve in 4 of our patients. By doubling the lansoprazole dose in 2 of these patients, acid steatocrit values decreased slightly. Although we have no clear explanation for the lack of response of these patients, poor efficacy could hypothetically be due to host factors including poor intestinal lansoprazole absorption or interindividual differences in bioavailability of lansoprazole (34). Further, since the halflife of lansoprazole is between 1 and 2 hours and inhibition will be limited to proton pumps active during the effective plasma levels of the drugs, Sachs et al. suggested to give proton pump inhibitors twice a day whenever effective pH control is desired (33). However, studies hereover have yet to be done. Contrary to our expectations, acid steatocrit values increased in only 8 out of 14 patients one month after lansoprazole was stopped. We have no explanation for this apparent prolonged effect of lansoprazole on fecal fat since proton pump restoration has been reported to occur 50 to 72 hours after the inhibitor was interrupted (33). Further studies are necessary in order to clarify this point.

Because of the normal growth proces in children, Z-scores should be used for the evaluation of anthropometric parameters. Improving Z-scores reflect catch-up growth and consequently improvement in the nutritional status while the reverse is true for deteriorating Z-scores. The recent introduction of DXA methodology makes it possible to evaluate FM, LBM and BMC rapidly and non invasively in children (27). In agreement with previous studies, all except 2

of our CF children showed significant decreases in weight, length, arm circumference and 4 skinfolds Z-scores before treatment with lansoprazole. FM, LBM and BMC were also significantly decreased despite pancreatic enzyme substitution and hypercaloric supplementation. The catabolic process could be reversed after 3 months of lansoprazole resulting in Z-score improvement of all anthropometric parameters, FM, BMC and to a lesser extent LBM. The improved nutritional condition could be due either to higher energy intakes or to improved absorption. As we found somewhat lower energy intakes during the lansoprazole treatment period, a higher intake probably is not responsible for the improved nutritional condition. Further, the fact that lower fecal acid steatocrit results were found in our patients with the best nutritional response as assessed by subscapular Z-score improvements, supports the idea that improved absorption is the main factor responsible for the improvement of the nutritional status in our patients. However increased FM, LBM and BMC are difficult to interpret because results for reference populations expressed as Z-scores have not yet been reported. As Z-score of BMD did not change on lansoprazole, the increased BMC found in our CF children, is probably linked to the growth process. Our results showing deterioration of the nutritional condition after interruption of lansoprazole "intervention", are in agreement with those of Bertrand et al., who reported nutritional deterioration after stopping elemental enteral alimentation (14). Oral hypercaloric diets have not been shown to improve Z-scores of weight, length and skinfolds as parameters for nutritional status and growth process of CF children (8). Only parenteral or elemental "predigested" enteral nutrition have been shown to reverse the catabolic process in these children (9-15). This indicates that persisting maldigestion or malabsorption is mainly responsible for malnutrition in CF. As alkalinization of duodenal pH improves malabsorption (16,22), it could also, as elemental diets do, improve the nutritional status of CF children. This hypothesis is confirmed by the results of our study. In contrast with O' Loughlin (10), Shepherd (12) and Levy (13), who found significant improvement in LBM after 6 to 12 months of elemental diets, no significant increase in LBM was seen in our study. The 3 months of treatment in our study could be too short for a significant change in LBM to be noticed. Our results are in agreement with those of other authors, who evaluated the effect of gluten free diet on body compartments by DXA. Only FM and BMC improved after a 6 months gluten free diet while LBM did not (35). As H<sub>2</sub> - receptor antagonists have been shown to significantly decrease nitrogen malabsorption in CF patients (36), we do not

think that the poor improvement of LBM in our patients can be ascribed to a selective improvement of fat and not of protein malabsorption.

In conclusion, most of our CF children maintained steatorrhea and were malnourished despite optimal treatment with hypercaloric diets and pancreatic enzymes. Lansoprazole as adjuvant therapy resulted in decreased fat malabsorption and improved nutritional status in these CF children after 3 months of treatment. Longterm evaluation of the effect of lansoprazole on both the nutritional status and lung function parameters have yet to be performed.

#### *Acknowledgment*

we are gratefull to Liesbeth van der Ploeg, Lianne Schoorlemmer dieticians and Piet Willems, Sandra Zimny from the department of nuclear medicine for their invaluable assistance. We also thank all nurses of the pediatric polyclinic for their welwilling support.

## REFERENCES

- (1) M. Welsh, A. Smith. Molecular mechanisms of CFTR chloride channel dysfunction in cystic fibrosis. *Cell* 1993; 73:1251-1254.
- (2) J. Tomezsko, V. Stallings, D. Kawchak, J. Goin, G. Diamond, T. Scanlin. Energy expenditure and genotype of children with cystic fibrosis. *Pediatr Res* 1994; 35: 451- 460.
- (3) M. Bronstein, P. Davies, K. Hambidge, F. Accurso. Normal energy expenditure in the infant with presymptomatic cystic fibrosis. *J Pediatr* 1995; 126: 28-33.
- (4) R.Kraemer, A. Rudeberg, B. Hadorn, E. Rossi. Relative underweight in cystic fibrosis and its prognostic value. *Acta Paediatr Scand* 1978; 67: 33-37.
- (5) P. Robinson, P. Sly. High dose pancreatic enzymes in cystic fibrosis. *Arch Dis Child*. 1990; 65: 311-312.
- (6) E. Lebenthal. High strength pancreatic exocrine enzyme capsules associated with colonic strictures in patients with cystic fibrosis: "more is not necessarily better". *J Pediatr Gastroenterol Nutr*. 1994; 18: 423-425.
- (7) R. Smyth, D. van Velzen, A. Smyth, D. Lloyd, D. Heaf. Strictures of ascending colon in cystic fibrosis and high strength pancreatic enzymes. *The Lancet*. 1994; 343: 85-86.
- (8) A. Rettammel, M. Marcus, P. Farrell, S. Sondel, R. Kosciak, E. Mischler. *J Am Diet Assoc*. 1995; 95: 454-459.
- (9) K. Gaskin, D. Waters, L. Baur, V. Soutter, M. Gruca. *Acta Paediatr Scand [Suppl]*. 1990; 366: 106-110.
- (10) E. O' Loughlin, D. Forbes, H. Parsons, B. Scott, D. Cooper, G. Gall. Nutritional rehabilitation of malnourished patients with cystic fibrosis. *Am J Clin Nutr*. 1986: 43: 732-737.
- (11) R. Shepherd, T. Holt, B. Thomas et al. Nutritional rehabilitation in cystic fibrosis: Controlled studies of effects on nutritional growth retardation, body protein turnover, and course of pulmonary disease. *J Pediatr*. 1986; 109: 788-94.
- (12) R. Shepherd, B. Thomas, D. Bennett, W. Cooksley, L. Ward. Changes in body composition and muscle protein degradation during nutritional supplementation in nutritionally growth-retarded children with cystic fibrosis. *J Pediatr Gastroenterol Nutr*. 1983; 2: 439-446.
- (13) L. Levy, P. Durie, P. Pencharz, M. Corey. Effects of long-term nutritional rehabilitation on body composition and clinical status in malnourished children and adolescents with cystic

fibrosis. *J Pediatr* 1985; 107: 225-230.

(14) J. Bertrand, C. Morin, R. Lasalle, J. Patrick, A. Coates. Short-term clinical, nutritional, and functional effects of continuous elemental enteral alimentation in children with cystic fibrosis. *J Pediatr*. 1984; 104: 41-46.

(15) R. Shepherd, W. Cooksley, and W. Domville. Improved growth and clinical, nutritional, and respiratory changes in response to nutritional therapy in cystic fibrosis. *J Pediatr*. 1980; 97: 351-357.

(16) P. Robinson, A. Smith, and P. Sly. Duodenal pH in Cystic fibrosis and its relationship to fat malabsorption. *Dig Dis Sci*. 1990; 35: 1299-1304.

(17) A. Carroccio, F. Pardo, G. Montalto et al. Use of famotidine in severe exocrine pancreatic insufficiency with persistent maldigestion on enzymatic replacement therapy: A long-term study in cystic fibrosis. *Dig Dis Sci* 1992; 37: 1441-1446.

(18) D. Chalmers, R. Brown, M. Miller et al. The influence of longterm cimetidine as an adjuvant to pancreatic enzyme therapy in cystic fibrosis. *Acta Paediatr Scand*. 1985; 74: 114-117.

(19) P. Robinson and P. Sly. Placebo-controlled trial of misoprostol in cystic fibrosis. *J Pediatr Gastroenterol Nutr*. 1990; 11: 37-40.

(20) H. Heijerman, C. Lamers, J. Dijkman, and W. Bakker. Ranitidine compared with the dimethylprostaglandin E2 analogue enprostil as adjunct to pancreatic enzyme replacement in adult cystic fibrosis. *Scand J Gastroenterol*. 1990; 25 (Suppl 178): 26-31.

(21) M. Schöni, R. Kraemer, E. Rossi. Cimetidine and fat malabsorption in children with cystic fibrosis. *Helv Paediat Acta*. 1981; 36: 359-369.

(22) B. Boyle, W. Long, W. Balistreri, S. Widzer, and N. Huang. Effect of cimetidine and pancreatic enzymes on serum and fecal bile acids and fat absorption in cystic fibrosis. *Gastroenterology*. 1980; 78: 950-953.

(23) H. Heijerman, C. Lamers, W. Bakker. Omeprazole enhances the efficacy of pancreatin (pancrease) in cystic fibrosis. *Ann Intern Med*. 1991; 114: 200-201.

(24) J. van de Kamer, H. Huinink, H. Weyers. Rapid method for determination of fat in feces. *J Biol Chem*. 1949; 177: 349-55.

(25) M. Tran, P. Forget, A. Van den Neucker, J. Strik, B. van Kreel, and R. Kuijten. The acid steatocrit: A much improved method. *J Pediatr Gastroenterol Nutr*. 1994; 19: 299-303.

- (26) W. Gerver, R. de Bruin. *Paediatric Morphometrics: A reference manual*. 1th ed. Utrecht: Bunge, 1995.
- (27) R. Mazess, H. Barden, J. Bisek, and J. Hanson. Dual-energy x-ray absorptiometry for total-body and regional bone-mineral and soft-tissue composition. *Am J Clin Nutr*. 1990; 51:1106-12.
- (28) G. Ogle, J. Allen, I. Humphries et al. Body-composition assessment by dual-energy x-ray absorptiometry in subjects aged 4-26 y. *Am J Clin Nutr*. 1995; 61:746-53.
- (29) A. Weber, C. Roy. Intraduodenal events in cystic fibrosis. *J Pediatr Gastroenterol Nutr*. 1984; 3 (Suppl. 1): S113-S119.
- (30) P. Regan, J. Malagelada, E. Dimagno, and V. Go. Reduced intraluminal bile acid concentrations and fat maldigestion in pancreatic insufficiency: Correction by treatment. *Gastroenterology*. 1979; 77: 285-289.
- (31) P. Zentler-Munro, W. Fitzpatrick, J. Batten, and T. Northfield. Effect of intrajejunal acidity on aqueous phase bile acid and lipid concentrations in pancreatic steatorrhoea due to cystic fibrosis. *Gut*. 1984; 25: 500-507.
- (32) P. Zentler-Munro, D. Fine, J. Batten, and T. Northfield. Effect of cimetidine on enzyme inactivation, bile acid precipitation, and lipid solubilisation in pancreatic steatorrhoea due to cystic fibrosis. *Gut*. 1985; 26: 892-901.
- (33) G. Sachs, J. Shin, C. Briving, B. Wallmark, S. Hersey. The pharmacology of the gastric acid pump: The H<sup>+</sup>, K<sup>+</sup> ATPase. *Annu Rev Pharmacol Toxicol*. 1995; 35: 277-305.
- (34) C. Spencer and D. Faulds. *Drugs: Focus on Lansoprazole*. 1994; 48: 404-430.
- (35) G. Barera, P. Brambilla, P. Manzoni, S. Acciuffi, G. Caccia, C. Bianchi. Changes in body composition evaluated by DXA during gluten free diet in celiac children. *J Pediatr Gastroenterol Nutr*. 1995; 20: 476 "abstr".
- (36) K. Cox, J. Isenberg, A. Osher, R. Dooley. The effect of cimetidine on maldigestion in cystic fibrosis. *J. Pediatr*. 1979; 94: 488-492.

## CHAPTER 7

### ANTHROPOMETRY AND BODY COMPOSITION IN CHILDREN WITH CYSTIC FIBROSIS: EFFECTS OF A PROTON - PUMP INHIBITOR

<sup>(1)</sup>My-Dung T. Tran, <sup>(1)</sup>Anita Van den Neucker, <sup>(1)</sup>Han J. Hendriks, <sup>(2)</sup>Bernard van Kreel,  
<sup>(1)</sup>Patricia Forget, <sup>(3)</sup>Guido Heidendal, <sup>(1)</sup>Pierre-Philippe Forget

<sup>(1)</sup>Department of Pediatrics, <sup>(2)</sup> Clinical Chemistry and <sup>(3)</sup>Nuclear Medicine, University Hospital  
Maastricht, Maastricht, the Netherlands.

---

Submitted for publication

#### *Abstract*

We studied the body composition of 18 CF children making use of dual-energy X-ray absorptiometry (DEXA), deuterium-bromide and skinfold methods and evaluated the efficacy of these body composition methods for the detection of body composition changes during 3 months therapeutic intervention with lansoprazole. Our CF patients were malnourished with decreased mean Z-scores for armcircumference (-1.62), biceps (-0.77), subscapular (-0.92), suprailiac skinfolds (-1.66), weight (-1.03) and height (-1.31). Their fatmass was significantly depleted as shown by DEXA, skinfold and total body water (TBW) methods. Extracellular volume (%) was increased, while intracellular volume (%) was normal. Only the lean body mass (LBM) as measured by DEXA was decreased ( $p = 0.02$ ). Decreased bone mineral content and bone mineral density Z-scores were also found ( $p = 0.005$  and  $p = 0.03$  respectively). After treatment with lansoprazole, significant increases in fatmass was found by DEXA and skinfold methods (53% and 97% of weight changes respectively) whereas weight increase was exclusively ascribed to an increase in LBM with the TBW method. Changes in body-weight however, were not correlated with either fatmass and fat free mass changes as measured by any of these methods. We conclude that results of DEXA, TBW and skinfold methods are not interchangeable and that the methods used are not accurate enough for the differential detection of small changes in fatmass and fat free mass as found in the present study.

## INTRODUCTION

Due to malabsorption (1), chronic lung infections with increased energy expenditure (2,3) and poor appetite, most cystic fibrosis patients show signs of malnutrition. As malnutrition can affect pulmonary function and shorten survival (4), feeding interventions are sometimes necessary to restore normal growth and body composition. Assessment of body composition changes is necessary for the precise evaluation of nutritional interventions. While different body composition methods have been described, only few studies have, to our knowledge, compared different measurement techniques in pediatric subjects and there are no reports on the efficacy of these methods for the detection of body composition changes during therapeutic interventions. In children, Dual-energy X-ray absorptiometry (DEXA), Total Body Water (TBW) and Skinfold methods are frequently used for the determination of body composition since they are all noninvasive. In this age group, methods used for measuring body composition have to be very precise in order to detect small changes in body composition. The precision for repeated measurements has been reported to be 1-2 % for DEXA, 1.6% for TBW and either 5% (intraobserver) or 15% (interobserver) for the skinfold method (5). In the present study we first evaluated the body composition of our 18 CF children making use of DEXA, skinfolds and TBW (deuterium-bromide) methods and subsequently evaluated the agreement between these results. Secondly, we investigated the sensitivity of these 3 methods for the detection of small changes in body composition of 15 CF children whose nutritional condition improved significantly after intervention with lansoprazole for 3 months. For the purpose of the present study we defined fat free mass by DEXA (FFM-DEXA) as the sum of lean body mass (LBM) and bone mineral content (BMC) and total mass (TM) as DEXA constructed weight (LBM + FM + BMC).

## SUBJECTS

### *Population studied for the comparison of body composition methods*

23 CF children were recruited from the Academic Hospital Maastricht, The Netherlands. Of these 2 patients were too ill to take part in the study and 3 children refused to participate. 18

children who had no exacerbation 4 weeks before the study were included. Thirteen of them were prepubertal and younger than ten years, 3 children were postpubertal and were between 11.6 - 14.1 years. Two subjects were adolescents of 16.1 and 22.6 years. Fourteen of these 18 children were diagnosed during the first year of life while 16 of them had pancreatic insufficiency (abnormal fecal chymotrypsin and 72h fecal fat balance). Their nutritional status was moderate with a mean BMI (body mass index) of 15.6 (range: 13.2 - 23.2). Mean age, weight and height were respectively 9.0 y (range: 2.9 - 22.6 y); 27.4 kg (range: 13.6 - 67.6 kg) and 127.5 cm (range: 96.2 - 184.9 cm). Mean FEV1 (forced expiratory volume in 1 second) and FVC (forced vital capacity) were respectively 84% (range: 39 - 117%) and 86% (range: 44 - 109%) of predicted values. Mean energy intake was 113% RDA (recommended daily allowance). All patients were on conventional physiotherapy, pancreatic enzymes and some of them received antibiotics regularly for pulmonary exacerbations. Weight, height, arm circumferences, TBW, DEXA and skinfolds were measured on the same day. All usual CF medications were continued during the study.

#### *Population studied for the evaluation of changes in body composition*

We included 15 out of 16 children with pancreatic insufficiency as described above who maintained steatorrhea despite pancreatic enzymes and were treated with lansoprazole for 3 months with significant improvement of anthropometric parameters (results will be published separately). Mean age, weight and height of these 15 children were 9.5 y (range: 3.1 - 22.6 y); 29.3 kg (range: 13.6 - 67.6 kg) and 131 cm (range: 97.7 - 184.9 cm) respectively. Their nutritional status was moderately altered with a mean Body Mass Index (BMI) of 15,6 (range: 13.2 - 18.3). Mean FEV1 and FVC were respectively 81.3% (range: 39 - 114%) and 85.5% (range: 44 - 108%) of predicted values.

Anthropometry, DEXA and TBW were measured on the same day just before starting and 3 months after treatment with lansoprazole (15mg / day). Other usual CF medications were continued throughout the study. Informed patient and parental consent were obtained from all study subjects.

## METHODS

### Growth parameters

Weight, height, upper armcircumferences and 4 skinfold thicknesses (biceps, triceps, subscapular and suprailiac) were measured on the left side of the body in triplicate, using the Harpenden caliper. Average of three measures was taken and was expressed as standard deviation scores of the normal population for age and sex using the growth charts from Gerver and de Bruin (6). BMI was calculated as  $\text{weight}/\text{height}^2$ . Results of BMI were compared to the reference population described by Westrate and Deurenberg et al. (7). Mid upper arm muscle area was calculated from the mid upper armcircumference and the sum of biceps and triceps skinfolds (6).

### Body composition

Body composition results obtained from all three methods were compared to those of a recently reported pediatric reference population (8). The percentage of fatmass and fat free mass measured by the skinfold method were also compared to those of the reference population described by Gerver and de Bruin (6).

### Body composition by anthropometry

It has been found that subcutaneous fat as measured by skinfolds is related to the body density (9). This latter is itself related to the body fatmass. From these theoretical principles, Gerver and de Bruin have constructed a chart, expressing the relationship between the 4 skinfolds (biceps, triceps, subscapular and suprailiac) and the percent fat free mass (6). In our study, fat free mass determined by this method was derived from these charts and fatmass was then calculated by subtracting FFM from bodyweight.

### Body composition by dual-energy x-ray absorptiometry

The theoretical principles for DEXA measurement of body composition and the precision of this method have been described previously (10-12). All DEXA measurements were performed with a Dual Photon X-ray ( Lunar Radiation Corp, Madison, WI ) total body scanner. These results were analysed with a pediatric software programme, version 1.5e. Daily quality assurance tests were performed according to the manufacturer 's directions. Total body analysis was performed in all children using a fast scan mode with a sample size of 4.8 x 9.6mm, sample interval of 0.03s and source collimation of 1.68mm. The following body compartments were assessed: total non bone lean body mass, total bone mineral content, total bone mineral density (BMD), total body fatness and Z-score of BMD.

### Body composition by total body water and bromide space

TBW and ECV were measured by deuterium oxide (13) and bromide dilution respectively (14). Each subject received orally 20 ml (40 ml was given to the 2 adolescent patients) of a mixture of D<sub>2</sub>O (99.9% purity) and Bromide salt (150mMol/L) solution in a volume ratio of 1:1. Saliva and plasma samples were taken before intake of D<sub>2</sub>O - NaBr solution and 4 hours thereafter when an "plateau" has been reached. To prevent saliva dilution by fluid intake which can result in a higher TBW content, patients were told not to take any fluid orally half an hour before saliva samples were taken. Urine and fecal loss of bromide and D<sub>2</sub>O during the equilibration period were considered negligible as the D<sub>2</sub>O and bromide T<sub>1/2</sub> are about 8 days (14). Saliva samples were obtained making use of dental cotton-wool, that was dried overnight at 100 °C and kept in a gas-tight tube until use. The cotton-wools and the blood samples were centrifuged and the saliva and serum thus obtained were kept in a stoppered glass vial and stored in a freezer at -20 °C until analysis. Results of TBW, ECV and ICV were compared to the reference values described by Friis-Hansen (15).

#### 1. TBW ANALYSIS

D<sub>2</sub>O concentrations of saliva samples were determined as described by van Kreel (14): Calcium carbide (CaC<sub>2</sub>) was placed in the siliconized vacutainer tube and evacuated for 30 sec. with a rotatory vane pump to a total pressure of 0,01 atm. Thereafter, 25µl of salivary sample

was injected in the vacutainer tube. This was done in duplicate.  $\text{CaC}_2$  react with  $\text{D}_2\text{O}$  forming acetylene gas. A  $25\mu\text{l}$  sample of this gas was subsequently injected in duplicate into the GC/CF - IRMS system (gas chromatography/continuous flow isotope ratio mass spectrometry) at 2 min. intervals. The mass 27/26 ratio ( $R_{27/26}$ ) was measured on a Isotope Ratio Mass Spectrometer configured for Acetylene (Finnigan MAT 252 for CF-IRMS). The mean value of 4 determinations was calculated for each sample. By inserting the tracer/tracee ratio, defined as  $R_{27/26}(\text{T4}) - R_{27/26}(\text{T0})$ , into the regression equation obtained from the standards, we get the dilution factor of  $\text{D}_2\text{O}$ . TBW is calculated as ingested  $\text{D}_2\text{O}$  volume/dilution factor. FFM and FM are then calculated by the following formulae:

$$\text{FFM (kg)} = \text{TBW} / (1,04 \times d)$$

$$\text{FM (kg)} = \text{Weight} - \text{FFM}$$

The 1,04 factor is a correction for the estimated 4% nonaqueous hydrogen exchange and  $d$  is the hydration factor of LBM which varies with age and sex. Because our CF population was young, we used the age dependent hydration factors described by Fomon (16) for children younger than 10 year and by Boileau and Lohman (17) for older children.

## 2. BROMIDE DILUTION ANALYSIS

Because bromide resides mainly in the extracellular space, the measurement of bromide dilution gives an estimate of the extracellular volume. Bromide was determined by using a Gas Chromatograph type CP 9000 (Chrompack) equipped with an ECD detector after it was converted into bromoacetone gas (14). First, perchloric acid was added to the serum sample and centrifuged for deproteinisation. An aliquot of the supernatant was then added to silver nitrate ( $\text{AgNO}_3$ ) for precipitation of silver bromide and chloride. After centrifugation, the precipitate was taken up in  $\text{NH}_3$  after adding  $\text{Na}_2\text{S}$  and  $\text{NaOH}$  in order to eliminate the silver. After agitation and centrifugation, the supernatant was heated until dry.  $\text{H}_2\text{O}$  was added followed by  $\text{H}_2\text{O}_2$  in order to oxidize sulfide. After drying,  $\text{H}_2\text{O}$  was then added and dried again. This was repeated several times. Thereafter, perchloric acid and acetone were added and the reaction was started by addition of  $\text{KmnO}_4$  with Bromoacetone formed. The solution

is then extracted with benzene. The organic phase was separated from the water phase by shaking and centrifugation. The water phase was then removed. An aliquot of the organic solution is then applied to the gas chromatograph for measuring of the bromoacetone/internal standard ratio. The bromide concentration was then derived from the bromoacetone standard curves. Because the distribution of bromide depends on the potential difference between the in- and extra-cellular compartments and on the total body volume, the corrected bromide space was calculated as follows:

$$\text{ECV (L)} = \frac{\text{Bromide administered (mmol)}}{\text{Bromide change T4 - T0 (mmol/L)}} - 0.036\text{TBW}$$

Where 0.036TBW is the correction factor for the cell potential and for the total body volume (14). Body cell mass (BCM) was then calculated by subtracting ECV from TBW.

### Statistics

All data were analysed with SPSS statistic program version 6.0. The sign test was used to compare the growth parameters and body composition results with those of the reference population. The Pearson correlation coefficient was used to determine the relationship between measurements obtained by the various body composition methods. Partial correlation coefficients, controlling for age, were used for the evaluation of the relationships between measured ICV, ECV, mid-upper-arm muscle area and the various body composition results. The between method differences were compared, using the Wilcoxon rank test. The agreement between methods were evaluated by the Statistical method of Bland and Altman (18).

## RESULTS

### *Body composition of 18 cf children*

Mean age, nutritional parameters expressed as standard deviation scores (Z-scores) and results of body composition measured by skinfold, DEXA and TBW from 18 CF children are shown in table 1. Compared to the reference population, mean Z-scores for weight, height, BMI, arm circumference and skinfolds (except for the triceps) were significantly decreased (fig 1). The mid-upper-arm muscle area was significantly decreased in our CF population ( $p = 0.005$ ). In absolute terms, all body composition components measured by DEXA such as FM, LBM, BMC and BMD Z-scores were significantly decreased ( $p = 0.01$ ;  $p = 0.02$ ;  $p = 0.005$  and  $p = 0.03$  respectively) compared to the control population described by Oggle (8). When compared to the normal DEXA body composition data reported by Oggle (8), results obtained with either the TBW or the skinfold methods showed only fat mass to be significantly decreased in our patients ( $p = 0.03$  and  $p = 0.05$  respectively). In relative terms, FM-DEXA (compared to normal DEXA data reported by Oggle) and FM-skinfolds (compared to normal skinfolds data reported by Gerber) were also significantly decreased. The BMI was correlated with both FM and FFM measured by all three methods (FM-DEXA  $r = 0.90$ ; FM-skinfold  $r = 0.87$ ; FM-TBW  $r = 0.75$ ; FFM-DEXA  $r = 0.79$ ; FFM-skinfold  $r = 0.81$ ; FFM-TBW  $r = 0.8$  with  $p = 0.001$  for all correlations). As expected strong correlations were found between age on the one hand and FM-DEXA ( $r = 0.67$   $p = 0.003$ ), FFM-DEXA ( $r = 0.95$   $p = 0.001$ ), FM-skinfolds ( $r = 0.59$   $p = 0.01$ ), FFM-skinfolds ( $r = 0.95$   $p = 0.001$ ), FM-TBW ( $r = 0.47$   $p = 0.047$ ), FFM-TBW ( $r = 0.94$   $p = 0.001$ ) and BMC-DEXA ( $r = 0.94$   $p = 0.001$ ). No sex differences in body composition data were found. When compared to the reference values described by Fris-Hansen (15), the ECV and TBW as a percentage of bodyweight were significantly increased ( $p < 0.005$  both) while the ICV as percent of bodyweight was normal. The partial correlations (controlling for age) between ECV, ICV, the mid-upper-arm muscle area LBM and FM are shown in table 2.

**Table 1 Characteristics of 18 CF children.**

	MEAN	SEM	RANGE
AGE (Yr)	8.97	1.16	2.9 - 22.6
BW (Kg)	27.43	3.66	13.6 - 67.6
BW (SDS)	-1.03	0.25	-2.33 - 1.36
TM (Kg)	27.82	3.84	12.96 - 67.33
HEIGHT (cm)	128	6.0	96.2 - 184.9
HEIGHT (SDS)	-1.31	0.25	-2.89 - 0.40
BMI (Kg / m <sup>2</sup> )	15.6	0.57	13.21 - 23.17
ARMCIRCUMFER. (SDS)	-1.62	0.31	-3.14 - 1.43
BICEPS (SDS)	-0.77	0.23	-1.86 - 1.33
TRICEPS (SDS)	-0.33	0.23	-2 - 2.33
SUBSCAPULAR (SDS)	-0.92	0.23	-2 - 1.6
SUPRAILIIACA (SDS)	-1.66	0.09	2.43 - -0.57
SUM 4 SKINFOLDS (SDS)	-1.17	0.20	-2.2 - 1.2
FM-DXA (%)	12.11	1.48	6.1 - 28.8
FM-TBW (%)	10.39	1.61	0.0 - 22.2
FM-SKINFOLD (%)	14.44	0.94	8 - 23
FFM-DXA (%)	87.9	1.5	71.2 - 93.9
FFM-TBW (%)	89.8	1.6	77.8 - 101
FFM-SKINFOLD (%)	85.6	0.9	77 - 92
BMC-DXA (Kg)	1.02	0.17	0.37 - 2.68
TBW (L)	19.06	2.38	9.9 - 47.4
TBW (%)	70.82	1.32	59.62 - 79.70
ICV (L)	10.96	1.78	3.71 - 31.93
ICV (%)	38.41	1.42	27.28 - 51.01
ECV (L)	8.10	0.65	4.45 - 15.47
ECV (%)	32.44	1.75	19.01 - 47.69

BW: Body weight

FM: Fat mass

TBW: Total body water

TM: DEXA constructed weight

FFM: Fat free mass

ECV: Extracellular volume

BMI: Body mass index

BMC: Bone mineral content

ICV: Intracellular volume

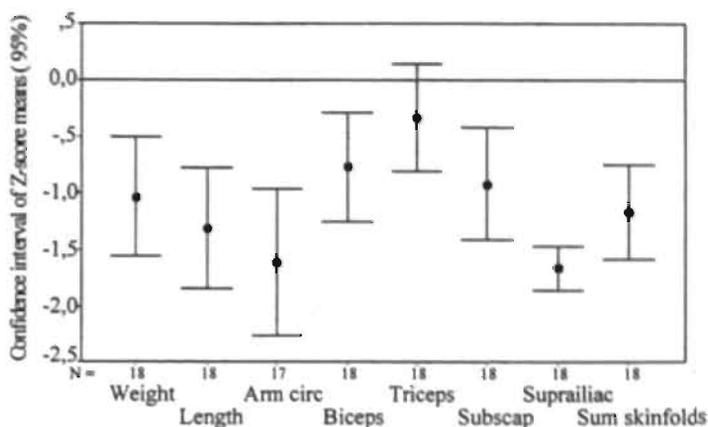


Figure 1 Confidence interval of Z-score means for various anthropometric parameters in cystic fibrosis children showing significantly lower values for all parameters except for the triceps skinfold.

Table 2 Correlation coefficients between muscle area, ECV, ICV and body composition results

	MUSCLE AREA (cm <sup>2</sup> )	ECV (L)	ICV (L)
LBM-DEXA (Kg)	0.84 ( p = 0.001 )	0.67 ( p = 0.004 )	0.91 ( p = 0.001 )
LBM-SKINF (Kg)	0.90 ( p = 0.001 )	0.58 ( p = 0.02 )	0.92 ( p = 0.001 )
LBM-TBW (Kg)	0.86 ( p = 0.001 )	0.63 ( p = 0.007 )	0.95 ( p = 0.001 )
FM-DEXA (Kg)	0.62 ( p = 0.02 )	0.15 ( p = 0.58 )	0.53 ( p = 0.04 )
FM-SKINF (Kg)	0.61 ( p = 0.02 )	0.23 ( p = 0.38 )	0.53 ( p = 0.03 )
FM-TBW (Kg)	0.33 ( p = 0.21 )	-0.004 ( p = 0.99 )	0.20 ( p = 0.43 )
ECV (L)	0.45 ( p = 0.08 )		
ICV (L)	0.86 ( p = 0.001 )		

LBM-DEXA: Lean body mass by DEXA method

LBM-SKINF: Lean body mass by skinfolds method

LBM-TBW: Lean body mass by TBW method

ECV: Extracellular volume

FM-DEXA: Fatmass by DEXA

FM-SKINF: Fatmass by skinfolds method

FM-TBW: Fatmass by TBW method

ICV: Intracellular volume

**Table 3 Changes in bodyweight and body composition after 3 months on lansoprazole in 15 CF children.**

	T3 - T0		
	MEAN ± SEM	MINIMUM	MAXIMUM
BW (Kg)	0.97 ± 0.13	0.4	2.1
TM (Kg)	0.97 ± 0.15	-0.05	1.9
FM-DXA (Kg)	0.52 ± 0.19	-0.46	2.18
FM-TBW (Kg)	-0.23 ± 0.52	-4.85	3.44
FM-SKINFOLD (Kg)	0.94 ± 0.26	0.05	3.52
LBM-DXA (Kg)	0.43 ± 0.18	-0.96	1.46
BMC-DXA (Kg)	0.03 ± 0.01	-0.03	0.09
FFM-DXA (Kg)	0.41 ± 0.17	-0.98	1.03
FFM-TBW (Kg)	1.27 ± 0.49	-1.84	5.35
FFM-SKINFOLD (Kg)	0.04 ± 0.26	-3.02	0.98
TBW (L)	0.99 ± 0.39	-1.4	4.1
ICV (L)	0.09 ± 0.41	-3.49	3.17
ECV (L)	0.92 ± 0.46	-2.38	4.91

T0: Before start lansoprazole

T3: 3 months after lansoprazole

TM: Total mass (DEXA)

BW: Body weight

FFM: Fat free mass

FM: Fatmass

BMC: Bone mineral content

LBM: Lean body mass

TBW: Total body water

ECV: Extracellular water

ICV: Intracellular water

### Limits of agreement between methods

As only 5 of our patients 3 girls and 2 boys were postpubertal, all results of both boys and girls were analysed together. There was a high correlation between the 3 body composition methods for measuring of FM and FFM (fig 2 and 3). The best correlation for FM determination was between DEXA and the skinfold method ( $r = 0.98$ ). As DEXA is a 3 compartments model, BMC was not included in the lean body mass. After correction for BMC, the correlation coefficient was unchanged. Plots of the paired differences for FM and FFM measured in kilogram versus their mean, with indication of the limits of agreement are shown in figure 4 and figure 5 respectively. Since our population was small, we preferred to use the 10th and 90th centile values instead of  $\pm 2$  SD for defining the limits of agreement. No intermethod correlations were found between means and differences as shown in figures 4 and 5. The 50th centile of the differences between FM-TBW versus FM-skinfolds was -1.68kg (-2.64 - -0.46kg); FM-skinfolds versus FM-DEXA was 0.75kg (-3.01 - 1.21kg); FM-TBW versus FM-DEXA was -0,96kg (-5.05 - -0.03kg). The 50th centile of the differences between FFM-TBW versus FFM-DEXA was 0.45kg (-0.70 - 4.85kg); FFM-skinfolds versus FFM-DEXA was -0.17kg (-0.77 - 3.46); FFM-TBW versus FFM-skinfolds was 1.68kg (0.46 - 2.96kg). The DEXA constructed weight was highly correlated with scale weight ( $r = 0.999$   $p = 0.001$ ). However, the DEXA weight was significantly lower than scale weight ( $p = 0.003$ ); the 50th centile of the differences between bodyweight constructed from DEXA (TM) and scale weight was -0.52kg (-0.79 - 0.12). Significant differences were found between the means of FFM measured by the TBW and the skinfold methods ( $p = 0.02$ ), the skinfolds and DEXA methods ( $p = 0.001$ ) as well as the TBW and DEXA methods ( $p = 0.001$ ). Only FM results from TBW and skinfolds were significantly different ( $p = 0.01$ ).

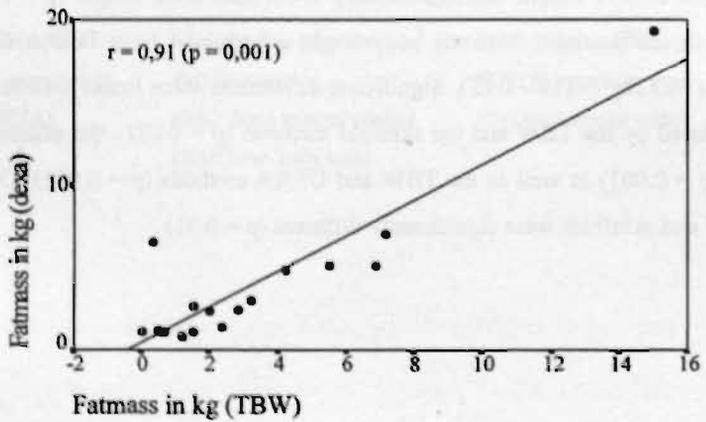
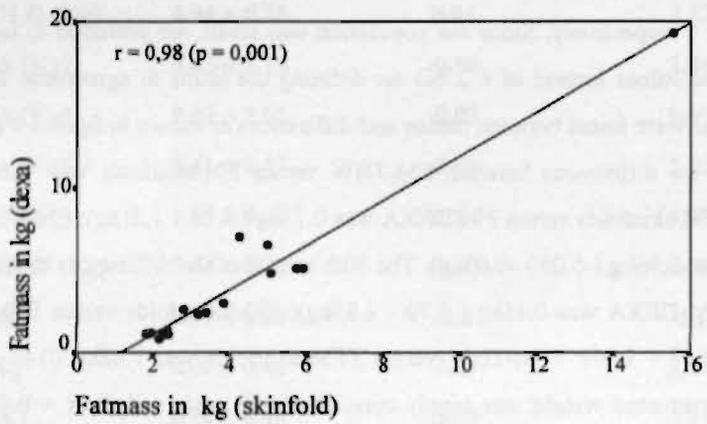
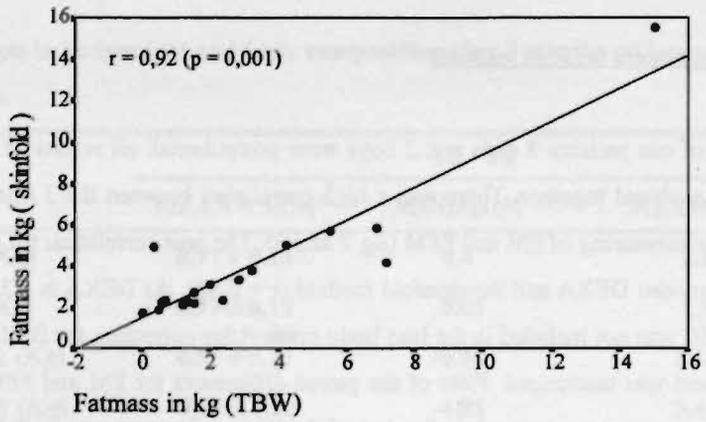


Figure 2 Intermethod fatmasses correlation coefficients.

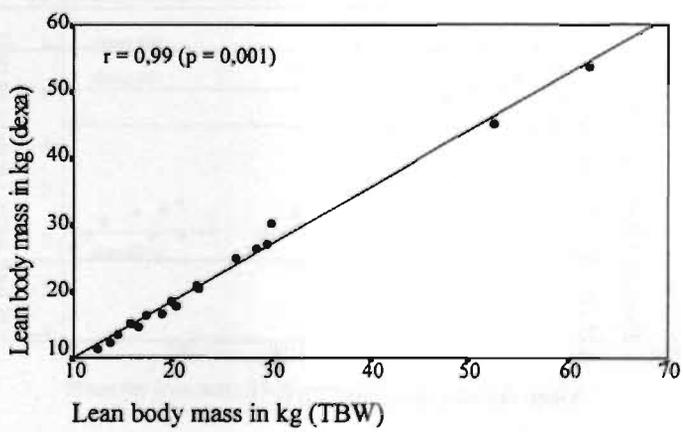
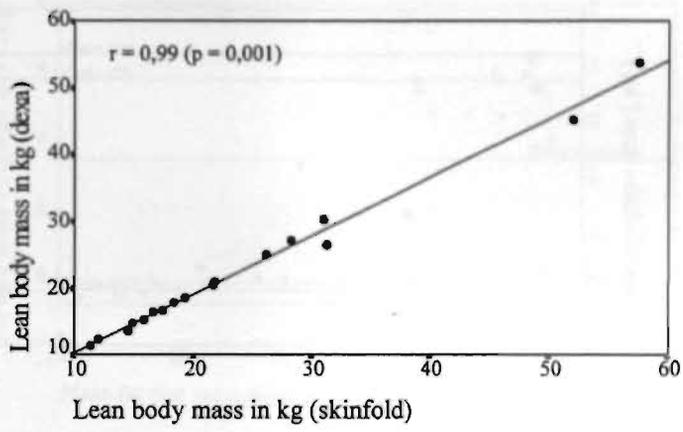
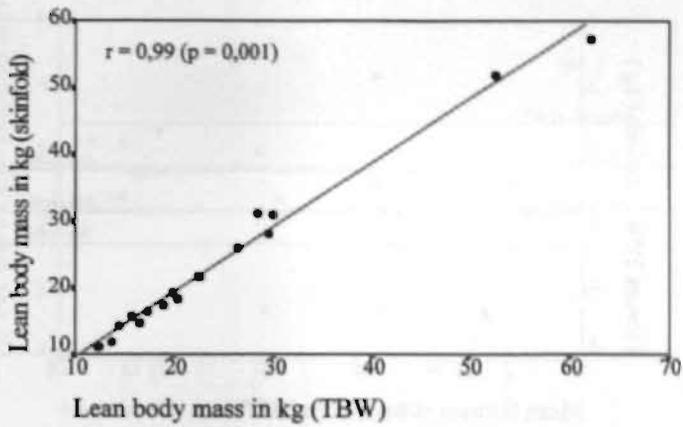


Figure 3 Intermethod lean body mass correlation coefficients.

