Maastricht University

Clinical use of acid steatocrit

Citation for published version (APA):

van den Neucker, A. M. (2001). *Clinical use of acid steatocrit*. [Doctoral Thesis, Maastricht University]. Universitaire Pers Maastricht. https://doi.org/10.26481/dis.20011122an

Document status and date:
Published: 01/01/2001

DOI:
10.26481/dis.20011122an

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

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

repository@maastrichtuniversity.nl

providing details and we will investigate your claim.

Download date: 23 May. 2025

Clinical Use of Acid Steatocrit

Cover: Detail of "Children's Games" of Pieter Bruegel de Oudere, Kunsthistorisches Museum Vienna
English correction of the Introduction, Discussion and Conclusion by Mr. B. Wilkinson, Worldneth / Talencentrum UM

Datawyse / Universitaire Pers Maastricht
ISBN 90 5278 324 1
© A.M. Van den Neucker, Lanaken - België 2001

CLINICAL USE OF ACID STEATOCRIT

PROEFSCHRIFT

ter verkrijging van de graad van doctor
aan de Universiteit Maastricht,
op gezag van de Rector Magnificus,
Prof.dr. A.C. Nieuwenhuijzen Kruseman,
volgens het besluit van het College van Decanen,
in het openbaar te verdedigen
op donderdag 22 november 2001 om 14.00 uur

door

Anita Maria Van den Neucker

Promotor
Prof.dr. R.A.M.G. Donckerwolcke

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

Beoordelingscommissie
Prof.dr. M.P. van Dieijen-Visser (voorzitter)
Dr. W. Hameeteman
Prof.dr. E. Robberecht (Universitair Ziekenhuis, Gent, België)
Dr. M. Sinaasappel (Sophia Kinderziekenhuis, Rotterdam)
Prof.dr. R.W. Stockbrügger

Leva in signum super nos lumen vultus Tui
Psalm 4

Voor mijn moeder,
ter nagedachtenis aan mijn vader

CONTENTS

CHAPTER 1	**Introduction.**	9
	Lipid metabolism	
	Lipid malabsorption	
	Methods for determination of faecal lipids	17
	Chemical methods	
	Gravimetric method	
	Titrimetric method of Van de Kamer	
	Method of Jeejeebhoy	
	Microscopic examination of stool specimen for fat droplets	
	Near-infrared reflectance analysis	
	NMR	
	Steatocrit	
	Fat absorption tests	19
	Triglycerides in the serum / Fatty meal test	
	Beta-carotene	
	Vitamin A	
	Radioactive and isotope labeled fat absorption tests	
	References	21
	Aim of the thesis	25
CHAPTER 2	**The acid steatocrit: a much improved method.**	27
	J Paediatr Gastroenterol nutr 1994; 19: 299 - 303.	
CHAPTER 3	**Comparison of near infrared reflectance analysis of fecal fat, nitrogen and water with conventional methods, and fecal energy content.**	37
	(submitted)	

CHAPTER 4 **Lipid, nitrogen, water and energy content of a single stool sample in healthy children and children with cystic fibrosis.** 47
(submitted)

CHAPTER 5 **Acid steatocrit during infancy.** 55
Acta Paediatr 1996; 85: 1153 - 5.

CHAPTER 6 **Clinical use of acid steatocrit.** 63
Acta Paediatr 1997; 86: 466 - 9.

CHAPTER 7 **Acid steatocrit a reliable screening tool for steatorrhoea.** 71
Acta Paediatrica 2001; 90:1-3

CHAPTER 8 **Discussion and conclusion.** 79

SUMMARY 85

SAMENVATTING 87

DANKWOORD 89

CURRICULUM VITAE 91

CHAPTER 1

Introduction

Obesity is considered the most common health problem of the new millennium (1) and vascular diseases are the main cause of death (2). Nutritional lipids, considered to be the most important contributing factor to these diseases, are not popular among nutritionists (3, 4), while fat is the cream of our food and gives taste to it (5).

Lipids are essential for the nutrition of children, not only as a source of essential fatty acids (6, 7) and as a vehicle for lipid soluble vitamins, but mostly to provide sufficient nutritional energy. Lipids are important for cellular metabolic processes as well as for cell membrane composition, fluidity, peroxidation, prostaglandin and leukotriene synthesis. Further, lipids are implicated in the mechanisms of brain development, inflammatory processes, atherosclerosis, carcinogenesis, ageing and cell renewal (8, 9). Children will fail to thrive if the intake, digestion or absorption of lipids is insufficient; therefore dietary fat restriction is not recommended in infancy (10, 11, 12).

While the intake of lipids is easy to calculate (13), the evaluation of lipid digestion and absorption is more laborious. Different laboratory techniques have been designed to assess fat absorption (14, 15).

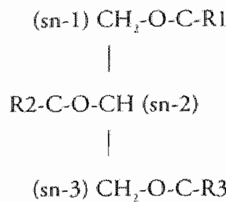
At present, the gold standard to evaluate fat absorption is the fat balance technique, based on fat intake and faecal fat excretion and expressed as fat absorption coefficient. Faecal fat is usually determined by the titrimetric method as described by Van de Kamer in 1949 (16). This method is laborious and unpleasant for patients and laboratory staff and for this reason more simplified techniques have been proposed (17, 18, 19, 20, 21).

Before describing those various techniques, it is worth giving a short review on lipid absorption and malabsorption to allow adequate interpretation of the results obtained by these different methods.

Lipid Metabolism

Fats in the human diet consist of triglycerides, phospholipids, and cholesterol. Approximately 97% of dietary lipids consist of triglycerides (9). Each triglyceride molecule consists of three fatty acids esterified to one molecule of glycerol. The fatty acid chains have different chain lengths and different degrees of saturation.