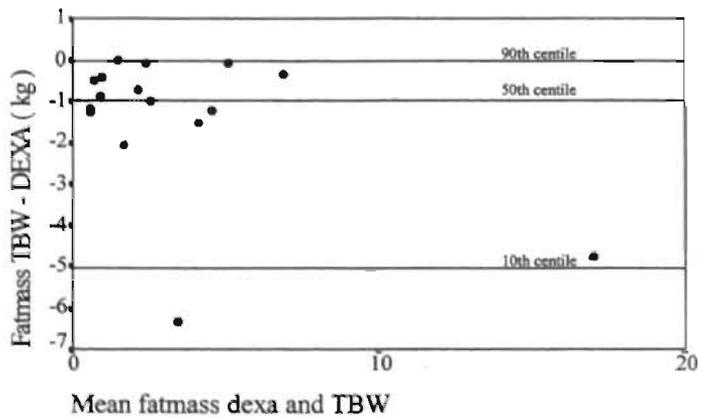
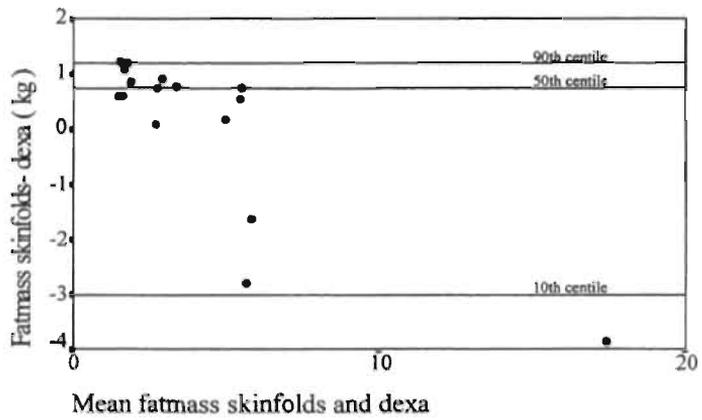
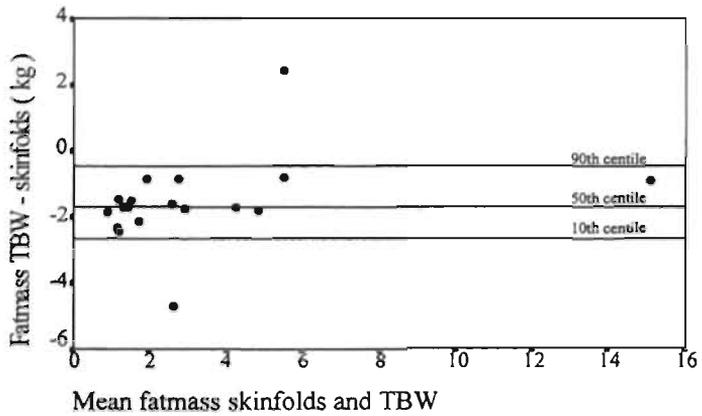


Figure 4 Limits of agreement for fatmass measured by the various methods.

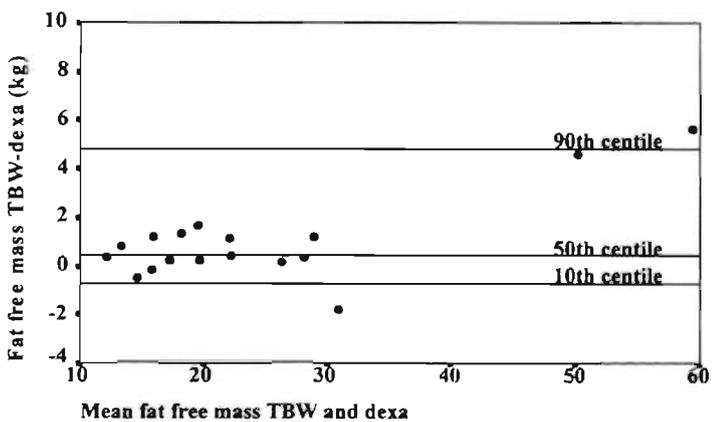
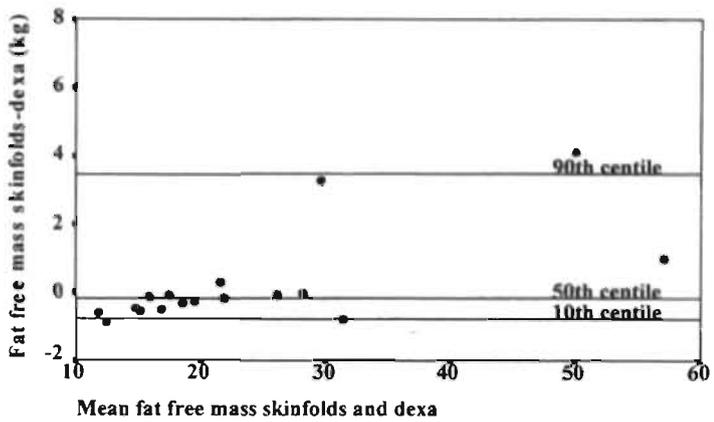
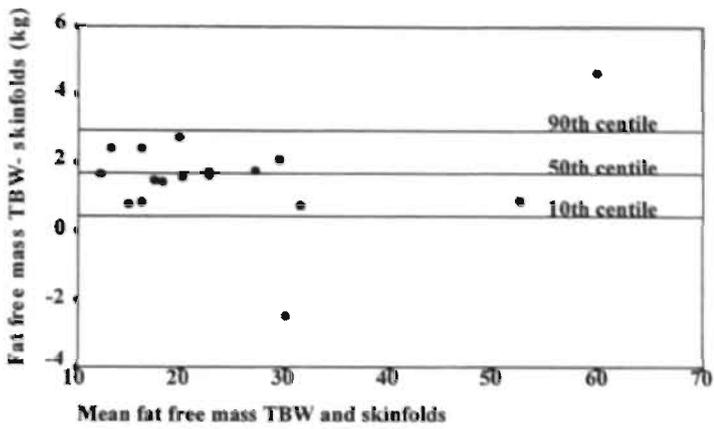


Figure 5 Limits of agreement for fat free mass measured by the various methods.

### Body composition changes

The changes in bodyweight, FM and FFM measured by skinfold, DEXA and TBW are shown in table 3. The increase in bodyweight was the same for both DEXA constructed weight and scale weight. However changes both in FM and FFM were different between methods. Both changes in FM and FFM measured by skinfold were highly correlated with those measured by DEXA ( $r=0.91$   $p = 0.001$  and  $r = 0.84$   $p = 0.001$  for FM and FFM respectively). Changes in FM measured by the TBW method were not correlated with changes of the same parameter measured by either the DEXA or the skinfold method, whereas changes in FFM-TBW were negatively correlated with those measured by skinfold and DEXA (FFM-TBW versus FFM-skinfold:  $r = -0.56$   $p = 0.03$  and FFM-TBW versus FFM-DEXA:  $r = -0.52$   $p = 0.05$ ). No correlation was found between changes in bodyweight and changes in FM or FFM measured by any method. Changes in ECV and ICV did not correlate with bodyweight changes. No correlation was found between changes in ICV and changes in LBM by any method.

## DISCUSSION

In children, effective evaluation of deterioration or catch-up growth can only be achieved by using the Z-score method. Despite high caloric polymeric intake, treatment of steatorrhea and support of pulmonary function, significantly lowered Z-scores for arm circumference, biceps, subscapular, suprailiac, sum of the 4 skinfolds weight and height were found in our patients. As our patients showed decreased weight, height Z-scores and mid upper arm muscle area, we expected both FM and FFM to be decreased. Since all body composition methods are based on assumptions, we used 3 noninvasive methods (DEXA, TBW and skinfolds) to evaluate the body composition of our patients. However, interpretation of the body composition results is difficult due to the lack of reference values for several measuring methods. The results of various methods used were strongly correlated with each other but still showed differences. In absolute terms, only DEXA results were as expected; showing a decrease in all 3 body components measured. Results of the TBW and skinfolds method could not be assessed accurately due to the lack of reference data expressed in absolute terms. When compared to the DEXA reference values, TBW and skinfolds methods only showed a decrease in FM. In relative terms, our CF populations showed an increase in TBW and ECV while the ICV (body cell water mass) appeared well maintained. These results imply a decrease of fatmass, associated with a relative increase in TBW, ECV and consequently FFM (19). In agreement with these data, our results showing an increased percentage of FFM as evaluated by the skinfold method also implies a decrease in fatmass percentage in our CF patients. According to these results, we believe our CF patients mainly have a depletion of fatmass and bone mineral content with a slight decreased in lean body mass in absolute terms. Comparison of our results with other body composition studies in CF children is difficult: First, the general condition of the studied populations differed between studies and second, methods used for the assessment of the nutritional condition were different. Tomezsko et al. found no significant decrease in body FM and FFM in their CF children with only significantly decreased suprailiac skinfold thicknesses and subscapular Z-scores. However their CF population was very young and showed only mild symptoms (2). In another study concerning older CF children with abnormal pulmonary function, Johnston et al. did find a significantly lower percentage of body fat (FFM not reported) compared to matched control children similar to our findings with all 3

methods (20). In agreement with our study, Miller et al. who studied the body composition and muscle protein metabolism in a group undernourished CF children with Z-scores for weight and height similar to those of our CF population, found a significant decrease in FM, FFM and muscle mass (21). The strong correlation we found between components of body composition and age are well known (8,22,23). The lack of sex differences can probably be explained by the prepubertal age of most patients (22). A high correlation was found between mid-upper-arm muscle area, ECV, ICV and LBM and FM. As expected, correlation coefficients between mid-upper-arm muscle area, ECV, ICV and LBM remained high while only weak correlations were found with FM. Correlations were also evaluated after "homogenizing" our patient group by excluding the 2 adolescent patients. Highly significant correlations were again found between the above parameters and LBM while none were found with FM. As expected the between methods results differed significantly. Despite a high correlation between DEXA constructed weight and scale weight, mean DEXA weight was significantly and about 520 gram lower than scale weight. This is in agreement with results from Oggle et al. (8). FM measured by TBW was lower than that measured by either skinfolds or DEXA methods whereas FM-skinfold was often higher than FM-DEXA. The low values of FM when measured by the TBW method might be due to overestimation of FFM by this method. The FFM calculated by TBW is based on the assumption that a fraction of FFM is water. As the water content of FFM decreases with age (17), we used the age dependent FFM hydration fraction to calculate the FFM (17). The mean FFM hydration fraction used in our study was 76.27% (range: 73.7 - 77.5%). In a study of body composition of CF prepubertal children, making use of skinfolds and TBW methods, Tomezco et al. also found a significantly lower body fat percentage with the TBW when compared to the skinfold method, which showed normal results (2). In two compartment models such as the TBW and the skinfolds methods, the densities of FFM is assumed to be constant in the range 18 - 67 years but the density does vary depending on the concentrations of water and mineral in FFM (24,25). Although in our study the variation in water and mineral content was taken into account in the regression equations of skinfolds and TBW methods for the calculation of FFM and FM in the age range below 18 years, the water and mineral content are still population specific depending on the presence of illnesses. The percentage of TBW in CF children has been reported to be increased compared to control children (19,26). Theoretically, the DEXA method has the advantage

of being independent of biological assumptions about the densities and level of tissue hydration but the accuracy of the method still depends on the internal calibration (27,28). It has been reported that when compared to chemical analysis, DEXA overestimate fat measured in meat blocks with lower fat content and underestimate the content in those with high fat content (29). Moreover, studies comparing DEXA results with those obtained from chemical analysis, using piglets, showed slight inadequacies in the estimation of fatmass and lean body mass (27,30). We think that the between methods differences are most likely related to the various body compartments measured by these 3 methods rather than to inherent inaccuracies in the techniques themselves. This means that results obtained from each of these methods are not interchangeable. An important question to answer is whether or not any of the used methods is capable of detecting body composition changes occurring during nutritional interventions. DEXA has been introduced as direct method with very good reproducibility (12,31). In this study we compared the sensitivity of DEXA, TBW and skinfolds for detecting small body composition changes in children. For this purpose, we assessed the body composition of 15 CF children before and 3 months after they were treated with lansoprazole as an adjunct therapy for pancreatic enzymes in order to decrease steatorrhea. All 15 CF children showed significant increases in Z-scores for weight, height and skinfolds (unpublished observations). The evaluation of body composition changes differed depending on the method used. With the DEXA method, 53% of the weight increase was ascribed to FM, 44% to FFM and 3% to BMC. Both DEXA and skinfolds methods showed significant increases in fatmass but the increased FFM was not significant. In contrast, weight increase was exclusively ascribed to an increase in FFM with the TBW method. However, no significant correlations were found between weight changes and either FM, LBM or BMC changes by any method. The correlation coefficient of 0.40 found between weight changes and changes in FFM by DEXA just failed to reach statistical significance ( $p = 0.07$ ). There were also no significant changes in ECV and ICV after intervention. This is in contrast to results reported by Going et al., who studied the changes in body compartments induced by dehydration - rehydration with oral fluid using DEXA method for assessment of body composition changes. They found a correlation between bodyweight changes and changes in TM, soft tissue mass (LBM + FM) and LBM. However, the total weight changes induced in their study was higher than in our study (approximately 1.2 kg versus 0.97 kg in our study) and as the changes in bodyweight were

induced by water content, the total bodyweight changes were exclusively ascribed to changes in the water content of STM, reflected by the exclusive increase in LBM (32). Since fatmass was mostly depleted in our patients, it is likely that this body compartment will normalize first as a result of an effective intervention.

From the results of this study, we conclude that results measured by different methods are not interchangeable. It is consequently important to use the same method for longitudinal evaluation of body composition. However, the use of DEXA, TBW and skinfolds methods is limited in children in whom only slight changes in bodyweight after intervention are expected (3% in this study) since the sensitivity is apparently not high enough for the detection of small differential changes in FM and FFM.

*Acknowledgment:* The authors wish to thank Mia Meers from the department of clinical laboratory, Sandra Zimny and Piet Willems from the department of nuclear medicine for their kind and expert technical assistance.

## REFERENCES

- (1) M. Aitken, S. Fiel. Cystic Fibrosis. *Dis Mon* 1993;39: 1-52.
- (2) J. Tomezsko, T. Scanlin, V. Stallings. Body composition of children with cystic fibrosis with mild clinical manifestations compared with normal children. *Am J Clin Nutr* 1994; 59: 123-8.
- (3) M. Bronstein, P. Davies, K. Hambidge, F. Accurso. Normal energy expenditure in the infant with presymptomatic cystic fibrosis. *J Pediatr* 1995; 126: 28-33.
- (4) R. Kraemer, A. Rudeberg, B. Hadorn, E. Rossi. Relative underweight in cystic fibrosis and its prognostic value. *Acta Paediatr Scand* 1978; 67: 33-37.
- (5) R. Branson, Y. Vaucher, G. Harrison, M. Vargas, C. Thies. Inter- and intra-observer reliability of skinfold thickness measurements in newborn infants. *Hum Biol* 1982; 54: 137-143.
- (6) W. Gerver, R. de Bruin. *Paediatric Morphometrics: A reference manual*. 1th ed. Utrecht: Bunge, 1996.
- (7) J. Westrate, P. Deurenberg, H. Van Tinteren. *Int J Obesity*. 1989; 13: 465-477.
- (8) G. Ogle, J. Allen, I. Humphries et al. Body-composition assessment by dual-energy x-ray absorptiometry in subjects aged 4-26 y. *Am J Clin Nutr*. 1995; 61:746-53.
- (9) J. Westrate, P. Deurenberg. Body composition in children: proposal for a method for calculating body fat percentage from total body density or skinfold-thickness measurements. *Am J Clin Nutr* 1989; 50: 1104-15.
- (10) R. Mazess, B. Collick, J. Trempe, H. Barden, J. Hanson. Performance evaluation of a dual-energy x-ray bone densitometer. *Calcif Tissue Int* 1989; 44: 228-232.
- (11) W. Peppler, R. Mazess. Total body bone mineral and lean body mass by dual-photon absorptiometry. I. Theory and measurement procedure. *Calcif Tissue Int* 1981; 33: 353-359.
- (12) R. Mazess, H. Barden, J. Bisek, J. Hanson. Dual-energy x-ray absorptiometry for total-body regional bone-mineral and soft-tissue composition. *Am J Clin Nutr* 1990; 51: 1106-12.
- (13) B. Van Kreel, F. Van der Vegt, M. Meers, T. Wagenmakers, K. Westerterp, A. Coward. Determination of total body water by a simple and rapid mass spectrometric method. *J Mass Spectrom* 1996; 31: 108-111.
- (14) B. Van Kreel. An improved bromide assay for the estimation of extracellular water

- volume by capillary gas chromatography. *Clinica Chimica Acta* 1994; 231: 117-128.
- (15) B. Friis-Hansen. Body water compartments in children: Changes during growth and related changes in body composition. *Pediatrics* 1961; 28: 169-181.
- (16) S. Fomon, F. Haschke, E. Ziegler, S. Nelson. Body composition of reference children from birth to age 10 years. *Am J Clin Nutr* 1982; 35: 1169-1175.
- (17) R. Boileau, T. Lohman, M. Slaughter, T. Ball, S. Going and M. Hendrix. Hydration of the fat-free body in children during maturation. *Hum Biol* 1984; 56: 651-666.
- (18) J. Bland, D. Altman. Statistical methods for assessing agreement between two methods of clinical measurement. *The Lancet* 1986; 8: 307-310.
- (19) M. Miller, D. Kornhauser. Bromide pharmacokinetics in cystic fibrosis. *Arch Pediatr Adolesc Med* 1994; 148:266-271.
- (20) J. Johnston, M. Leong, E. Checkland, P. Zuberbuhler, P. Conger, A. Quinney. Body fat assessed from body density and estimated from skinfold thickness in normal children and children with cystic fibrosis. *Am J Clin Nutr* 1988; 48: 1362-6.
- (21) M. Miller, L. Ward, B. Thomas, W. Cooksley, R. Shepherd. Altered body composition and muscle protein degradation in nutritionally growth-retarded children with cystic fibrosis. *Am J Clin Nutr* 1982; 36: 492-499.
- (22) H. Rico, M. Revilla, L.F. Villa, E. Hernández, M. Alvarez de Buergo and M. Villa. Body composition in children and Tanner's stages: A study with Dual-energy X-ray absorptiometry. *Metabolism* 1993; 42: 967-970.
- (23) R. Faulkner, D. Bailey, D. Drinkwater, A. Wilkinson, C. Houston and H. McKay. Regional and total body bone mineral content, bone mineral density and total body tissue composition in children 8 - 16 years of age. *Calcif Tissue Int* 1993; 53: 7-12.
- (24) G. Forbes. *Human body composition*. New York: Springer-Verlag, 1987.
- (25) T. Lohman. *Advances in body composition assessment*. Champaign, IL: Human Kinetics, 1992.
- (26) M. Newby, N. Keim, D. Brown. Body composition of adult cystic fibrosis patients and control subjects as determined by densitometry, bioelectrical impedance, total body electrical conductivity, skinfold measurements, and deuterium oxide dilution. *Am J Clin Nutr* 1990; 52: 209-13.
- (27) K. Ellis, R. Shypailo, J. Pratt, W. Pond. Accuracy of dual-energy x-ray absorptiometry

for body composition measurements in children. *Am J Clin Nutr* 1994; 60: 660-5.

(28) R. Wellens, W. Chumlea, S. Guo, A. Roche, N. Reo, R. Siervogel. Body composition in white adults by dual-energy x-ray absorptiometry, densitometry, and total body water. *Am J Clin Nutr* 1994; 59: 547-55.

(29) M. Jensen, J. Kanaley, L. Roust et al. Assessment of body composition with use of dual-energy x-ray absorptiometry: Evaluation and comparison with other methods. *Mayo Clin Proc* 1993; 68: 867-873.

(30) J. Brunton, H. Bayley, S. Atkinson. Validation and application of dual-energy x-ray absorptiometry to measure bone mass and body composition in small infants. *Am J Clin Nutr* 1993; 58: 839-45.

(31) P. Chilibeck, A. Calder, D. Sale, C. Webber. Reproducibility of dual-energy x-ray absorptiometry. *Can Assoc Radiol J* 1994; 45: 297-302.

(32) S. Going, M. Massett, M. Hall et al. Detection of small changes in body composition by dual-energy x-ray absorptiometry. *Am J Clin Nutr* 1993; 57: 845-50.

## CHAPTER 8

### GENERAL DISCUSSION

Chronic pulmonary infections and poor appetite together with fat malabsorption are the main causes of malnutrition and growth retardation in CF children (1-3). The ideal treatment of CF should be the correction of the underlying defect by introduction of a normal copy of the defective gene into these patients genetic material. Although gene therapy is presently under intensive scrutiny (4-6), the role of this treatment in CF patients is not yet settled. Until then, treatment of these patients has to focus on improving the nutritional condition, since malnutrition can adversely affect survival (7). As 85% of CF patients have pancreatic insufficiency (8), improved absorption by pancreatic enzymes substitution is one of the main goals. Diagnosis and regular monitoring of fecal fat loss along with close evaluation of growth and the nutritional condition are consequently necessary in the follow up of these patients. Although the fat balance method is considered to be the golden standard for the evaluation of steatorrhea, it is too cumbersome to be used for the frequent monitoring of fat losses in these children. Several studies have shown the measurement of fecal fat concentration to be a valuable alternative to fat excretion studies for the diagnosis of fat malabsorption (9). These studies also shown that the differences in fat excretion between either 3 or 1 day collections are mainly due to day to day variation in stool volume, the stool fat concentration being much more constant. These studies led us to suppose that the repeated measurement of stool fat concentration in stool samples would be a valuable aid to the monitoring of steatorrhea. As chemical measurement of stool fat is time consuming, we looked for an alternative easy measure of fat content. Although the steatocrit looked quite attractive (10) our first results and also results reported by others (11,12) disappointingly often showed low steatocrit results in stools of high fat content.

By acidification of stool homogenates, we could show fat extraction to be much improved and to result in a satisfactory correlation coefficient between chemically measured fecal fat and "acid steatocrit" results. We consequently decided to use the acid steatocrit in an intervention study (proton pump inhibitor) aiming at improving both steatorrhea and the nutritional condi-

tion in children with CF.

Both anthropometric parameters and body composition methods were used for the evaluation of the nutritional condition. Difficulties arise due to the fact that weight, height and skinfolds are age and sex specific. Although several authors have overcome this problem by expressing results of these parameters as a percentage of the predicted values for age and sex, the use of Z-scores is the preferred method for most authors. Z-scores measure deviations from the median value expressed in standard deviation units. Improving Z-scores reflect catch-up growth while the reverse is true for deteriorating Z-scores. Recently, Gerver and de Bruin have constructed growth charts with standard deviation for weight, height armcircumferences and the 4 skinfolds (biceps, triceps, subscapular and suprailiac) (13). Anthropometric parameters can be easily converted into Z-scores through the use of these reference data for normal children. As weight changes could be due to either changes in fatmass, fat free mass or both, we measured body composition by several methods in order to evaluate body composition before and after our intervention (proton pump inhibitor) study.

Our study results show significant decreases of most measured anthropometric parameters in children with cystic fibrosis. Decrease in skinfold thicknesses were most significant and contrary to a commonly held belief triceps skinfolds were often normal while subscapular and suprailiac skinfolds were very sensitive indicators of chronic malnutrition in these patients. Our findings support the use of these simple anthropometric measurements for the evaluation of the nutritional condition in children. As far as body composition results are concerned, interpretation of results is uneasy due to the lack of reference values for several measuring methods. Notwithstanding these drawbacks, results of the various methods used were strongly correlated with each other but, still showed differences which preclude the use of these various methods interchangeably. Results should be looked at both in relative and in absolute terms.

In absolute terms, the DEXA method showed a severe decrease of fatmass and a slight decrease of fat free mass and of bone mineral content. Results of the total body water and skinfold method agreed with the DEXA results but could not be accurately assessed due to the lack of reliable reference data.

In relative terms, the deuterium - bromide results showed a relatively increased total body water and extracellular water compartment while the relative body cell water mass appeared

well maintained. These results imply a decrease of fatmass as percent of bodyweight. Likewise, the fat free mass (%) measured by the skinfold method was increased in our CF children. All these results agree with each other rather well and show that children with CF have a lowered bodyweight accompanied by a decreased fatmass (%), an increased fat free mass (%) and an increased extracellular water compartment (%) while the intracellular water compartment (%) appears to be well maintained (table 1).

**Table 1** Body composition in children with cystic fibrosis.

	<b>FM</b>	<b>FFM</b>	<b>TBW</b>	<b>ECV</b>	<b>ICV</b>
	<b>kg (%)</b>	<b>kg (%)</b>	<b>(%)</b>	<b>(%)</b>	<b>(%)</b>
<b>DEXA</b>	↓ (↓)	↓ (↑)			
<b>Skinfold</b>	? (↓)	? (↑)			
<b>Deuterium</b>					
<b>Bromide</b>	? (↓↓)	? (↑)	(↑)	(↑)	(n)

DEXA: Dual energy X - Ray Absorptiometry

FM: Fatmass

FFM: Fat free mass

TBW: Total Body Water

ECV: Extracellular volume

ICV: Intracellular volume

A positive effect of omeprazole on fat absorption has been found in adults with CF (14). However the role of proton pump inhibitors on steatorrhea and its effects on the nutritional condition has not been evaluated in children. We have studied the effect of 3 months treatment of lansoprazole on fat malabsorption and body composition in 15 CF children, maintaining steatorrhea while on pancreatic enzymes. These children showed significant improvements of both fat absorption (as measured by the acid steatocrit) and Z-scores for all parameters except for the biceps and triceps skinfolds and deteriorated again 3 months after lansoprazole was stopped. The increase in skinfold thicknesses Z-scores were accompanied by signifi-

cant increases in fatmass as measured by the skinfold and the DEXA methods.

Different body composition methods have been described but, only few studies have compared different measurement techniques in pediatric subjects. An important question to answer is whether or not any of these methods is capable of detecting body composition changes occurring during nutritional interventions. Our study comparing the changes in body composition measured by DEXA, TBW and skinfolds methods in 15 CF children, whose nutritional condition improved significantly after intervention with lansoprazole for 3 months, showed different results for each method. Both DEXA and skinfolds methods showed significant increases in fatmass but not in lean body mass in absolute terms. Likewise, the percentage of body cell water mass did not increase significantly after nutritional intervention. On the other hand, the increases in bodyweight were completely ascribed to increases in lean body mass but not in fatmass when evaluated by the TBW method. Since fatmass was mostly depleted in our CF children (as shown by DEXA, skinfolds and total body water methods), it is likely that this body compartment will normalize first as a result of an effective intervention. Our results do not allow firm conclusions as to the effect of lansoprazole on FFM while a significant increase in bone mineral content was found. The bodyweight changes occurring during lansoprazole intervention were unrelated to either fatmass or FFM changes measured by any of the three methods used. We think the weight changes in the various body compartments were too small to be accurately measured by body composition methods.

In conclusion, the acid steatocrit is a reliable, cheap and noninvasive alternative method for the monitoring of fat malabsorption. Most cystic fibrosis patients are malnourished even when lung functions are stable and a hypercaloric diet is used. Body composition studies in these patients mainly show a loss of fat mass and bone mineral content with a relative increase in extracellular water and a normal intracellular water mass (%). Inhibition of gastric acid secretion by a proton pump inhibitor improved both fat absorption and the nutritional condition of our patients. Methods for the assessment of body composition are not interchangeable and not accurate enough for detecting small changes in fatmass and fat free mass such as measured in our 3 months study. A longterm study is needed in order to better evaluate the effects of lansoprazole on body composition in children with cystic fibrosis.

## REFERENCES

- (1) J. Dodge, J. Yassa. Food intake and supplementary feeding programs. In: J. Sturgess, ed. perspectives in cystic fibrosis. Toronto: Canadian Cystic Fibrosis Foundation; 1980: 125-136.
- (2) M. Bronstein, R. Sokol, S. Abman et al. Pancreatic insufficiency, growth, and nutrition in infants identified by newborn screening as having cystic fibrosis. *J Pediatr* 1992; 120: 533-40.
- (3) J. Tomezsco, V. Stallings, D. Kawchak, J. Goin, G. Diamond, T. Scanlin. Energy expenditure and genotype of children with cystic fibrosis. *Pediatr Res* 1994; 35: 451-460.
- (4) M. Rosenfeld, W. Siegfried, K. Yoshimura et al. Adenovirus-mediated transfer of a recombinant alpha 1-antitrypsin gene to the lung epithelium in vivo. *Science*. 1991;252: 431-4
- (5) B. Pitt, M. Schwarz, J. Pilewski et al. Retrovirus-mediated gene transfer in lungs of living fetal sheep. *Gene Ther* 1995; 2: 344-50.
- (6) M. Rosenfeld, K. Yoshimura, B. Trapnell et al. In vivo transfer of the human cystic fibrosis transmembrane conductance regulator gene to the airway epithelium. *Cell*. 1992; 68: 143-55.
- (7) R. Kraemer, A. Rudeberg, B. Hadorn, E. Rossi. Relative underweight in cystic fibrosis and its prognostic value. *Acta Paediatr Scand* 1978; 67: 33-37.
- (8) M. Aitken, S. Fiel. Cystic fibrosis. *Dis Mon* 1993; 39: 1-52.
- (9) N. Thorsgaard Pedersen, H. Halgreen, H. Worning. Estimation of the 3-day faecal fat excretion and fat concentration as a differential test of malabsorption and maldigestion. *J Gastroenterol* 1987; 22: 91-96.
- (10) P. Phuapradit, A. Narang, P. Mendonca, D. Harris, J. Baum. The steatocrit: a simple method for estimating stool fat content in newborn infants. *Arch Dis Child* 1981; 56: 725-727.
- (11) M. Walters, J. Kelleher, J. Gilbert, J. Littlewood. Clinical monitoring of steatorrhea in cystic fibrosis. *Arch Dis Child* 1990; 65: 99-102.
- (12) E. Sugai, G. Srur, H. Vazquez et al. Steatocrit: a reliable semiquantitative method for detection of steatorrhea. *J Clin Gastroenterol* 1994; 19: 206-9.
- (13) W. Gerver, R. de Bruin. *Paediatric Morphometrics: A reference manual*. 1th ed. Utrecht: Bunge, 1996.
- (14) H. Heijerman, C. Lamers, W. Bakker. Omeprazole enhances the efficacy of pancreatin (pancrease) in cystic fibrosis. *Ann Intern Med*. 1991; 114: 200-201.

## SUMMARY

**In chapter one**, the pathogenesis, clinical manifestations and treatment modalities of cystic fibrosis are briefly summarized. CF is a multisystem disease, the basic defect is a mutation of the CFTR gene. Until now, more than 200 mutations have been characterized. CFTR has been found in epithelial cells of several organs with the lung and pancreas being mostly affected. The role of gene therapy in the management of CF patients is not yet settled. Until then treatment of these patients has to focus on support of lung function and improved fat absorption in order to maintain a normal nutritional status. From our literature review, only predigested foods such as (semi)elemental diets and very high-energy polymeric diets, have been reported to improve the nutritional condition in CF patients. Low duodenal pH is thought to be at least partly responsible for the persisting maldigestion. Inhibition of gastric acid secretion by a proton pump inhibitor has been shown to improve steatorrhea in CF adults. The effect of proton pump inhibitors on fat absorption and on the nutritional status of children with CF has not been reported. The effect of treatment on steatorrhea can only be evaluated by regular monitoring of fecal fat loss. The fat balance method being too cumbersome for the repeated evaluation of steatorrhea, we first aimed at developing an alternative test suitable for our purpose. This test (acid steatocrit) was subsequently used to evaluate the effect of lansoprazole (proton pump inhibitor) on steatorrhea in CF patients showing persisting malabsorption while on pancreatic enzymes. The effects of therapy on the nutritional condition of our patients was evaluated simultaneously.

**In chapter two**, the methods used in this study are described. For the determination of fecal fat, the titrimetric method described by van de Kamer and the Sudan staining method were used for the comparison of steatocrit and acid steatocrit methods. Anthropometry, dual-energy X-ray absorptiometry, total body water and bromide dilution techniques were used to assess body composition.

**In chapter three, four and five**, we describe the steatocrit test as an alternative method for the 3 days fecal fat balance method for the monitoring of steatorrhea. Although the steatocrit

test has been reported to be cheap, simple and noninvasive test, its reliability has been questioned. As this might be due to inadequate fat extraction during the centrifugation step of the steatocrit procedure, we aimed at improving fat extraction by acidification of the fecal homogenate. Results obtained by our modified steatocrit method, called the "acid steatocrit", were highly correlated with those obtained by chemical analysis. We found a high sensitivity and specificity for the acid steatocrit.

Results of the evaluation of the nutritional condition of our patients as well as results concerning the presence of persisting steatorrhea in patients on pancreatic enzymes are described in **chapter six**. Despite hypercaloric intake and the use of pancreatic enzymes, our CF patients maintained steatorrhea and showed signs of malnutrition with significantly decreased Z-scores for weight, height, armcircumference, biceps, subscapular and suprailiac skinfolds. Moreover, their fatmass, lean body mass and bone mineral content were significantly decreased when compared to the reference population described by Oggle et al. Treatment of these CF children with lansoprazole as an adjunct therapy of pancreatic enzymes, resulted in a significant decrease in steatorrhea accompanied by a significant improvement in their nutritional condition.

In **chapter seven**, we describe results of our body composition studies in our patients before and after treatment with lansoprazole. Although highly correlated, results from these various methods were shown not to be interchangeable. In absolute terms, the DEXA, the TBW and the skinfold methods showed children with CF to have a severe depletion of fatmass and a slight decrease of FFM. In relative terms, the above results point to lower body fat percentage accompanied by a higher percentage of LBM. Our results with deuterium - bromide do confirm the above results by showing a high relative TBW content and consequently a low relative fat content. Bromide results further show the relative increase of water percentage to be due to a relatively increased extracellular water compartment with a maintained relative body cell water mass. Although small changes in bodyweight were correctly detected by DEXA examination, the latter method was not accurate enough for the differential detection of small changes in FM and FFM. The usefulness of DEXA, TBW and skinfold methods for the assessment of small body composition changes in children is therefore limited.

## SAMENVATTING

**In hoofdstuk een,** zijn de pathogenese, de klinische manifestaties en de therapeutische mogelijkheden voor cystic fibrosis (CF) kort samengevat. Cystic fibrosis is een multisysteem ziekte, waarvan mutatie van de CFTR (cystic fibrosis transmembrane regulator) gene is het basis defect.

Tot dus ver, zijn er meer dan 200 mutaties beschreven. CFTR werd in de epitheel cellen van verschillende organen gevonden. De longen en de pancreas zijn het meest betrokken by deze erfelijke aandoening. De rol van de gen therapie is nog niet bevestigd in de behandeling van CF patienten. De behandeling van deze patienten is er dan ook gericht op de long functies te ondersteunen en een normale voedingstoestand te behouden door het verbeteren van de vet malabsorptie.

Uit het litteratuur overzicht blijkt dat de voedingsstatus van CF patienten alleen effectief te verbeteren is met voorverteerd voedsel zoals (semi)elementaire voeding, of met een zeer hoge energie inname. Een lage duodenale pH is mede verantwoordelijk voor het slechte verteringsproces. Het is bij volwassen CF patienten bekend dat de vet absorptie significant te verbeteren is door remming van de maagzuur secretie met een proton pomp remmer. Er is nog geen studie gedaan naar het effect van dit middel op de vet vertering en de voedings status bij CF kinderen.

Regelmatig monitoring van vet in de ontlasting is noodzakelijk voor de behandeling van vet malabsorptie. De gebruikelijke vet balans methode is hiervoor te omslachtig. Ons eerste doel was het ontwikkelen van een alternatieve test die snel en makkelijk uitvoerbaar is. Deze test (zure steatocriet) werd dan gebruikt om het effect van een proton pomp remmer (lansoprazol) op steatorrhoea in CF patienten met persisterende malabsorptie onder pancreas enzymen, te evalueren. Daarnaast, werd het effect van lansoprazol op de voedingstoestand van onze patienten geevalueerd.

**In hoofdstuk twee,** beschrijven we de methoden die we gebruikt hebben in deze studie. Voor de bepaling van vet in de ontlasting, werden de titrimetrische methode, beschreven door van de Kamer, en de Sudan kleurings techniek gebruikt om de klassieke steatocriet te vergelijken

met de zure steatocriet. De anthropometrische methode, de dual-energy X-ray absorptiometry (DEXA), het totale lichaamswater (TBW) en de bromide dilutie technieken werden toegepast om de lichaamsamenstelling te beoordelen.

In hoofdstuk drie, vier en vijf, beschrijven we de steatocriet test als een alternatieve methode voor de 3 dagen vet balans ter monitoring van vet in de ontlasting. Hoewel de steatocriet test werd gezien als een goedkope, simpele en noninvasieve test, de betrouwbaarheid van deze test wordt betwist. Dit is mogelijk toe te schrijven aan de inadequate vet extractie tijdens het centrifugeren van de steatocriet procedure. Ons doel was de vet extractie te verbeteren door het aanzuren van het faeces homogenaat. De resultaten verkregen met deze gemodificeerde steatocriet genaamd "zure steatocriet", correleerden goed met de resultaten van de chemische vet analyse. We vonden een hoge sensitiviteit en specificiteit voor de zure steatocriet test.

In hoofdstuk zes, bestuderen we de mate van vet malabsorptie en de voedingstoestand van onze CF kinderen behandeld met pancreas enzymen. Ondanks de hypercalorische voeding en de behandeling met pancreas enzymen, hadden onze patiënten aanhoudende steatorrhoea en toonden tekenen van malnutritie met significante verslechtering van de gemiddelde Z-scores voor gewicht, lengte, armomtrek, biceps, subscapulaire en suprailiacale huidplooiën. Bovendien, hun vetmassa, spiermassa en botmineral is significant lager dan die van de normale kinderen, beschreven door Oggle. Na de behandeling van deze kinderen met een proton pomp remmer (lansoprazol) als supplementaire therapie by pancreas enzymen, vonden we een significante vermindering van steatorrhoea met verbetering van de voedingstoestand.

In hoofdstuk zeven, beschrijven we de resultaten van de lichaamsamenstelling van onze patiënten voor en na de behandeling met lansoprazol. Ondanks de hoge correlatie tussen de resultaten van de gebruikte lichaamsamenstelling methodes, zijn deze technieken niet uitwisselbaar. In absolute zin, toonden de DEXA, de TBW en de huidplooi methode een ernstige depletie van de vetmassa en een lichte afname van de vet-vrije massa by CF kinderen. In relatieve zin, wijzen deze resultaten in de richting van een afname van het vet percentage gepaard aan een hoger percentage van lean body mass (LBM). Dit komt overeen met de resultaten van deuterium-bromide, waarbij een hoog TBW percentage en dus een laag vet

percentage gevonden werd. De toename in het TBW percentage is toe te schrijven aan het verhoogde percentage extracellulair water terwijl intracellulair water normaal blijft. Alhoewel de verandering in lichaamsgewicht door het DEXA onderzoek correct werd geschat, was geen van de gebruikte lichaamssamenstelling methodes nauwkeurig genoeg voor het schatten van kleine veranderingen in de vetmassa en vet-vrije massa. De bruikbaarheid van DEXA, TBW en huidplooi methoden voor het schatten van kleine veranderingen in de lichaamssamenstelling bij kinderen is daarom beperkt.

## DANKWOORD

Woorden schieten tekort om mijn dank uit te drukken. Ik ben niet zo goed in taal expressie, toch hoop ik met enkele eenvoudige zinnen iedereen te kunnen bedanken, die het mij mogelijk hebben gemaakt dit proefschrift vorm te geven.

Zonder iemand tekort te willen doen, richt ik een speciaal dankwoord tot de volgende personen:

**Prof. Dr. C. Blanco**, promotor, beste Carlos, ondanks je drukke taak, heb je toch heel snel en kritisch mijn werkstukken doorgenomen. Hiervoor dank ik je extra.

**Dr. P. Ph. Forget**, copromotor, beste Philippe, het lukte mij nooit je te tutoyeren, niet vanwege onze persoonlijke contacten, maar vanwege mijn respect voor jou. De manier waarop je het onderzoek stuurde waarbij je mij geheel in mijn waarde en vrijheid liet, was van buitengewoon hoog niveau. Je leerde mij wetenschappelijk denken. Waar nodig was bood je hulp aan, soms ook met het verwerken van de resultaten. De correctie van het manuscript was binnen korte tijd klaar. Zelfs in je vakantie, nam je mijn werkstukken mee en was je bereid hiervoor terug te komen. Ik heb genoten van je onuitputtelijke bron van nieuwe ideeën.

Ook in het persoonlijk contact was je aangenaam. Je heb altijd in mij geloofd en stond altijd achter mij. Beste Philippe, zonder jouw inzet en je vertrouwen als begeleider, zou dit proefschrift nooit deze vorm hebben gekregen.

**Dr. B. van Kreel**, copromotor, de helft van mijn tijd als onderzoeker heb ik in uw laboratorium doorgebracht. Uw deur stond altijd voor mij open. Als het niet lukte met de steatocrit-bepaling, heb u altijd nieuwe suggesties. Uw deskundigheid en eerlijkheid was onmisbaar voor het slagen van dit onderzoek.

**Prof. Dr. R. H. Kuijten**, bedankt voor de mogelijkheden die u hebt gecreeërd voor dit onderzoek.

**Drs. A. Van den Neucker**, beste Anita, al die jaren ben je voor mij een goede vriendin geweest. Ook als het mij tegen zat, wist je met je nuchtere kijk en eerlijkheid mijn problemen te relativeren. Ik heb genoten van onze discussies en van je gezelschap op verscheidene congressen. Je interesse in anderen en je brede algemene kennis maakte het zeer boeiend. Anita, je hebt mijn "gat" in de Westerse cultuur opgevuld.

**Hooggeleerde leden van de beoordelingscommissie**, bedankt voor uw vlotte en kritische beoordeling van dit manuscript.

**Alle kinderartsen, neonatologen en arts-assistenten** kindergeneeskunde in het AZM dank ik hierbij voor de aanspraak in de afgelopen jaren.

**Dr. W. J. M. Gerver en Dr. R. De Bruin**, jullie groeicurven hebben grote waarde toegevoegd aan dit onderzoek. Bedankt voor jullie voortreffelijke bijdrage.

**Jolanda van Golde en Rony Neefjes**, beste Rony en Jolanda, bedankt voor het meelevens en de gezellige uren in het AIO-hok, in het restaurant, in het theater aan het Vrijthof, in de bioscoop, bij een van ons thuis of in het zwembad. Bedankt voor het aanhoren van mijn "gezeurd". We hebben goede en slechte tijden met elkaar doorgemaakt. Ik hoop dat onze vriendschap hierdoor alleen maar sterker is geworden.

**Alle medewerkers van het klinisch chemisch laboratorium** van het AZM, met name **Serva, Lou, Michel, Mia, Theo, Peter en Marian**, bedankt voor jullie inzet en betrokkenheid tijdens het onderzoek. Jullie wetenschappelijke interesse was van niveau. Als ik hulp nodig had waren jullie bereid, soms ook ongevroegd, het eigen werk neer te leggen en mij bij te staan. Bedankt voor de aangename sfeer en de gezellige samenwerking.

**Liesbeth van der Ploeg en Lianne Schoorlemmer**, dietisten, wil ik danken voor het uitrekenen van de calorieën bij mijn patiënten populatie.

**Dr. G. A. K. Heidendal, Piet Willems en Sandra Zimny** van de nucleaire afdeling, bedankt voor jullie fijnzinnige instructies over de DEXA scan.

**Alle poli-assistenten en de secretaresses** van de kindergeneeskunde, wil ik danken voor de samenwerking in de afgelopen jaren.

**Oom Wim en tante Margriet van der Avoort**, bedankt voor jullie steun en betrokkenheid in de afgelopen 15 jaren van mijn leven in Nederland.

Ik ben de firma's **Hoechst Marion Roussel B.V.** (Hoevelaken) en **Janssen-Cilag B.V.** (Tilburg) dankbaar voor hun financiële ondersteuning in de drukkosten van dit proefschrift.

Tenslotte, zou dit boek niet volledig zijn zonder hulp en meelevens van mijn familie. Lieve mama, oom Kiet, Manh Hung en Manh Cong, terwijl ik rustig aan mijn proefschrift werkte, hebben jullie voor mijn verhuizing gezorgd.

**Manh Cong**, bedankt voor het ter beschikbaar stellen van je computer en Manh Hung voor je

deskundige steun. Als ik met de computer problemen had, kon ik altijd op jullie terugvalen. Oom Kiet, bedankt voor je inzet en betrokkenheid. Nooit hoefde ik je om hulp te vragen, je was er gewoon.

Lieve Mama, zonder jou zou dit boek er nooit zijn gekomen. Heel je leven lang heb je voor ons klaar gestaan. Jouw droom is een goede toekomst voor je kinderen. Daarvoor heb je 15 jaar geleden je leven op het spel gezet. Je stimuleerde ons om te studeren. Rijkdom is niet belangrijk, maar kennis, dat is de beste bagage die je op onze weg aan ons hebt kunnen meegeven. Mama, bedankt voor je betrokkenheid en het aanhoren van mijn frustraties. Met een glimlach en een schouderklop wist je al mijn problemen op te lossen. Mama, ik hou van jou en ik ben trots dat jij mijn moeder ben.

## CURRICULUM VITAE

Thi My Dung Tran werd op 27 april 1967 te Dinh Tuong in Vietnam geboren.

Na het doorlopen van het basisonderwijs volgde zij, eveneens in Vietnam, drie jaren vervolgonderwijs op voorbereidend wetenschappelijk niveau.

In 1981 kwam zij met haar familie in Nederland.

Op 2 juni 1986 verwierf zij aan de Rijksscholengemeenschap "Den Hulster" te Venlo het diploma Atheneum B. met als eindexamenpakket de vakken: Nederlands, Engels, Wiskunde I en II, Natuurkunde, Scheikunde en Biologie.

Vanaf het najaar 1986 studeerde zij geneeskunde aan de Rijksuniversiteit te Maastricht. Zij behaalde op 13 augustus 1990 haar doctoraal getuigschrift. Op 1 februari 1993 werd het diploma basisarts aldaar aan haar uitgereikt.

Tijdens haar studie verrichte zij wetenschappelijk onderzoek onder leiding van Dr. P. PH. Forget op de afdeling kindergeneeskunde van het Academisch Ziekenhuis Maastricht: "Singel Stool analysis for fat, alfa-animo nitrogen and electrolyt".

Vanaf 1 maart tot 3 november 1993 werkte zij als arts-onderzoeker bij de vakgroep kindergeneeskunde van het A.Z.M. aan het project "Effect of ranitidine in children with chronic abdominal pain".

Van 1 december 1993 tot 1 december 1994 was zij werkzaam als AGNIO kindergeneeskunde in het A.Z.M.

In de periode 1 december 1994 tot 1 april 1996 werkte zij onder leiding van Dr. P. PH. Forget, kinder-gastroenteroloog in het A.Z.M., aan haar promotie-onderzoek.



**Steatorrhea and nutritional condition  
in cystic fibrosis children effects  
of a proton-pump inhibitor**



T.M.D. Tran

**STEATORRHEA AND NUTRITIONAL CONDITION IN CYSTIC FIBROSIS CHILDREN  
EFFECTS OF A PROTON - PUMP INHIBITOR**

## CONTENTS

<b>Chapter 1</b>	<b>General introduction - literature review - Aims of the study</b>	<b>1-31</b>
	1. Genetics of cystic fibrosis	
	2. Pathogenesis	
	3. Clinical manifestations	
	4. Diagnosis	
	5. Therapy	
	6. Evaluation of steatorrhea	
	<b>Aims of the study</b>	
	References	
<b>Chapter 2</b>	<b>Methods</b>	<b>32-38</b>
	1. Methods used for fecal fat determination	
	2. Methods used for assessment of nutritional condition	
<b>Chapter 3</b>	<b>The acid steatocrit: A much improved method</b>	<b>39-49</b>
	Tran M., Forget P., Van den Neucker A., Strik J., van Kreeel B., Kuijten R.	
	J Pediatr Gastroenterol Nutr 1994; 19: 299-303	
<b>Chapter 4</b>	<b>Improved steatocrit results obtained by acidification of fecal homogenates are due to improved fat extraction</b>	<b>50-58</b>
	M. Tran, P. Forget, A. Van den Neucker, B. Van Kreeel	
	J Pediatr Gastroenterol Nutr 1996; 22: 157-160	
<b>Chapter 5</b>	<b>Clinical use of acid steatocrit</b>	<b>59-66</b>
	A. Van den Neucker, N. Pestel, T. My Dung Tran, P. Ph. Forget, H. J. Veeze, J. Bouquet, M. Sinaasappel	
	Submitted for publication	
<b>Chapter 6</b>	<b>Role of lansoprazole in children with cystic fibrosis: Evidence for improved fat malabsorption and nutritional status</b>	<b>67-83</b>
	Tran TMD, Van den Neucker A, Hendriks JJE, Forget P (junior), Forget P (senior)	
	Submitted for publication	

<b>Chapter 7</b>	<b>Anthropometry and body composition methods in children with cystic fibrosis: Effects of nutritional intervention</b>	84-107
	My-Dung T. Tran, Anita Van den Neucker, Han J. Hendriks, Bernard van Kreel, Patricia Forget, Guido Heidendal, Pierre-Philippe Forget	
	Submitted for publication	
<b>Chapter 8</b>	<b>General discussion</b>	108-112
<b>Summary</b>		113-114
<b>Samenvatting</b>		115-117
<b>Dankwoord</b>		118-120
<b>Curriculum vitae</b>		121

**STEATORRHEA AND NUTRITIONAL CONDITION IN CYSTIC FIBROSIS CHILDREN**  
**EFFECTS OF A PROTON-PUMP INHIBITOR**

**PROEFSCHRIFT**

Ter verkrijging van de graad van doctor  
aan de Rijksuniversiteit Limburg te Maastricht,  
op gezag van de Rector Magnificus, Prof.Mr. M.J. Cohen,  
volgens het besluit van het College van Dekanen,  
in het openbaar te verdedigen  
op donderdag 17 oktober 1996 om 16.00 uur

door

Therese Marie Pascale Thi My Dung Tran  
geboren op 27 april 1967 te Dinh Tuong, Vietnam

**Promotor:** Prof. Dr. C. Blanco

**Co-promotores:** Dr. P-Ph. Forget  
Dr. B. van Kreel

**Beoordelingscommissie:** Prof. Dr. P.B. Soeters, ( voorzitter )  
Prof. Dr. H.S.A. Heymans, ( Universiteit van Amsterdam )  
Prof. Dr. R.W. Stockbrugger  
Prof. Dr. J.M. Wit, ( Rijksuniversiteit Leiden )  
Prof. Dr. E.F.M. Wouters

Steatorrhea and nutritional condition in cystic fibrosis children:  
Effects of a proton - pump inhibitor /  
Therese Marie Pascale Thi My Dung Tran.  
Proefschrift Maastricht - Met lit. Opg. - Met samenvatting in het Nederlands.  
ISBN 90-5681-011-1

Trefw.: Steatocriet / steatorrhoe / cystic fibrosis / voedingstoestand / proton -  
pomp remmer / lichaamsamenstelling.

Vormgeving: My Dung Tran  
Omslagillustratie: Vetbollen in een microscopische faeces preparaat,  
met Soudan kleuring.

STEVENS-REISSNER SYNDROOM

*Aan mijn lieve moeder  
Voor alle cystic fibrosis kinderen . . .*

## CHAPTER 1

### GENERAL INTRODUCTION

## **1. GENETICS OF CYSTIC FIBROSIS (CF)**

Cystic fibrosis was first described in 1928 by Fanconi (1). It is an autosomal recessive disease and is reported in all racial groups with varying prevalence. In caucasians, CF occurs in 1 out of 2000 live births. Males and females are equally affected. The basic defect is a mutation of the Cystic Fibrosis Transmembrane Regulator (CFTR), a protein responsible for chloride ion transport in response to cAMP mediated signals. The most frequent CF mutation in the caucasian population is a deletion of 3 nucleotides, encoding for phenylalanine at position 508 in the CFTR protein amino acid sequence. Its overall frequency reported by the CF Genetic Analysis Consortium is 68% (2). Until now, over 200 mutations have been characterized and account for the remaining mutations.

## **2. PATHOGENESIS**

It is generally accepted that cAMP stimulated chloride conductance is a function of the CFTR (3). This function is deficient in epithelial cells of CF patients. The inability to secrete chloride and secondarily secrete water results in viscous secretions. Poor clearance of these viscid secretions from the epithelium often results in obstruction of excretory ducts. CFTR has been found in epithelial cells of several organs such as the airways, the sweat glands, the genitourinary system and the gastrointestinal tract including the pancreas and the biliary tract (4). Dysfunction of these organ systems are therefore possibly related to the same underlying defect in the CFTR-gene product.

## **3. CLINICAL MANIFESTATIONS**

CF is a multisystem disease with lungs and pancreas mostly affected in young patients.

### **3.1 Respiratory tract**

Lung disease accounts for more than 95% of the morbidity and mortality in CF (5,6). The desiccated mucus in the respiratory tract causes stasis and bronchiolar obstructions, resulting

in bacterial overgrowth and chronic lung infection. This gives rise to the production of proteases by bacteria and neutrophils. These enzymes hydrolyze important structural proteins of the lung and airways such as elastin, proteoglycans and collagen, leading to instability of bronchial walls and bronchiectasis. Furthermore, these enzymes also alter the mucosal function by increasing the secretion of macromolecular glycoconjugates contributing to a high viscosity of the mucus (7). Bronchiolitis with wheezing is frequent during the first year of life. Some patients remain however, asymptomatic for long periods. When pulmonary disease progresses, exercise intolerance occurs and finally, progressive pulmonary deterioration is the main cause of death in these patients (6,8). As a consequence of improved supportive therapy, survival has increased from 6 months at the end of the fifties (9) to nearly 30 years currently (10,11). Sinusitis and nasal polyposis sometimes occur in CF (12,13).

### **3. 2 Pancreas**

In the pancreas, obstruction of the ductules is the cause of acinar / ductular distention, followed by disruption with release of proteolytic enzymes and autodigestion of the pancreas resulting in pancreatic insufficiency with steatorrhea. The changes in the pancreas can occur early during gestation, compromising the normal maturation of the pancreas. Approximately, 85% of CF patients have steatorrhea (11). In 85 - 90% of these cases, exocrine pancreatic insufficiency develops during the first year of life. Decreased secretion of bicarbonate and water first occurs before a decrease of pancreatic enzyme concentration in duodenal fluid can be detected (14-17). Recurrent acute pancreatitis occurs in approximately 10% of CF patients (18).

Because of fat malabsorption, serum concentrations of fat soluble vitamins are often lowered. Since Vit A consists of esters of long chain fatty acids, it cannot be absorbed in the absence of pancreatic esterases. Due to its short half life, low serum levels of Vit A are often found in early untreated CF patients (19,20). Vit D deficiency resulting in decreased bone mineralization has also been reported in CF patients (21-23). Due to frequent antibiotic therapy, suppression of endogenous vit K synthesis by anaerobic intestinal bacteria often contribute to a low vit K serum level in CF patients with steatorrhea. Although Vit B12 is water soluble, serum levels may also be low in CF patients. Binding to intrinsic factor, necessary for absorption,

can only take place after cobalamin has been released from the R-protein binding by pancreatic enzymes. Decreased pancreatic bicarbonate secretion may play a role herein since the binding affinity of cobalamin for R-protein decreases at neutral or slightly alkaline pH (24). However, pancreatic enzymes supplements will normalize the Vit B12 serum level.

Abnormal glucose tolerance occurs in 30 - 75% of CF patients while diabetes mellitus develops in 10% (11). Several diabetogenic factors including increased passive sugar transport (25), increased mucosal absorption of D-glucose (26), decreased beta cell mass (27) and delayed insulin secretion (28) are present in CF. On the contrary, several antidiabetogenic factors such as an increased tissue insulin sensitivity (29) and an increased number of insulin receptors on monocytes (30) have been reported in cystic fibrosis. Moran et al. reported a decreased alpha-, beta- and pancreatic polypeptide- cell function in CF patients with exocrine disease compared to those without this disorder. Due to this finding, they suggest that either exocrine disease causes endocrine dysfunction or that a common pathogenic process simultaneously and independently impairs exocrine and endocrine function in CF patients (31). However, the exact etiology of diabetes in CF is still unknown.

### **5. 5 Malnutrition**

CF children are malnourished when compared to normal controls (32,33). Both, malabsorption accompanying pancreatic insufficiency (34) and high energy expenditure due to chronic lung infection (35,36) are thought to be responsible for the poor nutritional condition in these patients. Moreover, in CF, several intraluminal factors other than pancreatic insufficiency are also considered responsible for fat malabsorption:

1) Increased gastric acid secretion after stimulation with pentagastrin (37):

A high postprandial acid secretion could, by lowering the duodenal pH, contribute to fat malabsorption.

2) Decrease pancreatic bicarbonate secretion (13-16):

Higher gastric acid secretion after meals together with a decrease in pancreatic bicarbonate secretion, has been shown to result in a prolonged postprandial lowering of duodenal pH with

inactivation of the remaining pancreatic lipase. Moreover, low duodenal pH also results in bile acid precipitation,, fecal loss of bile acids and a decrease in the bile acid pool, contributing to fat malabsorption

### 3) Increased glycine to taurine conjugated bile acid ratios:

Due to a relatively deficient supply of taurine compared to glycine and to continuous fecal loss of bile acids, newly formed bile acids are mainly conjugated with glycine, leading to high glycine-/taurine- bile acid conjugation ratios (38). The glycine conjugated bile acids precipitate in an acidic environment contributing to the luminal bile acid deficiency in these patients.

## 3. 4 Intestinal tract

Gastroesophageal reflux and esophagitis are frequent causes of epigastric pain in CF patients (39,40) and can be responsible for decreased pulmonary functions (41). Peptic ulcers are found in up to 13% of CF patients (42) and are thought to be related to the low duodenal pH (43). Meconium ileus occurs in 15% of newborn infant with CF (44); 10% of CF patients have meconium ileus "equivalents" at a later age with a peak incidence at 20-25 years of age (45). Protein precipitation as a result of decreased duodenal pH and high secretion viscosity all probably contribute to these obstructive events (11). Up to 25% of the CF patients have rectal prolapse occurring mostly in children aged 6 - 36 months (46) while intussusception has been shown to occur in 1% (47).

## 3. 5 Biliary and Hepatic tracts

An increased incidence of cholelithiasis has been reported in CF patients (48) and is thought to be related to hypokinesia and increased fasting gallbladder volumes (49). Biliary cirrhosis with hepatosplenomegaly leading to portal hypertension occurs in 25% of CF patients. Liver steatosis has been reported in 30% of patients with CF.

## 3. 6 Genitourinary tract

More than 95% of males are infertile due to obstruction of the reproductive tracts (50). Active spermatogenesis does occur but produced spermatozoa are abnormal or immature (51). In CF women, the reproductive tracts are anatomically normal but fertility is decreased (52). Increased viscosity of the cervical mucus is thought to interfere with sperm penetration (53).

### 3.7 Sweat gland

Decreased sodium and chloride reabsorption due to dysregulation of sweat gland duct cells results in susceptibility of CF patients to salt depletion during warm weather and during gastroenteritis.

### 4. Diagnosis

The standard diagnostic procedure is the sweat test based on increased concentration of electrolytes in the sweat of the patients (54). The sweat test was developed by Gibson and Cooke (55), whereby the sweat production is stimulated by pilocarpine iontophoresis. The then collected sweat is analysed for its chloride and sodium content. However, chloride content has a better diagnostic value than sodium content, since abnormal sodium secretion can also be found in other endocrine diseases. Sweat chloride concentration higher than 60 mM or sodium above 70 mM measured minimal on two conditions is considered as abnormal, whereas chloride values under 50mM and sodium value under 30mM are found in normal persons. Chloride concentrations between 50 and 70 mM are inconclusive. For reliable results, at least 50mg of sweat should be collected. Low sweat production in the first few weeks of life is the reason for unreliable test results in this age group. In cases of doubt, identification of CFTR-mutation or measurement of intestinal current in a rectal biopsiate have been reported to be conclusive (56).

### 5. Therapy

The ideal treatment of CF should be the correction of the underlying defect by introduction of a normal copy of the defective gene into these patients genetic material. Gene therapy is

presently under intensive scrutiny. Adenovirus and recently also the retrovirus seem promising as an effective vector for normal gene transport into the target cells (57,58). Recently, transfer of the CFTR gene to the rat airways epithelia has been successfully performed (59). However, the role of gene therapy in the management of CF patients is not yet settled. Until then, treatment of CF patients has to focus on improving the nutritional condition, since malnutrition can adversely affect survival (60). The nutritional status of CF patients can be improved by firstly ameliorating the respiratory function, thereby minimizing energy expenditure and secondly, by increasing energy supply either by increasing nutrient intake or by improving nutrient digestion and absorption.

### **5.1 Respiratory function support**

Since viscous mucus in the lung is the cause of chronic lung infection, efforts should be made to improve mucus clearance. Although most patients on mucolytics such as acetylcysteine have the feeling of decreased sputum viscosity, studies with acetylcysteine either orally or as aerosol have failed to support this finding (61-63). Alternative methods such as chest percussion combined with postural drainage (64), positive expiratory pressure mask (65,66) and forced expiratory pressure (67) have been suggested to improve mucus clearance. Moreover,  $\beta_2$ -agonists as aerosol can increase sputum clearance (68) and some bronchodilating effect has been experienced in CF patients on this regimen (69,70). Corticosteroids, have been found to delay disease progression and to improve lung function in CF patients (71-74) but, short-term adverse effects such as hyperglycemia and long-term adverse effects such as development of cataract and growth retardation preclude the routine use of corticosteroids in these patients (73).

Treatment with antibiotics can reduce the progression of lung infection. Colonisation with *Ps. aeruginosa* often occur in CF patients and various regimes have failed to eradicate the bacteria (75). *Ps. aeruginosa* vaccines are presently being evaluated (76). In the end stage, lung transplantation can offer an outcome. The one and two year survival rates approach 70% and 54% respectively (77). Amiloride inhalation by blocking sodium reabsorption in the respiratory epithelium, has been shown to increase sputum clearance in a placebo-controlled cross-

ver study (78,79). Although improvement in pulmonary function was not found in one study (78), a delay in the deterioration of forced vital capacity (FVC) was reported by an other author (79). Dornase (Pulmozyme), a recombinant human desoxyribonuclease which breaks off the sputum DNA, has been reported to increase the forced expiratory volume (FEV1) and FVC safely in CF patients (80-82). Inhalation of  $\alpha$ 1-antitrypsine inhibits neutrophil elastase (83), which is released from the neutrophils and causes lung damage. Chloride channel facilitators, which directly stimulate a CFTR protein independent anion channel, are presently being evaluated (84).

## 5.2 Increase energy supply

In the past, restricted diets with **low fat content** were often prescribed for CF patients in order to minimize steatorrhea, abdominal cramps and stool bulk (85-87). Due to both unpalatability and low caloric density, these diets often resulted in **malnutrition and growth failure** in these patients (87-89). In the early 1970s, Crozier introduced the use of **high fat diets** in combination with pancreatic enzymes in order to increase the energy intake of CF patients. This regimen resulted in **better growth** with evident steatorrhea (90). Moreover, CF children from clinics using low fat diets were reported to show poorer growth (87-89) than those from clinics, encouraging the use of high fat diets (91). In order to further improve the nutritional status and growth of CF patients, feeding intervention studies have been done with different kinds of nutrients such as hypercaloric polymeric, semielemental or elemental diets. It has been shown that interventions making use of very high caloric intakes of polymeric diets (150 - 180% Recommended Daily Allowance) by overnight nasogastric tube resulted in improved nutritional status in CF adults. In children with CF, the effects of interventions with hypercaloric polymeric diets up to 130% of RDA are however unconvincing. Luder and coworkers, studying the effects of a 4 year period of nonrestricted fat diet in CF children, found improved Z-scores for weight, height and BMI for their CF patients when compared to the national population of CF patients on fat restricted diets, while no changes were seen when compared to normal children without CF (92). More recently, studies with hypercaloric polymeric diets with high fat content did not result in significant improvements of Z-scores for weight, height and skinfolds in CF patients (93), whereas **parenteral nutrition** and either oral or enteral

**elemental and semielemental** nutrition have been shown to **significantly improve the nutritional condition** of these patients (94-106). The results of short-term and long-term studies of feeding interventions on the nutritional status in CF children are summarized in table 1 and 2. The fact that predigested food can improve the nutritional status better than standard diets, strongly suggests that nutrient maldigestion plays a crucial role in the poor response to oral hypercaloric polymeric diets. The latter hypothesis is further supported by the known inactivation of pancreatic enzymes and bile acids precipitation accompanying the low duodenal pH due to low bicarbonate secretion in CF patients (107-110) . **Enteric-coated pancreatic enzyme** preparations have therefore been introduced but the low duodenal pH interferes with the release of enzymes through the acid resistant coating (111). **High doses of pancreatic enzymes** did not solve the problems of malabsorption (112) and recently, colon strictures have been observed in CF children on high doses of pancreatic enzymes (113-115). Attempts have been made to inhibit gastric acid production in the hope to improve the digestion and absorption of nutrients. However, the reported effects of **H2-receptor antagonists and prostaglandine E2** on steatorrhea have been variable and unconvincing (116-125). Results of short-term studies of cimetidine and misoprostol on fat excretion have not been consistent (table 3). This may be partly due to the lack of control of dietary fat intake. In long-term studies cimetidine showed no significant changes in fat excretion and nutritional status in CF children. on the contrary, **famotidine**, a more potent inhibitor of gastric acid secretion, showed both a significant improved fat absorption coefficient and improved growth parameters (table 4). However, interpretation of growth effects in the latter study is rather difficult because Z-score methods have not been used to evaluate growth. Further, in a double blind study, a significant improvement in steatorrhea was found when a **proton pump inhibitor** was added as adjuvant therapy in pancreatic enzyme treated CF adults (112). In children with CF, the effects of proton pump inhibitors on fat absorption and on the nutritional condition have not been reported.

**Table 1 short-term feeding intervention studies in cystic fibrosis.**

Authors	<sup>10</sup> Shepherd et al., '80	<sup>11</sup> Shepherd et al., '83	<sup>12</sup> Bertrand et al., '84	<sup>13</sup> Mansell et al., '84	<sup>14</sup> Ciociani et al., '85	<sup>15</sup> Loughlin et al., '86	<sup>16</sup> Remmel et al., '95
Number of cases	12	7	10	11	21	10	15
Age range	0.5 - 11 y	5 - 13 y	3 - 12 y	10 - 17 y	1 - 14 m	7 - 28 y	5 - 27 y
Nutritional status	malnutrition	malnutrition	malnutrition	malnutrition	normal	malnutrition	malnutrition
Study duration	1 month	6 months	1 month	1 month	5 days	6 months	3 months
Type of study	prospective own control	prospective own control	prospective own control	prospective own control	prospective CF control	prospective CF elemental CF polymeric	prospective own control
Feeding intervention	TPN 90 - 100 % RDA	elemental 20 - 40 % RDA (extra)	elemental 110 - 150 % RDA	TPN 120 % RDA	semielemental 142 kcal/kg	elemental 35% RDA (extra) versus hypercaloric	hypercaloric polymeric 130 % RDA
Route	parenteral	enteral	nasogastric	parenteral	oral	enteral	oral
Effect	SDS weight ↓ SDS height ↓ MUAC% std ↓ clinical score ↓ FVC, FeV1 ↓ PEF ↓	SDS weight ↓ SDS height ↓ MUAC % std. ↓ FM ,LBM (kg) ↓ muscle mass (kg) ↓ clinical score ↓ 3-meHis excre ↓	weight/height ↓ skinfold % std. ↓ MUAC % std. ↓ fat excretion (ns) MAMC % std. = FeV1, FEF = final work load =	weight (kg) ↓ height (cm) ↓ skinfold (mm) ↓ MAMC (cm) ↓ MIP, MEP ↓ FeV1, FEF =	Weight (kg) ↓ N-excretion ↓ fat absorption coefficient ↓	Weight/height % std. ↓ SDS height ↓ FM,LBM (kg) ↓ fat excretion ↓ clinical score ↓ FeV1, FEF, FVC ↓	SDS weight (ns) SDS height (ns) growth velocity (ns) skinfold (ns) weight (kg) ↓
Follow up (duration)	all parameters improve further (after months)	NR	all parameters ↓ (after 2 months)	MIP, MEP = FeV1, FEF = (after 2 months) all other parameters ↓ (after 1-6months)	NR	NR	NR

MIP : Maximal Inspiratory Pressure

MEP : Maximal Expiratory Pressure

FeV1 : Forced expiratory volume in 1 sec.

SDS : Standard deviation score

N-excretion : Fecal Nitrogen excretion

MUAC : Mid Upper Arm Circumference

MAMC : Mid Arm Muscle Circumference

FVC : Forced Vital Capacity

FEF : Forced Expiratory Flow

PEF : Peak Expiratory Flow

LBM : Lean Body Mass

FM : Fatmass

RDA: Recommended daily allowance

3-meHis excre : 3 methylhistidine excretion in urine

↑ : Improved

↓ : Decreased

= : unchanged

(ns) : not significant

NR: not reported

**Table 2 Long-term feeding intervention studies in cystic fibrosis.**

Authors	<sup>10</sup> Allan et al, '73	<sup>11</sup> Berry et al, '75	<sup>12</sup> Yassa et al, '78	<sup>13</sup> Levy et al, '85	<sup>14</sup> Boland et al, '86	<sup>15</sup> Sheperd et al, '86	<sup>16</sup> Farrell et al, '87	<sup>17</sup> Luder et al, '89
Number of cases	17	15	43	14	10	10	36	37
Age range	2 - 21 y	10m -18y	3 - 16 y	5 - 22 y	5 - 20 y	3 - 13 y	3 - 4 m	2 - 27 y
Nutrition status	malnutrit	malnutrit	malnutrit	malnutrit	malnutrit	malnutrit	normal	malnutrit
Study duration	3 months to 3 years	1 year	1 year	1,1 year	10 - 36 months	1 year	8 months	4 years
Type of study	prospect own control	prospect CF control	prospect CF control	prospect CF control	prospect own control	prospect CF control	prospect CF control	prospect own control
Feeding intervent	elemental 50-100% RDA	elemental 100 % RDA	elemental 100 % RDA	(semi) elemental 30 % RDA (extra)	predigest non-elemental 1000 to 2000Kcal	semielemental 120-140 % RDA	pregestimil versus standard 120Kcal / kg	hypercal polymeric 120 % RDA
Route	oral	oral	oral	gastrostomy	jejunostomy	enteral	oral	oral
Effect	SDSwei ↓ SDShei ↓ clinical score ↓	SDSwei ↓ clinical score ↓ SDS hei (ns)	SDSwei ↓ SDShei ↓ SDSskf ↓ boneage ↓	weight (kg) ↓ height (cm) ↓ wei/hei % std. growth velocity ↓ BF % ↓ FFM ↓ TBK ↓ TBN ↓ ‡FVC = ‡FeV1 =	SDSwei ↓ MAMC ↓ FVC =	SDSwei ↓ SDS hei ↓ protein synthes ↓ protein catabol ↓ FeV1 ↓ FVC ↓ FEF ↓	weight (kg) ↓ height (cm) ↓ growth velocity ↓	SDSwei (ns) SDS hei (ns) FEF = BMI ↓
Follow up (duration)	NR	NR	all parameters ↓ except bone age (1 year)	NR	NR	NR	NR	NR

↓ : Improved

↓ : Decreased

= : unchanged

RDA : Recommended Daily Allowance

SDSwei : SDS weight

SDShei : SDS height

SDSskf : SDS skinfolds

LBM : Lean Body Mass

FFM : Fat Free Mass

TBN : Total body nitrogen

TBK : Total Body Kalium

FVC : Forced Vital Capacity

FeV1 : Forced expiratory volume ‡ FVC and FeV1 decrease in CF control

FEF : Forced Expiratory Flow

BMI : Body Mass Index

BF : Body fat

N-excret : Nitrogen excretion

(ns) non significant

Abs. coeff : absorption coefficient

‡ FVC and FeV1 decrease in CF control

MAMC : Mid Arm Muscle Circumference

**Table 3 Effect of short-term use of gastric acid inhibitors on steatorrhea and nutritional status in CF children.**

Authors	<sup>117</sup> Cox et al., '79	<sup>118</sup> Boyle et al., '80	<sup>122</sup> Durie et al., '80	<sup>126</sup> Gow et al., '81	<sup>128</sup> Schöni et al., '81	<sup>129</sup> Cleghorn et al., '83	<sup>131</sup> Robinson et al., '90
number of cases	10	8	15	10	10	11	15
age range	6 - 27 y	12 - 25 y	10 - 17 y	6 - 13 y	11 - 17 y	2 - 17 y	0.5 - 13.8 y
type of study	prospective crossover open	prospective randomized crossover	prospective randomized crossover	prospective randomized crossover	prospective open	prospective open	double-blind placebo controlled crossover
pancreatic enzyme	Cotazym or Viokase	Viokase	Cotazym	Pancrease	Eurobiol	Pancrease	Pancrease
intervention	cimetidine 150-200mg / day	cimetidine 300 mg / day	cimetidine 20 mg / kg / day	cimetidine 20 mg / kg / day	cimetidine 600 mg / m <sup>2</sup> / day	misoprostol 400 µg / day	misoprostol 400 µg / day
duration	1 week ?	5 days	7 days	14 days	6 days	1 week	3 weeks
effect on steatorrhea	fat excretion ↓ N-excretion ↓ fat abs coeff ↓	fat excretion ↓ fat abs coeff ↓ fecal weight ↓	fat excretion ↓ N-excretion ↓ fecal weight ↓	fat excretion (ns) N-excretion (ns) fecal weight(ns)	fat abs. coeff. (ns) N abs coeff (ns)	fat excretion normalized fat abs coeff (ns)	fat excretion ↓
effect on nutritional status	ND	ND	ND	ND	ND	ND	ND
comments	no diet evaluation per treatment period	results of diet evaluation not given	no effect on steatorrhea in patients with fat intake > 120 g / day	-	-	fat intake not controlled	fat absorption not improved in patients with < 10% fat malabsorption

abs coeff : fat absorption coefficient

N-excretion : fecal Nitrogen excretion

(ns) : not significant

**Table 4 Effect of long-term use of gastric acid inhibitors on steatorrhea and nutritional status in CF children.**

Authors	<sup>123</sup> Schöni et al, '84	<sup>124</sup> Chalmers et al, '85	<sup>125</sup> Carroccio et al, '92
number of cases	38	16	10
age range	mean 13 y	5 - 19 y	7 - 18 y
type of study	prospective randomized doubleblind	double-blind crossover	double-blind crossover
pancreatic enzyme	Pancrease	Cotazym	Pancrease
intervention	cimetidine 600mg/m <sup>2</sup> /day	cimetidine 25mg/kg/ day	famotidine 1mg/kg/day
duration	4 months	6 months	6 months
effect on steatorrhea	plasma lipid and lipoprotein (ns)	fat excretion ↓ N-excretion (ns) fecal weight (ns)	fat absorption coeff. ↓ fecal weight ↓
effect on nutritional status	weight, height (ns) skinfolds (ns) TLC, TGV (ns) Raw, sGaw ↓	SDSweight (ns) SDSheight (ns) skinfolds (ns) bone age (ns) clinical score (ns)	weight (kg) ↓ height (cm) ↓ clinical score (ns)
comments	no diet evaluation	results of diet evaluation was not given	results of diet evaluation was not given

SDS : Standard Deviation Score for age and sex

TLC : Total Lung Capacity

TGV : Thoracic Gas volume

Raw : airway resistance

sGaw : specific conductance

(ns) : not significant

## 1.6 EVALUATION OF STEATORRHEA

### 1.6.1 Fecal fat balance

The 3 days fecal fat excretion while patients are on a standard fat diet is the most reliable method for quantitative determination of fecal fat loss. The fat absorption coefficient is calculated by the following formula:

$$(\text{Fat ingested} - \text{fat excreted}) / \text{fat ingested} \times 100$$

Normal fat absorption coefficient at different ages have been reported as follows:

Age > 1 year :  $\geq 95\%$  (126-128)

Age < 1 year :  $> 83\%$  if formula fed and  $> 93\%$  if breast fed (126)

Premature infants : 38 - 73 % depending on the formula used (129)

Fecal fat can be determined by either Gravimetric or Titrimetric methods. For both methods, fecal fat is extracted with an organic solvent, the fat content is subsequently measured either by weighing (Gravimetric method) or by titration (Titrimetric method). The Gravimetric method determine all fecal lipid components, resulting in erroneously high results. On the contrary, the titrimetric method only measures fatty acids. Fecal lipids are first saponified and subsequently acidified to liberate fatty acids which are then extracted. Since its first description in 1949 (130), the **titrimetric procedure of van de Kamer** has been used as a reference method for the evaluation of malabsorption. The fat balance method is **reliable** for the quantification of fecal fat loss with a coefficient of variation of 4,6 % (131). However, the determination procedure is **time consuming, expensive and necessitates sophisticated apparatus**. Further, since the fat excretion is dependent on fat intake, patients have to keep up a **strictly fat constant diet** of more than 80 gram per day. Moreover, **fecal collection** have to be done very accurately. The balance method consequently is poorly reliable in outpatients, especially in children and infants. When fat balance is not possible, measuring **fecal fat concentration** in a fecal sample can be used for the screening of fat malabsorption. Results are then expressed as percent of wet fecal weight (fecal fat concentration). Using the  $^{14}\text{C}$ -triolein/ $^3\text{H}$ -oleic acid test as a reference method, Pedersen et al. have studied the diagnostic

value of fecal fat concentration as measured by the titrimetric method of van de Kamer in a 72 hours fecal collection without controlling for dietary fat (132). In this study, a **similar diagnostic value** was found for both **fecal fat concentration (FFC)** and **fecal fat excretion (FFE)**: The sensitivity, specificity, positive predictive value and negative predictive value of FFC versus FFE were respectively 93,1% versus 90%; 92,4% versus 89,4%; 90% versus 93% and 89% versus 90% with a **day to day coefficient of variation of 29% for FFC and 64% for FFE**. In only 6% of the patients studied, the FFC when measured in a single day sample differed from the mean 3-day fecal fat concentration value whereas the FFE differed from the mean 3-day fecal fat excretion in 37% of the patients. FFC correlated weakly but significantly with FFE ( $r = 0,55$ ;  $p < 0,01$ ) (133). FFC results in pancreatic steatorrhea being higher than in nonpancreatic steatorrhea, Bolinn et al. have suggested that FFC could be used for the differentiation of both types of steatorrhea (134). This has however not been confirmed by other investigators; who found much overlap in FFC results between pancreatic and nonpancreatic steatorrhea (132,133,135,136). Results of these studies are shown in table 5.

The utility of FFC as an screening method for fat malabsorption has been limited because of the high interday variation (29%). This interday variation might be due to the **varying fecal water content** as reported by Weijers et al. (137). This suggests that if the effect of varying water content could be eliminated, the interday variation of FFC would be much lower. A new method for the semiquantitative determination of FFC, which eliminates the influence of varying fecal water content is the **steatocrit**.

### 1. 6. 2 Steatocrit

This procedure is based on the fact that fecal fat is extracted by centrifugation of diluted stool in a hematocrit capillary at 13000 rpm for 15 minutes (138). After centrifugation, three layers are distinguished in the capillary; the upper fatty layer (FL), the middle fluid layer and the bottom solid layer (SL). The fecal fat measured by steatocrit is expressed as fecal fat concentration and is calculated as  $FL / (FL + SL)$ . Reported normal values are  $< 2\%$  (139). The steatocrit method is very suitable for use in children and infants since it is **simple, noninvasive** and can be performed on **small fecal samples** (0,5 gram). Moreover, it is inexpensive and the whole test takes only 20 minutes. Although several authors have reported this method to

be satisfactory for the evaluation of steatorrhea (138-142), some have reported the steatocrit to be **quite unreliable** (143). Sugai reported a specificity of 97% for steatocrit but a sensitivity of 98%, 79% and 29% for samples with respectively high, moderate and low fat content (144). This low sensitivity observed for samples of low fat content may be due to difficulties with either fat extraction or with obtaining a clear separation between the fatty, aqueous and solid layer, resulting in erroneous results.

### 1. 6. 3 Sudan staining method

The presence of fecal fat can be screened for by microscopic examination of stools. The fecal preparation is first acidified and stained with Sudan staining. After heating, fecal fatty acids and triglycerides are seen as fatty globules under the microscope. Dependent on the number and the size of the globules, the fatty globules are classified as normal, slightly increased or definitely increased (145). Weijers et al. studied the agreement between results of the Sudan staining method and chemically measured fecal fat (137). Satisfactory **agreement** between both methods was found in **(60 - 70 % cases) for fecal samples with very low or very high fat content ( < 3 % or > 9 % )** but the agreement dropped to **40% for samples of moderate fat content (3 - 9%)**.