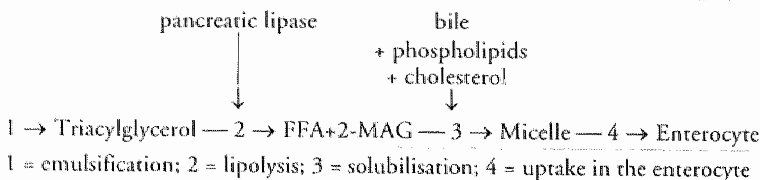
Figure 1. Triglyceride molecule with stereospecific numbering (sn) of the C-atoms of the glycerol-part; R= hydrocarbon chain.



Other dietary lipids are phospholipids, free fatty acids (FFA), mono- and diglycerides (MAG, DAG), cholesterol and cholesterolesters; cholesterol is the main dietary sterol (22).

After ingestion, lipids must be digested before absorption in the intestine and metabolization by the liver. The digestion of lipids can be described as a chain of events: emulsification, lipolysis, solubilisation, and uptake into the enterocyte, reesterification in the enterocyte and transport via the lymph into the circulation (fig 2).

Figure 2.



Defects at any level of the lipid metabolism can cause steatorrhoea, defined as an excess of faecal fat. Faecal lipids originate normally from unabsorbed dietary fat and for a small part from bacteria and from endogenous sources such as bile and

breakdown of desquamated epithelial cells (23). In childhood the normal amount of faecal fat depends on the age of the child. In adults faecal fat exceeding 6g per day is considered abnormal. Daily faecal fat excretion in normal children ranges between 1.3g and 3.8g with a fat absorption coefficient greater than 95%. The fat absorption coefficient of infants younger than 9-10 months ranges from 80-90% and these higher faecal fat losses are considered to be normal at this age (24, 25, 26, 27, 28).

Emulsification
After ingestion dietary lipids are emulsified by chewing, peristaltic movements of the stomach and forceful transport through the pyloric channel into the duodenum. Chemical modification by lingual and gastric lipase adds to the emulsification (22).

Lipolysis
Lipolysis is the result of the interaction of different enzymes on dietary lipid; some enzymes are secreted as pro-enzyme and have to be activated, others work in combination with bile acids (table 1). Different synonyms for these enzymes are reported in the literature (29).

Table 1. Enzymes involved in lipolysis (30, 31, 32, 33, 34, 35).

Source	Enzyme	Activator	Main substrate
Mammary gland	Carboxyl ester lipase	Bile salts	Monoglycerides+ cholesterylester
Lingual glands	Lingual lipase	–	Triglycerides
Stomach	Gastric lipase	–	Triglycerides
Pancreas	Co-lipase	Trypsin	Fat droplets
	Pancreatic lipase	–	Triglycerides
	Carboxyl ester lipase	Bile salts	Cholesterylester
	Phospholipase A_2	Trypsin	Phospholipids

Digestion of lipid starts in the stomach with hydrolysis by acid lipases, called pre-duodenal lipases also known as lingual, salivary, pharyngeal, gastric lipase and pre-gastric esterase (32, 36).

Duodenal hydrolysis of dietary lipids is carried out by pancreatic co-lipase-dependent lipase, carboxyl ester hydrolase and phospholipase A2; the structure of those enzymes has recently been described (33, 34, 35).

Pancreatic lipase acts in the duodenum in collaboration with bile acids (34). Co-lipase is secreted by the pancreas as a pro-enzyme. Co-lipase has affinity for

bile acid micelles as well as for phospholipid covered emulsion. The pancreatic lipase-co-lipase interaction is still a matter of intensive study (33).

Carboxyl ester hydrolase, known as cholesterol esterase, is also activated by bile acids. It is believed that this enzyme is only active in the upper intestine. Carboxyl ester hydrolase is secreted by the exocrine pancreas and the mammary gland and is also detectable in human milk (30). Carboxyl ester hydrolase hydrolyses sn-2 monoglycerides, which originate from lipolysis by pancreatic lipase in the intestinal lumen, and completes in this manner the hydrolysis of triacylglycerols (37, 36).

Phospholipids are the substrate for phospholipase A2. Phospholipase A2 is secreted by the pancreas as pro-enzyme and is activated in the intestinal lumen by tryptic cleavage (38, 39).

Transport of lipids from intestinal lumen to the enterocyte

After hydrolysis of lipids, free fatty acids and monoglycerides are absorbed from the intestinal lumen. The duodenum and jejunum are the intestinal segments where fat absorption occurs, while most ingested fat is absorbed by the mid-jejunum (39). For long chain fatty acids and cholesterol, passage across the unstirred water layer is rate limiting, whereas passage of short and medium chain fatty acids is limited by the brush border membrane (8). Bile acids are necessary for the absorption of lipids by increasing their solubility into the unstirred water layer. Nearly all bile acids have a hydrophobic site and a hydrophylic site (40). The hydrophobic site binds to the lipids and surrounds them with a hydrophylic wall by forming micelles: bilayer discs with a lipid core and a hydrophylic surrounding which makes them soluble in water (22). The lipids migrate through the unstirred water layer and through the cell membrane. This transport is not yet elucidated but it is accepted that lipids migrate through this water layer as micelles, and then reach the cell membrane followed by passive transport through the membrane. Some investigators, however, mention active transport of lipids by a specific protein in the jejunal enterocyte, the MVM-FABP (micro villous membrane fatty acid binding protein) (31, 41).