### 1. 6. 4 C- Triolein absorption test

After ingestion,  $^{14}\text{C}$ -Triolein is digested by pancreatic lipase in the duodenum liberating fatty acids, which on further oxidation yield  $^{14}\text{CO}_2$  which can be detected in expired air. Although this method is **simple, rapid** to perform and gives a **direct evaluation of pancreatic function**, it is not appropriate for use in children because of the **radioactivity**. Recently, a new non radioactive substrate  $^{13}\text{C}$ -Triolein has been introduced but this is however **expensive** and a **mass spectrometer** is needed in order to use this test (131).

### 1. 6. 5 Near Infrared Reflectance Analysis

This method is based on the analysis of the infrared spectrum radiation, reflected by the

surface of the material under study. Specific peaks for the component to be investigated can be identified and their heights can be related to the concentration of the component studied by using computerised multilinear regression analysis. Besides **measuring fecal fat**, this apparatus can also be used for the determination of **fecal nitrogen and water content**. The analysis lasts less than 1 minute and can be performed on **small samples** ( 2 - 3 gram ). The variation coefficient is 2,1 % and the correlation coefficient with the van de Kamer method is 0,92 (146). However this high correlation is possibly due to the fact that this method is calibrated by the titrimetric method described by van de Kamer. The **calibration procedure is difficult** and this sophisticated instrument is **expensive** (147). Further studies are necessary in order to better evaluate the usefulness of near infrared reflectance analysis in clinical practice.

**Table 5 Diagnostic value of fecal fat concentration (FFC) and fecal fat excretion (FFE) in studies of fat malassimilation.**

Authors	<sup>133</sup> Pedersen '84	<sup>133</sup> Bolinn '84	<sup>133</sup> Roberts '86	<sup>133</sup> Lembcke '87	<sup>133</sup> Bai et al. '89
Number of cases	87	50	125	369	538
Aims of study	diagnostic value of FFC versus FFE				
Method used	†titrimetric method (72h fecal collection)	†titrimetric method (72h fecal collection)	†titrimetric method (72h fecal collection)	†titrimetric method (72h fecal collection)	†titrimetric method (72h fecal collection)
	- <sup>14</sup> C-triolein/ <sup>3</sup> H-oleic acid test as reference				
Population (n)	I. pancreatic steatorrhea (21) II. non pancreatic steatorrhea (12) III. no steatorrhea (54)	I. pancreatic steatorrhea (19) II. nonpancreatic steatorrhea (31)	I. pancreatic steatorrhea (24) II. nonpancreatic steatorrhea (70) III. no steatorrhea (31)	I. pancreatic steatorrhea (59) II. nonpancreatic steatorrhea (53) III. no steatorrhea: sick and normal controls (257)	I. pancreatic steatorrhea (88) II. nonpancreatic steatorrhea (525)
Results	I + II versus III: - pos. pred. value: FFE 0.93 FFC 0.90 - neg. pred. value: FFE 0.90 FFC 0.89 - if based on single day sampling, FFC more reliable (6% errors) than FFE (37% errors) - overlap of FFC between I and II	- FFC-pancreatic $\geq 9.5\%$ - FFC-nonpancreat $< 9.5\%$ - no overlap of FFC between I and II	- FFC-pancreatic $>$ nonpancreatic $>$ control - correlation between FFC and FFE ( $r=0.55; p<0.01$ ) - overlap of FFC between I and II	- FFC-pancreatic $>$ non-pancreatic steatorrhea $>$ pancreat. control - Overlap of FFC between I and II	- FFCsens 58% - FFCspec 70% - overlap of FFC between I and II
Comments	- free fat intake - <sup>14</sup> C excretion $\geq 10\%$ for steatorrhea - <sup>14</sup> C/ <sup>3</sup> H $> 1.3$ for pancreatic steatorrhea	- fat diet 90-100g/day	- fat diet 100g/day - 10% as cutoff for pancreatic steatorrhea	- fat diet $\geq 80$ g/day	- 10% as cutoff for pancreatic steatorrhea. - fat diet 100g/day

FFC: Fecal fat concentration

FFE: Fecal fat excretion

† Method as described by van de Kamer (11)

pos. pred. value: positive predictive value

neg. pred. value: negative predictive value

sens: sensitivity

spec: specificity

## **AIMS OF THE STUDY**

With age, children with CF show progressing malnutrition mainly attributed to either persisting malabsorption notwithstanding the use of high oral doses of pancreatic enzymes or increased energy consumption secondary to respiratory disease. Prospective studies in young children have shown malnutrition to occur only in patients with pancreatic insufficiency (34). Efforts to either maintain or restore the nutritional condition have shown that, notwithstanding the use of pancreatic enzymes, high nutrient intakes only seems to be effective when administered "digested" either as total parenteral nutrition or as (semi)elemental feedings orally or by tube feeding. The apparent insufficient effect of pancreatic enzymes does not seem to be due to too low administered doses and recently very high doses have been used with the hope of correcting malabsorption. Suggestions have been made that these high doses might be responsible for the recently reported occurrence of colitis in these patients (113-115).

Our hypothesis was that persisting malabsorption in these patients is likely to be linked to a low duodenal pH which interferes with several digestive and absorptive processes such as impeding transport of split fatty acids from the luminal lipid globules to the absorptive area through the mediation of bile salt micelles. If this was correct, antacid treatment should improve fat malabsorption in these patients. The fact that most studies hereover have been inconclusive might be due to the short and inefficient control of duodenal pH with the drugs used. A recent double-blind control study in adults patients has shown malabsorption to normalize in several patients treated with a proton pump inhibitor (omeprazol) (112). Until now, no studies with proton pump inhibitor have been reported in children.

### **The aims of the present work were:**

1. Develop an easy, noninvasive, cheap and reliable test for the monitoring of fecal fat loss in pancreatic malabsorption.
2. Evaluate the nutritional condition, the body composition and the presence or persistence of fat malabsorption in our patients with exocrine pancreatic insufficiency accompanying cystic fibrosis.

3. Evaluate whether or not the use of a proton pump inhibitor (lansoprazole) in our patients with persisting malabsorption improves both the fat malabsorption and the nutritional condition.

## REFERENCES

- (1) G. Fanconi, E. Uehlinger, C. Knauer. Das Coeliaksyndrom bei Angeborener Zystischer Pankreas fibromatose und Bronchiektasien. *Wein Med Wschr* 1936; 86: 753-756.
- (2) CF Genetic analysis consortium. Worldwide survey of the  $\Delta F$  508 mutation. *Am J Hum Genet* 1990; 47: 354-359.
- (3) M. Welsh, A. Smith. Molecular mechanisms of CFTR chloride channel dysfunction in cystic fibrosis. *Cell* 1993; 73: 1251-1254.
- (4) C. Marino, L. Matovecik, F. Gorelick, J. Cohn. Localization of the cystic fibrosis transmembrane conductance regulator in pancreas. *J Clin Invest* 1991; 88: 712-716.
- (5) T. Boat, M. Welsh, A. Beaudet. (1989). In *The Metabolic Basis of inherited Disease*, C. Scriver, A. Beaudet, W. Sly, D. Valle, eds. (New York: McGraw-Hill,inc.), pp. 2649-2680.
- (6) E. Kerem, J. Reisman, M. Corey, G. Canny, H. Levison. Prediction of mortality in patients with cystic fibrosis. *N Engl J Med* 1992; 326: 1187-91.
- (7) C. Sommerhoff, J. Nadel, C. Basbaum et al. Neutrophil elastase and cathepsin G stimulate secretion from culture bovine airway gland serous cells. *J Clin Invest* 1990;85: 682-689.
- (8) L. Sharples, T. Hathaway, C. Dennis, N. Caine, T. Higenbottam, J. Wallwork. Prognosis of patients with cystic fibrosis awaiting heart and lung transplantation. *J Heart-Lung-Transplant* 1993; 12: 669-74.
- (9) J. Britton. Effects of social class, sex, and region of residence on age at death from cystic fibrosis. *Br Med J* 1989; 298: 483-487.
- (10) M. Corey, F. McLaughlin, M. Williams, H. Levison. Comparison of survival, growth and pulmonary function in patients with cystic fibrosis in Boston and Toronto. *J Clin Epidemiol* 1988; 41: 583-591.
- (11) M. Aitken, S. Fiel. Cystic fibrosis. *Dis Mon* 1993; 39: 1-52.
- (12) P. Brihaye, P. Clement, I. Dab, B. Desprechin. Pathological changes of the lateral nasal wall in patients with cystic fibrosis. *Int J Pediatr Otorhinolaryngol* 1994; 28: 141-7.
- (13) I. Mackay, B. Djazaeri. Chronic sinusitis in cystic fibrosis. *J Roy Soc Med* 1994; 87 (Suppl 21): 17-19.
- (14) B. Hadorn, P. Johansen, C. Anderson. Pancreozymin secretin test of exocrine pancreatic function in cystic fibrosis and the significance of the result for the pathogenesis of the disease.

Can Med Assoc J 1968; 98: 377-385.

(15) B. Hadorn, G. Zoppi, D. Shmerling, A. Prader, I. McIntyre, C. Anderson. Quantitative assessment of exocrine pancreatic function in infants and children. *J Pediatr* 1968; 73: 39-50.

(16) H. Schachman, E. Lebenthal, K. Khat. Recurrent acute pancreatitis in patients with normal pancreatic enzymes. *Pediatrics* 1975; 55: 86-95.

(17) K. Gaskin, P. Durie, M. Corey, P. Wei, G. Forstner. Evidence for a primary defect of pancreatic HCO<sub>3</sub><sup>-</sup> secretion in cystic fibrosis. *Pediatr Res* 1982; 16: 554-557.

(18) A. Atlas, S. Orenstein, D. Orenstein. Pancreatitis in young children with cystic fibrosis. *J Pediatr* 1992; 120: 756-9.

(19) F. Ahmed, J. Ellis, J. Murphy, S. Wooton, A. Jackson. Excessive faecal loss of vitamin A (retinol) in cystic fibrosis. *Arch Dis Child* 1990; 65: 589-593.

(20) R. Sokol, M. Reardon, F. Accurso et al. Fat-soluble-vitamin status during the first year of life in infants with cystic fibrosis identified by screening of newborns. *Am J Clin Nutr* 1989; 50: 1064-71.

(21) V. Hubbard, P. Farrell, P. di Sant 'Agnese. 25-hydroxylcholecalciferol levels in patients with cystic fibrosis. *J Pediatr* 1979; 94: 84-86.

(22) E. Mischler, PJ Chesney, PW Chesney, R. Mazess. Demineralization in cystic fibrosis detected by direct protein absorptiometry. *Am J Dis Child* 1979; 133: 632-635.

(23) N. Solomons, J. Wagonfeld, C. Rieger et al. Some biochemical indices of nutrition in treated cystic fibrosis patients. *Am J Clin Nutr* 1981; 34: 462-474.

(24) R. Allen, B. Seetharam, E. Podell, D. Alpers. Effect of proteolytic enzymes on the binding of cobalamin to R protein and intrinsic factor. *J Clin Invest* 1978; 61: 47-54.

(25) M. Murphy, W. Sheldon, A. Brunetto et al. Active and passive sugar absorption in pancreatic insufficiency. *J Pediatr Gastroenterol Nutr* 1989; 8: 189-194.

(26) L. Frase, A. Strickland, G. Kachel, G. Krejs. Enhanced glucose absorption in the jejunum of patients with cystic fibrosis. *Gastroenterology* 1985; 88: 478-484.

(27) M. Lohr, P. Goertchen, H. Nizze et al. Cystic fibrosis associated islet changes may provide a basis for diabetes. An immunocytochemical and morphometrical study. *Virchows Arch [A]* 1989; 414: 179-185.

(28) L. Krueger, A. Lerner, S. Katz, R. Mack, D. Holsclaw, E. Lebenthal. Cystic fibrosis and diabetes mellitus: interactive or idiopathic. *J Pediatr Gastroenterol Nutr* 1991; 13: 209-219.

- (29) E. Wilmshurst, J. Soeldner, D. Holsclaw. Endogeneous and exogeneous insulin responses in patients with cystic fibrosis. *Pediatrics* 1975; 55: 75-82.
- (30) O. Andersen, S. Garner, C. Heilmann, K. Petersen, W. Petersen, C. Koch. Glucose tolerance and insulin receptor binding to monocytes and erythrocytes in patients with cystic fibrosis. *Acta Paediatr Scand* 1988; 77: 67-71.
- (31) A. Moran, P. Diem, D. Klein, M. Levitt, R. Robertson. pancreatic endocrine function in cystic fibrosis. *J Pediatr* 1991; 118: 715-723.
- (32) H. Berry, F. Kellogg, M. Hunt, R. Ingberg, L. Richter, C. Gutjahr. Dietary supplement and nutrition in children with cystic fibrosis. *Am J Dis Child* 1975; 129: 165-171.
- (33) J. Dodge, J. Yassa. Food intake and supplementary feeding programs. In: J. Sturgess, ed. *perspectives in cystic fibrosis*. Toronto: Canadian Cystic Fibrosis Foundation; 1980: 125-136.
- (34) M. Bronstein, R. Sokol, S. Abman et al. Pancreatic insufficiency, growth, and nutrition in infants identified by newborn screening as having cystic fibrosis. *J Pediatr* 1992; 120: 533-40.
- (35) J. Tomezsco, V. Stallings, D. Kawchak, J. Goin, G. Diamond, T. Scanlin. Energy expenditure and genotype of children with cystic fibrosis. *Pediatr Res* 1994; 35: 451-460.
- (36) M. Bronstein, P. Davies, K. Hambidge, F. Accurso. Normal energy expenditure in the infant with presymptomatic cystic fibrosis. *J Pediatr* 1995; 126: 28-33.
- (37) K. Cox, J. Isenberg, M. Ament. Gastric acid hypersecretion in cystic fibrosis. *J Pediatr Gastroenterol Nutr* 1982; 1: 559-565.
- (38) C. Roy, A. Weber, C. Morin et al. Abnormal biliary lipid composition in cystic fibrosis. *N Engl J Med* 1977; 297: 1301-1305.
- (39) A. Malfroot, I. Dab. New insights on gastro-oesophageal reflux in cystic fibrosis by longitudinal follow up. *Arch Dis Child* 1991; 66: 1339-1345.
- (40) S. Cucchiara, F. Santamaria, M. Andreotti et al. Mechanisms of gastro-oesophageal reflux in cystic fibrosis. *Arch Dis Child* 1991; 66: 617-622.
- (41) P. Gustafsson, S. Fransson, N. Kjellman, L. Tibbling. Gastro-oesophageal reflux and severity of pulmonary disease in cystic fibrosis. *Scand J Gastroenterol* 1991; 26: 449-456.
- (42) S. Fiedorek, R. Shulman, W. Klish. Endoscopic detection of peptic ulcer disease in cystic fibrosis. *Clin Pediatr (Phila)* 1986; 25: 243-246.
- (43) P. Robinson, A. Smith, P. Sly. Duodenal pH in cystic fibrosis and its relationship to fat malabsorption. *Dig Dis Sci* 1990; 35: 1299-1304.

- (44) J. McPartin, J. Dickson, V. Swain. Meconium ileus, immediate and longterm survival. *Arch Dis Child* 1972; 47: 207-210.
- (45) H. Andersen, K. Hjelt, E. Waever, K. Overgaard. The age related incidence of meconium ileus equivalent in a cystic fibrosis population: the impact of high energy intake. *J Pediatr Gastroenterol Nutr* 1990; 11: 356-360.
- (46) L. Kulczyki, H. Schwachman. Studies in cystic fibrosis of the pancreas: occurrence of rectal prolapse. *N Engl J Med* 1958;259: 409-412.
- (47) D. Holsclaw, C. Rocmans, H. Schwachman. Intussusception in patients with cystic fibrosis. *Pediatrics* 1971; 48:51-58.
- (48) H. Rovsing, K. Sloth. Microgallbladder and biliary calculi in mucoviscidosis. *Acta Radiol [Onco]* 1973; 14: 588-592.
- (49) F. Santamaria, P. Vajro, V. Oggero et al. Volume and emptying of the gallbladder in patients with cystic fibrosis. *J Pediatr Gastroenterol Nutr* 1990; 10: 303-306.
- (50) L. Taussig, C. Lobeck, P. Ackerman, J. Kattwinkel: Fertility in males with cystic fibrosis. *N Engl J Med* 1972; 287: 586-589.
- (51) E. Kaplan, H. Shwachman, A. Perlmutter et al.: Reproductive failures in males with cystic fibrosis. *N Engl J Med* 1968; 279: 65-69.
- (52) S. Fitzsimmons: Cystic Fibrosis Foundation Patient Registry 1990 Annual Report. Bethesda, Cystic Fibrosis Foundation, 1991.
- (53) P. Tam, P. Verdugo: Control of mucus hydration as a Donnan equilibrium process. *Nature* 1981; 292: 340-342.
- (54) P. Di Sant 'Agnese, R. Darling, G. Perera, E. Shea. Abnormal electrolyte composition of sweat in cystic fibrosis of the pancreas. Clinical significance and relationship to the disease. *Pediatrics* 1953; 12: 549-563.
- (55) L. Gibson, R. Cooke. A test for concentration of electrolytes in sweat in cystic fibrosis of the pancreas utilizing in pilocarpine by iontophoresis. *Pediatrics* 1959; 23: 545-549.
- (56) H. Veeze, A. Van den Ouweland, D. Halley et al. The diagnosis of cystic fibrosis: intestinal current measurements, a highly accurate method in case of a borderline phenotype. Submitted.
- (57) M. Rosenfeld, W. Siegfried, K. Yoshimura et al. Adenovirus-mediated transfer of a recombinant alpha 1-antitrypsin gene to the lung epithelium in vivo. *Science*.1991; 252: 431-4

- (58) B. Pitt, M. Schwarz, J. Pilewski et al. Retrovirus-mediated gene transfer in lungs of living fetal sheep. *Gene Ther* 1995; 2: 344-50.
- (59) M. Rosenfeld, K. Yoshimura, B. Trapnell et al. In vivo transfer of the human cystic fibrosis transmembrane conductance regulator gene to the airway epithelium. *Cell*. 1992; 68: 143-55.
- (60) R. Kraemer, A. Rudeberg, B. Hadorn, E. Rossi. Relative underweight in cystic fibrosis and its prognostic value. *Acta Paediatr Scand* 1978; 67: 33-37.
- (61) S. Rao, D. Wilson, R. Brooks et al. Acute effects of nebulization of n-acetylcysteine on pulmonary mechanics and gas exchange. *Am Rev Respir Dis*. 1970; 102: 17-22.
- (62) W. Waring. Current management of cystic fibrosis. *Adv Pediatr*. 1976; 23: 401-38.
- (63) M. Gotz, R. Kraemer, K. Kerrebijn et al. Oral acatylsysteine in cystic fibrosis. A cooperative study. *Eur J Respir Dis*. 1980;61: (Suppl 111): 122-6.
- (64) J. Reisman, B. Rivington-Law, M. Corey et al. Role of conventional physiotherapy in cystic fibrosis. *J Pediatr* 1988; 113: 632-6.
- (65) H. Steen, A. Redmond, D. O'Neil et al. Evaluation of the PEP mask in cystic fibrosis. *Acta Paediatr Scand*. 1991; 80: 51-6.
- (66) C. Braggion, L. Cappelletti, M. Cornacchia, L. Zanolla, G. Mastella. Short-term effects of three chest physiotherapy regimens in patients hospitalized for pulmonary exacerbations of cystic fibrosis: A cross-over randomized study. *Pediatr Pulmonol* 1995; 19: 16-22.
- (67) J. Pryor, B. Webber, M. Hobson et al. Evaluation of the forced expiratory technique as an adjunct to postural drainage in the treatment of cystic fibrosis. *J Pediatr*. 1983; 103: 538-42.
- (68) P. Sutton, H. Gemmell, N. Innes et al. Use of nebulized saline and nebulized terbutaline as an adjunct to chest physiotherapy. *Thorax*. 1988; 43: 57-60.
- (69) E. Pattishall. Longitudinal response of pulmonary function to bronchodilators in cystic fibrosis. *Pediatr Pulmonol*. 1990; 9: 80-5.
- (70) P. Konig, D. Gayer, J. Shaffer et al. Bronchodilator responsiveness and spontaneous diurnal variation of PEFr in patients with cystic fibrosis. Poster presented at the North American Cystic Fibrosis Conference. Washington, DC: 1992 Oct. Abstract.
- (71) M. Konstan, P. Byrard, C. Hoppel, P. Davis: Effect of high dose ibuprofen in patients with cystic fibrosis. *N Engl J Med* 1995; 332: 848.
- (72) H. Auerbach, M. Williams, J. Kirkpatrick et al. Alternate-day prednisone reduces morbi-

- dity and improves pulmonary function in cystic fibrosis. *Lancet*. 1985; 2: 686-8.
- (73) B. Rosenstein, H. Eigen. Risk of alternate-day prednisone in patients with cystic fibrosis. *Pediatrics*. 1991; 87: 245-6.
- (74) C. Pantin, R. Stead, M. Hodson et al. Prednisolone in the treatment of airflow obstruction in adults with cystic fibrosis. *Thorax*. 1986; 41: 34-38.
- (75) M. Zach. Pathogenesis and management of lung disease in cystic fibrosis. *J R Soc Med*. 1991; 84 (Suppl 18): 10-7.
- (76) McNeil Pharmaceutical. Pseudomonas vaccine tests start. *Cystic Fibrosis Currents*. 1991; 6(4).
- (77) A. Khaghani, B. Madden, M. Hodson et al. Heart-lung transplantation for cystic fibrosis. Paper presented at the North American Cystic Fibrosis Conference. Dallas, TX: 1991 Oct 4.
- (78) E. App, M. King, R. Helfesrieder et al. Acute and longterm amiloride inhalation in cystic fibrosis lung disease. *Am Rev Respir Dis*. 1990; 141: 605-12.
- (79) M. Knowles, N. Church, W. Waltner et al. A pilot study of aerosolized amiloride for the treatment of lung disease in cystic fibrosis. *N Engl J Med*. 1990; 322: 1189-94.
- (80) M. Hodson. Clinical studies of rhDNase in moderately and severely affected patients with cystic fibrosis - An Overview. *Respiration* 1995; 62 (suppl 1); 29-32.
- (81) M. Aitken, W. Burke, G. McDonald et al. Recombinant human Dnase inhalation in normal subjects and patients with cystic fibrosis. A phase I study. *JAMA*. 1992; 267: 1947-51.
- (82) R. Hubbard, N. McElvaney, P. Steven et al. A preliminary study of aerosolized recombinant human deoxyribonuclease I in the treatment of cystic fibrosis. *N Engl J Med*. 1992; 326:812-5.
- (83) N. McElvaney, R. Hubbard, P. Birrer et al. Aerosol alpha-1-antitrypsin treatment for cystic fibrosis. *Lancet*. 1991; 337: 392-4.
- (84) R. Boucher, E. Cheng, A. Paradiso et al. Chloride secretory response of cystic fibrosis airway epithelia: presentation of calcium but not protein kinase C and A-dependent mechanism. *J Clin Invest*. 1989;84: 1424-31.
- (85) H. Chase, M. Long, M. Lavin. Cystic fibrosis and malnutrition. *J Pediatr* 1979; 95: 337-47.
- (86) C. Roy, A. Silverman, F. Cozzetto. *Pediatric Clinical Gastroenterology*. 2nd ed. St.

Louis: CV Mosby, 1975: 615-35.

(87) J. Dodge, J. Yassa. Food intake and supplementary feeding programs. In: Sturgess JM, ed. Perspectives in cystic fibrosis. Proceedings of the 8th International Cystic Fibrosis Congress. Toronto: Canadian Cystic Fibrosis Foundation, 1980: 125-36.

(88) H. Parsons, P. Beaudry, A. Dumas, P. Pencharz. Energy needs and growth in children with cystic fibrosis. *J Pediatr Gastroenterol Nutr* 1983; 2: 44-9.

(89) H. Schwachman, R. Dooley, F. Guilmette, P. Patterson, C. Weil, H. Leubner. Cystic fibrosis of the pancreas with varying degrees of pancreatic insufficiency. *Am J Dis Child* 1956; 92: 347-68.

(90) D. Crozier. Cystic fibrosis a not so fatal disease. *Pediatr Clin North Am* 1974; 21: 935-950.

(91) D. Gurwitz, M. Corey, P. Francis, D. Crozier, H. Levison. Perspectives in cystic fibrosis. *Pediatr Clin North Am*. 1979; 26: 603-615.

(92) E. Luder, M. Kattan, J. Thornton, K. Koehler, R. Bonforte. Efficacy of a nonrestricted fat diet in patients with cystic fibrosis. *AJDC*. 1989; 143: 458-464.

(93) A. Rettammel, M. Marcus, P. Farrell, S. Sondel, R. Kosciak, E. Mischler. Oral supplementation with a high-fat, high-energy product improves nutritional status and alters serum lipids in patients with cystic fibrosis. *J Am Diet Assoc*. 1995; 95: 454-459.

(94) K. Gaskin, D. Waters, L. Baur, V. Soutter, M. Gruca. Nutritional status, growth and development in children undergoing intensive treatment for cystic fibrosis. *Acta Paediatr Scand [Suppl]*. 1990; 366: 106-110.

(95) E. O' Loughlin, D. Forbes, H. Parsons, B. Scott, D. Cooper, G. Gall. Nutritional rehabilitation of malnourished patients with cystic fibrosis. *Am J Clin Nutr*. 1986; 43: 732-737.

(96) R. Shepherd, T. Holt, B. Thomas et al. Nutritional rehabilitation in cystic fibrosis: Controlled studies of effects on nutritional growth retardation, body protein turnover, and course of pulmonary disease. *J Pediatr*. 1986; 109: 788-94.

(97) R. Shepherd, B. Thomas, D. Bennett, W. Cooksley, L. Ward. Changes in body composition and muscle protein degradation during nutritional supplementation in nutritionally growth-retarded children with cystic fibrosis. *J Pediatr Gastroenterol Nutr*. 1983; 2: 439-446.

(98) L. Levy, P. Durie, P. Pencharz, M. Corey. Effects of long-term nutritional rehabilitation on body composition and clinical status in malnourished children and adolescents with cystic

fibrosis. *J Pediatr* 1985; 107: 225-230.

(99) J. Bertrand, C. Morin, R. Lasalle, J. Patrick, A. Coates. Short-term clinical, nutritional, and functional effects of continuous elemental enteral alimentation in children with cystic fibrosis. *J Pediatr*. 1984; 104: 41-46.

(100) R. Shepherd, W. Cooksley, and W. Domville. Improved growth and clinical, nutritional, and respiratory changes in response to nutritional therapy in cystic fibrosis. *J Pediatr*. 1980; 97: 351-357.

(101) J. Yassa, R. Prosser, J. Dodge. Effects of an artificial diet on growth of patients with cystic fibrosis. *Arch Dis Child*. 1978; 53: 777-783.

(102) J. Allan, A. Mason, A. Moss. Nutritional supplementation in treatment of cystic fibrosis of the pancreas. *Am J Dis Child*. 1973; 126: 22-26.

(103) A. Mansell, J. Andersen, C. Muttart et al. Short-term pulmonary effects of total parenteral nutrition in children with cystic fibrosis. *J Pediatr* 1984; 104: 700-705.

(104) M. Canciani, G. Mastella. Absorption of a new semielemental diet in infants with cystic fibrosis. *J Pediatr Gastroenterol Nutr*. 1985; 4: 735-740.

(105) M. Boland, D. Stoski, N. Macdonald, P. Soucy, J. Patrick. Chronic jejunostomy feeding with a non-elemental formula in undernourished patients with cystic fibrosis. *Lancet* 1986; 1: 232-234.

(106) P. Farrell, E. Mischler, S. Sondel, M. Palta. Predigested formula for infants with cystic fibrosis. *Research*. 1987; 87: 1353-1356.

(107) P. Regan, J. Malagelada, E. Dimagno, and V. Go. Reduced intraluminal bile acid concentrations and fat maldigestion in pancreatic insufficiency: Correction by treatment. *Gastroenterology*. 1979; 77: 285-289.

(108) P. Zentler-Munro, W. Fitzpatrick, J. Batten, and T. Northfield. Effect of intrajejunal acidity on aqueous phase bile acid and lipid concentrations in pancreatic steatorrhea due to cystic fibrosis. *Gut*. 1984; 25: 500-507.

(109) P. Zentler-Munro, D. Fine, J. Batten, and T. Northfield. Effect of cimetidine on enzyme inactivation, bile acid precipitation, and lipid solubilisation in pancreatic steatorrhea due to cystic fibrosis. *Gut*. 1985; 26: 892-901.

(110) A. Weber, C. Roy. Intraduodenal events in cystic fibrosis. *J Pediatr Gastroenterol Nutr*. 1984; 3 (Suppl. 1): S113-S119.

- (111) S. Dutta, V. Hubbard, M. Appler. Critical examination of therapeutic efficacy of a pH-sensitive enteric-coated pancreatic enzyme preparation in treatment of exocrine pancreatic insufficiency secondary to cystic fibrosis. *Dig Dis Sci* 1988; 33: 1237-44.
- (112) H. Heijerman, C. Lamers, W. Bakker. Omeprazole enhances the efficacy of pancreatin (pancrease) in cystic fibrosis. *Ann Intern Med.* 1991; 114: 200-201.
- (113) S. Schwarzenberg, C. Wielinski, I. Shamieh et al. Cystic fibrosis-associated colitis and fibrosing colonopathy. *J Pediatr* 1995; 127: 565-70.
- (114) R. Smyth, D. van Velzen, A. Smyth, D. Lloyd, D. Heaf. Strictures of ascending colon in cystic fibrosis and high strength pancreatic enzymes. *The Lancet.* 1994; 343: 85-86.
- (115) M. Pettei, J. Leonidas, J. Levine, J. Gorvoy. Pancolonic disease in cystic fibrosis and high-dose pancreatic enzyme therapy. *J Pediatr* 1994; 125: 587-9.
- (116) G. Cleghorn, R. Shepherd, T. Holt. The use of a synthetic prostaglandin E<sub>1</sub> analogue (Misoprostol) as an adjunct to pancreatic enzyme replacement in cystic fibrosis. *Scand J Gastroenterol.* 1988; 23(Suppl 143): 142-147.
- (117) K. Cox, J. Isenberg, A. Osher, R. Dooley. The effect of cimetidine on maldigestion in cystic fibrosis. 1979; 94: 488-492.
- (118) M. Schöni, R. Kraemer, E. Rossi. Cimetidine and fat malabsorption in children with cystic fibrosis. *Helv Paediat Acta.* 1981; 36: 359-369.
- (119) B. Boyle, W. Long, W. Balistreri, S. Widzer, and N. Huang. Effect of cimetidine and pancreatic enzymes on serum and fecal bile acids and fat absorption in cystic fibrosis. *Gastroenterology.* 1980; 78: 950-953.
- (120) R. Gow, R. Bradbear, P. Francis, R. Shepherd. Comparative study of varying regimens to improve steatorrhoea and creatorrhoea in cystic fibrosis: effectiveness of an enteric-coated preparation with and without antacids and cimetidine. *Lancet* 1981; 14: 1071-1074.
- (121) P. Robinson and P. Sly. Placebo-controlled trial of misoprostol in cystic fibrosis. *J Pediatr Gastroenterol Nutr.* 1990; 11: 37-40.
- (122) P. Durie, L. Bell, W. Linton, M. Corey, G. Forstner. Effect of cimetidine and sodium bicarbonate on pancreatic replacement therapy in cystic fibrosis. *Gut* 1980; 21: 778-786.
- (123) A. Carroccio, F. Pardo, G. Montalto et al. Use of famotidine in severe exocrine pancreatic insufficiency with persistent maldigestion on enzymatic replacement therapy: A long-term study in cystic fibrosis. *Dig Dis Sci* 1992; 37: 1441-1446.

- (124) D. Chalmers, R. Brown, M. Miller et al. The influence of longterm cimetidine as an adjuvant to pancreatic enzyme therapy in cystic fibrosis. *Acta Paediatr Scand.* 1985; 74: 114-117.
- (125) M. Schöni, R. Kraemer, A. Ruedeberg et al. Long-term cimetidine in children with cystic fibrosis: a randomized double blind study.
- (126) J. van de Kamer. Standard methods of clinical chemistry, edited by Seligson D. New York, Academic Press, 1958, Vol 2, p 34.
- (127) E. Wollaeger, M. Comfort, A. Osterberg. Total solids, fat and nitrogen in feces: Study of normal persons taking test diets containing moderate amount of fat; comparison with results obtained with normal persons taking test diet containing large amount of fat. *Gastroenterology* 1947; 9: 272-283.
- (128) D. Woodman, W. Yeoman. A simplified method of investigating steatorrhoea. *J Clin Pathol* 1955; 8:79-80.
- (129) M. Davidson, C. Bauer. Patterns of fat excretion in feces of premature infants fed various preparations of milk. *Pediatrics* 1960; 25: 375-84.
- (130) J. van de Kamer, H. Huinink, A. Weyers. Rapid method for the determination of fat in feces. *J Biol Chem* 1949; 177: 349-55.
- (131) B. Lembeke, B. Braden, J. Stein. Diagnostik der steatorrhoe. *Z Gastroenterol* 1994; 32: 256-261.
- (132) N. Thorsgaard Pedersen, H. Halgreen, H. Worning. Estimation of the 3-day faecal fat excretion and fat concentration as a differential test of malabsorption and maldigestion. *J Gastroenterol* 1987; 22: 91-96.
- (133) I. Roberts, C. Poturich, A. Wald. Utility of fecal fat concentrations as screening test in pancreatic insufficiency. *Dig Dis Sci* 1986; 31: 1021-4.
- (134) G. Bo-Linn, J. Fordtran. Fecal fat concentration on patients with steatorrhea. *Gastroenterology* 1984; 87: 319-322.
- (135) J. Bai, A. Andriush, G. Matelo et al. Fecal fat concentration in the differential diagnosis of steatorrhea. *Am J Gastroenterol* 1989; 84: 27-30.
- (136) B. Lembeke, K. Grimm, P. Lankisch. Raised fecal fat concentration is not a valid indicator of pancreatic steatorrhea. *Am J Gastroenterol* 1987; 82: 526-531.
- (137) H. Weijers. Fat absorption in normal and abnormal infants and children with special

reference to coeliac disease (proefschrift) 1950: 19-23.

(138) P. Phuapradit, A. Narang, P. Mendonca, D. Harris, J. Baum. The steatocrit: a simple method for estimating stool fat content in newborn infants. *Arch Dis Child* 1981; 56: 725-727.

(139) G. Iacono, A. Carroccio, G. Montalto et al. Steatocrit: normal range and physiological variations in preterm and low-birth-weight full-term newborns. *Acta Paediatr* 1992; 81: 933-4.

(140) A. Guarino, L. Tarallo, L. Greco, L. Cesarano, S. Guandalini, A. Rubino. Reference values of the steatocrit and its modifications in diarrheal diseases. *J Pediatr Gastroenterol Nutr* 1992; 14: 268-274.

(141) C. Colombo, R. Maiavacca, M. Ronchi, E. Consalvo, M. Amoretti, A. Giunta. The steatocrit: a simple method for monitoring fat malabsorption in patients with cystic fibrosis. *J Pediatr Gastroenterol Nutr* 1987; 6: 926-930.

(142) G. Iacono, A. Carroccio, F. Cavataio et al. Steatocrit test: normal range and physiological variations in infants. *J Pediatr Gastroenterol Nutr* 1990; 11: 53-57.

(143) M. Walters, J. Kelleher, J. Gilbert, J. Littlewood. Clinical monitoring of steatorrhoea in cystic fibrosis. *Arch Dis Child* 1990; 65: 99-102.

(144) E. Sugai, G. Srur, H. Vazquez et al. Steatocrit: a reliable semiquantitative method for detection of steatorrhea. *J Clin Gastroenterol* 1994; 19: 206-9.

(145) G. Drummey, J. Benson, C. Jones. Microscopical examination of the stool for steatorrhea. *N Engl J Med* 1961; 264: 85-7.

(146) L. Benini, S. Caliarì, G. Guodi. Near infrared spectrometry for faecal fat measurement: comparison with conventional gravimetric and titrimetric methods. *Gut* 1989; 30: 1344-1347.

(147) O. Bekers, C. Postma, A. Lombarts. Determination of faecal fat by Near-Infrared Spectroscopy. *Eur J Chem Clin Biochem* 1995; 33: 83-86.

## **CHAPTER 2**

### **METHODS**

The following methods were used in our studies:

## **1. Methods used for fecal fat determination:**

### **1.1 Steatocrit and Acid Steatocrit**

About 0,5 g solid stool was weighed and diluted with a volume of deionized water, equal to two times the weight of stool. The stool and water were premixed using a Vortex mixer. Subsequently, the mixture was homogenized using a 5 ml Potter Elvehjem tissue homogenizer. The fecal homogenate was aspirated into a 75  $\mu$ l plain haematocrit capillary. This capillary was sealed with wax at one end and centrifuged horizontally (13000 rpm, 15 min) in a standard haematocrit centrifuge. After centrifugation, the upper fatty layers (FL) and the bottom solid layers (SL) were measured with a graduated magnifying lens. The steatocrit was calculated as  $(FL / (FL + SL)) \times 100\%$ . Since the fat extraction was not optimal in the steatocrit procedure, we have try to increase this step by adding the perchloric acid (5N) to the fecal homogenate in a volume equal to 1/5 of the homogenate after homogenization. This acid homogenate was then mixed for 30 seconds using a Vortex mixer and the following steps were the same as the classical steatocrit. This "Acid Steatocrit" is used in our further study.

### **1.2 Titrimetric method**

The 72 hours fecal collection was first homogenized and about 5 gram of feces was weighed. The feces was saponified with concentrated potassium hydroxide (33% KOH) in ethanol, giving a solution which contains the soaps derived from neutral fats, fatty acids and also soaps originally present in the stool. By adding HCl (2N), fatty acids were obtained. After adding 125 ml toluene, the mixture was shaken vigorously for 2 - 3 minutes. 25ml of the toluene layer containing the fatty acids was then transferred to an erlenmeyer for titration with 0,1N tetrabutylammoniumhydroxide solution in propanol/methanol and thymol blue as indicator. The titration was done three times and the mean of this was used for the calculation of fecal fat excretion, which was calculated as followed:

$$\frac{125\text{ml} + 4.5\text{ml}}{25\text{ml}} (X - X_0) \times 0,1\text{N} \times 1/3 \times \frac{\text{total feces weight(g)}}{\text{sample weight (5g)}} \times 891\text{g} = \text{Total fecal fat (g/72h)}$$

with 125ml representing the toluene volume used for extraction of the fatty acids, 25ml representing the titration volume, 4,5ml is the correction for the volume interaction, X representing the number of meq of fatty acids titrated,  $X_0$  the correction for the acids present in toluene, 0,1N the concentration of tetrabutylammoniumhydroxide solution, 1/3 is the conversion factor from fatty acid to fat molecule (3 molecule fatty acids derived from 1 molecule fat) and 891 is the molecular weight of stearic acid (C-18-fat).

### 1. 3 Sudan staining method

We used the split fat stain, which identifies both triglyceride and fatty acid (1). Several drops of 100% acetic acid and several drops of Sudan III solution were added. The preparation was subsequently mixed with the coverslip, which was then applied. The slide was gently heated on a lighter until bubbling. All preparations were examined while still warm under high magnification (magnification of 400). For quantification of the amount of fat detected microscopically, we used the criteria established by Drummey et al.(2). They are as follows: normal (+): up to 100 fat globules per high power field, varying in a diameter between 1 and 4  $\mu\text{m}$ , as noted on the micrometer scale always using a magnification of 400; Increased (2+): up to 100 fat globules per high power field, the diameter of fat globules varying between 1 and 8  $\mu\text{m}$ ; Markedly increased (3+): more than 100 fat globules per high power field, varying in size from 6 to 75  $\mu\text{m}$  in diameter.

## 2. Methods used for assessment of nutritional condition:

### 2.1 Anthropometry

Weight, height and 4 skinfolds (biceps, triceps, subscapular and suprailiac) were expressed as standard deviation scores of the normal population for age and sex by using the growth charts from Gerver and de Bruin (3).

It has been found that subcutaneous fat as measured by skinfolds is related to the body density (4). This latter is again related to the body fatmass. From these theoretical principles, Gerber and de Bruin have constructed a chart, expressing the relationship between the 4 skinfolds (biceps, triceps, subscapular and suprailiac) and the percent fat free mass. In our study, the fatmass and fat free mass determined with the anthropometric method were derived from these charts.

## **2.2 Dual energy X-ray absorptiometry (DXA)**

This method first developed by Mazess et al., measures simultaneously bone mineral, fat and nonbone lean tissue. For a DXA scan, subjects lied supine on a padded table while the scintillation counter moved in a raster pattern across the body from head to foot. The Lunar DPX uses a constant x-ray source and a filter that converts the polychromatic x-ray beam into one that has two main energy peaks (40 kV and 70 kV). The ratio of soft tissue attenuation ( $R_{ST}$ ) at the two energies is measured. The attenuation of pure fat ( $R_f$ ) and of bone free lean tissue ( $R_L$ ) are known from both theoretical calculations and calibration. From this, the fatmass and lean tissue mass were calculated. The bone mineral content was calculated after correction of the overlying soft tissue (5). Body composition measurements in our study were made by a DPX with a pediatric software programme, Lunar version 1.5e. Daily quality assurance test were performed according to the manufacturer's directions. Total body analysis was performed in all children using a fast scan mode with a sample size of  $4,8 \times 9,6$ mm, sample interval of 0,03s and source collimation of 1,68mm.