Lipid metabolism in the enterocyte

In the enterocyte the free fatty acids bind to the intestinal fatty acid binding protein (I-FABP), and cholesterol, monoglycerides, lysophosphatides as well as fatty acids bind to the liver fatty acid binding protein (L-FABP). Both proteins exist in the cytosol of epithelial cells of the upper small intestine. I-FABP was first isolated from the intestine, while L-FABP was first found in the liver. Cholesterol and other sterols bind to two isoforms of sterol carrier proteins, SCP-1 and SCP-2. Binding of lipids to intracellular binding proteins may prevent lipids

from forming oil droplets in the cytosol and transport lipids from the brush border to the smooth endoplasmic reticulum. In the smooth endoplasmic reticulum monoglycerides are re-esterified into triacylglycerols, lysophospholipids are re-converted to phospholipids and cholesterol is re-esterified, although some free cholesterol remains. The reprocessed lipids, along with the new lipids that are synthesized in the epithelial cell, accumulate in the smooth endoplasmic reticulum. Phospholipids tend to cover the external surfaces of these lipid droplets, with their polar head groups toward the aqueous exterior. These lipid droplets are known as pre-chylomicrons. These droplets fuse with the lipoprotein precursor released from the ribosomes and chylomicrons originate. Chylomicrons, lipid globules, migrate to the basolateral side of the enterocyte where they are secreted into the interstitial fluid and reach the bloodstream via the lymph. The exit phase of lipid absorption is still incompletely understood (8).

The lipoprotein precursor is a dense emulsion particle originating from the apoB synthesizing ribosomes by translation of the amino-terminal of apoB in combination with microsomal triglyceride transfer protein (MTP) that continues to be enlarged during translation. In the absence of MTP, apoB is translated without undergoing lipidation. Moreover underlipidated particles are generated that are too small to accommodate the proper folding of apo-B. Misfolded apoB is degraded mainly by the ubiquitin-proteasome pathway. Other proteolytic systems of the endoplasmic reticulum and other post-endoplasmic reticulum compartments are also involved in the presecretory degradation of apoB. This results in accumulation of fat in the enterocyte and causes lipid malabsorption. (42).

Lipid malabsorption

Lipid malabsorption can occur when there is a disturbance in either lipid digestion or absorption. Lipid digestion and absorption are the result of the interaction of various complicated mechanisms as described above. Different causes can be responsible for lipid malabsorption, of which the most important are: bile deficiency, pancreatic insufficiency and mucosal atrophy.

Even with severe malabsorption there always remains some degree of absorption because of the complexity of lipid digestion and the presence of alternative absorptive pathways (39). Severe fat malabsorption, however, results in malnutrition, in vitamin or essential fatty acid deficiency (8). The diseases most frequently associated with lipid malabsorption in childhood are cystic fibrosis, celiac disease and disturbances in bile acid metabolism due to cholestasis or interrupted enterohepatic circulation.

Fat that is not absorbed leaves the intestines with the stools and gives them a greasy colour or constitutes sometimes an oil phase distinct from, or surrounding, otherwise normal stools (43, 44). When the amount of faecal fat exceeds a certain level, depending on the age of the child, it is called steatorrhoea (25, 26, 27). Different causes of steatorrhoea are listed in table 2 according to the localization of the defect in fat maldigestion and malabsorption, and in table 3 according to different diseases. Sometimes there are different causes in one disease responsible for the fat malabsorption e.g. in patients with untreated celiac disease the fat malabsorption may be due to both the reduced lipid absorption because of villous atrophy and to exocrine pancreatic insufficiency (45). In cystic fibrosis fat malabsorption may be due to exocrine pancreatic insufficiency, to absorption problems through the unstirred water layer, and if associated liver disease is present, to bile salt deficiency (46).

Table 2. Defects leading to steatorrhoea I: (44)

Defect of lipolysis
Defect of forming of micelles
Defect of absorption in the enterocyte
Defect in the re-esterification
Defect in the chylomicron release into the lymphatics.

Table 3. Causes of steatorrhoea II: (47)

Small intestinal abnormalities:

- **Anatomic abnormalities:**
  - Intestinal malrotation
  - Atresia
  - Stenosis
  - Web
  - Duplication
  - Short bowel syndrome
  - Intestinal Lymphangiectasia
- **Inflammation**
  - Microbial overgrowth
    - Anaerobes
    - Parasites: Giardia Lamblia
  - Regional enteritis
- **Metabolic disorders**
  - Abetalipoproteinemia
  - Acrodermatitis enteropathica

Immunologic disorders
- Agammaglobulinemia
- Hypogammaglobulinemia
- Dysgammaglobulinemia
- Thymic displasia
- Alpha chain disease
- Nodular lymphoid hyperplasia of the small intestine

Sensitivity reactions
- Milk protein sensitivity
- Soybean protein sensitivity
- Allergic gastroenteropathy
- Transient gluten intolerance

Drugs
- Antibiotics: neomycine
  kanamycine
- Paraaminosalicylic acid
- Calcium carbonate
- Cathartics (podophylin)
- Cholestyramine
- Colchicine
- Methotrexate

Miscellaneous
- Celiac disease
- Tropical sprue
- Intractable diarrhoea of infancy
- Protein-calorie malnutrition
- Intestinal lymphoma

Exocrine pancreatic insufficiency:

Generalized pancreatic insufficiency
- Cystic fibrosis
- Schwachman-Diamond syndrome
- Congenital rubella
- Chronic pancreatitis / relapsing acute pancreatitis
- Familial hereditary pancreatitis
- Protein-calorie malnutrition

Isolated enzyme deficiencies
- Lipase deficiency
- Trypsinogen deficiency
- Enterokinase deficiency

Liver disease

Cirrhosis

Hepatitis

Biliary atresia

Steatorrhoea is the result of lipid malabsorption and this can be measured, irrespective of the cause of steatorrhoea. For the diagnosis of steatorrhoea the amount of faecal fat has to be measured quantitatively or semi-quantitatively. Some investigators believe that the eye of a good clinician, the weight of the daily stool or the faecal water content allows a diagnosis of steatorrhoea. The majority of clinicians, however prefer to rely on the results of the faecal fat balance for the diagnosis of steatorrhoea (16, 44, 48, 49).

The **faecal fat balance technique** is still considered the gold standard for the diagnosis of fat malabsorption (16, 25). The oral fat intake of at least 3 days (some authors recommend 5 or 6 days) has to be recorded. The total faecal excretion has to be collected during this recorded period and frozen at -18°C. All collected stools have to be homogenized thoroughly and the faecal fat content determined by the method of Van de Kamer. The fat absorption coefficient is calculated by the following equation and expressed as a percentage:

$$\frac{\text{ingested fat (g/day)} - \text{excreted fat (g/day)}}{\text{ingested fat (g/day)}} \times 100$$

This method is laborious and unpleasant for patients and for laboratory personnel. Furthermore, there are many possibilities for inaccuracies. First, oral fat intake has to be recorded carefully during 3 or more days. The total faecal production during this 3-day period has to be collected thoroughly. This can be managed by the use of a non-absorbable dye at the start and at the end of the collection period. The total amount of stools between the appearance of these two markers has to be collected. This 3-day stool collection is necessary to minimize the normal daily variation in fat absorption (25). During this collection period a minimal (> 80g/day) and constant daily fat intake is necessary. Normal daily faecal fat output is 1 to 2 g / day due to endogenous fat excretion even without oral fat intake (23, 44). With fat malabsorption a small increase of dietary fat will result in steatorrhoea, whereas in patients with normal fat absorption a rise in dietary fat will have no influence on the faecal fat excretion (50, 51)

In his thesis in 1950 Weijers had already mentioned many difficulties associated with the faecal fat balance technique and described a way of collecting faecal material in infants and young children while staying in hospital and using a pot specially made for this purpose (21). The main problem at that time, however, was the lack of a rapid method for faecal fat determination.

Since the introduction in 1949 of the titrimetric method of Van de Kamer for the measurement of faecal fat, the faecal fat balance technique gained popularity

(12). But the collection of stools of infants and children today is as difficult as it was at that time, and the determination of faecal fat by the method of Van de Kamer is as laborious as it was in 1949. Patients and parents are unwilling to collect stools. For these reasons new methods for evaluating steatorrhoea are desired.

A break-through came with the idea that faecal fat concentration could give clinically useful information on the etiology of steatorrhoea. In 1984 Bo-Linn and Fordtran suggested that faecal fat concentration could differentiate steatorrhoea due to pancreatic insufficiency from that due to intestinal abnormalities (52). For this purpose they used 48 or 72-hour stool collections. In 1987 Pederson et al. demonstrated that the faecal fat concentration in a random stool sample gave the same clinical information as the 3-day faecal fat excretion (53). Other investigators subsequently demonstrated that measurement of faecal fat concentration could give similar information as a 3-day stool collection, and this initiated the use of simpler tests to screen, diagnose and monitor fat malabsorption in clinical practice (54, 55, 56). However to assess the absorption coefficient, the 72-hour balance technique remains indispensable.

Methods for the determination of faecal lipids

Chemical methods

Gravimetric determination of total faecal fat

In this method a pre-weighed, emulsified stool specimen is acidified to decrease the ionisation of fatty acids (present as free acids, or soaps). The lipids are then extracted from the stool specimen with an organic solvent, the supernatant evaporated, and the residue measured by gravimetry (57). By this method 98-99% of the fat is extracted (28). This method is laborious and time consuming and fat extraction depends on the solvent (16, 44)

Titrimetric method of Van de Kamer (16)

In 1949 Van de Kamer described a chemical method for determining faecal fat on wet faeces; faeces are saponified with potassium hydroxide in ethanol, hydrochloric acid is added to the solution and fatty acids are liberated. Ethanol is then added and fatty acids are extracted with petroleum ether. After shaking of the homogenate, there is a delineation in different layers. The fatty acids in the petroleum ether layer are subsequently titrated with alkali. This method was at that time an improvement on the existing methods, but is nowadays considered imprecise, complex, tedious and time consuming (58). Eighty percent of total faecal lipids are determined by this method (28).

The method described by Jeejeebhoy in 1970 gives a better fat recovery compared to the method of Van de Kamer. Through the use of a different solvent 99% of faecal lipids are extracted (28, 59).

Microscopic examination of the stool specimen for fat droplets
In patients with steatorrhoea the microscopic examination of stool reveals fat droplets. This test can be performed by mixing a small amount of stool with several drops of water or Sudan red stain. Drummy et al. showed a correlation between the diameter of the fat globules and the total faecal lipids (18). But this good correlation exists mainly when there is severe steatorrhoea, and is less evident in patients with moderate steatorrhoea (25). This method is more qualitative than quantitative, but Khouri et al have ameliorated the Sudan stain by acidification and heating and concluded that they provided specific quantitative information about the faecal fat content (60). Some investigators have reported favourably about this test, but others have not (44, 61, 62).