## **2.3 Total body water (TBW) and extra cellular volume (ECV)**

TBW and ECV were measured by deuterium oxide (6) and bromide dilution respectively (7). Each subject received orally 20 ml of a mixture of  $D_2O$  (99,9% purity) and Bromide salt (150mMol/L) solution in a volume ratio of 1:1. Saliva and plasma samples were taken before intake of  $D_2O$  - NaBr solution and 4 hours thereafter when "an equilibrium" has been reached. To prevent saliva dilution by fluid intake which can result in a higher TBW content, patients were told not to take any fluid orally half an hour before saliva samples were taken. Urine and

fecal loss of bromide and D<sub>2</sub>O were negligible during the test since 1/2 of D<sub>2</sub>O and Bromide is 8 days (7). Saliva samples were obtained making use of dental cotton-wool, that was dried overnight at 100 °C and kept in a gas-tight tube until use. The cotton-wools and the blood samples were centrifuged and the saliva and serum thus obtained were kept in a stoppered glass vial and stored in a freezer until analysis.

### 2. 3. 1 Total body water

D<sub>2</sub>O concentrations of saliva samples were determined as follows: Calcium carbide (CaC<sub>2</sub>) was placed in the siliconized vacutainer tube and evacuated for 30 sec. with a rotatory vane pump to a total pressure of 0,01 atm. Thereafter, 25µl of salivary sample was injected in the vacutainer tube. This was done in duplicate. CaC<sub>2</sub> react with D<sub>2</sub>O forming acetylene gas. A 25µl of this gas was subsequently injected in duplicate into the GC/CF - IRMS system (gas chromatography/continous flow isotope ratio mass spectrometry) at 2 min. intervals. The mass 27/26 ratio (R<sub>27/26</sub>) was measured on a Isotope Ratio Mass Spectrometer configured for Acetylene (Finnigan MAT 252 for CF-IRMS) (6). The mean value of 4 determinations was calculated for each sample. By inserting of the tracer/tracee ratio, defined as R<sub>27/26</sub> (T<sub>4</sub>) - R<sub>27/26</sub> (T<sub>0</sub>), into the regression equation obtained from the standards, we get the dilution factor of D<sub>2</sub>O. TBW is calculated as ingested D<sub>2</sub>O volume/ dilution factor. From the TBW, LBM and FM can calculated by the following formulés:

$$\text{LBM (kg)} = \text{TBW} / ( 1,04 \times d )$$

$$\text{BF (kg)} = \text{Weight} - \text{LBM}$$

The 1,04 factor is a correction for the estimated 4% nonaqueous hydrogen exchange and d is the hydration factor of LBM which varies with age. Because our CF population was young, we used the age dependent hydration factors described by Fomon (8) for children younger than 10 year and by Boileau and Lohman (9) for older children.

### 2. 3. 2 Bromide space

Because Bromide resides mainly in the extracellular space, measured of Bromide dilution in serum give us an estimation of the extracellular volume. Bromide was determined by using a Gas Chromatograph type CP 9000 (Chrompack) equipped with an ECD detector after it was converted into a bromoacetone gas. First, perchloric acid was added to the serum sample and centrifugated for deproteinisation. An aliquot of the supernatant was then added to silver nitrate ( $\text{AgNO}_3$ ) for precipitating of silver bromide and chloride. After centrifugation, the precipitate was taken up in  $\text{NH}_3$  after that  $\text{Na}_2\text{S}$  and  $\text{NaOH}$  were added to precipitate the silver as  $\text{Ag}_2\text{S}$ . After agitation and centrifugation, the supernatant was heated until dry,  $\text{H}_2\text{O}$  was added followed by  $\text{H}_2\text{O}_2$  to oxidize sulfide. After drying,  $\text{H}_2\text{O}$  was then added and dried again. This was repeated several times. Thereafter, perchloric acid and acetone were added and the reaction is started by addition of  $\text{KmnO}_4$  with Bromoacetone formed. The solution is then extracted with benzene. The organic phase was separated from the water phase by shaking and centrifugation. The water phase was then removed. An aliquot of the organic solution is then applied to the gas chromatograph for measuring of bromoacetone/internal standard ratio. The bromide concentration was then derived from the bromoacetone standard curves. Because the distribution of Bromide depend on the potential difference between in- and extra-cellular and on the quantity of total body volume, corrected bromide space was calculated as follow:

$$\text{ECV (L)} = \frac{\text{Bromide administered (mmol)}}{\text{Bromide change T4 - T0 (mmol/L)}} - 0,036\text{TBW}$$

Where 0,036TBW is the correction factor for the cell potential and for the total body volume (7).

## REFERENCES

- (1) M. Khouri, G. Huang, Y. Shiau. Sudan stain of fecal fat : New insight into an old test. *Gastroenterology* 1989; 96: 421-7.
- (2) G. Drummey, J. Benson, C. Jones. Microscopical examination of the stool for steatorrhea. *N Engl J Med* 1961; 264: 85-7.
- (3) W. Gerver, R. de Bruin. *Paediatric Morphometrics: a reference manual*. 1th ed. Utrecht: Bunge, 1996.
- (4) J. Weststrate, P. Deurenberg. Body composition in children: proposal for a method for calculating body fat percentage from total body density or skinfold-thickness measurements. *Am J Clin Nutr* 1989; 50: 1104-15.
- (5) S. Heymsfield, J. Wang, S. Heshka, J. Kehayias, R. Pierson. Dual-photon absorptiometry: comparison of bone mineral and soft tissue mass measurements in vivo with established methods. *Am J Clin Nutr* 1989; 49: 1283-9.
- (6) B. Van Kreei, F. Van der Vegt, M. Meers, T. Wagenmakers, K. Westerterp, A. Coward. Determination of total body water by a simple and rapid mass spectrometric method. *J Mass Spectrom* 1996; 31: 108-111.
- (7) B. Van Kreei. An Improved bromide assay for the estimation of extracellular water volume by capillary gas chromatography. *Clinica Chimica Acta* 1994; 231: 117-128.
- (8) S. Fomon, F. Haschke, E. Ziegler, S. Nelson. Body composition of reference children from birth to age 10 years. *Am J Clin Nutr* 1982; 35: 1169-1175.
- (9) Boileau, R. Lohman, M. Slaughter, T. Ball, S. Going, M. Hendrikx. Hydration of the fat-free body in children during maturation. *Hum Biol.* 56: 651-666.

## CHAPTER 3

### THE ACID STEATOCRIT : A MUCH IMPROVED METHOD

Tran M, Forget P, Van den Neucker A, Strik J, van Kreel B, Kuijten R.

Departments of Pediatrics and Clinical Chemistry, University Hospital Maastricht,  
Maastricht, The netherlands.

---

J Pediatr Gastroenterol Nutr 1994; 19: 299-303

#### *Abstract*

The steatocrit method has recently been introduced as a simple screening test for steatorrhea. As it seemed likely that separation of fecal homogenate by centrifugation into a lipid phase, a watery phase and a solid phase would be pH-dependent, we evaluated the effect of fecal acidification on steatocrit results in healthy children and in patients with cystic fibrosis and studied the relationship between two steatocrit methods and fecal fat content as measured by a reference chemical method. Steatocrit results increased with the degree of fecal acidification, and maximal results were obtained at the lowest fecal pH values. Means and SEM for classical and acid steatocrit values were  $1.1 \pm 0.4\%$  (classical) versus  $3.8 \pm 1\%$  (acid) in controls ( $n = 6$ ) and  $5.4 \pm 1.9\%$  (classical) versus  $26.9 \pm 4.3\%$  (acid) in cystic fibrosis ( $n = 9$ ). The correlations between fecal fat content measured chemically and steatocrit results were 0.18 ( $p = 0.35$ ) and 0.81 ( $p < 0.0001$ ) for classical and acid steatocrit, respectively. We conclude that acidification of fecal homogenates leads to a marked improvement in the steatocrit method.

## INTRODUCTION

The diagnosis of fat malabsorption still mainly relies on the 72-hour faecal fat quantitation in which daily stool fat loss is evaluated by collecting stools for 3 days and determining stool fat content by chemical methods. The most widely used chemical method is the titrimetric method as described by van de Kamer in 1949 (1).

Work by Kouri et al has suggested that the titrimetric method largely overestimates nutritional faecal fat losses because it measures not only malabsorbed exogenous fat but also endogenous fat of various origins such as biliary lipids and lipids derived from the turnover of intestinal epithelial cells and gut bacteria (2).

Making use of the staining properties of purified lipids in an artificial matrix, Khouri et al. have suggested the fat absorption coefficient in normal adults is much higher than usually believed (2). Although the microscopic evaluation of steatorrhea by the Sudan stain provides a satisfactory screening method for steatorrhea, it is at best semiquantitative.

The steatocrit has been introduced in recent years as a simple test for the evaluation of fat malabsorption (3-6). Although several authors have reported the method to be satisfactory for the evaluation of steatorrhea, some have reported the steatocrit to be quite unreliable (6).

As it has been shown that faecal acidification results in an enhanced sensitivity of the Sudan faecal staining method (2), we wondered whether the same modification could improve fat extraction by centrifugation as performed in the steatocrit determination.

Consequently, we evaluated the effects of stool sample acidification on steatocrit determinations and to compare results from previously reported methods with acid steatocrit results in healthy children and in children with cystic fibrosis. We also determined the correlation between steatocrit results and faecal fat concentrations as measured by the reference chemical method of van de Kamer et to evaluate which of the two steatocrit methods gave the best estimate of faecal fat content.

## METHODS

### "classic" steatocrit method

Stool ( 0.5 gr ) was diluted (1/3) with deionized water and thoroughly homogenized in a 5 ml Potter Elvehjem tissue homogenizer (Heidolph Elektro KG Kelheim, no. 170-1700/20-200) stamper, tissue grind pestle (size 20 from Kontes Scientific Glassware Instruments, no. 885451-0020). The homogenate was aspirated into a 75  $\mu$ l plain glass haematocrit tube. The capillary tube was subsequently centrifuged horizontally (13,000 rpm for 15 min) in a standard haematocrit centrifuge.

After centrifugation, the upper (fat) and bottom (solid) layers were measured with a graduated magnifying lens. Steatocrit was calculated as  $FL/(FL + SL)$ , where FL is the fatty-layer length and SL is the solid-layer length.

#### "Acid" steatocrit method

The method used was exactly the same as the classic steatocrit method except that, before aspirating the homogenate in the capillary tube, perchloric acid in various concentrations (5N for maximal acidification) was added to the homogenate in a volume equal to 1/5 of the homogenate volume. The resulting acid homogenate was mixed for 30 seconds with a standard Vortex mixer.

#### Chemical determination of stool fat concentration.

The method of van de Kamer et al. was used to determine stool fat content (1).

## EXPERIMENTAL DESIGN

#### Effect of stool homogenate acidification on fat extraction

To evaluate the effect of stool acidification and thus stool pH on the length of the fat column obtained by centrifugation, several stool samples from patients with and without steatorrhea were centrifuged after addition of perchloric acid solutions of various concentrations.

### Classic and acid steatocrit

To compare classic and acid steatocrit results in children with and without steatorrhea, we measured fecal steatocrit by both methods in 6 control children (mean age: 5.8 years, range 3 to 12 years; five boys and one girl) and in 9 children with cystic fibrosis (mean age: 6.9 years; range 0.5 to 20; nine boys). The control children were patients with chronic aspecific respiratory disease without gastrointestinal symptoms and with a normal sweat test. The cystic fibrosis patients all had abnormal sweat tests on several occasions and were being treated with pancreatic enzymes when steatocrit determinations were performed. As our purpose was to compare classical and acid steatocrit results in the same fecal samples, no attempt was made to quantify the fat content of the diet which was "normal" in all patients.

### Correlation between steatocrit results and fecal fat content

To further compare both steatocrit methods we looked at the relationship between results obtained by each method and fecal fat content results as measured by the method of van de Kamer et al. (1). Steatocrit measurements (classic and acid) and fecal fat content determinations (chemical method) were performed on 27 consecutive stool samples (from adults and children) sent to our laboratory for evaluation of malabsorption. No attempt was made to classify patients in disease categories as our only goal was to study the relationship between steatocrit results and fecal fat content independent of the presence of disease (clinical results will be published separately).

### Statistical methods

The coefficient of variation of each steatocrit method was determined with duplicate results of each sample for both methods. Pearson correlation coefficient was used to evaluate the relationship between steatocrit results and chemically measured fecal fat content.

## RESULTS

Effect of stool homogenate acidification on fat extraction

Several steatorrheal stool samples were analysed after acidification with various concentrations of perchloric acid.

A typical finding is shown in figure 1; The upper fat column was seen to increase in length with the degree of homogenate acidification. A typical normal stool sample result (no steatorrhea) is shown in figure 2. The acid steatocrit remained completely negative in normal samples.

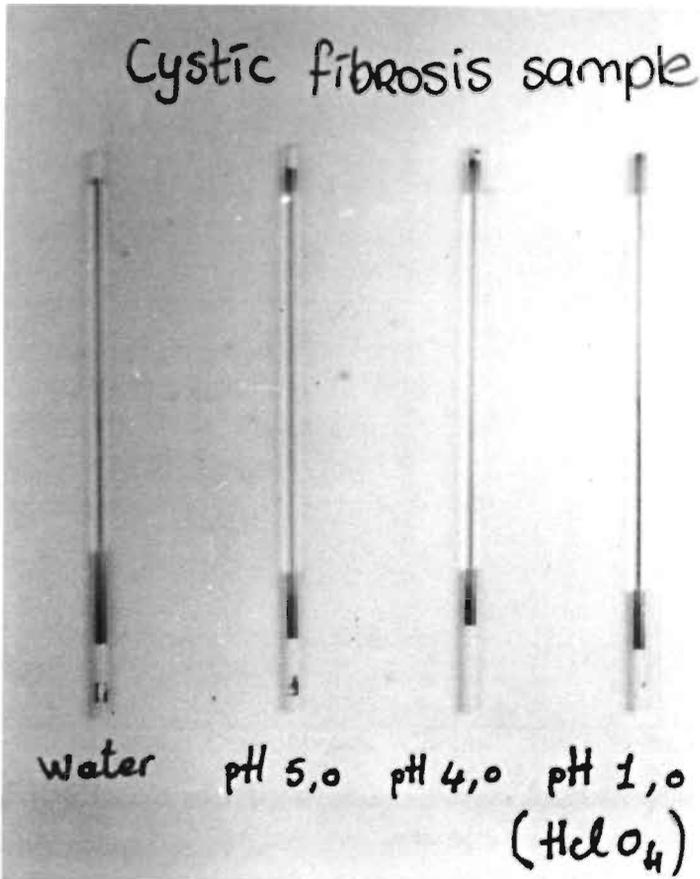
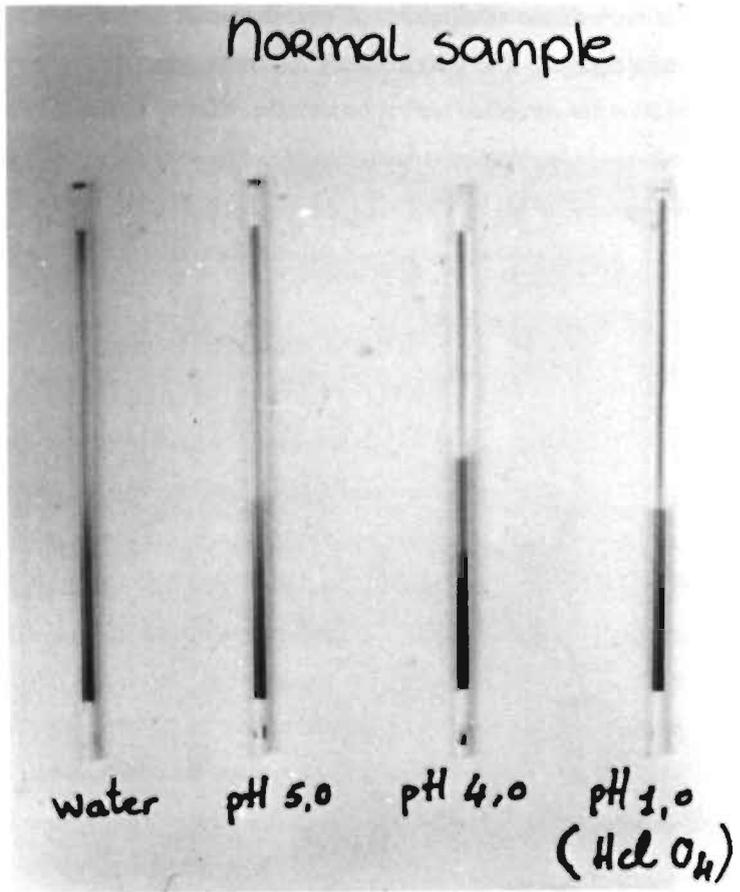


Figure 1 Effect of acidification with various concentrations of perchloric acid on the fat column length (upper part of picture) of a stool sample from a patient with cystic fibrosis.



**Figure 2** Effect of acidification with various concentrations of perchloric acid on fat extraction from a normal stool sample. Fat layer is absent at all pH values.

### Classic and acid steatocrit

Results of classic and acid steatocrit in 6 control and 9 cystic fibrosis patients (figure 3) were as follows : Steatocrit means and SEM in control patients were  $1.1 \pm 0.4$  and  $3.8 \pm 1\%$  for classic and acid steatocrit, respectively. This difference was not statistically significant. Steatocrit means and SEM in cystic fibrosis patients were  $5.4 \pm 1.9$  and  $26.9 \pm 4.3 \%$  for classic and acid steatocrit, respectively. This difference is significant ( $p < 0.01$ )

The precision of the methods was evaluated by comparing the variation coefficients; variation coefficients were 6.9 and 5.1 % for the classic and acid steatocrit methods, respectively.

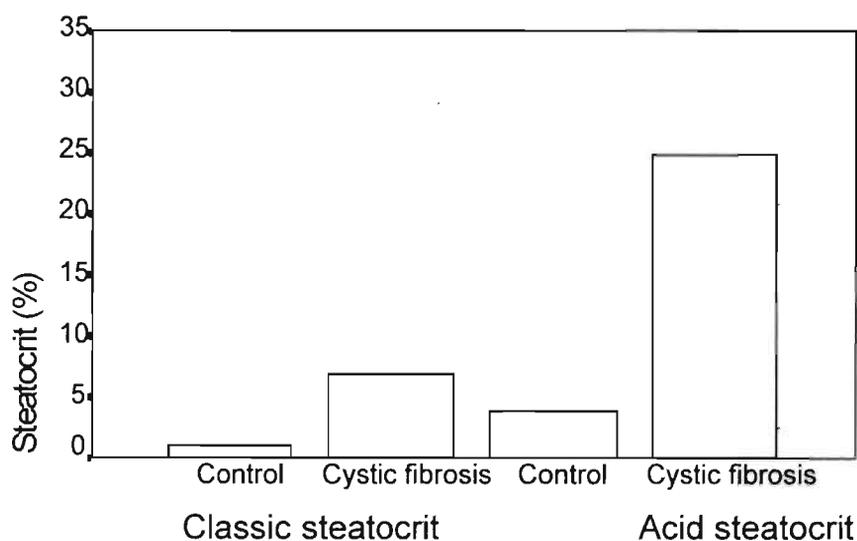


Figure 3 Classic and acid steatocrit results in six controls and nine patients with cystic fibrosis.



## DISCUSSION

Although several authors have reported the steatocrit method to be reliable for the screening of steatorrhea ( 3-5 ), Walters et al reported the method to be completely unreliable (6). Methodological inadequacies probably underlie these discrepancies. We have been using the "classic" steatocrit in our department for a few years and have found completely negative results in some patients with proven steatorrhea. We hypothesized that in some patients fat detection might be poor and that a possible solution to the problem would be an improved method of liberating fat during the centrifugation step. It has been shown in a recent study that fecal fat in patients with pancreatic insufficiency mainly consists of fatty acids and that the fecal triglyceride content does not differ from that of normal controls (7).

Fecal fatty acid molecules exist in the form of soaps (8). Further, since the pKa of most fatty acids is lower (about 4.8) than fecal pH, most fatty acids in stool would be present as ionized species or soaps. We speculated that fecal acidification would result in the conversion of ionized fatty acid species and soaps into the protonated species leading to easier separation into lipid and water phases during the centrifugation step of the steatocrit method.

Our results show that the effect of stool homogenate acidification on the length of the upper fatty layer very nicely confirms our predictions. Although we have not checked this point in detail, it can be expected that at the low PH values obtained after maximal acidification as performed in the present study, all fatty acids will be present in the protonated form.

Further, the fact that acidification of fecal samples from patients without steatorrhea and with completely negative steatocrit results did not result in the appearance of a fatty layer, probably indicating that the improved fat extraction is not a spurious artifactual finding but the result of better extraction of lost exogenous fat.

Khouri et al have suggested that ionized fatty acids are not readily stainable with Sudan stain, although staining does occur after acidification (2). By alkalization with sodium hydroxide, the same authors showed that fatty acids lost their ability to form fat droplets and to stain with Sudan red III (2). We suppose that similar mechanisms underlie the improvement of both the fat staining method and fat extraction by fecal acidification as shown in the present study.

A further advantage of acidification is that it enhances the visible boundaries between the

various layers, resulting in improved accuracy in the reading of layers lengths. Improved fecal fat extraction by acidification should therefore result in higher diagnostic sensitivity of the steatocrit method.

Our results show classic steatocrit in control children and in children with cystic fibrosis are similar to results published by other authors (4); However, acidified steatocrit results in both control children and cystic fibrosis patients were much higher than those obtained by classic steatocrit. Ongoing work in our laboratory aims at establishing normal population values for acid steatocrit in infants and children.

In order to better interpret the differences found between the steatocrit methods, we compared steatocrit results with fecal fat concentrations measured by the most accepted reference method. Our findings show that only acid steatocrit results correlate very significantly with fecal fat content as measured by the van de Kamer method. The literature is quite varied on this point. Several studies have looked for a correlation between steatocrit results and either the fat absorption coefficient or 3-day fecal fat excretion. A good correlation was reported by two studies (4,9) while a total lack of correlation was reported by a third author (6). As steatocrit is supposed to reflect fecal fat concentration we preferred to relate steatocrit results to fecal fat concentrations rather than daily excretion or fat absorption coefficients. To our knowledge only one study reporting results in a similar way found a significant relationship between steatocrit results and fecal fat content (3). We think our finding of a lack of correlation between classic steatocrit and fecal fat content results can best be explained by the small number of observations or by the lack of homogeneity in our patient material.

This lack of homogeneity was, however, purposely chosen as we were only interested in the correlation between steatocrit results and fecal fat content. We think a positive correlation between the two steatocrit methods and fecal fat content could have been found but the acid steatocrit method would always better correlate with fecal fat content.

We conclude that acidification of fecal homogenates led to a much better fat extraction by centrifugation, increased sensitivity of the steatocrit method and to a better prediction of fecal fat content as measured by chemical methods.

*Acknowledgment:* The authors thank the clinical laboratory staff for their kind and expert technical assistance. We are very grateful to Nutricia Netherlands for financial support.

## REFERENCES

- (1) van de Kamer JH, Huinink HTB, Weyers HA. Rapid method for determination of fat in feces. *J Biol Chem* 1949 ; 177 :349-55.
- (2) Khouri MR, Huang G, Shiau YF. Sudan stain of fecal fat : new insight into an old test. *Gastroenterology* 1989 ; 96 : 421-427.
- (3) Phuapradit P, Narang A, Mendonca P, Harris DA, Baum JD. The steatocrit : a simple method for estimating stool fat content in newborn infants. *Arch Dis Child* 1981 ; 56 : 725-727.
- (4) Colombo C, Maiavacca R, Ronchi M, Consalvo E, Amoretti M, Giunta A. The steatocrit : a simple method for monitoring fat malabsorption in patients with cystic fibrosis. *J Pediatr Gastroenterol Nutr* 1987 ; 6 : 926-930.
- (5) Iacono G, Carroccio A, Cavataio F et al. Steatocrit test : normal range and physiological variation in infants. *J Pediatr Gastroenterol Nutr* 1990 ; 11 : 53-57.
- (6) Walters MP, Kelleher J, Gilbert J, Littlewood JM. Clinical monitoring of steatorrhea in cystic fibrosis. *Arch Dis Child* 1990; 65 : 99-102.
- (7) Khouri MR, Huang G, Shiau YF. Fecal triglyceride excretion is not excessive in pancreatic insufficiency. *Gastroenterology* 1989; 96 : 848-852.
- (8) Shiau YF, Popper DA, Reed M, Umstetter C, Capuzzi D, Levine GM. Intestinal triglycerides are derived from both endogenous and exogenous sources. *Am J Physiol* 1985; 248 : G164-169.
- (9) Guarino A, Tarallo L, Greco L, Cesarano L, Guandalini S, Rubino A. Reference values of the steatocrit and its modifications in diarrheal diseases. *J Pediatr Gastroenterol Nutr* 1992; 14: 268-274.

## CHAPTER 4

### IMPROVED STEATOCRIT RESULTS OBTAINED BY ACIDIFICATION OF FECAL HOMOGENATES ARE DUE TO IMPROVED FAT EXTRACTION

M. Tran, P. Forget, A. Van den Neucker and B. Van Kreel

Department of Pediatrics and Clinical Chemistry, University Hospital Maastricht,  
Maastricht, The Netherlands

---

*J Pediatr Gastroenterol Nutr* 1996; 22: 157-160

#### *Abstract*

Conflicting results have been reported on the value of the steatocrit as a screening test for steatorrhea. We recently modified the test procedure by fecal acidification with the hope of improving fat extraction and consequently the sensitivity of the test. The aim of the present study was to ascertain, whether or not fecal acidification led to improved fat extraction, by comparing the fat content of both fatty and solid layers obtained by centrifugation of 12 acidified (acid steatocrit) and unacidified (classical steatocrit) steatorrheal stool samples.

The fat content of fatty and solid layers was evaluated using of the semiquantitative (+ = 1, ++ = 2, +++ = 3) scoring system described by Drummey, for the interpretation of the sudan microscopic method for fecal fat.

The fatty layers sum of scores for the 12 samples examined, was 31 and 16, for the acid and classical steatocrit respectively. The solid layers sum of scores for the 12 samples, was 13 and 24, for the acid and classical steatocrit respectively. Fat extraction from stool samples was significantly improved after fecal sample acidification ( $p < 0.005$ ). Acid steatocrit results agreed better with chemically measured fecal fat than classical steatocrit results.

We conclude that fecal acidification, by improving fat extraction, increases the reliability of the steatocrit method for the detection of steatorrhea.

## INTRODUCTION

Several methods are in use for the diagnosis of fat malabsorption. One of these is the 72 hour fecal fat quantitation method, which is regarded as the most accurate method to evaluate steatorrhea (1). However, there are several problems. First, it is a laborious method for laboratory technicians, and second, fecal collection for 3-5 days makes the method unpleasant for the patient and sometimes poorly reliable in non collaborating children.

Another well accepted test to screen for fat malabsorption is the Sudan staining method for fecal fat (2). Unfortunately this method is only semiquantitative.

In 1981 Phuapradit introduced the steatocrit method as a new, simple and easily repeatable method for measuring fecal fat content (3).

Although several authors have reported this method to be satisfactory for the evaluation of steatorrhea (3-5), some described it as quite unreliable (6). We have been using this method for years and have often found normal steatocrit values in patients, who, when measured chemically had steatorrhea with an increased fecal fat content.

As it has been shown that fecal acidification results in an enhanced sensitivity of the Sudan fecal staining method (7), we wondered whether fecal acidification could also be used to improve the sensitivity of the steatocrit method.

We consequently modified the reported (8) steatocrit method by adding perchloric acid to the fecal homogenate. Fat extraction was evaluated for classical and acid steatocrit methods, making use of the Sudan microscopic method for fecal fat.

We further compared calculated steatocrit results from acidified and unacidified samples, and related the results to fecal fat content of the same samples, measured by the reference chemical method of van de Kamer et al. (1).

## MATERIALS AND METHODS

### *Population studied*

Twelve stool samples from 4 premature babies, 3 boys and 1 girl, with a mean gestational age of 35,3 weeks (ranged from 27 5/7 to 35 5/7 weeks), were analysed by means of both

the classical and the acid steatocrit method.

Their postnatal age varied between 11 and 18 days. They received full oral formula feedings. Their weight ranged from 1810 g to 2360 g.

### Steatocrit methods

0,5 g solid stool was weighed and diluted with a volume of deionized water, equal to two times the weight of stool. The stool and water were premixed using a Vortex mixer. Subsequently, the mixture was homogenized using a 5 ml Potter Elvehjem tissue homogenizer. Then, the homogenate was aspirated into a 75  $\mu$ l plain haematocrit capillary. This capillary was sealed with wax at one end and centrifuged horizontally (13,000 rpm, 15 min) in a standard haematocrit centrifuge.

The method used for the acid steatocrit was exactly the same as that of the classical steatocrit, the only exception being, that after homogenizing, 5N perchloric acid was added to the homogenate in a volume equal to 1/5 of the homogenate. This acid homogenate was then mixed for 30 seconds using a Vortex mixer.

After centrifugation, three layers were distinguished: a basal solid layer, an intermediate liquid layer, and an upper fatty layer. After calculating the steatocrit results for both methods as usual, the capillaries were cut in the middle of the fatty, and of the solid layers using a special glass knife. Subsequently, the layers were removed from the capillaries, using a syringe. A standard amount of each of these fatty and solid layers was then transferred to different glass slides for staining with Sudan III dye. In this way, we acquired a total of 48 slides (24 from each steatocrit method, 12 fatty and 12 solid layers) for microscopic evaluation.

### Sudan stain method for fecal fat

For this purpose we used the split fat stain, which identifies both triglyceride and fatty acid (7). Several drops of 100% acetic acid and several drops of Sudan III solution were added. The preparation was subsequently mixed with the coverslip, which was then applied. The slide was gently heated on a lighter until bubbling. All preparations were examined while

still warm under high magnification (magnification of 400), by the same person who was blind to the steatocrit method used (classical or acid).

For quantification of the amount of fat detected microscopically, we used the criteria established by Drummey et al.(2). They are as follows: normal (+): up to 100 fat globules per high power field, varying in a diameter between 1 and 4  $\mu\text{m}$ , as noted on the micrometer scale always using a magnification of 400; Increased (2+): up to 100 fat globules per high power field, the diameter of fat globules varying between 1 and 8  $\mu\text{m}$ ; and markedly increased (3+): more than 100 fat globules per high power field, varying in size from 6 to 75  $\mu\text{m}$  in diameter.

The sum of the fatty and solid layer scores of all our samples, was calculated for both steatocrit methods, and results were compared. Fisher's exact probability test was used to test whether or not the solid layers microscopic fat content was dependent on sample acidification. Finally, calculated acidified and unacidified steatocrit results were compared and related to the chemically measured fecal fat content.

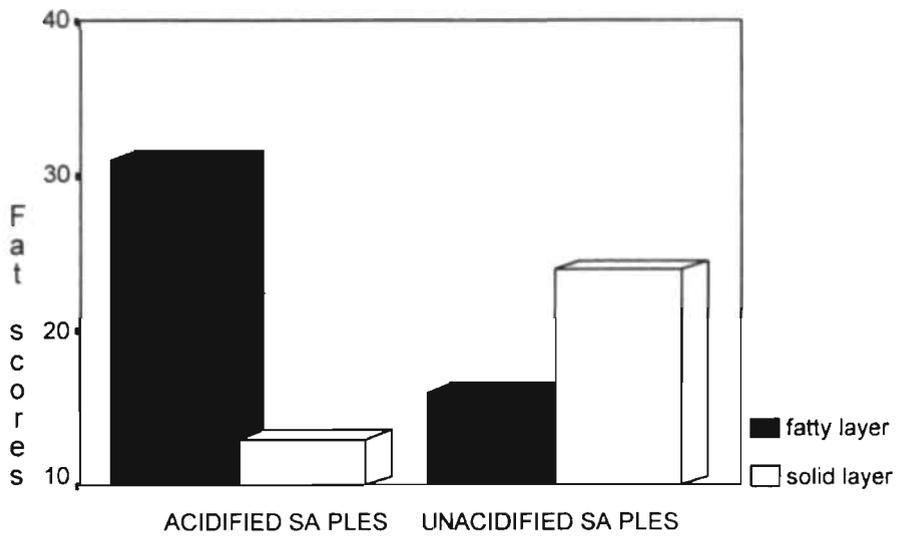
## RESULTS

Table 1 shows that acidification of the fecal homogenates before centrifugation (acid steatocrit method) results in a higher Drummey score in the fatty layers and a lower score in the solid layers. In four specimens (sample 6, 7, 8 and 10), the fatty layers obtained by the classical steatocrit method were so small that we did not succeed in making microscopical slides. Equal results were obtained by both the classical and the acid steatocrit method, in only one sample (sample 11).

**Table 1 Fatty and solid layer microscopical fat scores for both acid and classic steatocrit methods.**

SAMPLES	SCORES OF FAT GLOBULES (+, ++, +++)			
	FATTY LAYERS		SOLID LAYERS	
	ACID	CLASSIC	ACID	CLASSIC
2	+++	++	+	+
3	++	++	+	+++
4	+++	+	+	+++
5	+++	++	+	+++
6	+	-	+	++
7	++	-	+	++
8	+++	-	+	+
9	+++	+++	+	++
10	++	-	+	+
11	+++	+++	+	+
12	+++	++	+	+++
<b>SUM</b>	<b>31</b>	<b>16</b>	<b>13</b>	<b>24</b>

The sum of the fat scores for fatty and solid layers, and for both steatocrit methods is summarized in figure 1. The number of solid layer samples with low microscopic ( $\leq +$ ) fat content, was 11 of 12, and 4 of 12, for the acidified and unacidified samples respectively ( $p < 0.005$ , Fisher's exact probability test).



**Figure 1** Sum of 12 microscopical Sudan fat globule scores (1, 2, 3) performed on fatty and solid layers of acidified and unacidified fecal samples.

The calculated steatocrit results for both steatocrit methods, and the chemically measured fecal fat content for the 12 samples, are shown in table 2. Chemical fat measurements of two samples (1 and 2) were not performed. The chemically measured fecal fat concentration was very high in all samples and corresponded with high acid steatocrit results, while only 5 classical steatocrit results were elevated.

**Table 2 Classic and acid steatocrit results compared to chemically measured fecal fat in 12 steatorrheal fecal samples.**

SAMPLES	CLASSIC STEATOCRIT (%)	ACID STEATOCRIT (%)	FECAL FAT (GRAM %)
1	5.3	81.7	-
2	2.5	72.4	-
3	5.3	71.1	16.6
4	28.8	93.3	28.5
5	26	90.9	26.7
6	6.2	92.6	28.3
7	3.1	92.5	18.7
8	2.8	94.2	26.5
9	59.8	93.7	24.3
10	6.2	96.4	10.3
11	63.9	96	27.3
12	48.7	94.4	20.6

## DISCUSSION

There has always been a need for a simple, rapid and easy to perform screening test for fat malabsorption. Such a test would not only be useful for the detection of steatorrhea but also for the therapeutic monitoring of children treated for pancreatic insufficiency.

The steatocrit is a simple and rapid micromethod that can be repeated at short time intervals

(3). It is inexpensive and not invasive (5). Some authors have reported it as a very satisfactory screening test (3-5), but others have found it quite unreliable (6). Our experience with the method has shown the steatocrit to often be normal, in fecal samples with a very high chemically measured fecal fat content. This could be due to inefficient fecal fat extraction at the centrifugation step. Therefore we recently improved the steatocrit method by acidifying the fecal homogenate before centrifugation (8).

The present study was set up to study the effect of fecal homogenate acidification on fat extraction at the centrifugation step. If fat extraction improves by acidification, we would expect to find more fat globules in the fatty layer and less in the solid layer, after centrifugation of acidified fecal samples, when compared to unacidified samples. Our results are in agreement with our expectations and support the hypothesis that fecal acidification improves fat extraction, and should consequently improve the sensitivity of the steatocrit. Due to various reasons, the fat content of premature infants' stool, is known to be very high. Confirming the latter, chemical fat measurements of all our samples from 4 premature babies showed very high values. The acid steatocrit seems to reflect these very high fat contents, while classical steatocrit results were high in only 5 of 12 samples. The correlation between chemical fat measurement and acid steatocrit has been reported previously (8). Such a correlation cannot be shown in the present study where only high-fat-content stools were evaluated.

Results of the present study do support our previous findings, confirming, that fecal acidification improves fat extraction at the centrifugation step, and consequently increases the reliability of steatocrit results for the detection of fat malabsorption. Because the Sudan staining method for fecal fat is only semiquantitative, we suggest using the acid steatocrit as a good alternative to chemical fat measurement.

*Acknowledgement:* The authors wish to thank the clinical laboratory staff for their kind and expert technical assistance. We are very grateful to Nutricia Netherlands for financial support.

## REFERENCES

- (1) Van de Kamer JH, Huinink HTB, Weyers HA. Rapid method for determination of fat in feces. *J Biol Chem* 1949; 177: 349-55.
- (2) Drummey GD, Benson JA, Jones CM. Microscopical examination of the stool for steatorrhea. *N.Engl J Med* 1961; 264: 85-7.
- (3) Phuapradit P, Narang A, Mendonca P, Harris DA, Baum JD. The steatocrit: a simple method for estimating stool fat content in newborn infants. *Arch Dis Child* 1981; 56: 725-727.
- (4) Iacono G, Carroccio A, Cavataio F, Montalto G, Mancusco C, Balsamo V et al. Steatocrit test: normal range and physiological variation in infants. *J Pediatr Gastroenterol Nutr* 1990; 11: 53-57.
- (5) Columbo C, Maiavacca R, Ronchi M, Consalvo E, Amoretti M, Giunta A. The steatocrit: a simple method for monitoring fat malabsorption in patients with cystic fibrosis. *J Pediatr Gastroenterol Nutr* 1987; 6 : 926-930.
- (6) Walters MP, Kelleher J, Gilbert J, Littlewood JM. Clinical monitoring of steatorrhea in cystic fibrosis. *Arch Dis Child* 1990; 65: 99-102.
- (7) Khouri MR, Huang G, Shiao YF. Sudan stain of fecal fat: new insight into an old test. *Gastroenterology* 1989; 96: 421-427.
- (8) Tran M, Forget P, Van den Neucker A, Strik J, van Kreel B, Kuijten R. The acid steatocrit: a much improved method. *J. Pediatr. Gastroenterol Nutr.* 1994; 19: 299-303

## CHAPTER 5

### CLINICAL USE OF ACID STEATOCRIT

A. Van den Neucker<sup>1</sup>, N. Pestel<sup>1</sup>, T. My Dung Tran<sup>1</sup>, P.Ph. Forget<sup>1</sup>, H.J. Veeze<sup>2</sup>,  
J. Bouquet<sup>2</sup>, M. Sinaasappel<sup>2</sup>.

<sup>1</sup>Department of Pediatrics, University Hospital Maastricht and <sup>2</sup>Sophia Children's Hospital  
Rotterdam, The Netherlands

---

Submitted for publication

#### *Abstract*

Malabsorption of fat is an important gastrointestinal cause of malnutrition and growth retardation in childhood. The golden standard for the evaluation of fat malabsorption is the fecal fat balance method. The acid steatocrit method has recently been introduced as a simple method to evaluate fecal fat. The present study aimed at evaluating the acid steatocrit in clinical practice. Fecal fat excretion and acid steatocrit results were determined in 42 children, half with and half without fat malabsorption. Acid steatocrit results correlated significantly with both fecal fat excretion ( $p < 0.01$ ) and fecal fat concentration ( $p < 0.001$ ). Sensitivity and specificity of the acid steatocrit for the diagnosis of malabsorption was 90% and 100% respectively. We consider the acid steatocrit method useful for the screening and monitoring of patients with steatorrhea. Acid steatocrit, Steatorrhoea, Cystic Fibrosis.

## INTRODUCTION

Malabsorption of fat is the most important gastrointestinal cause of malnutrition and growth retardation in childhood. The detection of steatorrhea is useful for the diagnosis of intestinal and pancreatic disease. The gold standard for the evaluation of patients suspected of fat malabsorption is the fat balance method whereby fecal fat is chemically measured according to the method of van de Kamer (1). This method is cumbersome, laborious, expensive and often difficult to manage in children. In 1981 Phuapradit et al. introduced a simple test to evaluate fecal fat content (2). Although some authors found this test quite reliable (3), others did not (4). As previously reported, substantial improvement of the method was obtained by acidification of fecal samples, acid steatocrit (AS) (5).

The present study was designed to compare the fecal fat excretion with the acid steatocrit for the diagnosis of fat malabsorption in children.