The near-infrared reflectance analysis
The near-infrared reflectance analysis is based on the measurement of radiation in the near-infrared spectrum scattered by the surface of a spot faecal sample (63). The reflectance is matrix and substrate specific; the components measured have typical functional groups (CH, NH, OH...) with specific absorption bands in the near-infrared range (700-2500 nm). It is possible to determine dry weight, total nitrogen, total fat and hydrolyzed fat on a faecal sample in a few minutes. Although the manufacturer recommends no homogenization of the faecal sample, this is necessary since the reflectance is matrix specific. Another difficulty with this method is the need to perform one's own calibration curve (44, 58). Furthermore the cost of the apparatus is high, but that of personnel is low. Several investigators consider this method as a good alternative for the chemical determination of faecal fat in a stool sample (20, 21, 64, 65, 66).

NMR
Fat is the only liquid component of lyophilized faecal material after heating at 80°C. It is possible to measure this fat by nuclear magnetic resonance. This method is used in the food industry, but the costs, and preparation of the material makes it unsuitable for clinical use (44, 67).

The steatocrit
While searching for a simple and easy test to quantify faecal fat, Phuapradit described the steatocrit method in 1981 (19). The method resembles the haematocrit method, as the name indicates. Faeces diluted with deionized water is mixed, homogenized and sucked in a capillary tube and centrifuged in a

haematocrit centrifuge. After centrifugation, three layers are separated: a solid layer, a water layer and a fat layer at the top. The result is calculated by:

$$\frac{\text{length of fat layer (mm)}}{\text{length of fat layer (mm)} + \text{length of solid layer (mm)}} \times 100$$

Colombo et al. and Gurarino et al. were initially enthusiastic about this cheap, easy and simple test, but soon Walters et al. reported the inability to reproduce these results (68, 69, 70). Others reported the difficulty to measure the lengths of the different layers, because of the lack of sharp delineation and tried to ameliorate the test by adding Sudan stain and using a dial caliper (71). This problem may be solved by acidification of the homogenate: the acid steatocrit method (72). Acidification of the faecal material is a technique frequently used in methods determining faecal fat: Van de Kamer added HCL to the mixture after saponifying faecal fat (16). Furthermore, Bettinardi demonstrated that the type of faecal fat influences the results of the steatocrit and that acidification can overcome this problem (73). Also other investigators have confirmed this methodological improvement (74).

Fat absorption tests

All the tests reported above measure the result of fat malabsorption by evaluation of faecal fat loss. The following tests evaluate fat absorption by measuring absorbed lipids or fat-soluble products:

Triglycerides in the serum / Fatty meal test

Determination of triglycerides or chylomicrons in the serum before and after a meal gives an idea about the absorption of fat. The serum triglycerides are determined with the aid of nephelometry: estimations of light scattering performed on blood plasma (28).

Jonas et al. described an oral fat loading test for children. After a 6-hour fast in small children and a 12-hour fast in older ones, a meal consisting of fat (Blue Band margarine) 2g/kg is given to the children, orally or by nasogastric tube in babies or reluctant toddlers. Blood is sampled at periods of 0, 2, 4 hours after fat ingestion. Plasma trigyceride levels is determined fluorimetrically (75).

This is a semi-quantitative method and several other factors besides fat digestion and absorption interfere with the results: e.g. the amount and type of fat ingested and the emptying rate of the stomach. This technique is therefore mostly considered not quantitative enough, but is still used by several authors in addition to other tests (25, 73, 76).

Beta-carotene test

The determination of beta-carotene, a precursor of vitamin A, a fat-soluble vitamin, is a simple test based on the fact that a disturbed fat absorption influences the beta-carotene absorption. This test is however an indirect test with possible interference of liver diseases, as beta-carotene is converted to vitamin A in the liver (28). Furthermore, there is an important intra- and inter-individual variation in measurement of beta-carotene in plasma (44, 77).

Vitamin A test

Vitamin A is a fat-soluble vitamin, and on the assumption that vitamin A metabolism parallels fat metabolism, a vitamin A loading test was designed. A doses of 10.000 IU vitamin A per kg body weight is given orally and vitamin A is determined in the plasma before and 3½ and 7 hours after ingestion. The association with lipid metabolism is however not so clear and the correlation with fat absorption is poor (25). Furthermore, vitamin A serum levels appear to be controlled by intestinal regulation. Vitamin A is not passively absorbed with fat and the relation with the lipid metabolism is not so strict (28, 78).

Radioactive and isotope labeled fat absorption tests

Fat labelled with radioactive- markers or isotopes in particular ^{14}C and ^{13}C-enriched triglycerides and ^{13}C-cholesteryl ester were used to study lipolysis. For ethical reasons radioactive ^{14}C-labelled fat is not used in infants and children.
In 1989 Vantrappen et al. introduced 'The mixed triglyceride breath test' as a non-invasive test of pancreas lipase activity in the duodenum (14). ^{13}C-marked triglycerides, which contain a ^{13}C-marked medium chain fatty acid in the 2 position and long-chain fatty acids in the 1 and 3 position (1,3-distearyl,2 [carboxyl-^{13}C] octanoyl glycerol), is given orally. The 2 stearyl groups are split by lipolysis and the octanoyl group is metabolized after absorption into $^{13}CO_2$, which can be measured in the breath. Breath samples are taken before and every 30min after a ^{13}C-enriched meal for 5 hours and $^{13}CO_2$ is measured in the expired air by mass-spectrometry. When lipolysis fails, the triglycerides are not split and are eliminated by the stools, and the amount of recovered $^{13}CO_2$ is reduced (14, 44, 79, 80). Although this test is safe, non-invasive and useful in the study of lipolysis, the results are dependent on the different steps of lipid metabolism in the enterocyte and in the liver. Pulmonary problems or endogenous CO_2 production may also influence the test. For these reasons and because of the difficulty of collecting breath samples and the cost of detecting the isotope, this test is not often used for routine clinical purposes (43, 62, 81, 82, 83, 84).

References

1. Martinez JA. Obesity in young Europeans: genetic and environmental influences. Eur J Clin Nutr 2000 Mar; 54 Suppl 1: 56-60.
2. Dobson A J, Evans A, Ferrario M, Kuulasmaa K A, Moltchanov V A, Sans S, Tunstall Pedoe H, Tuomilehto J O, Wedel H, Yarnell J. Changes in estimated coronary risk in the 1980s: data from 38 populations in the WHO-Monica Project. World Health Organisation. Monitoring trends and determinants in cardiovascular diseases. Ann Med 1998; 30: 199-205.
3. WHO Study Group. Diet, nutrition and the prevention of chronic diseases. WHO Tech Rep Ser 797, Geneva: WHO 1990.
4. Milner J A, Allison R G. The role of dietary fat in child nutrition and development: summary of an ASNS workshop. J Nutr; 1999; 129: 2094-2105.
5. Drewnowski A. Energy density, palatability, and satiety: implications for weight control. Nutr Rev 1998 Dec; 56 (12); 347-53.
6. Makrides M, Neumann M, Simmer K, Pater J, Gibson R. Are long-chain polyunsaturated fatty acids essential nutrients in infancy? Lancet; 1995 June; 10; 1463-68.
7. Hornstra G. Essential fatty acids in mothers and their neonates. Am J Clin Nutr 2000 May; 71 (5 Suppl): 1262-9S.
8. Koletsko B, Demmelmair H, Socha P. Nutritional support of infants and children: supply and metabolism of lipids. Ballieres Clin Gastroenterol 1998; 12 (4): 671-96.
9. Thomson AB, Schoeller C, Keelan M, Smith L, Clandinin MT. Lipid absorption: passing through the unstirred layers, brush-border membrane, and beyond. Can J Physiol Pharmacol 1993: 71 (8): 531-55.
10. Kleinmann R. Towards a 'new beginning': dietary fat restrictions in infancy? Acta Paediatr 2000; 89: 2-3.
11. Koletzko B. Response to and range of acceptable fat intakes in infants and children. Eur J Clin Nutr 1999; 53: S78- 83.
12. Koletzko B. Lipid supply and metabolism in infancy. Curr Opin Clin Nutr Metab Care 1998; 1 (2): 171-7.
13. Nederlandse voedingsmiddelentabel. Voorlichtingsbureau voor voeding.1999.
14. Vantrappen G R, Rutgeerts P J, Ghoos Y F, Hiele M I. Mixed triglyceride breath test: a non invasive test of pancreatic lipase activity in the duodenum. Gastroenterology 1989; 96: 1126-34.
15. Goldstein R, Blondheim O, Levy E, Stankiewicz H, Freier S. The fatty meal test: an alternative to stool fat analysis. Am J Clin Nutr 1983; 38: 763-8.
16. Van de Kamer JH, Huinink HV, Weyers HA. Rapid method for the determination of fat in feces. J Biol Chem 1949; 177: 347-55.
17. Harrison GA, Sheldon WPH. Faecal fat analyses in children. Arch Dis Child 1926; 2: 338-48.
18. Drummey GD, Benson JA, Jones CM. Microscopical examination of the stool for steatorrhea. N Engl J Med 1961; 264: 85-7.
19. 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.
20. Fales FW. Evaluation of a spectrophotometric metod for determination of total fecal lipid. Clin Chem 1971; 11: 1103-8.
21. Benini L, Caliari S, Guidi GC, Vaona B, Talamini G, Vantini I and Scuro LA. Near infrared spectrometry for faecal fat measurement: comparison with conventional gravimetric and titrimetric methods. Gut 1989; 30: 1344-47.