## PATIENTS

Forty two children, 23 boys and 19 girls, aged between 6.5 months and 18 years (mean: 6.6 years) were selected for the study. All these children were suspected of fat malabsorption, on the basis of anamnestic and clinical parameters. The different diagnoses of our patients are shown in table 1.

## METHODS AND MATERIAL

Three days stool collections from each patient were collected in separate containers, one container for each day. The stools were frozen at  $-18^{\circ}\text{C}$  Celsius. Fat content in each collection was determined by the titrimetric method described by van de Kamer et al. (1). Acid steatocrit from a single stool sample on day 1 and from a sample out of the homogenized 72 hours collection were determined by the method of Tran et al. (5) Feces (0.5 gr.) was diluted (1/4) with deionized water and thoroughly homogenized making use of a 5ml. Potter Elvehjem tissue homogenizer. Perchloric acid 5N was added to the homogenate in a volume equal to 1/5 of the homogenate volume. The resulting acid homogenate was mixed for 30 seconds making

**Table 1 List of diagnosis (n = 42).**

DIAGNOSIS	NUMBER OF CASES
CYSTIC FIBROSIS	20
MENTAL RETARDATION	2
RECURRENT DIARRHEA	5
FAILURE TO THRIVE	5
COELIAC DISEASE	2
INFLAMMATORY BOWEL DISEASE	1
SHORT BOWEL	1
CHOLEDOCHAL CYSTE	1
SUCRASE-ISOMALTASE DEFICIENCE	1
RECURRENT ABDOMINAL PAIN	1
UNKNOWN	3

use of a standard Vortex mixer. The homogenate was aspirated into a 75  $\mu$ l plain glass haematocrit capillary. The capillary was subsequently centrifuged horizontally (13000 rpm. for 15 min.) in a standard centrifuge. After centrifugation, the lengths of the upper (fat) and the bottom (solid) layers were measured by means of a graduated magnifying lens. Steatocrit was calculated as follows: percentage of (the fatty layer length / (fatty layer length + solid layer length)).

In order to validate the diagnostic value of the acid steatocrit we studied two patients groups, one with and one without steatorrhea. We divided the patients according to previous clinical data and fat excretion results, whereby a fecal fat excretion  $\geq$  3gr./day was considered as abnormal (6).

## RESULTS

Correlation coefficients between acid steatocrit results from either a single stool sample or from the sample taken from the 72 hours homogenized collection, and both the fecal fat excretion and the fecal fat concentration are shown in table 2.

**Table 2** Correlation between the results of the acid steatocrit from either a single stool sample and a sample from the homogenised stool collection and the results of both fat excretion and fecal fat concentration in 42 children suspected of malabsorption.

ACID STEATOCRIT	FAT EXCRETION	FAT CONCENTRATION
SINGLE STOOL	$r = 0.4 (p \leq 0.01)$	$r = 0.82 (p \leq 0.001)$
COLLECTION	$r = 0.68 (p \leq 0.001)$	$r = 0.82 (p \leq 0.001)$

The sensitivity and the specificity of the acid steatocrit determination from either a single stool sample or a sample taken from the 72 hours homogenized collection, and of the fecal fat concentration, for the diagnosis of steatorrhea are shown in table 3.

**Table 3** Sensitivity and specificity of the acid steatocrit determination from a single stool sample and from a homogenised stool collection sample and of the fecal concentration, for the diagnosis of steatorrhea.

	SENSITIVITY	SPECIFICITY
AS SINGLE STOOL (%)	75%	84%
AS COLLECTION (%)	90%	100%
FAT CONCENTRATION (%)	100%	76%

AS: Acid steatocrit

Fig.1 shows our AS results from the homogenized fecal collection sample related to the fecal fat excretion (g/day). The reference line for AS was set at the level of 10% (5), and the cut off reference line for the daily fat excretion was set at the level of 3 gram per day (6). As can be seen from the figure, one false positive and three false negative acid steatocrit results were found in our study population. Regarding these results one should notice that they are very close to the reference lines: the false positive steatocrit result had a value of 16% and the results of the fecal fat excretion corresponding to the false negative steatocrit results were 4.9; 6.4 and 7.7g/day, and concern children aged 12. 6 and 13 years respectively.

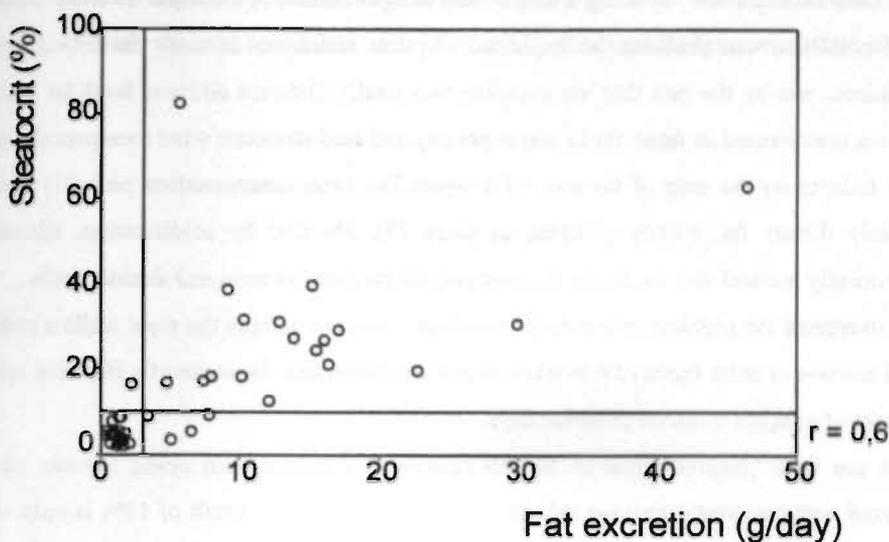


Figure 1 Relationship between acid steatocrit and fat excretion. Reference lines for acid steatocrit at 10 % and for fat excretion at 3 gram / day.

## DISCUSSION

Since the fecal fat balance excretion as described by van de Kamer is cumbersome, expensive and unpleasant for all involved, there is a need for a simple test. Some authors reported the steatocrit micromethod described by Phuapradit as a simple method for monitoring fat malabsorption (3), and reported a good correlation ( $r=0.93$ ) with the fecal fat excretion. Although others considered the steatocrit method of Phuapradit unreliable and mentioned the difficulty to delineate the fatty layer (4) and the impression that fat remains in the solid layer, as a problem of this method. This problem was solved by acidification of the fecal sample, whereby fat extraction is improved, and steatocrit results correlate much better with chemically measured fecal fat (5).

Our AS results correlate satisfactorily with chemically measured fecal fat concentrations and somewhat less, but still significantly, with fecal fat excretion. However, our correlation coefficient is lower than the correlation coefficient of the steatocrit without acidification as published in a previous study (3). We have no explanation for this discrepancy, and other

authors also failed to reproduce these results (4). The lesser correlation of the AS results with the fecal fat excretion, by using a single stool sample instead of a sample from the homogenized collection can probably be explained by the variability of daily fat consumption in children, and by the fact that we compare two totally different entities: fecal fat excretion, which is expressed as fecal fat in grams per day and acid steatocrit what measures the ratio of the fatlayer on the sum of fat and solid layers. The latter determination probably measures mainly dietary fat, mostly occurring as soaps (7), liberated by acidification, whereas the chemically method also measures the endogenous fat from bacteria and shedded cells.

To overcome the problem of the daily variability, one can sample the stool while a standardized amount of daily dietary fat is taken or one can determine the mean of a few acid steatocrit results of samples taken on different days.

The one false positive result of the AS concerned a patient with cystic fibrosis who was treated with pancreatic enzyme substitution therapy. This AS result of 16% is only slightly elevated considering the values obtained in cystic fibrosis patients on substitution therapy, which are mostly between 20 and 30%.

The three false negative results of the acid steatocrit can probably be explained by the choice of the reference line for the normal fecal fat excretion. Fecal fat excretion higher than 4.5g/24hours is considered pathologic (6,8) for children and adolescents, whereas other authors consider 7g/day the upper limit of normal fecal fat excretion in adults (9). The reference line for normal daily fecal fat excretion varies clearly with age and dietary fat intake as previously suggested by Williams (8). Taking account of these remarks, the fat excretion studies of 2 of our 3 patients with false negative steatocrit results could, due to their ages (12 and 13 years), still be considered "normal" and in agreement with AS results. This would reduce the disagreement between the methods to only a few ones.

## CONCLUSION

Acid steatocrit results are highly correlated with the chemically measured fecal fat concentration and significantly correlated with the fecal fat excretion. Although single sample acid steatocrit results are slightly less sensitive and specific than other measured parameters for the diagnosis of steatorrhoea, acid steatocrit measured in the stool samples taken from the homo-

genized collection compare favourably with the fecal fat concentration. We consider the acid steatocrit method useful for the screening and monitoring of patients with steatorrhea. If it is necessary to know the real coefficient of fat absorption, the fecal fat balance method is needed.

## REFERENCES

- (1) van de Kamer JH, Huinink HTB, Weyers HA. Rapid method for determination of fat in feces. *J Biol Chem* 1949; 177:349-55.
- (2) Phuapradit P, Narang A, Mendonca P, Harris DA, Baum JD. The steatocrit: a simple method for estimating stool fat content in newborn infants. *Arch Dis Child* 1981; 56:725-7.
- (3) Colombo C, Maiavacca R, Ronchi M, Consalvo E, Amoretti M, Giunta A. The steatocrit: a simple method for monitoring fat malabsorption in patients with cystic fibrosis. *J Pediatr Gastroenterol Nutr* 1987;6:926-30.
- (4) Walters MP, Kelleher J, Gilbert J, Littelwood JM. Clinical monitoring of steatorrhea in cystic fibrosis. *J Pediatr Gastroenterol Nutr* 1990; 65:99-102.
- (5) Tran M, Forget P, Van den Neucker A, Strik J, Kreel van B, Kuijten R. The acid steatocrit: a much improved method. *J Pediatr Gastroenterol Nutr* 1994; 19: 299-303.
- (6) Navarro J, Schmitz J. *Gastroenterologie pédiatrique*, Flammarion Médecine Sciences, Paris 1986.
- (7) Quinlan PT, Lockton S, Irwin J, Lucas AL. The relationship between stool hardness and stool composition in breast- and formula-fed infants. *J Pediatr Gastroenterol Nutr* 1995; 20:81-90.
- (8) Williams HH, Endicott EN, Shepherd ML, Galbraith H, Macy IG. Fat excretion by normal children. *J. of Nutrition* 1943; 25, 379.
- (9) Bai JC, Andrúsh A, Matelo G, Martinez C, Vazquez H, Boerr L, Sambuelli A. Fecal fat concentration in the differential diagnosis of steatorrhea. *Am. J. Gastroenterol.* 1989; 27-30.

## CHAPTER 6

### ROLE OF LANSOPRAZOLE IN CHILDREN WITH CYSTIC FIBROSIS: EVIDENCE FOR IMPROVED FAT ABSORPTION AND NUTRITIONAL STATUS

Tran TMD, Van den Neucker A, Hendriks JJE, Forget P ( junior ), Forget P ( senior )

Department of Pediatrics, University Hospital Maastricht, Maastricht, The Netherlands

---

Submitted for publication

#### *Abstract*

Stearrhea and nutritional parameters were investigated in 15 cystic fibrosis children before starting lansoprazole, after 3 months on lansoprazole (15mg/day) and 3 months after stopping lansoprazole. There were 5 girls and 10 boys with a mean age of 9.5 years (range: 3.1 - 22.6y). Patients were their own controls. Acid steatocrit, anthropometric methods and DXA were used to evaluate steatorrhea and the nutritional status respectively. On lansoprazole, mean  $\pm$  SD acid steatocrit values decreased from  $37.1 \pm 8.8$  % to  $28.5 \pm 10.6$  % ( $p = 0.02$ ). During lansoprazole therapy, significant mean Z-score changes were found for weight ( $+0.14$  /  $p = 0.02$ ), length ( $+0.15$  /  $p = 0.03$ ), subscapular ( $+ 0.61$  /  $p = 0.003$ ), suprailiaca ( $+0.8$  /  $p = 0.002$ ) and the sum of 4 skinfolds ( $+0.61$  /  $p = 0.002$ ) . Z-scores deteriorated again after stopping lansoprazole. Fatmass and bone mineral content increased significantly on lansoprazole ( $p = 0.008$  and  $p = 0.005$  resp.). Improvement of subscapular Z-score was related to improvement of acid steatocrit values ( $p = 0.01$ ) during treatment. We conclude that lansoprazole as adjuvant therapy significantly improves fat absorption and the nutritional status in CF children.

## INTRODUCTION

Cystic fibrosis (CF) is an autosomal recessive inherited disease. Defect in the chloride transepithelial transport system results in viscous mucus in various organs with lung and pancreas mostly affected (1). Both, pancreatic insufficiency resulting in malabsorption and high energy expenditure due to increased respiratory work (2-3), are thought to be responsible for the poor nutritional condition in CF patients. Since malnutrition can compromise absorptive and immune function resulting in a shortened survival (4), all efforts should be made in order to improve the nutritional status of these patients. Unfortunately, high doses of pancreatic enzymes did not solve the problems of malabsorption (5) and colon stricture has been observed in CF children on this regimen (6,7). Further, the use of hypercaloric diets did not result in significant improvements of Z-scores for weight, length and skinfolds in CF children (8). Only parenteral nutrition and either oral or enteral elemental and semielemental nutrition have been shown to significantly improve the nutritional condition of these children (9-15). The latter strongly suggests that nutrient maldigestion plays a crucial role in the poor response to oral hypercaloric diets. As cystic fibrosis patients have a low duodenal pH probably linked to fat maldigestion (16), inhibition of gastric acid production could improve absorption. The reported effects of H<sub>2</sub>-receptor antagonists and prostaglandine E<sub>2</sub> on steatorrhea have been variable (17-22). Insufficient inhibition of gastric acid could be responsible for these unconvincing results. Recently, in a double blind study, a significant improvement in steatorrhea was found when a proton-pump inhibitor was added as adjuvant therapy in pancreatic enzyme treated cystic fibrosis patients (23). The effect of proton pump inhibitors on the nutritional condition of children with CF have not yet been reported. The aims of our study were to evaluate the effects of lansoprazole (proton-pump inhibitor) on both steatorrhea and the nutritional condition of CF patients while on enzyme therapy.

## SUBJECTS AND METHODS

### *Study design*

As the effect of a proton pump inhibitor on fat balance has been convincingly proven in a

double blind study (23), we adapted a prospective open study design wherein patients were their own controls. In the month preceding the study, all patients were screened for steatorrhea by measuring fecal acid steatocrit once every 10 days. Patients with a mean acid steatocrit value higher than 25% (normal values in our laboratory < 20%) were invited to take part in the study. After evaluation of nutritional parameters by DXA and anthropometric methods, lansoprazole was added to their standard treatment in a dose of 15 mg day before breakfast for 3 months. When fat malabsorption did not change after 2 months, the dose was doubled in children older than 10 years and weighing more than 30 kg. During the lansoprazole treatment period 9 fecal samples were taken with an interval of 10 days for acid steatocrit measurements. The mean of these 9 measurements was used as a measure of steatorrhea during the treatment period. After 3 months on treatment, the nutritional condition assessment was repeated. All measurements of nutritional condition were performed on a single day. Three fecal samples for acid steatocrit determinations and anthropometric parameters were again measured respectively 1 month and 3 months after stopping lansoprazole therapy. Dietary evaluations were performed at the start, at the end and one month after stopping lansoprazole.

#### Patients population

23 CF out-clinic patients from the academic Hospital Maastricht were recruited. All patients were treated with pancreatic enzymes. Of these, 2 patients were too ill to participate in the study. 21 patients were screened for steatorrhea while on pancrease enzyme. 15 of them who had steatorrhea were included. In most children, the CF diagnosis had been made during the first year of life by repeated positive sweat tests, all 15 children were considered to have pancreatic insufficiency on the basis of abnormal fecal chymotrypsin, 72 hours fat balance (24) and increased acid steatocrit results (25). Mean energy intake was 113 % RDA (recommended daily allowance). The mean number of pancreas enzyme capsules (Pancrease) taken by 13 of these patients was 20 (range: 11 - 33), one patient took 3 Pancrease capsules (5000E lipase, 2900E amylase, 330E protease) and 6 Panzytrat tablets (25000E lipase, 22500E amylase, 1250E protease) and another one took 10 Creon capsules (8000E lipase, 9000E amylase, 450E protease) per day. Mean age, weight and length of those 15 children were 9.5 y (range: 3.1 - 22.6 y); 29.3 kg (range: 13.6 - 67.6 kg) and 131 cm (range: 97.7 - 184.9 cm)

respectively. Their nutritional status was moderately altered with a mean Body Mass Index (BMI) of 15.6 (range : 13.2 - 18.3). Mean predicted values of FEV1 and FVC were respectively 81.3% (range: 39 - 114%) and 85.5% (range: 44 - 108%). Informed patient and parental consent were obtained.

#### Evaluation of fat malabsorption by acid steatocrit

The acid steatocrit was determined as previously reported (25). In short, 0.5g solid stool was weighed and diluted with a volume of deionized water, equal to two times the weight of stool. The stool and water were premixed using a Vortex mixer. Subsequently, the mixture was homogenized using a 5 ml Potter Elvehjem tissue homogenizer. After then 5N perchloric acid was added to the homogenate in a volume equal to 1/5 of the homogenate. After mixing with the Vortex, the acidified homogenate was aspirated into a 75  $\mu$ l plain haematocrit capillary. This capillary was sealed with wax at one end and centrifuged horizontally (13000 rpm, 15 min). After centrifugation, 3 layers were distinguished: a basal solid layer (SL), an intermediate liquid layer and an upper fatty layer (FL). Acid steatocrit was calculated as  $(FL / (FL + SL)) \times 100\%$

### EVALUATION OF NUTRITIONAL STATUS

#### Anthropometry

The arm circumference, biceps, triceps, subscapular and suprailiac skinfolds were measured 3 times on the left side of the body using the Harpenden caliper. Average values were taken. Weight and length were also measured. BMI was calculated as  $\text{weight} / (\text{length}^2)$ . The Z-score

(Z-score is defined as  $(X - x) / S$  where X is the patients 's measurement, x is the mean value for age and sex and S is the standard deviation of x) of all these anthropometric parameters were calculated based on the reference data described by Gerver and de Bruin (26). A negative value indicates values under the mean reference value and a positive or negative change in Z-score means catch up or slowing down of growth respectively. All measurements were done

by the same investigator (TT).

#### Dual - energy x-ray absorptiometry (DXA)

DXA measurement is based on the differential tissue attenuation of photons of two energy levels from an X-ray source (27). All patients underwent total body scan performed with a DPX (Lunar Radiation Corp, Madison, WI) total body scanner. The results were analysed with a paediatric software programme, version 1.5e. Daily quality assurance test was performed according to the manufacturer's directions. Total non bone LBM (lean body mass), total BMC (bone mineral content), total body FM (fatmass) and BMD (bone mineral density) Z-score were measured by DXA procedure. These results were compared to those of the reference population, recently described by Ogle et al., who studied the body composition by DXA in 265 normal individuals aged 4 - 26 year (28).

#### Diet evaluation

At the beginning, at the end and one month after stopping lansoprazole nutrient intake was assessed by a specially trained clinical CF dietitian from consecutive 3 day food diaries including one weekend day. Intakes were expressed as kilocalorie per kg bodyweight for the energy intake and gram per kg bodyweight for fat-, carbohydrate- and protein-intakes, using the netherlands nutrients table "NEVO" 1993.

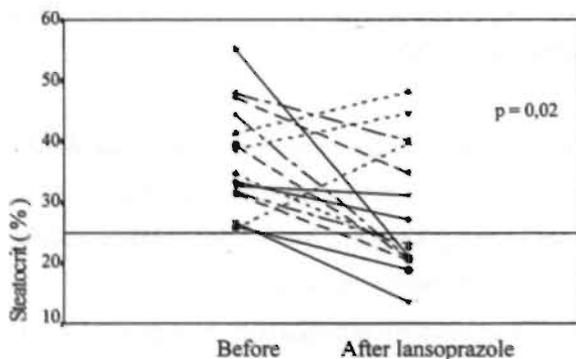
#### Statistic

All data were analysed by using SPSS statistic program. Anthropometric parameters and body composition results measured before the start and at the end of the trial were compared making use of Wilcoxon one sample test. The sign test was used to compare LBM, FM and BMC assessed by DXA, with the reference population described by Ogle.

## RESULTS

### Fat malabsorption

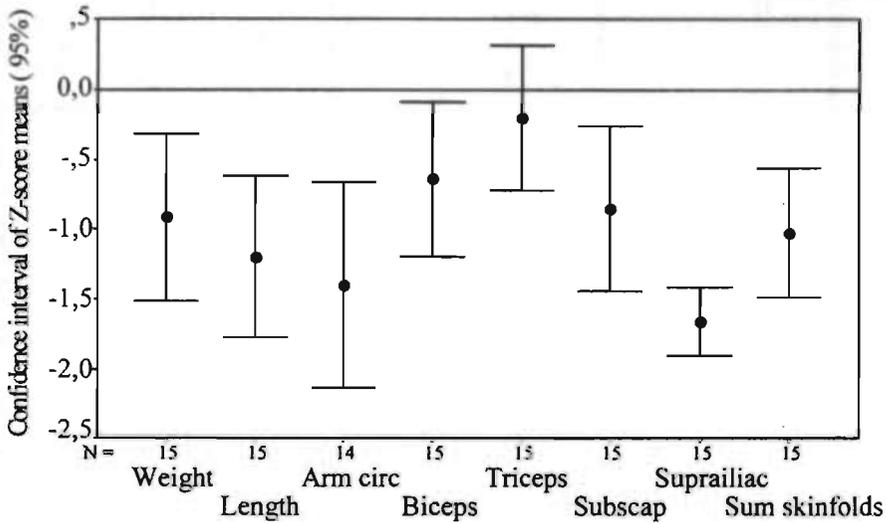
Despite standard pancreas enzyme, 15 of 21 children had steatorrhea with an average  $\pm$  SD pretreatment acid steatocrit value (mean of 3 determinations in each patient) of  $37.1\% \pm 8.8\%$ . After 3 months of treatment with lansoprazole, there was a significant ( $p = 0.02$ ) improvement in steatorrhea with a mean  $\pm$  SD acid steatocrit value of  $28.5\% \pm 10.6\%$ . Eight patients on lansoprazole had a mean acid steatocrit value lower than 25% (fig 1). In this group the mean decrease was 16% (44.2% of start value). In 3 children the acid steatocrit value decreased with 9% (20.6% of start value) but was not completely corrected. In 4 children fat malabsorption did not improve at all. Four children received a double dose of lansoprazole for 1 month, resulting in a decreased acid steatocrit results in 2 (decrease of 8.6% and 19%). Due to social problems one child was dropped out of the study after the lansoprazole period. Mean  $\pm$  SD acid steatocrit value for the remaining 14 children in the first month after stopping lansoprazole was  $29.7\% \pm 13.9\%$ , which was not significantly different from the values on lansoprazole. Of 4 children whose acid steatocrit was not changed on lansoprazole, 2 had higher acid steatocrit values after stopping.



**Figure 1** Acid steatocrit before and after 3 months treatment with lansoprazole in 15 CF children. A line through the 25 % value is drawn showing the study inclusion limit. Acid steatocrit values on lansoprazole are significantly decreased ( $p = 0.02$ ).

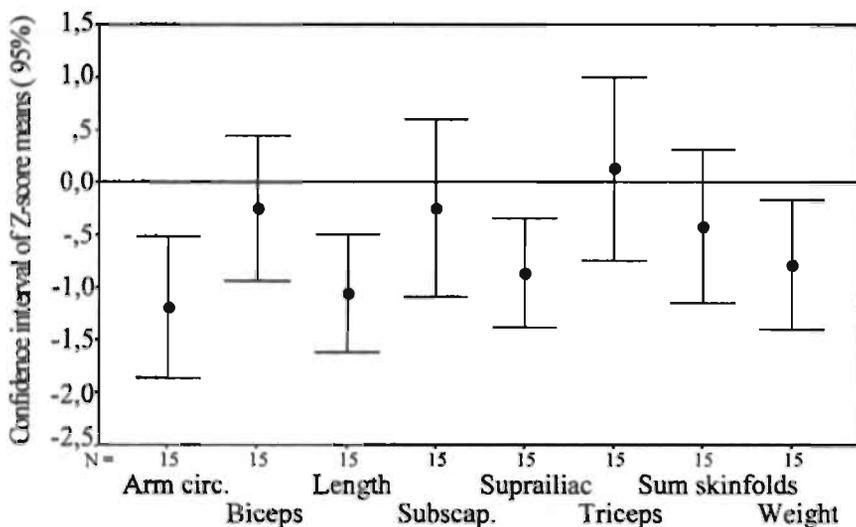
### Anthropometric parameters

Mean and 95% CI (confidence interval) for weight, length, arm circumference, 4 skinfolds and the sum of these 4 skinfolds expressed as Z-score are shown in fig.2. For all parameters except the triceps, the CI do not include the reference 50th centile line (Z-score 0), underscoring the fact that except for the triceps skinfold, all other anthropometric parameters mean Z-scores were significantly decreased in our CF children when compared to those of the normal population. The suprailiac skinfold was most abnormal and showed the smallest interindividual variation.



**Figure 2** Mean Z-scores and 95% confidence interval of anthropometric parameters in 15 CF children before lansoprazole therapy. The line through 0 represents the 50th centile of the reference population. The differences between the study group and the reference population are significant when the CI do not include the Z-score 0 line. All anthropometric parameters were significantly decreased in our CF children except for the triceps skinfold.

After treatment with lansoprazole, Z-scores of anthropometric parameters improved significantly. All parameters moved toward the Z-score 0 line (50th centile for reference population). The Z-scores of biceps, subscapular and sum of the 4 skinfolds did not significantly differ from the reference population any more (fig.3).



**Figure 3** Mean Z-scores and 95% confidence interval of anthropometric parameters in 15 CF children on lansoprazole for 3 months. The differences between the study group and the reference population are significant when the CI do not include the Z-score 0 line. Several parameters (biceps, subscapular and sum of 4 skinfolds) normalized during lansoprazole treatment (see figure 2).

Z-score changes for all anthropometric parameters studied are shown in table 1. Except for arm circumference, biceps and triceps skinfolds, Z-scores of all parameters improved significantly. Subscapular, suprailiac and the sum of the 4 skinfolds showed the most significant changes. The acid steatocrit results during lansoprazole treatment were significantly lower ( $p = 0.01$ ) in our patient subgroup with subscapular Z-score improvement  $\geq 0.5$  when compared to the subgroup showing lower Z-score changes. Three months after lansoprazole was stopped, 5 children were dropped out of the study; 2 because of the far distances from home, 1 had

taken lansoprazole again because of increased symptoms of steatorrhea and abdominal pain and 2 because of social problems. Nutritional parameters were therefore evaluated in only 10 children 3 months after stopping lansoprazole. Z-scores of all anthropometric parameters deteriorated, with weight and subscapular Z-score changes reaching statistical significance (table 1).

**Table 1** Mean Z-scores of anthropometric parameters in 15 CF children before (T0), after 3 months on lansoprazole (T3) and 3 months after stopping lansoprazole (T6).

Anthrop. Parameters	T0 Mean Z-score (n = 15)	T3 Mean Z-score (n = 15)	T6 Mean Z-score (n = 10)	T3 - T0 Mean Z-score (n = 15)	T6 - T3 Mean Z-score (n = 10)
Weight	-0.91	-0.78	-1.38	0.14 (p = 0.02)	-0.6 (p = 0.01)
Length	-1.2	-1.05	-1.23	0.15 (p = 0.03)	-0.18 (p = 0.16)
Armcircum	-1.4	-1.19	-1.74	0.22 (p = 0.05)	-0.55 (p = 0.52)
Biceps	-0.63	-0.24	-1	0.39 (p = 0.06)	-0.76 (p = 0.21)
Triceps	-0.2	0.14	-0.72	0.34 (p = 0.2)	-0.86 (p = 0.26)
Subscapular	-0.85	-0.24	-1.14	0.61 (p = 0.003)	-0.9 (p = 0.03)
Suprailiaca	-1.66	-0.86	-1.13	0.8 (p = 0.002)	-0.27 (p = 0.68)
Sum skinfolds	-1.02	-0.41	-1.16	0.61 (p = 0.002)	-0.75 (p = 0.31)

### Body composition

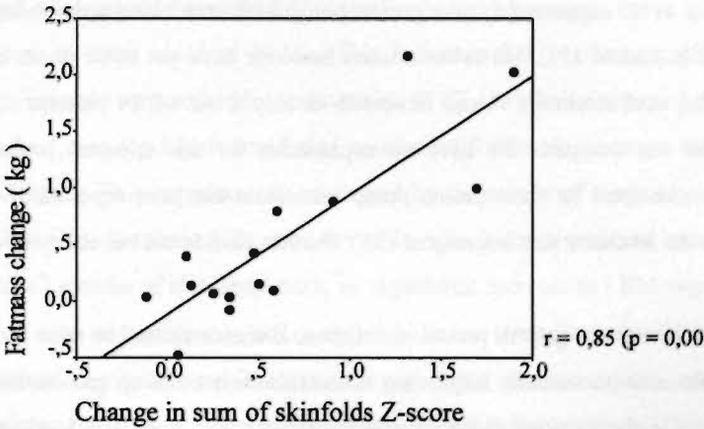
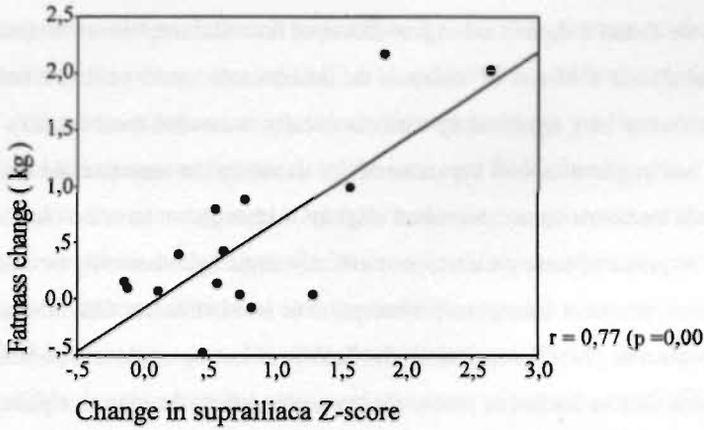
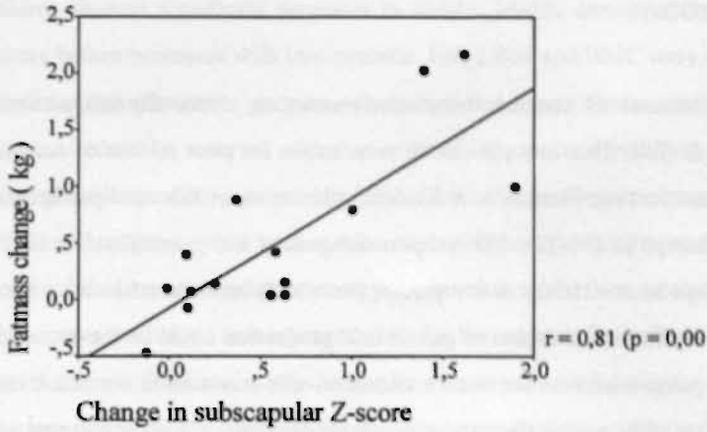
Body composition data before and after lansoprazole are given in table 2. All 3 components FM, LBM and BMC were significantly decreased in our 15 CF children when compared to the reference population described by Ogle et al. (p = 0.01; p = 0.02 and p = 0.005 respectively). Bone mineral density Z-score was significantly decreased (p ≤ 0.05). Significant increases of FM and BMC occurred after 3 months of treatment with lansoprazole. Changes in the subscapular, suprailiaca and sum of the 4 skinfolds Z-scores were highly correlated with changes in FM by DXA (r = 0.81 / r = 0.77 / r = 0.85 resp. with p = 0.001) (fig 4).

### Diet evaluation

Mean fat, protein, carbohydrate and energy intakes were 3.1 - 2.8 - 3.0 g / kg ; 3.0 - 2.7 - 2.8 g / kg ; 10.4 - 9.7 - 9.8 g / kg and 2095 - 1986 - 1977 kcal / kg bodyweight at the start, at the end and one month after lansoprazole trial respectively. None of these changes were significant.

**Table 2 Body composition before (T0) and after (T3) lansoprazole by DXA.**

<b>BODY COMPOSITION</b>	<b>T0 ( n = 15 )</b>	<b>T3 ( n = 15 )</b>	<b>SIGNIFICANCY ( p )</b>
<b>MEAN FATMASS ( Kg )</b>	<b>3.97</b>	<b>4.76</b>	<b>0.008</b>
<b>MEAN LBM ( Kg )</b>	<b>22.83</b>	<b>24.03</b>	<b>0.06</b>
<b>MEAN BMC ( Kg )</b>	<b>1.02</b>	<b>1.08</b>	<b>0.005</b>
<b>MEAN BMD ( Z-SCORE )</b>	<b>-0.55</b>	<b>-0.58</b>	<b>0.65</b>



**Figure 4** Relation between changes in subscapular skinfold Z-score, suprailiac skinfold Z-score, sum of 4 skinfolds Z-score and fatmass as measured by DXA (dual energy X-ray absorptiometry) in 15 CF children.

## DISCUSSION

Due to a decrease of pancreas bicarbonate secretion, cystic fibrosis patients have a low duodenal pH (29). This low pH can be responsible for poor release of enzymes through the acid resistant coating. Further, low duodenal pH can cause bile acid precipitation resulting in lipid malabsorption (30-32). H<sub>2</sub>-receptor antagonists and prostaglandine E<sub>2</sub> have been used with the hope to reverse the above proces, but results on steatorrhea have been controversial (17-22). Insufficient inhibition of gastric acid production could be the cause of these failures. As proton pump inhibitors are known to control acid secretion in a much more effective way (33), they could be more effective in increasing duodenal pH. In agreement with Heijermans et al. (23), we found a significant improvement of fat malabsorption as measured by the acid steatocrit in all but 4 of our CF children on lansoprazole. Acid steatocrit results have been shown to correlate very significantly with chemically measured fecal fat (25). Acid steatocrit values did not improve in 4 of our patients. By doubling the lansoprazole dose in 2 of these patients, acid steatocrit values decreased slightly. Although we have no clear explanation for the lack of response of these patients, poor efficacy could hypothetically be due to host factors including poor intestinal lansoprazole absorption or interindividual differences in bioavailability of lansoprazole (34). Further, since the halflife of lansoprazole is between 1 and 2 hours and inhibition will be limited to proton pumps active during the effective plasma levels of the drugs, Sachs et al. suggested to give proton pump inhibitors twice a day whenever effective pH control is desired (33). However, studies hereover have yet to be done. Contrary to our expectations, acid steatocrit values increased in only 8 out of 14 patients one month after lansoprazole was stopped. We have no explanation for this apparent prolonged effect of lansoprazole on fecal fat since proton pump restoration has been reported to occur 50 to 72 hours after the inhibitor was interrupted (33). Further studies are necessary in order to clarify this point.

Because of the normal growth proces in children, Z-scores should be used for the evaluation of anthropometric parameters. Improving Z-scores reflect catch-up growth and consequently improvement in the nutritional status while the reverse is true for deteriorating Z-scores. The recent introduction of DXA methodology makes it possible to evaluate FM, LBM and BMC rapidly and non invasively in children (27). In agreement with previous studies, all except 2

of our CF children showed significant decreases in weight, length, arm circumference and 4 skinfolds Z-scores before treatment with lansoprazole. FM, LBM and BMC were also significantly decreased despite pancreatic enzyme substitution and hypercaloric supplementation. The catabolic process could be reversed after 3 months of lansoprazole resulting in Z-score improvement of all anthropometric parameters, FM, BMC and to a lesser extent LBM. The improved nutritional condition could be due either to higher energy intakes or to improved absorption. As we found somewhat lower energy intakes during the lansoprazole treatment period, a higher intake probably is not responsible for the improved nutritional condition. Further, the fact that lower fecal acid steatocrit results were found in our patients with the best nutritional response as assessed by subscapular Z-score improvements, supports the idea that improved absorption is the main factor responsible for the improvement of the nutritional status in our patients. However increased FM, LBM and BMC are difficult to interpret because results for reference populations expressed as Z-scores have not yet been reported. As Z-score of BMD did not change on lansoprazole, the increased BMC found in our CF children, is probably linked to the growth process. Our results showing deterioration of the nutritional condition after interruption of lansoprazole "intervention", are in agreement with those of Bertrand et al., who reported nutritional deterioration after stopping elemental enteral alimentation (14). Oral hypercaloric diets have not been shown to improve Z-scores of weight, length and skinfolds as parameters for nutritional status and growth process of CF children (8). Only parenteral or elemental "predigested" enteral nutrition have been shown to reverse the catabolic process in these children (9-15). This indicates that persisting maldigestion or malabsorption is mainly responsible for malnutrition in CF. As alkalinization of duodenal pH improves malabsorption (16,22), it could also, as elemental diets do, improve the nutritional status of CF children. This hypothesis is confirmed by the results of our study. In contrast with O' Loughlin (10), Shepherd (12) and Levy (13), who found significant improvement in LBM after 6 to 12 months of elemental diets, no significant increase in LBM was seen in our study. The 3 months of treatment in our study could be too short for a significant change in LBM to be noticed. Our results are in agreement with those of other authors, who evaluated the effect of gluten free diet on body compartments by DXA. Only FM and BMC improved after a 6 months gluten free diet while LBM did not (35). As H<sub>2</sub> - receptor antagonists have been shown to significantly decrease nitrogen malabsorption in CF patients (36), we do not

think that the poor improvement of LBM in our patients can be ascribed to a selective improvement of fat and not of protein malabsorption.

In conclusion, most of our CF children maintained steatorrhea and were malnourished despite optimal treatment with hypercaloric diets and pancreatic enzymes. Lansoprazole as adjuvant therapy resulted in decreased fat malabsorption and improved nutritional status in these CF children after 3 months of treatment. Longterm evaluation of the effect of lansoprazole on both the nutritional status and lung function parameters have yet to be performed.

#### *Acknowledgment*

we are gratefull to Liesbeth van der Ploeg, Lianne Schoorlemmer dieticians and Piet Willems, Sandra Zimny from the department of nuclear medicine for their invaluable assistance. We also thank all nurses of the pediatric polyclinic for their welwilling support.

## REFERENCES

- (1) M. Welsh, A. Smith. Molecular mechanisms of CFTR chloride channel dysfunction in cystic fibrosis. *Cell* 1993; 73:1251-1254.
- (2) J. Tomezsko, V. Stallings, D. Kawchak, J. Goin, G. Diamond, T. Scanlin. Energy expenditure and genotype of children with cystic fibrosis. *Pediatr Res* 1994; 35: 451- 460.
- (3) M. Bronstein, P. Davies, K. Hambidge, F. Accurso. Normal energy expenditure in the infant with presymptomatic cystic fibrosis. *J Pediatr* 1995; 126: 28-33.
- (4) R.Kraemer, A. Rudeberg, B. Hadorn, E. Rossi. Relative underweight in cystic fibrosis and its prognostic value. *Acta Paediatr Scand* 1978; 67: 33-37.
- (5) P. Robinson, P. Sly. High dose pancreatic enzymes in cystic fibrosis. *Arch Dis Child*. 1990; 65: 311-312.
- (6) E. Lebenthal. High strength pancreatic exocrine enzyme capsules associated with colonic strictures in patients with cystic fibrosis: "more is not necessarily better". *J Pediatr Gastroenterol Nutr*. 1994; 18: 423-425.
- (7) R. Smyth, D. van Velzen, A. Smyth, D. Lloyd, D. Heaf. Strictures of ascending colon in cystic fibrosis and high strength pancreatic enzymes. *The Lancet*. 1994; 343: 85-86.
- (8) A. Rettammel, M. Marcus, P. Farrell, S. Sondel, R. Kosciak, E. Mischler. *J Am Diet Assoc*. 1995; 95: 454-459.
- (9) K. Gaskin, D. Waters, L. Baur, V. Soutter, M. Gruca. *Acta Paediatr Scand [Suppl]*. 1990; 366: 106-110.
- (10) E. O' Loughlin, D. Forbes, H. Parsons, B. Scott, D. Cooper, G. Gall. Nutritional rehabilitation of malnourished patients with cystic fibrosis. *Am J Clin Nutr*. 1986: 43: 732-737.
- (11) R. Shepherd, T. Holt, B. Thomas et al. Nutritional rehabilitation in cystic fibrosis: Controlled studies of effects on nutritional growth retardation, body protein turnover, and course of pulmonary disease. *J Pediatr*. 1986; 109: 788-94.
- (12) R. Shepherd, B. Thomas, D. Bennett, W. Cooksley, L. Ward. Changes in body composition and muscle protein degradation during nutritional supplementation in nutritionally growth-retarded children with cystic fibrosis. *J Pediatr Gastroenterol Nutr*. 1983; 2: 439-446.
- (13) L. Levy, P. Durie, P. Pencharz, M. Corey. Effects of long-term nutritional rehabilitation on body composition and clinical status in malnourished children and adolescents with cystic

fibrosis. *J Pediatr* 1985; 107: 225-230.