22. Devlin T M (1997). Textbook of biochemistry, 4th ed. Willey & sons. inc. New York.
23. Gompertz S M, Sammons H G. The origin of faecal lipids, the composition of faecal fats in human subjects. Clin Chim Acta; 1963; 8: 591-603.
24. Williams H H, Endicott E N, Shepherd M L, Galbraith H, Macy I G. Fat excretion by normal Children. J of Nutrition 1943; 25: 379-87.
25. Weijers H A. Fat absorption in normal and abnormal infants and children. Thesis 1950 Utrecht The Netherlands.
26. Shmerling D H, Forrer J C W, Prader B S C, Prader A. Fecal fat and nitrogen in healthy children and in children with malabsorption or maldigestion. Pediatrics 1970; 46: 690-95.
27. Fraisse F, Schmitz J, Rey J. Normal values of the main stool constituents from age 1 year to puberty. Arch Fr Pediatr 1981; 38: 667-70.
28. Burtis C A, Ashwood E R (1999). Tietz Textbook of Clinical Chemistry; 3th ed. Philadelphia, Pensylvenia, USA.
29. Verkade H J. Lipid Absorption and metabolism.Thesis;1993; Groningen The Netherlands.
30. Hamosh M. Lipid Metabolism in premature infants. Biol Neonate 1987; 52 (suppl 1): 50-64.
31. Berne R M, Levy M N. Physiology (1998); 4th ed. Mosby Inc. St Louis, Missouri, USA.
32. Canaan S, Roussel A, Verger R, Cambillau C. Gastric Lipase: crystal structure and activity. Biochim Biophys Acta 1441; 197-204.
33. Tilbeurgh van H, Bezzine S, Cambillau C, Verger R, Carriere F. Colipase: structure and interaction with pancreatic lipase. Biochim Biophys Acta 1999; 1441: 173-84.
34. Lidberg U, Kannius-Janson M, Nilsson J, Bjursell G. Transcriptional regulation of the human carboxyl ester lipase gene in exocrine pancreas. Evidence for a unique tissue-specific enhancer. J Biol Chem 1998; 273: 31417-26.
35. Yuan C, Tsai M-D. Pancreatic phospholipase A2: new views on old issues. Biochim Biophys Acta 1441; 215-22.
36. Carriere F, Barrowman J A, Verger R, Laugier R. Secretion and contribution to lipolysis of gastric and pancreatic lipases during a test meal in humans. Gastroenterology 1993; 105: 876-888.
37. Hernell O, Bläckberg L Digestion of human lipids: physiologic significance of sn-2 monoacyl glycerol hydrolysis by bile salt-stimulated lipase. Pediatr Res 1982; 16: 882-5.
38. Desnuelle P, Sjöström H, Norén O (1986). Molecular and Cellular Basis of Digestion. Elseviers Science Publishers BV, Amsterdam.
39. Ganong W F (1999). Review of Medical Physiology. Appleton & Lange, Stamford USA.
40. Agellon L B, Torchia E C. Intracellular transport of bile acids. Biochim Biophys Acta 2000; 1486: 198-209.
41. Stemmel W, Lotz G, Strohmeyer G, Berk P D. Identification, isolation, and partial characterisation of a fatty acid binding protein from rat jejunal microvillus membranes. J Clin Invest 1985; 75: 1068-76.
42. Davidson N O, Shelness G S. Apolipoprotein B: mRNA editing, lipoprotein assembly, and presecretory degradation. Annu Rev Nutr 2000; 20: 169-93.
43. Walker, Durie, Hamilton, Walker-Smith, Watkins (2000).Pediatric Gastrointestinal Disease. B C Decker Inc. Ontario Canada.
44. Lembke B, Braden B, Stein J. Diagnosis of Steatorrhea. Z Gastroenterol 1994; 32(5): 256-61.
45. Nousia-Arvanitakis S, Karagiozoglou-Lamboudes T, Aggouridaki C, Malaka-Lambrellis E, Galli-Tsinopoulou A, Xefteri M. Influence of jejunal morphology changes on exocrine pancreatic function in celiac disease. J Pediatr Gastroenterol Nutr 1999; 29: 81-5.

46. Kalivianakis M, Minich D M, Bijleveld C M A, Aalderen van W M C, Stellaard F, Laseur M, Vonk R, Verkade H J. Fat malabsorption in cystic fibrosis patients receiving enzyme replacement therapy is due to impaired intestinal uptake of long-chain fatty acids. Am J Clin Nutr 1999; 69: 127-34.
47. Lebenthal E.(1989) Textbook of Gastroenterology and Nutrition; second ed. Raven Press; New York.
48. De Curtis M, Kempson C, Ventura V, Carter N, McIntosh N. The relationship between fecal fat and water in very low birth weight infants. J Pediatr Gastroenterol Nutr 1990; 11: 63-5.
49. Thirup P. Steatorrhea cannot be excluded where there is a fecal weight below 0.200 kg per day and a high fecal consistency. Scand J Clin Lab Invest 1998; 58(7): 577-83.
50. Kasper H. Faecal fat excretion, diarrhea, and subjective complaints with highly dosed oral fat intake. Digestion 1970; 3: 321-30.
51. Hill E, Bloor W R. Fat excretion. J Biol Chem 1922; 53: 171-8.
52. Bo-Linn G W, Fordtran J S. Fecal fat concentration in patients with steatorrhea. Gastroenterology 1984; 87: 319-22.
53. Pedersen N T, Halgreen H, Worning H. Estimation of the 3-day faecal fat excretion and fat concentration as a differential test of malabsorption and maldigestion. Scand J Gastroenterol 1987; 22: 91-6.
54. Bai J C, Andrüsh A, Matelo G, Martinez C, Vazquez H, Boerr L, Sambuelli A. Fecal fat concentration in the differential diagnosis of steatorrhea. Am J Gastroenterol 1989; 84: 27-30.
55. Benini L, Caliari S, Bonfante F, Bardelli E, Castellani G, Sembenini C, Brentegani M T, Vantini I. Fecal fat concentration in the screening of steatorrhea. Digestion 1992; 53: 94-100.
56. Saavedra J M, Brown K H. Nonabsorbable marker and single, random stool samples used for measuring intestinal absorption of macronutrients in infants and children. Am J Clin Nutr 1991; 53: 790-4.
57. Zuckerman J L, Zymaris M C, Natelson B S, Natelson S. 1948; J Lab Clin Med:282-6.
58. Bekers O, Postma C, Lombarts A J P F. Determination of faecal fat by near-infrared spectroscopy. Eur J Clin Chem Clin Biochem 1995; 33: 83-6.
59. Jeejeebhoy K N, Ahmad S, Kozak C. Determination of fecal fats containing both medium and long chain triglycerides and fatty acids. Clin Biochem 1970; 3: 157-63.
60. Khouri M R, Huang G, Shiau Y F. Sudan stain of fecal fat: new insight into an old test. Gastroenterology 1989; 96: 421-7.
61. Newcomer A D, Hofmann A F, DiMagno E P. Triolein breath test. A sensitive and specific test for fat malabsorption. Gastroenterology; 1979; 76: 6-13.
62. Leus J, Van Biervliet S, Robberecht E. Detection and follow up of exocrine pancreatic insufficiency in cystic fibrosis: a review. Eur J Pediatr 2000; 159: 563-8.
63. Peuchant E, Salles C, Jensen R. Value of a spectroscopic 'fecalgram' in determining the etiology of steatorrhea. Clin Chem 1988; 34: 5-8.
64. Stein J, Purschian B, Bieniek U, Caspary W F, Lembke B. Near-infrared reflectance analysis: a new dimension in the investigation of malabsorption syndromes. Eur J Gastroenterol Hepatol 1994; 6: 889-94.
65. Neumeister V, Henker J, Kaltenborn G, Sprossig C, Jaross W. Simultaneous determination of fecal fat, nitrogen, and water by near-infrared reflectance spectroscopy. J Pediatr Gastroenterol Nutr 1997; 25: 388-93.
66. Picarelli A, Greco M, Di Giovambattista F, Ramazzoti A, Cedrone C, Corazziari E, Torsoli A. Quantitative determination of fecal fat, nitrogen and water by means of a spectrophotometric

technique: near-infrared reflectance analysis (NIRA). Assesment of its accuracy and reproducibility compared with chemical methods. Clin Chem Acta 1995; 234: 147-156.