(14) J. Bertrand, C. Morin, R. Lasalle, J. Patrick, A. Coates. Short-term clinical, nutritional, and functional effects of continuous elemental enteral alimentation in children with cystic fibrosis. *J Pediatr*. 1984; 104: 41-46.

(15) R. Shepherd, W. Cooksley, and W. Domville. Improved growth and clinical, nutritional, and respiratory changes in response to nutritional therapy in cystic fibrosis. *J Pediatr*. 1980; 97: 351-357.

(16) P. Robinson, A. Smith, and P. Sly. Duodenal pH in Cystic fibrosis and its relationship to fat malabsorption. *Dig Dis Sci*. 1990; 35: 1299-1304.

(17) A. Carroccio, F. Pardo, G. Montalto et al. Use of famotidine in severe exocrine pancreatic insufficiency with persistent maldigestion on enzymatic replacement therapy: A long-term study in cystic fibrosis. *Dig Dis Sci* 1992; 37: 1441-1446.

(18) D. Chalmers, R. Brown, M. Miller et al. The influence of longterm cimetidine as an adjuvant to pancreatic enzyme therapy in cystic fibrosis. *Acta Paediatr Scand*. 1985; 74: 114-117.

(19) P. Robinson and P. Sly. Placebo-controlled trial of misoprostol in cystic fibrosis. *J Pediatr Gastroenterol Nutr*. 1990; 11: 37-40.

(20) H. Heijerman, C. Lamers, J. Dijkman, and W. Bakker. Ranitidine compared with the dimethylprostaglandin E2 analogue enprostil as adjunct to pancreatic enzyme replacement in adult cystic fibrosis. *Scand J Gastroenterol*. 1990; 25 (Suppl 178): 26-31.

(21) M. Schöni, R. Kraemer, E. Rossi. Cimetidine and fat malabsorption in children with cystic fibrosis. *Helv Paediat Acta*. 1981; 36: 359-369.

(22) B. Boyle, W. Long, W. Balistreri, S. Widzer, and N. Huang. Effect of cimetidine and pancreatic enzymes on serum and fecal bile acids and fat absorption in cystic fibrosis. *Gastroenterology*. 1980; 78: 950-953.

(23) H. Heijerman, C. Lamers, W. Bakker. Omeprazole enhances the efficacy of pancreatin (pancrease) in cystic fibrosis. *Ann Intern Med*. 1991; 114: 200-201.

(24) J. van de Kamer, H. Huinink, H. Weyers. Rapid method for determination of fat in feces. *J Biol Chem*. 1949; 177: 349-55.

(25) M. Tran, P. Forget, A. Van den Neucker, J. Strik, B. van Kreel, and R. Kuijten. The acid steatocrit: A much improved method. *J Pediatr Gastroenterol Nutr*. 1994; 19: 299-303.

- (26) W. Gerver, R. de Bruin. *Paediatric Morphometrics: A reference manual*. 1th ed. Utrecht: Bunge, 1995.
- (27) R. Mazess, H. Barden, J. Bisek, and J. Hanson. Dual-energy x-ray absorptiometry for total-body and regional bone-mineral and soft-tissue composition. *Am J Clin Nutr*. 1990; 51:1106-12.
- (28) G. Ogle, J. Allen, I. Humphries et al. Body-composition assessment by dual-energy x-ray absorptiometry in subjects aged 4-26 y. *Am J Clin Nutr*. 1995; 61:746-53.
- (29) A. Weber, C. Roy. Intraduodenal events in cystic fibrosis. *J Pediatr Gastroenterol Nutr*. 1984; 3 (Suppl. 1): S113-S119.
- (30) P. Regan, J. Malagelada, E. Dimagno, and V. Go. Reduced intraluminal bile acid concentrations and fat maldigestion in pancreatic insufficiency: Correction by treatment. *Gastroenterology*. 1979; 77: 285-289.
- (31) P. Zentler-Munro, W. Fitzpatrick, J. Batten, and T. Northfield. Effect of intrajejunal acidity on aqueous phase bile acid and lipid concentrations in pancreatic steatorrhoea due to cystic fibrosis. *Gut*. 1984; 25: 500-507.
- (32) P. Zentler-Munro, D. Fine, J. Batten, and T. Northfield. Effect of cimetidine on enzyme inactivation, bile acid precipitation, and lipid solubilisation in pancreatic steatorrhoea due to cystic fibrosis. *Gut*. 1985; 26: 892-901.
- (33) G. Sachs, J. Shin, C. Briving, B. Wallmark, S. Hersey. The pharmacology of the gastric acid pump: The H<sup>+</sup>, K<sup>+</sup> ATPase. *Annu Rev Pharmacol Toxicol*. 1995; 35: 277-305.
- (34) C. Spencer and D. Faulds. *Drugs: Focus on Lansoprazole*. 1994; 48: 404-430.
- (35) G. Barera, P. Brambilla, P. Manzoni, S. Acciuffi, G. Caccia, C. Bianchi. Changes in body composition evaluated by DXA during gluten free diet in celiac children. *J Pediatr Gastroenterol Nutr*. 1995; 20: 476 "abstr".
- (36) K. Cox, J. Isenberg, A. Osher, R. Dooley. The effect of cimetidine on maldigestion in cystic fibrosis. *J. Pediatr*. 1979; 94: 488-492.

## CHAPTER 7

### ANTHROPOMETRY AND BODY COMPOSITION IN CHILDREN WITH CYSTIC FIBROSIS: EFFECTS OF A PROTON - PUMP INHIBITOR

<sup>(1)</sup>My-Dung T. Tran, <sup>(1)</sup>Anita Van den Neucker, <sup>(1)</sup>Han J. Hendriks, <sup>(2)</sup>Bernard van Kreel,  
<sup>(1)</sup>Patricia Forget, <sup>(3)</sup>Guido Heidendal, <sup>(1)</sup>Pierre-Philippe Forget

<sup>(1)</sup>Department of Pediatrics, <sup>(2)</sup> Clinical Chemistry and <sup>(3)</sup>Nuclear Medicine, University Hospital  
Maastricht, Maastricht, the Netherlands.

---

Submitted for publication

#### *Abstract*

We studied the body composition of 18 CF children making use of dual-energy X-ray absorptiometry (DEXA), deuterium-bromide and skinfold methods and evaluated the efficacy of these body composition methods for the detection of body composition changes during 3 months therapeutic intervention with lansoprazole. Our CF patients were malnourished with decreased mean Z-scores for armcircumference (-1.62), biceps (-0.77), subscapular (-0.92), suprailliac skinfolds (-1.66), weight (-1.03) and height (-1.31). Their fatmass was significantly depleted as shown by DEXA, skinfold and total body water (TBW) methods. Extracellular volume (%) was increased, while intracellular volume (%) was normal. Only the lean body mass (LBM) as measured by DEXA was decreased ( $p = 0.02$ ). Decreased bone mineral content and bone mineral density Z-scores were also found ( $p = 0.005$  and  $p = 0.03$  respectively). After treatment with lansoprazole, significant increases in fatmass was found by DEXA and skinfold methods (53% and 97% of weight changes respectively) whereas weight increase was exclusively ascribed to an increase in LBM with the TBW method. Changes in body-weight however, were not correlated with either fatmass and fat free mass changes as measured by any of these methods. We conclude that results of DEXA, TBW and skinfold methods are not interchangeable and that the methods used are not accurate enough for the differential detection of small changes in fatmass and fat free mass as found in the present study.

## INTRODUCTION

Due to malabsorption (1), chronic lung infections with increased energy expenditure (2,3) and poor appetite, most cystic fibrosis patients show signs of malnutrition. As malnutrition can affect pulmonary function and shorten survival (4), feeding interventions are sometimes necessary to restore normal growth and body composition. Assessment of body composition changes is necessary for the precise evaluation of nutritional interventions. While different body composition methods have been described, only few studies have, to our knowledge, compared different measurement techniques in pediatric subjects and there are no reports on the efficacy of these methods for the detection of body composition changes during therapeutic interventions. In children, Dual-energy X-ray absorptiometry (DEXA), Total Body Water (TBW) and Skinfold methods are frequently used for the determination of body composition since they are all noninvasive. In this age group, methods used for measuring body composition have to be very precise in order to detect small changes in body composition. The precision for repeated measurements has been reported to be 1-2 % for DEXA, 1.6% for TBW and either 5% (intraobserver) or 15% (interobserver) for the skinfold method (5). In the present study we first evaluated the body composition of our 18 CF children making use of DEXA, skinfolds and TBW (deuterium-bromide) methods and subsequently evaluated the agreement between these results. Secondly, we investigated the sensitivity of these 3 methods for the detection of small changes in body composition of 15 CF children whose nutritional condition improved significantly after intervention with lansoprazole for 3 months. For the purpose of the present study we defined fat free mass by DEXA (FFM-DEXA) as the sum of lean body mass (LBM) and bone mineral content (BMC) and total mass (TM) as DEXA constructed weight (LBM + FM + BMC).

## SUBJECTS

### *Population studied for the comparison of body composition methods*

23 CF children were recruited from the Academic Hospital Maastricht, The Netherlands. Of these 2 patients were too ill to take part in the study and 3 children refused to participate. 18

children who had no exacerbation 4 weeks before the study were included. Thirteen of them were prepubertal and younger than ten years, 3 children were postpubertal and were between 11.6 - 14.1 years. Two subjects were adolescents of 16.1 and 22.6 years. Fourteen of these 18 children were diagnosed during the first year of life while 16 of them had pancreatic insufficiency (abnormal fecal chymotrypsin and 72h fecal fat balance). Their nutritional status was moderate with a mean BMI (body mass index) of 15.6 (range: 13.2 - 23.2). Mean age, weight and height were respectively 9.0 y (range: 2.9 - 22.6 y); 27.4 kg (range: 13.6 - 67.6 kg) and 127.5 cm (range: 96.2 - 184.9 cm). Mean FEV1 (forced expiratory volume in 1 second) and FVC (forced vital capacity) were respectively 84% (range: 39 - 117%) and 86% (range: 44 - 109%) of predicted values. Mean energy intake was 113% RDA (recommended daily allowance). All patients were on conventional physiotherapy, pancreatic enzymes and some of them received antibiotics regularly for pulmonary exacerbations. Weight, height, armcircumferences, TBW, DEXA and skinfolds were measured on the same day. All usual CF medications were continued during the study.

#### *Population studied for the evaluation of changes in body composition*

We included 15 out of 16 children with pancreatic insufficiency as described above who maintained steatorrhea despite pancreatic enzymes and were treated with lansoprazole for 3 months with significant improvement of anthropometric parameters (results will be published separately). Mean age, weight and height of these 15 children were 9.5 y (range: 3.1 - 22.6 y); 29.3 kg (range: 13.6 - 67.6 kg) and 131 cm (range: 97.7 - 184.9 cm) respectively. Their nutritional status was moderately altered with a mean Body Mass Index (BMI) of 15,6 (range : 13.2 - 18.3). Mean FEV1 and FVC were respectively 81.3% (range: 39 - 114%) and 85.5% (range: 44 - 108%) of predicted values.

Anthropometry, DEXA and TBW were measured on the same day just before starting and 3 months after treatment with lansoprazole (15mg / day). Other usual CF medications were continued throughout the study. Informed patient and parental consent were obtained from all study subjects.

## METHODS

### Growth parameters

Weight, height, upper armcircumferences and 4 skinfold thicknesses (biceps, triceps, subscapular and suprailiac) were measured on the left side of the body in triplicate, using the Harpenden caliper. Average of three measures was taken and was expressed as standard deviation scores of the normal population for age and sex using the growth charts from Gerver and de Bruin (6). BMI was calculated as  $\text{weight}/\text{height}^2$ . Results of BMI were compared to the reference population described by Westrate and Deurenberg et al. (7). Mid upper arm muscle area was calculated from the mid upper armcircumference and the sum of biceps and triceps skinfolds (6).

### Body composition

Body composition results obtained from all three methods were compared to those of a recently reported pediatric reference population (8). The percentage of fatmass and fat free mass measured by the skinfold method were also compared to those of the reference population described by Gerver and de Bruin (6).

### Body composition by anthropometry

It has been found that subcutaneous fat as measured by skinfolds is related to the body density (9). This latter is itself related to the body fatmass. From these theoretical principles, Gerver and de Bruin have constructed a chart, expressing the relationship between the 4 skinfolds (biceps, triceps, subscapular and suprailiac) and the percent fat free mass (6). In our study, fat free mass determined by this method was derived from these charts and fatmass was then calculated by subtracting FFM from bodyweight.

### Body composition by dual-energy x-ray absorptiometry

The theoretical principles for DEXA measurement of body composition and the precision of this method have been described previously (10-12). All DEXA measurements were performed with a Dual Photon X-ray ( Lunar Radiation Corp, Madison, WI ) total body scanner. These results were analysed with a pediatric software programme, version 1.5e. Daily quality assurance tests were performed according to the manufacturer 's directions. Total body analysis was performed in all children using a fast scan mode with a sample size of 4.8 x 9.6mm, sample interval of 0.03s and source collimation of 1.68mm. The following body compartments were assessed: total non bone lean body mass, total bone mineral content, total bone mineral density (BMD), total body fatness and Z-score of BMD.

### Body composition by total body water and bromide space

TBW and ECV were measured by deuterium oxide (13) and bromide dilution respectively (14). Each subject received orally 20 ml (40 ml was given to the 2 adolescent patients) of a mixture of D<sub>2</sub>O (99.9% purity) and Bromide salt (150mMol/L) solution in a volume ratio of 1:1. Saliva and plasma samples were taken before intake of D<sub>2</sub>O - NaBr solution and 4 hours thereafter when an "plateau" has been reached. To prevent saliva dilution by fluid intake which can result in a higher TBW content, patients were told not to take any fluid orally half an hour before saliva samples were taken. Urine and fecal loss of bromide and D<sub>2</sub>O during the equilibration period were considered negligible as the D<sub>2</sub>O and bromide T<sub>1/2</sub> are about 8 days (14). Saliva samples were obtained making use of dental cotton-wool, that was dried overnight at 100 °C and kept in a gas-tight tube until use. The cotton-wools and the blood samples were centrifuged and the saliva and serum thus obtained were kept in a stoppered glass vial and stored in a freezer at -20 °C until analysis. Results of TBW, ECV and ICV were compared to the reference values described by Friis-Hansen (15).

#### 1. TBW ANALYSIS

D<sub>2</sub>O concentrations of saliva samples were determined as described by van Kreel (14): Calcium carbide (CaC<sub>2</sub>) was placed in the siliconized vacutainer tube and evacuated for 30 sec. with a rotatory vane pump to a total pressure of 0,01 atm. Thereafter, 25µl of salivary sample

was injected in the vacutainer tube. This was done in duplicate.  $\text{CaC}_2$  react with  $\text{D}_2\text{O}$  forming acetylene gas. A  $25\mu\text{l}$  sample of this gas was subsequently injected in duplicate into the GC/CF - IRMS system (gas chromatography/continuous flow isotope ratio mass spectrometry) at 2 min. intervals. The mass 27/26 ratio ( $R_{27/26}$ ) was measured on a Isotope Ratio Mass Spectrometer configured for Acetylene (Finnigan MAT 252 for CF-IRMS). The mean value of 4 determinations was calculated for each sample. By inserting the tracer/tracee ratio, defined as  $R_{27/26}(\text{T4}) - R_{27/26}(\text{T0})$ , into the regression equation obtained from the standards, we get the dilution factor of  $\text{D}_2\text{O}$ . TBW is calculated as ingested  $\text{D}_2\text{O}$  volume/dilution factor. FFM and FM are then calculated by the following formulae:

$$\text{FFM (kg)} = \text{TBW} / (1,04 \times d)$$

$$\text{FM (kg)} = \text{Weight} - \text{FFM}$$

The 1,04 factor is a correction for the estimated 4% nonaqueous hydrogen exchange and  $d$  is the hydration factor of LBM which varies with age and sex. Because our CF population was young, we used the age dependent hydration factors described by Fomon (16) for children younger than 10 year and by Boileau and Lohman (17) for older children.

## 2. BROMIDE DILUTION ANALYSIS

Because bromide resides mainly in the extracellular space, the measurement of bromide dilution gives an estimate of the extracellular volume. Bromide was determined by using a Gas Chromatograph type CP 9000 (Chrompack) equipped with an ECD detector after it was converted into bromoacetone gas (14). First, perchloric acid was added to the serum sample and centrifuged for deproteinisation. An aliquot of the supernatant was then added to silver nitrate ( $\text{AgNO}_3$ ) for precipitation of silver bromide and chloride. After centrifugation, the precipitate was taken up in  $\text{NH}_3$  after adding  $\text{Na}_2\text{S}$  and  $\text{NaOH}$  in order to eliminate the silver. After agitation and centrifugation, the supernatant was heated until dry.  $\text{H}_2\text{O}$  was added followed by  $\text{H}_2\text{O}_2$  in order to oxidize sulfide. After drying,  $2\text{H}_2\text{O}$  was then added and dried again. This was repeated several times. Thereafter, perchloric acid and acetone were added and the reaction was started by addition of  $\text{KmnO}_4$  with Bromoacetone formed. The solution

is then extracted with benzene. The organic phase was separated from the water phase by shaking and centrifugation. The water phase was then removed. An aliquot of the organic solution is then applied to the gas chromatograph for measuring of the bromoacetone/internal standard ratio. The bromide concentration was then derived from the bromoacetone standard curves. Because the distribution of bromide depends on the potential difference between the in- and extra-cellular compartments and on the total body volume, the corrected bromide space was calculated as follows:

$$\text{ECV (L)} = \frac{\text{Bromide administered (mmol)}}{\text{Bromide change T4 - T0 (mmol/L)}} - 0.036\text{TBW}$$

Where 0.036TBW is the correction factor for the cell potential and for the total body volume (14). Body cell mass (BCM) was then calculated by subtracting ECV from TBW.

### Statistics

All data were analysed with SPSS statistic program version 6.0. The sign test was used to compare the growth parameters and body composition results with those of the reference population. The Pearson correlation coefficient was used to determine the relationship between measurements obtained by the various body composition methods. Partial correlation coefficients, controlling for age, were used for the evaluation of the relationships between measured ICV, ECV, mid-upper-arm muscle area and the various body composition results. The between method differences were compared, using the Wilcoxon rank test. The agreement between methods were evaluated by the Statistical method of Bland and Altman (18).

## RESULTS

### *Body composition of 18 cf children*

Mean age, nutritional parameters expressed as standard deviation scores (Z-scores) and results of body composition measured by skinfold, DEXA and TBW from 18 CF children are shown in table 1. Compared to the reference population, mean Z-scores for weight, height, BMI, arm circumference and skinfolds (except for the triceps) were significantly decreased (fig 1). The mid-upper-arm muscle area was significantly decreased in our CF population ( $p = 0.005$ ). In absolute terms, all body composition components measured by DEXA such as FM, LBM, BMC and BMD Z-scores were significantly decreased ( $p = 0.01$ ;  $p = 0.02$ ;  $p = 0.005$  and  $p = 0.03$  respectively) compared to the control population described by Oggle (8). When compared to the normal DEXA body composition data reported by Oggle (8), results obtained with either the TBW or the skinfold methods showed only fat mass to be significantly decreased in our patients ( $p = 0.03$  and  $p = 0.05$  respectively). In relative terms, FM-DEXA (compared to normal DEXA data reported by Oggle) and FM-skinfolds (compared to normal skinfolds data reported by Gerber) were also significantly decreased. The BMI was correlated with both FM and FFM measured by all three methods (FM-DEXA  $r = 0.90$ ; FM-skinfold  $r = 0.87$ ; FM-TBW  $r = 0.75$ ; FFM-DEXA  $r = 0.79$ ; FFM-skinfold  $r = 0.81$ ; FFM-TBW  $r = 0.8$  with  $p = 0.001$  for all correlations). As expected strong correlations were found between age on the one hand and FM-DEXA ( $r = 0.67$   $p = 0.003$ ), FFM-DEXA ( $r = 0.95$   $p = 0.001$ ), FM-skinfolds ( $r = 0.59$   $p = 0.01$ ), FFM-skinfolds ( $r = 0.95$   $p = 0.001$ ), FM-TBW ( $r = 0.47$   $p = 0.047$ ), FFM-TBW ( $r = 0.94$   $p = 0.001$ ) and BMC-DEXA ( $r = 0.94$   $p = 0.001$ ). No sex differences in body composition data were found. When compared to the reference values described by Fris-Hansen (15), the ECV and TBW as a percentage of bodyweight were significantly increased ( $p < 0.005$  both) while the ICV as percent of bodyweight was normal. The partial correlations (controlling for age) between ECV, ICV, the mid-upper-arm muscle area LBM and FM are shown in table 2.

**Table 1 Characteristics of 18 CF children.**

	MEAN	SEM	RANGE
AGE (Yr)	8.97	1.16	2.9 - 22.6
BW (Kg)	27.43	3.66	13.6 - 67.6
BW (SDS)	-1.03	0.25	-2.33 - 1.36
TM (Kg)	27.82	3.84	12.96 - 67.33
HEIGHT (cm)	128	6.0	96.2 - 184.9
HEIGHT (SDS)	-1.31	0.25	-2.89 - 0.40
BMI (Kg / m <sup>2</sup> )	15.6	0.57	13.21 - 23.17
ARMCIRCUMFER. (SDS)	-1.62	0.31	-3.14 - 1.43
BICEPS (SDS)	-0.77	0.23	-1.86 - 1.33
TRICEPS (SDS)	-0.33	0.23	-2 - 2.33
SUBSCAPULAR (SDS)	-0.92	0.23	-2 - 1.6
SUPRAILIACA (SDS)	-1.66	0.09	2.43 - -0.57
SUM 4 SKINFOLDS (SDS)	-1.17	0.20	-2.2 - 1.2
FM-DXA (%)	12.11	1.48	6.1 - 28.8
FM-TBW (%)	10.39	1.61	0.0 - 22.2
FM-SKINFOLD (%)	14.44	0.94	8 - 23
FFM-DXA (%)	87.9	1.5	71.2 - 93.9
FFM-TBW (%)	89.8	1.6	77.8 - 101
FFM-SKINFOLD (%)	85.6	0.9	77 - 92
BMC-DXA (Kg)	1.02	0.17	0.37 - 2.68
TBW (L)	19.06	2.38	9.9 - 47.4
TBW (%)	70.82	1.32	59.62 - 79.70
ICV (L)	10.96	1.78	3.71 - 31.93
ICV (%)	38.41	1.42	27.28 - 51.01
ECV (L)	8.10	0.65	4.45 - 15.47
ECV (%)	32.44	1.75	19.01 - 47.69

BW: Body weight

FM: Fat mass

TBW: Total body water

TM: DEXA constructed weight

FFM: Fat free mass

ECV: Extracellular volume

BMI: Body mass index

BMC: Bone mineral content

ICV: Intracellular volume

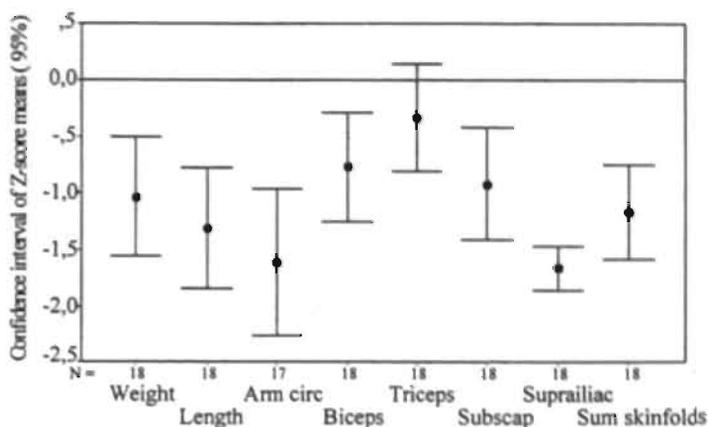


Figure 1 Confidence interval of Z-score means for various anthropometric parameters in cystic fibrosis children showing significantly lower values for all parameters except for the triceps skinfold.

Table 2 Correlation coefficients between muscle area, ECV, ICV and body composition results

	MUSCLE AREA (cm <sup>2</sup> )	ECV (L)	ICV (L)
LBM-DEXA (Kg)	0.84 ( p = 0.001 )	0.67 ( p = 0.004 )	0.91 ( p = 0.001 )
LBM-SKINF (Kg)	0.90 ( p = 0.001 )	0.58 ( p = 0.02 )	0.92 ( p = 0.001 )
LBM-TBW (Kg)	0.86 ( p = 0.001 )	0.63 ( p = 0.007 )	0.95 ( p = 0.001 )
FM-DEXA (Kg)	0.62 ( p = 0.02 )	0.15 ( p = 0.58 )	0.53 ( p = 0.04 )
FM-SKINF (Kg)	0.61 ( p = 0.02 )	0.23 ( p = 0.38 )	0.53 ( p = 0.03 )
FM-TBW (Kg)	0.33 ( p = 0.21 )	-0.004 ( p = 0.99 )	0.20 ( p = 0.43 )
ECV (L)	0.45 ( p = 0.08 )		
ICV (L)	0.86 ( p = 0.001 )		

LBM-DEXA: Lean body mass by DEXA method

LBM-SKINF: Lean body mass by skinfolds method

LBM-TBW: Lean body mass by TBW method

ECV: Extracellular volume

FM-DEXA: Fatmass by DEXA

FM-SKINF: Fatmass by skinfolds method

FM-TBW: Fatmass by TBW method

ICV: Intracellular volume

**Table 3 Changes in bodyweight and body composition after 3 months on lansoprazole in 15 CF children.**

	T3 - T0		
	MEAN ± SEM	MINIMUM	MAXIMUM
BW (Kg)	0.97 ± 0.13	0.4	2.1
TM (Kg)	0.97 ± 0.15	-0.05	1.9
FM-DXA (Kg)	0.52 ± 0.19	-0.46	2.18
FM-TBW (Kg)	-0.23 ± 0.52	-4.85	3.44
FM-SKINFOLD (Kg)	0.94 ± 0.26	0.05	3.52
LBM-DXA (Kg)	0.43 ± 0.18	-0.96	1.46
BMC-DXA (Kg)	0.03 ± 0.01	-0.03	0.09
FFM-DXA (Kg)	0.41 ± 0.17	-0.98	1.03
FFM-TBW (Kg)	1.27 ± 0.49	-1.84	5.35
FFM-SKINFOLD (Kg)	0.04 ± 0.26	-3.02	0.98
TBW (L)	0.99 ± 0.39	-1.4	4.1
ICV (L)	0.09 ± 0.41	-3.49	3.17
ECV (L)	0.92 ± 0.46	-2.38	4.91

T0: Before start lansoprazole

T3: 3 months after lansoprazole

TM: Total mass (DEXA)

BW: Body weight

FFM: Fat free mass

FM: Fatmass

BMC: Bone mineral content

LBM: Lean body mass

TBW: Total body water

ECV: Extracellular water

ICV: Intracellular water

### Limits of agreement between methods

As only 5 of our patients 3 girls and 2 boys were postpubertal, all results of both boys and girls were analysed together. There was a high correlation between the 3 body composition methods for measuring of FM and FFM (fig 2 and 3). The best correlation for FM determination was between DEXA and the skinfold method ( $r = 0.98$ ). As DEXA is a 3 compartments model, BMC was not included in the lean body mass. After correction for BMC, the correlation coefficient was unchanged. Plots of the paired differences for FM and FFM measured in kilogram versus their mean, with indication of the limits of agreement are shown in figure 4 and figure 5 respectively. Since our population was small, we preferred to use the 10th and 90th centile values instead of  $\pm 2$  SD for defining the limits of agreement. No intermethod correlations were found between means and differences as shown in figures 4 and 5. The 50th centile of the differences between FM-TBW versus FM-skinfolds was -1.68kg (-2.64 - -0.46kg); FM-skinfolds versus FM-DEXA was 0.75kg (-3.01 - 1.21kg); FM-TBW versus FM-DEXA was -0,96kg (-5.05 - -0.03kg). The 50th centile of the differences between FFM-TBW versus FFM-DEXA was 0.45kg (-0.70 - 4.85kg); FFM-skinfolds versus FFM-DEXA was -0.17kg (-0.77 - 3.46); FFM-TBW versus FFM-skinfolds was 1.68kg (0.46 - 2.96kg). The DEXA constructed weight was highly correlated with scale weight ( $r = 0.999$   $p = 0.001$ ). However, the DEXA weight was significantly lower than scale weight ( $p = 0.003$ ); the 50th centile of the differences between bodyweight constructed from DEXA (TM) and scale weight was -0.52kg (-0.79 - 0.12). Significant differences were found between the means of FFM measured by the TBW and the skinfold methods ( $p = 0.02$ ), the skinfolds and DEXA methods ( $p = 0.001$ ) as well as the TBW and DEXA methods ( $p = 0.001$ ). Only FM results from TBW and skinfolds were significantly different ( $p = 0.01$ ).

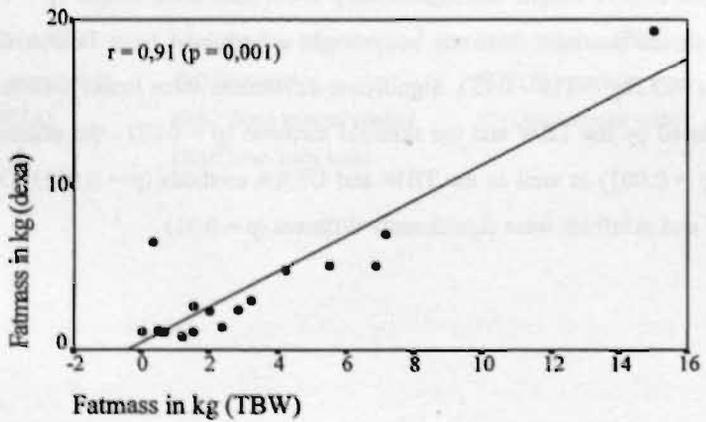
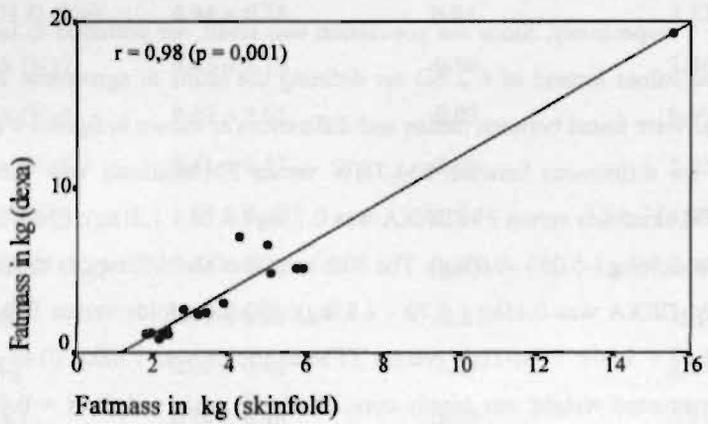
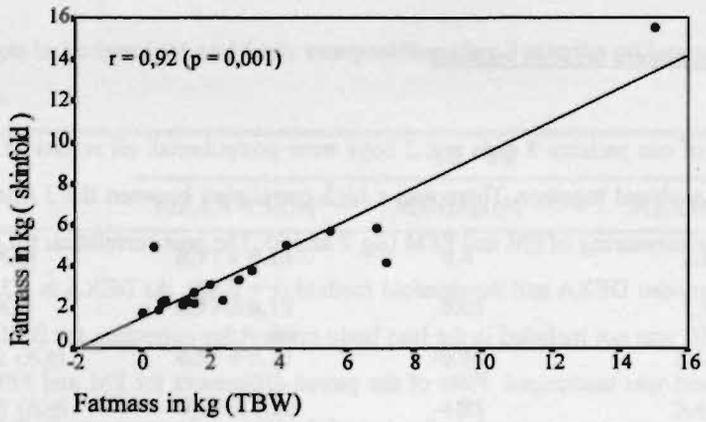


Figure 2 Intermethod fatmasses correlation coefficients.

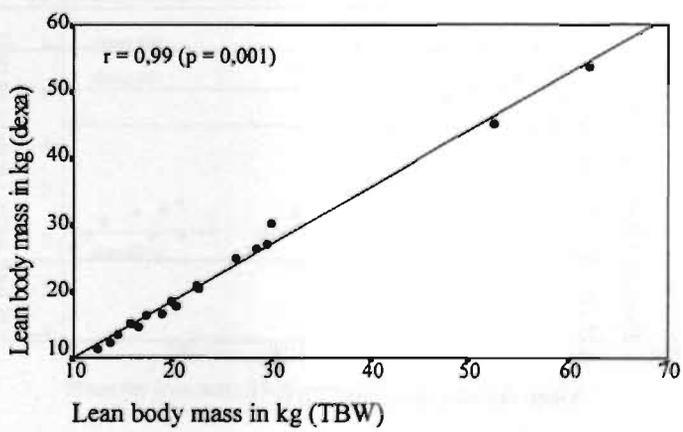
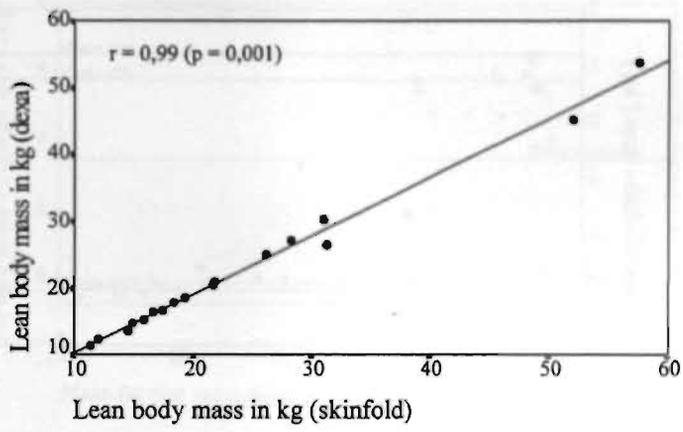
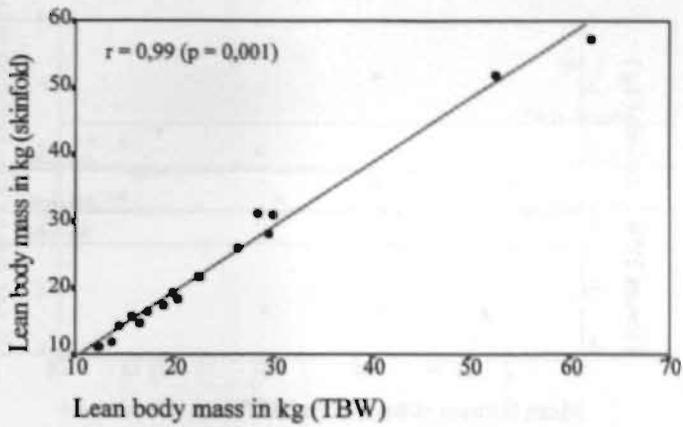


Figure 3 Intermethod lean body mass correlation coefficients.

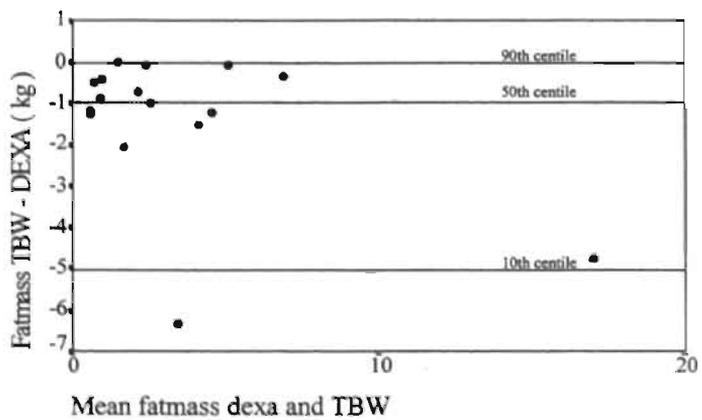
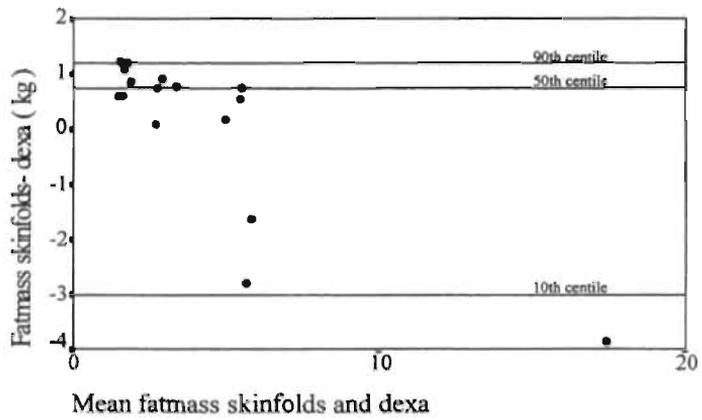
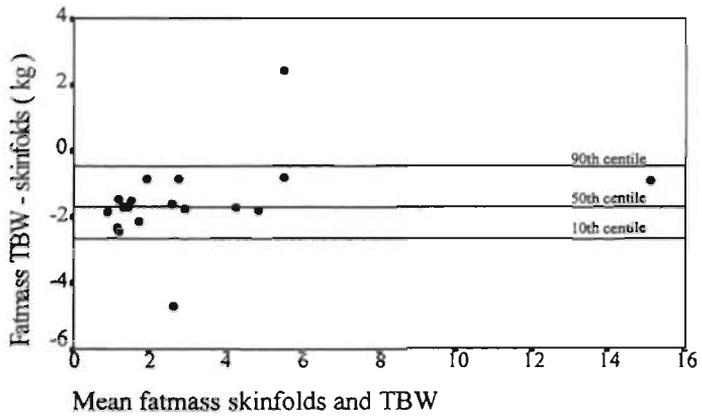


Figure 4 Limits of agreement for fatmass measured by the various methods.

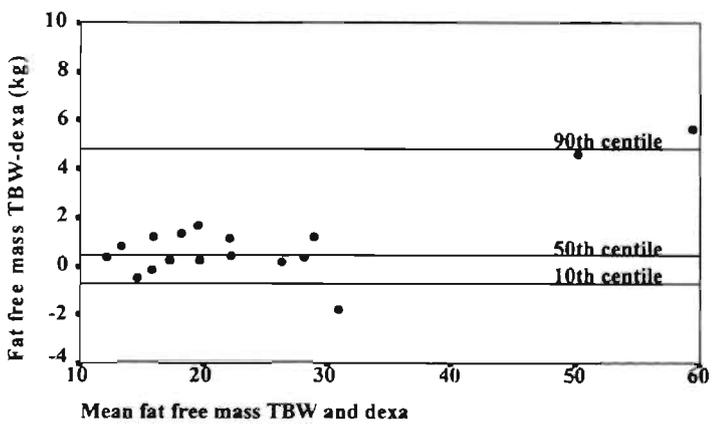
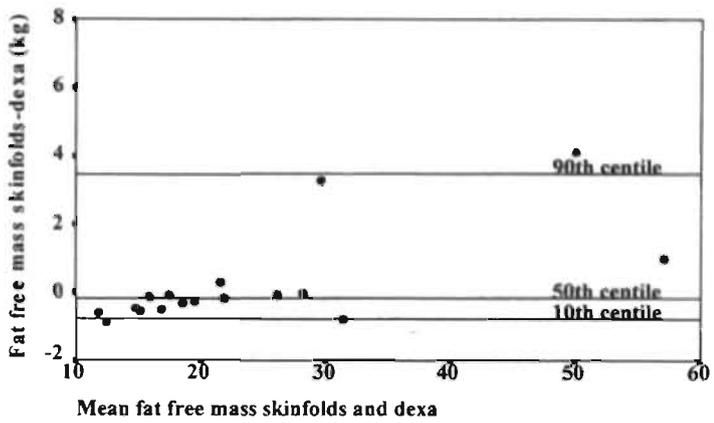
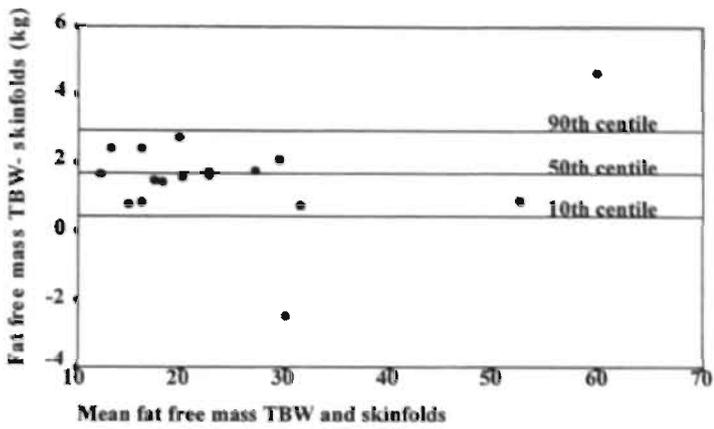


Figure 5 Limits of agreement for fat free mass measured by the various methods.

### Body composition changes

The changes in bodyweight, FM and FFM measured by skinfold, DEXA and TBW are shown in table 3. The increase in bodyweight was the same for both DEXA constructed weight and scale weight. However changes both in FM and FFM were different between methods. Both changes in FM and FFM measured by skinfold were highly correlated with those measured by DEXA ( $r=0.91$   $p = 0.001$  and  $r = 0.84$   $p = 0.001$  for FM and FFM respectively). Changes in FM measured by the TBW method were not correlated with changes of the same parameter measured by either the DEXA or the skinfold method, whereas changes in FFM-TBW were negatively correlated with those measured by skinfold and DEXA (FFM-TBW versus FFM-skinfold:  $r = -0.56$   $p = 0.03$  and FFM-TBW versus FFM-DEXA:  $r = -0.52$   $p = 0.05$ ). No correlation was found between changes in bodyweight and changes in FM or FFM measured by any method. Changes in ECV and ICV did not correlate with bodyweight changes. No correlation was found between changes in ICV and changes in LBM by any method.

## DISCUSSION

In children, effective evaluation of deterioration or catch-up growth can only be achieved by using the Z-score method. Despite high caloric polymeric intake, treatment of steatorrhea and support of pulmonary function, significantly lowered Z-scores for arm circumference, biceps, subscapular, suprailiac, sum of the 4 skinfolds weight and height were found in our patients. As our patients showed decreased weight, height Z-scores and mid upper arm muscle area, we expected both FM and FFM to be decreased. Since all body composition methods are based on assumptions, we used 3 noninvasive methods (DEXA, TBW and skinfolds) to evaluate the body composition of our patients. However, interpretation of the body composition results is difficult due to the lack of reference values for several measuring methods. The results of various methods used were strongly correlated with each other but still showed differences. In absolute terms, only DEXA results were as expected; showing a decrease in all 3 body components measured. Results of the TBW and skinfolds method could not be assessed accurately due to the lack of reference data expressed in absolute terms. When compared to the DEXA reference values, TBW and skinfolds methods only showed a decrease in FM. In relative terms, our CF populations showed an increase in TBW and ECV while the ICV (body cell water mass) appeared well maintained. These results imply a decrease of fatmass, associated with a relative increase in TBW, ECV and consequently FFM (19). In agreement with these data, our results showing an increased percentage of FFM as evaluated by the skinfold method also implies a decrease in fatmass percentage in our CF patients. According to these results, we believe our CF patients mainly have a depletion of fatmass and bone mineral content with a slight decreased in lean body mass in absolute terms. Comparison of our results with other body composition studies in CF children is difficult: First, the general condition of the studied populations differed between studies and second, methods used for the assessment of the nutritional condition were different. Tomezsko et al. found no significant decrease in body FM and FFM in their CF children with only significantly decreased suprailiac skinfold thicknesses and subscapular Z-scores. However their CF population was very young and showed only mild symptoms (2). In another study concerning older CF children with abnormal pulmonary function, Johnston et al. did find a significantly lower percentage of body fat (FFM not reported) compared to matched control children similar to our findings with all 3

methods (20). In agreement with our study, Miller et al. who studied the body composition and muscle protein metabolism in a group undernourished CF children with Z-scores for weight and height similar to those of our CF population, found a significant decrease in FM, FFM and muscle mass (21). The strong correlation we found between components of body composition and age are well known (8,22,23). The lack of sex differences can probably be explained by the prepubertal age of most patients (22). A high correlation was found between mid-upper-arm muscle area, ECV, ICV and LBM and FM. As expected, correlation coefficients between mid-upper-arm muscle area, ECV, ICV and LBM remained high while only weak correlations were found with FM. Correlations were also evaluated after "homogenizing" our patient group by excluding the 2 adolescent patients. Highly significant correlations were again found between the above parameters and LBM while none were found with FM. As expected the between methods results differed significantly. Despite a high correlation between DEXA constructed weight and scale weight, mean DEXA weight was significantly and about 520 gram lower than scale weight. This is in agreement with results from Oggle et al. (8). FM measured by TBW was lower than that measured by either skinfolds or DEXA methods whereas FM-skinfold was often higher than FM-DEXA. The low values of FM when measured by the TBW method might be due to overestimation of FFM by this method. The FFM calculated by TBW is based on the assumption that a fraction of FFM is water. As the water content of FFM decreases with age (17), we used the age dependent FFM hydration fraction to calculate the FFM (17). The mean FFM hydration fraction used in our study was 76.27% (range: 73.7 - 77.5%). In a study of body composition of CF prepubertal children, making use of skinfolds and TBW methods, Tomezco et al. also found a significantly lower body fat percentage with the TBW when compared to the skinfold method, which showed normal results (2). In two compartment models such as the TBW and the skinfolds methods, the densities of FFM is assumed to be constant in the range 18 - 67 years but the density does vary depending on the concentrations of water and mineral in FFM (24,25). Although in our study the variation in water and mineral content was taken into account in the regression equations of skinfolds and TBW methods for the calculation of FFM and FM in the age range below 18 years, the water and mineral content are still population specific depending on the presence of illnesses. The percentage of TBW in CF children has been reported to be increased compared to control children (19,26). Theoretically, the DEXA method has the advantage

of being independent of biological assumptions about the densities and level of tissue hydration but the accuracy of the method still depends on the internal calibration (27,28). It has been reported that when compared to chemical analysis, DEXA overestimate fat measured in meat blocks with lower fat content and underestimate the content in those with high fat content (29). Moreover, studies comparing DEXA results with those obtained from chemical analysis, using piglets, showed slight inadequacies in the estimation of fatmass and lean body mass (27,30). We think that the between methods differences are most likely related to the various body compartments measured by these 3 methods rather than to inherent inaccuracies in the techniques themselves. This means that results obtained from each of these methods are not interchangeable. An important question to answer is whether or not any of the used methods is capable of detecting body composition changes occurring during nutritional interventions. DEXA has been introduced as direct method with very good reproducibility (12,31). In this study we compared the sensitivity of DEXA, TBW and skinfolds for detecting small body composition changes in children. For this purpose, we assessed the body composition of 15 CF children before and 3 months after they were treated with lansoprazole as an adjunct therapy for pancreatic enzymes in order to decrease steatorrhea. All 15 CF children showed significant increases in Z-scores for weight, height and skinfolds (unpublished observations). The evaluation of body composition changes differed depending on the method used. With the DEXA method, 53% of the weight increase was ascribed to FM, 44% to FFM and 3% to BMC. Both DEXA and skinfolds methods showed significant increases in fatmass but the increased FFM was not significant. In contrast, weight increase was exclusively ascribed to an increase in FFM with the TBW method. However, no significant correlations were found between weight changes and either FM, LBM or BMC changes by any method. The correlation coefficient of 0.40 found between weight changes and changes in FFM by DEXA just failed to reach statistical significance ( $p = 0.07$ ). There were also no significant changes in ECV and ICV after intervention. This is in contrast to results reported by Going et al., who studied the changes in body compartments induced by dehydration - rehydration with oral fluid using DEXA method for assessment of body composition changes. They found a correlation between bodyweight changes and changes in TM, soft tissue mass (LBM + FM) and LBM. However, the total weight changes induced in their study was higher than in our study (approximately 1.2 kg versus 0.97 kg in our study) and as the changes in bodyweight were

induced by water content, the total bodyweight changes were exclusively ascribed to changes in the water content of STM, reflected by the exclusive increase in LBM (32). Since fatmass was mostly depleted in our patients, it is likely that this body compartment will normalize first as a result of an effective intervention.

From the results of this study, we conclude that results measured by different methods are not interchangeable. It is consequently important to use the same method for longitudinal evaluation of body composition. However, the use of DEXA, TBW and skinfolds methods is limited in children in whom only slight changes in bodyweight after intervention are expected (3% in this study) since the sensitivity is apparently not high enough for the detection of small differential changes in FM and FFM.

*Acknowledgment:* The authors wish to thank Mia Meers from the department of clinical laboratory, Sandra Zimny and Piet Willems from the department of nuclear medicine for their kind and expert technical assistance.

## REFERENCES

- (1) M. Aitken, S. Fiel. Cystic Fibrosis. *Dis Mon* 1993;39: 1-52.
- (2) J. Tomezsko, T. Scanlin, V. Stallings. Body composition of children with cystic fibrosis with mild clinical manifestations compared with normal children. *Am J Clin Nutr* 1994; 59: 123-8.
- (3) M. Bronstein, P. Davies, K. Hambidge, F. Accurso. Normal energy expenditure in the infant with presymptomatic cystic fibrosis. *J Pediatr* 1995; 126: 28-33.
- (4) R. Kraemer, A. Rudeberg, B. Hadorn, E. Rossi. Relative underweight in cystic fibrosis and its prognostic value. *Acta Paediatr Scand* 1978; 67: 33-37.
- (5) R. Branson, Y. Vaucher, G. Harrison, M. Vargas, C. Thies. Inter- and intra-observer reliability of skinfold thickness measurements in newborn infants. *Hum Biol* 1982; 54: 137-143.
- (6) W. Gerver, R. de Bruin. *Paediatric Morphometrics: A reference manual*. 1th ed. Utrecht: Bunge, 1996.
- (7) J. Westrate, P. Deurenberg, H. Van Tinteren. *Int J Obesity*. 1989; 13: 465-477.
- (8) G. Ogle, J. Allen, I. Humphries et al. Body-composition assessment by dual-energy x-ray absorptiometry in subjects aged 4-26 y. *Am J Clin Nutr*. 1995; 61:746-53.
- (9) J. Westrate, P. Deurenberg. Body composition in children: proposal for a method for calculating body fat percentage from total body density or skinfold-thickness measurements. *Am J Clin Nutr* 1989; 50: 1104-15.
- (10) R. Mazess, B. Collick, J. Trempe, H. Barden, J. Hanson. Performance evaluation of a dual-energy x-ray bone densitometer. *Calcif Tissue Int* 1989; 44: 228-232.
- (11) W. Peppler, R. Mazess. Total body bone mineral and lean body mass by dual-photon absorptiometry. I. Theory and measurement procedure. *Calcif Tissue Int* 1981; 33: 353-359.
- (12) R. Mazess, H. Barden, J. Bisek, J. Hanson. Dual-energy x-ray absorptiometry for total-body regional bone-mineral and soft-tissue composition. *Am J Clin Nutr* 1990; 51: 1106-12.
- (13) B. Van Kreel, F. Van der Vegt, M. Meers, T. Wagenmakers, K. Westerterp, A. Coward. Determination of total body water by a simple and rapid mass spectrometric method. *J Mass Spectrom* 1996; 31: 108-111.
- (14) B. Van Kreel. An improved bromide assay for the estimation of extracellular water

- volume by capillary gas chromatography. *Clinica Chimica Acta* 1994; 231: 117-128.
- (15) B. Friis-Hansen. Body water compartments in children: Changes during growth and related changes in body composition. *Pediatrics* 1961; 28: 169-181.
- (16) S. Fomon, F. Haschke, E. Ziegler, S. Nelson. Body composition of reference children from birth to age 10 years. *Am J Clin Nutr* 1982; 35: 1169-1175.
- (17) R. Boileau, T. Lohman, M. Slaughter, T. Ball, S. Going and M. Hendrix. Hydration of the fat-free body in children during maturation. *Hum Biol* 1984; 56: 651-666.
- (18) J. Bland, D. Altman. Statistical methods for assessing agreement between two methods of clinical measurement. *The Lancet* 1986; 8: 307-310.
- (19) M. Miller, D. Kornhauser. Bromide pharmacokinetics in cystic fibrosis. *Arch Pediatr Adolesc Med* 1994; 148:266-271.
- (20) J. Johnston, M. Leong, E. Checkland, P. Zuberbuhler, P. Conger, A. Quinney. Body fat assessed from body density and estimated from skinfold thickness in normal children and children with cystic fibrosis. *Am J Clin Nutr* 1988; 48: 1362-6.
- (21) M. Miller, L. Ward, B. Thomas, W. Cooksley, R. Shepherd. Altered body composition and muscle protein degradation in nutritionally growth-retarded children with cystic fibrosis. *Am J Clin Nutr* 1982; 36: 492-499.
- (22) H. Rico, M. Revilla, L.F. Villa, E. Hernández, M. Alvarez de Buergo and M. Villa. Body composition in children and Tanner's stages: A study with Dual-energy X-ray absorptiometry. *Metabolism* 1993; 42: 967-970.
- (23) R. Faulkner, D. Bailey, D. Drinkwater, A. Wilkinson, C. Houston and H. McKay. Regional and total body bone mineral content, bone mineral density and total body tissue composition in children 8 - 16 years of age. *Calcif Tissue Int* 1993; 53: 7-12.
- (24) G. Forbes. *Human body composition*. New York: Springer-Verlag, 1987.
- (25) T. Lohman. *Advances in body composition assessment*. Champaign, IL: Human Kinetics, 1992.
- (26) M. Newby, N. Keim, D. Brown. Body composition of adult cystic fibrosis patients and control subjects as determined by densitometry, bioelectrical impedance, total body electrical conductivity, skinfold measurements, and deuterium oxide dilution. *Am J Clin Nutr* 1990; 52: 209-13.
- (27) K. Ellis, R. Shypailo, J. Pratt, W. Pond. Accuracy of dual-energy x-ray absorptiometry

for body composition measurements in children. *Am J Clin Nutr* 1994; 60: 660-5.

(28) R. Wellens, W. Chumlea, S. Guo, A. Roche, N. Reo, R. Siervogel. Body composition in white adults by dual-energy x-ray absorptiometry, densitometry, and total body water. *Am J Clin Nutr* 1994; 59: 547-55.

(29) M. Jensen, J. Kanaley, L. Roust et al. Assessment of body composition with use of dual-energy x-ray absorptiometry: Evaluation and comparison with other methods. *Mayo Clin Proc* 1993; 68: 867-873.

(30) J. Brunton, H. Bayley, S. Atkinson. Validation and application of dual-energy x-ray absorptiometry to measure bone mass and body composition in small infants. *Am J Clin Nutr* 1993; 58: 839-45.

(31) P. Chilibeck, A. Calder, D. Sale, C. Webber. Reproducibility of dual-energy x-ray absorptiometry. *Can Assoc Radiol J* 1994; 45: 297-302.

(32) S. Going, M. Massett, M. Hall et al. Detection of small changes in body composition by dual-energy x-ray absorptiometry. *Am J Clin Nutr* 1993; 57: 845-50.

## CHAPTER 8

### GENERAL DISCUSSION

Chronic pulmonary infections and poor appetite together with fat malabsorption are the main causes of malnutrition and growth retardation in CF children (1-3). The ideal treatment of CF should be the correction of the underlying defect by introduction of a normal copy of the defective gene into these patients genetic material. Although gene therapy is presently under intensive scrutiny (4-6), the role of this treatment in CF patients is not yet settled. Until then, treatment of these patients has to focus on improving the nutritional condition, since malnutrition can adversely affect survival (7). As 85% of CF patients have pancreatic insufficiency (8), improved absorption by pancreatic enzymes substitution is one of the main goals. Diagnosis and regular monitoring of fecal fat loss along with close evaluation of growth and the nutritional condition are consequently necessary in the follow up of these patients. Although the fat balance method is considered to be the golden standard for the evaluation of steatorrhea, it is too cumbersome to be used for the frequent monitoring of fat losses in these children. Several studies have shown the measurement of fecal fat concentration to be a valuable alternative to fat excretion studies for the diagnosis of fat malabsorption (9). These studies also shown that the differences in fat excretion between either 3 or 1 day collections are mainly due to day to day variation in stool volume, the stool fat concentration being much more constant. These studies led us to suppose that the repeated measurement of stool fat concentration in stool samples would be a valuable aid to the monitoring of steatorrhea. As chemical measurement of stool fat is time consuming, we looked for an alternative easy measure of fat content. Although the steatocrit looked quite attractive (10) our first results and also results reported by others (11,12) disappointingly often showed low steatocrit results in stools of high fat content.

By acidification of stool homogenates, we could show fat extraction to be much improved and to result in a satisfactory correlation coefficient between chemically measured fecal fat and "acid steatocrit" results. We consequently decided to use the acid steatocrit in an intervention study (proton pump inhibitor) aiming at improving both steatorrhea and the nutritional condi-

tion in children with CF.

Both anthropometric parameters and body composition methods were used for the evaluation of the nutritional condition. Difficulties arise due to the fact that weight, height and skinfolds are age and sex specific. Although several authors have overcome this problem by expressing results of these parameters as a percentage of the predicted values for age and sex, the use of Z-scores is the preferred method for most authors. Z-scores measure deviations from the median value expressed in standard deviation units. Improving Z-scores reflect catch-up growth while the reverse is true for deteriorating Z-scores. Recently, Gerver and de Bruin have constructed growth charts with standard deviation for weight, height armcircumferences and the 4 skinfolds (biceps, triceps, subscapular and suprailiac) (13). Anthropometric parameters can be easily converted into Z-scores through the use of these reference data for normal children. As weight changes could be due to either changes in fatmass, fat free mass or both, we measured body composition by several methods in order to evaluate body composition before and after our intervention (proton pump inhibitor) study.

Our study results show significant decreases of most measured anthropometric parameters in children with cystic fibrosis. Decrease in skinfold thicknesses were most significant and contrary to a commonly held belief triceps skinfolds were often normal while subscapular and suprailiac skinfolds were very sensitive indicators of chronic malnutrition in these patients. Our findings support the use of these simple anthropometric measurements for the evaluation of the nutritional condition in children. As far as body composition results are concerned, interpretation of results is uneasy due to the lack of reference values for several measuring methods. Notwithstanding these drawbacks, results of the various methods used were strongly correlated with each other but, still showed differences which preclude the use of these various methods interchangeably. Results should be looked at both in relative and in absolute terms.

In absolute terms, the DEXA method showed a severe decrease of fatmass and a slight decrease of fat free mass and of bone mineral content. Results of the total body water and skinfold method agreed with the DEXA results but could not be accurately assessed due to the lack of reliable reference data.

In relative terms, the deuterium - bromide results showed a relatively increased total body water and extracellular water compartment while the relative body cell water mass appeared

well maintained. These results imply a decrease of fatmass as percent of bodyweight. Likewise, the fat free mass (%) measured by the skinfold method was increased in our CF children. All these results agree with each other rather well and show that children with CF have a lowered bodyweight accompanied by a decreased fatmass (%), an increased fat free mass (%) and an increased extracellular water compartment (%) while the intracellular water compartment (%) appears to be well maintained (table 1).

**Table 1** Body composition in children with cystic fibrosis.

	<b>FM</b>	<b>FFM</b>	<b>TBW</b>	<b>ECV</b>	<b>ICV</b>
	<b>kg (%)</b>	<b>kg (%)</b>	<b>(%)</b>	<b>(%)</b>	<b>(%)</b>
<b>DEXA</b>	↓ (↓)	↓ (↑)			
<b>Skinfold</b>	? (↓)	? (↑)			
<b>Deuterium</b>					
<b>Bromide</b>	? (↓↓)	? (↑)	(↑)	(↑)	(n)

DEXA: Dual energy X - Ray Absorptiometry

FM: Fatmass

FFM: Fat free mass

TBW: Total Body Water

ECV: Extracellular volume

ICV: Intracellular volume

A positive effect of omeprazole on fat absorption has been found in adults with CF (14). However the role of proton pump inhibitors on steatorrhea and its effects on the nutritional condition has not been evaluated in children. We have studied the effect of 3 months treatment of lansoprazole on fat malabsorption and body composition in 15 CF children, maintaining steatorrhea while on pancreatic enzymes. These children showed significant improvements of both fat absorption (as measured by the acid steatocrit) and Z-scores for all parameters except for the biceps and triceps skinfolds and deteriorated again 3 months after lansoprazole was stopped. The increase in skinfold thicknesses Z-scores were accompanied by signifi-

cant increases in fatmass as measured by the skinfold and the DEXA methods.

Different body composition methods have been described but, only few studies have compared different measurement techniques in pediatric subjects. An important question to answer is whether or not any of these methods is capable of detecting body composition changes occurring during nutritional interventions. Our study comparing the changes in body composition measured by DEXA, TBW and skinfolds methods in 15 CF children, whose nutritional condition improved significantly after intervention with lansoprazole for 3 months, showed different results for each method. Both DEXA and skinfolds methods showed significant increases in fatmass but not in lean body mass in absolute terms. Likewise, the percentage of body cell water mass did not increase significantly after nutritional intervention. On the other hand, the increases in bodyweight were completely ascribed to increases in lean body mass but not in fatmass when evaluated by the TBW method. Since fatmass was mostly depleted in our CF children (as shown by DEXA, skinfolds and total body water methods), it is likely that this body compartment will normalize first as a result of an effective intervention. Our results do not allow firm conclusions as to the effect of lansoprazole on FFM while a significant increase in bone mineral content was found. The bodyweight changes occurring during lansoprazole intervention were unrelated to either fatmass or FFM changes measured by any of the three methods used. We think the weight changes in the various body compartments were too small to be accurately measured by body composition methods.

In conclusion, the acid steatocrit is a reliable, cheap and noninvasive alternative method for the monitoring of fat malabsorption. Most cystic fibrosis patients are malnourished even when lung functions are stable and a hypercaloric diet is used. Body composition studies in these patients mainly show a loss of fat mass and bone mineral content with a relative increase in extracellular water and a normal intracellular water mass (%). Inhibition of gastric acid secretion by a proton pump inhibitor improved both fat absorption and the nutritional condition of our patients. Methods for the assessment of body composition are not interchangeable and not accurate enough for detecting small changes in fatmass and fat free mass such as measured in our 3 months study. A longterm study is needed in order to better evaluate the effects of lansoprazole on body composition in children with cystic fibrosis.

## REFERENCES

- (1) J. Dodge, J. Yassa. Food intake and supplementary feeding programs. In: J. Sturgess, ed. perspectives in cystic fibrosis. Toronto: Canadian Cystic Fibrosis Foundation; 1980: 125-136.
- (2) M. Bronstein, R. Sokol, S. Abman et al. Pancreatic insufficiency, growth, and nutrition in infants identified by newborn screening as having cystic fibrosis. *J Pediatr* 1992; 120: 533-40.
- (3) J. Tomezsco, V. Stallings, D. Kawchak, J. Goin, G. Diamond, T. Scanlin. Energy expenditure and genotype of children with cystic fibrosis. *Pediatr Res* 1994; 35: 451-460.
- (4) M. Rosenfeld, W. Siegfried, K. Yoshimura et al. Adenovirus-mediated transfer of a recombinant alpha 1-antitrypsin gene to the lung epithelium in vivo. *Science*. 1991;252: 431-4
- (5) B. Pitt, M. Schwarz, J. Pilewski et al. Retrovirus-mediated gene transfer in lungs of living fetal sheep. *Gene Ther* 1995; 2: 344-50.
- (6) M. Rosenfeld, K. Yoshimura, B. Trapnell et al. In vivo transfer of the human cystic fibrosis transmembrane conductance regulator gene to the airway epithelium. *Cell*. 1992; 68: 143-55.
- (7) R. Kraemer, A. Rudeberg, B. Hadorn, E. Rossi. Relative underweight in cystic fibrosis and its prognostic value. *Acta Paediatr Scand* 1978; 67: 33-37.
- (8) M. Aitken, S. Fiel. Cystic fibrosis. *Dis Mon* 1993; 39: 1-52.
- (9) N. Thorsgaard Pedersen, H. Halgreen, H. Worning. Estimation of the 3-day faecal fat excretion and fat concentration as a differential test of malabsorption and maldigestion. *J Gastroenterol* 1987; 22: 91-96.
- (10) P. Phuapradit, A. Narang, P. Mendonca, D. Harris, J. Baum. The steatocrit: a simple method for estimating stool fat content in newborn infants. *Arch Dis Child* 1981; 56: 725-727.
- (11) M. Walters, J. Kelleher, J. Gilbert, J. Littlewood. Clinical monitoring of steatorrhea in cystic fibrosis. *Arch Dis Child* 1990; 65: 99-102.
- (12) E. Sugai, G. Srur, H. Vazquez et al. Steatocrit: a reliable semiquantitative method for detection of steatorrhea. *J Clin Gastroenterol* 1994; 19: 206-9.
- (13) W. Gerver, R. de Bruin. *Paediatric Morphometrics: A reference manual*. 1th ed. Utrecht: Bunge, 1996.
- (14) H. Heijerman, C. Lamers, W. Bakker. Omeprazole enhances the efficacy of pancreatin (pancrease) in cystic fibrosis. *Ann Intern Med*. 1991; 114: 200-201.

## SUMMARY

**In chapter one**, the pathogenesis, clinical manifestations and treatment modalities of cystic fibrosis are briefly summarized. CF is a multisystem disease, the basic defect is a mutation of the CFTR gene. Until now, more than 200 mutations have been characterized. CFTR has been found in epithelial cells of several organs with the lung and pancreas being mostly affected. The role of gene therapy in the management of CF patients is not yet settled. Until then treatment of these patients has to focus on support of lung function and improved fat absorption in order to maintain a normal nutritional status. From our literature review, only predigested foods such as (semi)elemental diets and very high-energy polymeric diets, have been reported to improve the nutritional condition in CF patients. Low duodenal pH is thought to be at least partly responsible for the persisting maldigestion. Inhibition of gastric acid secretion by a proton pump inhibitor has been shown to improve steatorrhea in CF adults. The effect of proton pump inhibitors on fat absorption and on the nutritional status of children with CF has not been reported. The effect of treatment on steatorrhea can only be evaluated by regular monitoring of fecal fat loss. The fat balance method being too cumbersome for the repeated evaluation of steatorrhea, we first aimed at developing an alternative test suitable for our purpose. This test (acid steatocrit) was subsequently used to evaluate the effect of lansoprazole (proton pump inhibitor) on steatorrhea in CF patients showing persisting malabsorption while on pancreatic enzymes. The effects of therapy on the nutritional condition of our patients was evaluated simultaneously.

**In chapter two**, the methods used in this study are described. For the determination of fecal fat, the titrimetric method described by van de Kamer and the Sudan staining method were used for the comparison of steatocrit and acid steatocrit methods. Anthropometry, dual-energy X-ray absorptiometry, total body water and bromide dilution techniques were used to assess body composition.

**In chapter three, four and five**, we describe the steatocrit test as an alternative method for the 3 days fecal fat balance method for the monitoring of steatorrhea. Although the steatocrit

test has been reported to be cheap, simple and noninvasive test, its reliability has been questioned. As this might be due to inadequate fat extraction during the centrifugation step of the steatocrit procedure, we aimed at improving fat extraction by acidification of the fecal homogenate. Results obtained by our modified steatocrit method, called the "acid steatocrit", were highly correlated with those obtained by chemical analysis. We found a high sensitivity and specificity for the acid steatocrit.

Results of the evaluation of the nutritional condition of our patients as well as results concerning the presence of persisting steatorrhea in patients on pancreatic enzymes are described in **chapter six**. Despite hypercaloric intake and the use of pancreatic enzymes, our CF patients maintained steatorrhea and showed signs of malnutrition with significantly decreased Z-scores for weight, height, armcircumference, biceps, subscapular and suprailiac skinfolds. Moreover, their fatmass, lean body mass and bone mineral content were significantly decreased when compared to the reference population described by Oggle et al. Treatment of these CF children with lansoprazole as an adjunct therapy of pancreatic enzymes, resulted in a significant decrease in steatorrhea accompanied by a significant improvement in their nutritional condition.

In **chapter seven**, we describe results of our body composition studies in our patients before and after treatment with lansoprazole. Although highly correlated, results from these various methods were shown not to be interchangeable. In absolute terms, the DEXA, the TBW and the skinfold methods showed children with CF to have a severe depletion of fatmass and a slight decrease of FFM. In relative terms, the above results point to lower body fat percentage accompanied by a higher percentage of LBM. Our results with deuterium - bromide do confirm the above results by showing a high relative TBW content and consequently a low relative fat content. Bromide results further show the relative increase of water percentage to be due to a relatively increased extracellular water compartment with a maintained relative body cell water mass. Although small changes in bodyweight were correctly detected by DEXA examination, the latter method was not accurate enough for the differential detection of small changes in FM and FFM. The usefulness of DEXA, TBW and skinfold methods for the assessment of small body composition changes in children is therefore limited.

## SAMENVATTING

**In hoofdstuk een**, zijn de pathogenese, de klinische manifestaties en de therapeutische mogelijkheden voor cystic fibrosis (CF) kort samengevat. Cystic fibrosis is een multisysteem ziekte, waarvan mutatie van de CFTR (cystic fibrosis transmembrane regulator) gene is het basis defect.

Tot dus ver, zijn er meer dan 200 mutaties beschreven. CFTR werd in de epitheel cellen van verschillende organen gevonden. De longen en de pancreas zijn het meest betrokken by deze erfelijke aandoening. De rol van de gen therapie is nog niet bevestigd in de behandeling van CF patienten. De behandeling van deze patienten is er dan ook gericht op de long functies te ondersteunen en een normale voedingstoestand te behouden door het verbeteren van de vet malabsorptie.

Uit het litteratuur overzicht blijkt dat de voedingsstatus van CF patienten alleen effectief te verbeteren is met voorverteerd voedsel zoals (semi)elementaire voeding, of met een zeer hoge energie inname. Een lage duodenale pH is mede verantwoordelijk voor het slechte verteringsproces. Het is bij volwassen CF patienten bekend dat de vet absorptie significant te verbeteren is door remming van de maagzuur secretie met een proton pomp remmer. Er is nog geen studie gedaan naar het effect van dit middel op de vet vertering en de voedings status bij CF kinderen.

Regelmatig monitoring van vet in de ontlasting is noodzakelijk voor de behandeling van vet malabsorptie. De gebruikelijke vet balans methode is hiervoor te omslachtig. Ons eerste doel was het ontwikkelen van een alternatieve test die snel en makkelijk uitvoerbaar is. Deze test (zure steatocriet) werd dan gebruikt om het effect van een proton pomp remmer (lansoprazol) op steatorrhoea in CF patienten met persisterende malabsorptie onder pancreas enzymen, te evalueren. Daarnaast, werd het effect van lansoprazol op de voedingstoestand van onze patienten geevalueerd.

**In hoofdstuk twee**, beschrijven we de methoden die we gebruikt hebben in deze studie. Voor de bepaling van vet in de ontlasting, werden de titrimetrische methode, beschreven door van de Kamer, en de Sudan kleurings techniek gebruikt om de klassieke steatocriet te vergelijken

met de zure steatocriet. De anthropometrische methode, de dual-energy X-ray absorptiometry (DEXA), het totale lichaamswater (TBW) en de bromide dilutie technieken werden toegepast om de lichaamsamenstelling te beoordelen.

In hoofdstuk drie, vier en vijf, beschrijven we de steatocriet test als een alternatieve methode voor de 3 dagen vet balans ter monitoring van vet in de ontlasting. Hoewel de steatocriet test werd gezien als een goedkope, simpele en noninvasieve test, de betrouwbaarheid van deze test wordt betwist. Dit is mogelijk toe te schrijven aan de inadequate vet extractie tijdens het centrifugeren van de steatocriet procedure. Ons doel was de vet extractie te verbeteren door het aanzuren van het faeces homogenaat. De resultaten verkregen met deze gemodificeerde steatocriet genaamd "zure steatocriet", correleerden goed met de resultaten van de chemische vet analyse. We vonden een hoge sensitiviteit en specificiteit voor de zure steatocriet test.

In hoofdstuk zes, bestuderen we de mate van vet malabsorptie en de voedingstoestand van onze CF kinderen behandeld met pancreas enzymen. Ondanks de hypercalorische voeding en de behandeling met pancreas enzymen, hadden onze patiënten aanhoudende steatorrhoea en toonden tekenen van malnutritie met significante verslechtering van de gemiddelde Z-scores voor gewicht, lengte, armomtrek, biceps, subscapulaire en suprailiacale huidplooiën. Bovendien, hun vetmassa, spiermassa en botmineral is significant lager dan die van de normale kinderen, beschreven door Oggle. Na de behandeling van deze kinderen met een proton pomp remmer (lansoprazol) als supplementaire therapie by pancreas enzymen, vonden we een significante vermindering van steatorrhoea met verbetering van de voedingstoestand.

In hoofdstuk zeven, beschrijven we de resultaten van de lichaamsamenstelling van onze patiënten voor en na de behandeling met lansoprazol. Ondanks de hoge correlatie tussen de resultaten van de gebruikte lichaamsamenstelling methodes, zijn deze technieken niet uitwisselbaar. In absolute zin, toonden de DEXA, de TBW en de huidplooi methode een ernstige depletie van de vetmassa en een lichte afname van de vet-vrije massa by CF kinderen. In relatieve zin, wijzen deze resultaten in de richting van een afname van het vet percentage gepaard aan een hoger percentage van lean body mass (LBM). Dit komt overeen met de resultaten van deuterium-bromide, waarbij een hoog TBW percentage en dus een laag vet

percentage gevonden werd. De toename in het TBW percentage is toe te schrijven aan het verhoogde percentage extracellulair water terwijl intracellulair water normaal blijft. Alhoewel de verandering in lichaamsgewicht door het DEXA onderzoek correct werd geschat, was geen van de gebruikte lichaamssamenstelling methodes nauwkeurig genoeg voor het schatten van kleine veranderingen in de vetmassa en vet-vrije massa. De bruikbaarheid van DEXA, TBW en huidplooi methoden voor het schatten van kleine veranderingen in de lichaamssamenstelling bij kinderen is daarom beperkt.

## DANKWOORD

Woorden schieten tekort om mijn dank uit te drukken. Ik ben niet zo goed in taal expressie, toch hoop ik met enkele eenvoudige zinnen iedereen te kunnen bedanken, die het mij mogelijk hebben gemaakt dit proefschrift vorm te geven.

Zonder iemand tekort te willen doen, richt ik een speciaal dankwoord tot de volgende personen:

**Prof. Dr. C. Blanco**, promotor, beste Carlos, ondanks je drukke taak, heb je toch heel snel en kritisch mijn werkstukken doorgenomen. Hiervoor dank ik je extra.

**Dr. P. Ph. Forget**, copromotor, beste Philippe, het lukte mij nooit je te tutoyeren, niet vanwege onze persoonlijke contacten, maar vanwege mijn respect voor jou. De manier waarop je het onderzoek stuurde waarbij je mij geheel in mijn waarde en vrijheid liet, was van buitengewoon hoog niveau. Je leerde mij wetenschappelijk denken. Waar nodig was bood je hulp aan, soms ook met het verwerken van de resultaten. De correctie van het manuscript was binnen korte tijd klaar. Zelfs in je vakantie, nam je mijn werkstukken mee en was je bereid hiervoor terug te komen. Ik heb genoten van je onuitputtelijke bron van nieuwe ideeën.

Ook in het persoonlijk contact was je aangenaam. Je heb altijd in mij geloofd en stond altijd achter mij. Beste Philippe, zonder jouw inzet en je vertrouwen als begeleider, zou dit proefschrift nooit deze vorm hebben gekregen.

**Dr. B. van Kreel**, copromotor, de helft van mijn tijd als onderzoeker heb ik in uw laboratorium doorgebracht. Uw deur stond altijd voor mij open. Als het niet lukte met de steatocrit-bepaling, heb u altijd nieuwe suggesties. Uw deskundigheid en eerlijkheid was onmisbaar voor het slagen van dit onderzoek.

**Prof. Dr. R. H. Kuijten**, bedankt voor de mogelijkheden die u hebt gecreeërd voor dit onderzoek.

**Drs. A. Van den Neucker**, beste Anita, al die jaren ben je voor mij een goede vriendin geweest. Ook als het mij tegen zat, wist je met je nuchtere kijk en eerlijkheid mijn problemen te relativeren. Ik heb genoten van onze discussies en van je gezelschap op verscheidene congressen. Je interesse in anderen en je brede algemene kennis maakte het zeer boeiend. Anita, je hebt mijn "gat" in de Westerse cultuur opgevuld.

**Hooggeleerde leden van de beoordelingscommissie**, bedankt voor uw vlotte en kritische beoordeling van dit manuscript.

**Alle kinderartsen, neonatologen en arts-assistenten** kindergeneeskunde in het AZM dank ik hierbij voor de aanspraak in de afgelopen jaren.

**Dr. W. J. M. Gerver en Dr. R. De Bruin**, jullie groeicurven hebben grote waarde toegevoegd aan dit onderzoek. Bedankt voor jullie voortreffelijke bijdrage.

**Jolanda van Golde en Rony Neeffes**, beste Rony en Jolanda, bedankt voor het meelevende en de gezellige uren in het AIO-hok, in het restaurant, in het theater aan het Vrijthof, in de bioscoop, bij een van ons thuis of in het zwembad. Bedankt voor het aanhoren van mijn "gezeurd". We hebben goede en slechte tijden met elkaar doorgemaakt. Ik hoop dat onze vriendschap hierdoor alleen maar sterker is geworden.

**Alle medewerkers van het klinisch chemisch laboratorium** van het AZM, met name **Serva, Lou, Michel, Mia, Theo, Peter en Marian**, bedankt voor jullie inzet en betrokkenheid tijdens het onderzoek. Jullie wetenschappelijke interesse was van niveau. Als ik hulp nodig had waren jullie bereid, soms ook ongevroegd, het eigen werk neer te leggen en mij bij te staan. Bedankt voor de aangename sfeer en de gezellige samenwerking.

**Liesbeth van der Ploeg en Lianne Schoorlemmer**, dietisten, wil ik danken voor het uitrekenen van de calorieën bij mijn patiënten populatie.

**Dr. G. A. K. Heidendal, Piet Willems en Sandra Zimny** van de nucleaire afdeling, bedankt voor jullie fijnzinnige instructies over de DEXA scan.

**Alle poli-assistenten en de secretaresses** van de kindergeneeskunde, wil ik danken voor de samenwerking in de afgelopen jaren.

**Oom Wim en tante Margriet van der Avoort**, bedankt voor jullie steun en betrokkenheid in de afgelopen 15 jaren van mijn leven in Nederland.

Ik ben de firma's **Hoechst Marion Roussel B.V.** (Hoevelaken) en **Janssen-Cilag B.V.** (Tilburg) dankbaar voor hun financiële ondersteuning in de drukkosten van dit proefschrift.

Tenslotte, zou dit boek niet volledig zijn zonder hulp en meelevende van mijn familie. Lieve mama, oom Kiet, Manh Hung en Manh Cong, terwijl ik rustig aan mijn proefschrift werkte, hebben jullie voor mijn verhuizing gezorgd.

**Manh Cong**, bedankt voor het ter beschikbaar stellen van je computer en **Manh Hung** voor je

deskundige steun. Als ik met de computer problemen had, kon ik altijd op jullie terugvalen. Oom Kiet, bedankt voor je inzet en betrokkenheid. Nooit hoefde ik je om hulp te vragen, je was er gewoon.

Lieve Mama, zonder jou zou dit boek er nooit zijn gekomen. Heel je leven lang heb je voor ons klaar gestaan. Jouw droom is een goede toekomst voor je kinderen. Daarvoor heb je 15 jaar geleden je leven op het spel gezet. Je stimuleerde ons om te studeren. Rijkdom is niet belangrijk, maar kennis, dat is de beste bagage die je op onze weg aan ons hebt kunnen meegeven. Mama, bedankt voor je betrokkenheid en het aanhoren van mijn frustraties. Met een glimlach en een schouderklop wist je al mijn problemen op te lossen. Mama, ik hou van jou en ik ben trots dat jij mijn moeder ben.

## CURRICULUM VITAE

Thi My Dung Tran werd op 27 april 1967 te Dinh Tuong in Vietnam geboren.

Na het doorlopen van het basisonderwijs volgde zij, eveneens in Vietnam, drie jaren vervolgonderwijs op voorbereidend wetenschappelijk niveau.

In 1981 kwam zij met haar familie in Nederland.

Op 2 juni 1986 verwierf zij aan de Rijksscholengemeenschap "Den Hulster" te Venlo het diploma Atheneum B. met als eindexamenpakket de vakken: Nederlands, Engels, Wiskunde I en II, Natuurkunde, Scheikunde en Biologie.

Vanaf het najaar 1986 studeerde zij geneeskunde aan de Rijksuniversiteit te Maastricht. Zij behaalde op 13 augustus 1990 haar doctoraal getuigschrift. Op 1 februari 1993 werd het diploma basisarts aldaar aan haar uitgereikt.

Tijdens haar studie verrichte zij wetenschappelijk onderzoek onder leiding van Dr. P. PH. Forget op de afdeling kindergeneeskunde van het Academisch Ziekenhuis Maastricht: "Singel Stool analysis for fat, alfa-animo nitrogen and electrolyt".

Vanaf 1 maart tot 3 november 1993 werkte zij als arts-onderzoeker bij de vakgroep kindergeneeskunde van het A.Z.M. aan het project "Effect of ranitidine in children with chronic abdominal pain".

Van 1 december 1993 tot 1 december 1994 was zij werkzaam als AGNIO kindergeneeskunde in het A.Z.M.

In de periode 1 december 1994 tot 1 april 1996 werkte zij onder leiding van Dr. P. PH. Forget, kinder-gastroenteroloog in het A.Z.M., aan haar promotie-onderzoek.