67. Schneider M U, Demling L, Domschke S, Heptner G, Merkel I, Domschke W. NMR spectrometric stool fat analysis, a new technique for quantifying steatorrhea and establihing the indication for enzyme replacement in chronic pancreatitis. Hepatogastroenterology 1985; 32: 210-15.
68. 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.
69. 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: 268-74.
70. Walters M P, Kelleher J, Gilbert J, Littlewood J M. Clinical monitoring of steatorrhea in cystic fibrosis. Arch Dis Child 1990; 65: 99-102.
71. Rawashdeh M O, Lloyd D R, Puntis J W L, Brown G A, Booth I W. Using the steatocrit to determine optmal fat content in modular feeds. Arch Dis Child 1992; 67: 608-12.
72. 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.
73. Bettinardi N, Colombo C, Corbetta C. In vitro influence of different kinds of fats on results and imprecision of classic and acid steatocrits. Clin Chem 1999; 45: 406-7.
74. Amann S T, Josephon S A, Toskes P P. Acid steatocrit: a simple, rapid gravimetric method to determine steatorrhea. Am J Gastroenterol 1997; 92: 2280-4.
75. Jonas A, Weiser S, Segal P, Katznelson D. Oral fat loading test. Arch Dis Child 1979; 54, 770-2.
76. Robards M F. Changes in plasma nephelometry after oral fat loading in children with normal and abnormal small intestinal morphology. Arch Dis Child 1975; 50: 631-36.
77. Tangney CC, Shekelle RB, Raynor W, Gale M, Betz EP. Intra- and interindividual variation in measurements of beta-carotene, retinol, and tocopherols in diet and plasma. Am J Clin Nutr 1987; 45: 764-9.
78. Heller FR, Vandenplas C, Desager JP, Harvengt C. The vitamin A fat-loadind test in young normolipidemic subjects. Clin Chim Acta 1993; 219: 167-76.
79. Harper G D, Wheeler D C, Wicks A C. Butterfat absorption a valuable screening test in malabsorption. Postgrad Med J 1994; 70: 23-6.
80. Benini L, Scuro L A, Menini E, et al. Is the ^{14}C-triolein breath test useful in the assessment of malabsorption in clinical practice? Digestion 1984; 29: 91-7.
81. Kalivianakis M, Verkade H J, Stellaard F, Van der Werf M, Elzinga H, Vonk R J. The ^{13}C-mixed triglyceride breath test in healthy adults: determinants of the $^{13}CO^2$ response. Eur J Clin Invest 1997; 27: 434-442.
82. Perri F, Pastore M, Festa V, Clemente R, Quitadamo M, D'Altilia M R, Niro G, Paolucci P, Andriulli A. Intradoudenal lipase activity in celiac disease assessed by means of ^{13}C mixed-triglyceride breath test. J Pediatr Gastroenterol Nutr 1998; 27: 407-10.
83. Weaver L T. Stable isotope breath test. Nutrition 1998; 14: 826-9.
84. Murphy M S, Eastham E J, Nelson R, Aynsley-Green A. Non-invasive assessment of intraluminal lipolysis using a $^{13}CO^2$ breath test. Arch Dis Child 1990; 65: 574-8.

Aim of the thesis

The aim of this thesis was to evaluate the clinical usefulness of the acid steatocrit method, a non-invasive, simple and cheap method for determining faecal fat in a single stool sample. If this method is accurate, clinicians would be able to diagnose and monitor steatorrhoea in an easier, cheaper and faster way, with less discomfort for patients, parents, nurses and laboratory personnel. For this purpose the following investigations were performed:

- Comparison of the results obtained by the AS method with those obtained by the original method as described by Phuapradit, as well as with the results obtained by the titrimetric chemical method of Van de Kamer, in order to demonstrate whether or not the method can be improved by acidification of faecal samples – *chapter 2.*

- Comparison of the results of the AS method with those obtained by the gold standard method for determining faecal fat concentration: the titrimetric method of Van de Kamer; and with results obtained by the near-infrared analysis (NIRA) – *chapter 3.*

- Comparison of the results of lipid, nitrogen, water and energy measurements from a single stool sample with those of three-day stool collections in order to demonstrate whether or not a single stool sample can yield similar information to the more laborious stool collection method – *chapter 4.*

- Determination of age-related normal values of AS, because normal neonates and infants excrete larger amounts of faecal fat – *chapter 5.*

- Comparison of the results of single sample AS determinations with the results obtained by the faecal fat balance for the clinical diagnosis of steatorrhoea – *chapter 6.*

Evaluation of the value of the AS method in screening for steatorrhoea in children – *chapter 7.*

CHAPTER 2

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. We also compared classical and acid steatocrit result 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 ± 0.4% (classical) versus 3.8 ± 1% (acid) in controls (n = 6) and 5.4 ± 1.9% (classical) versus 26.9 ± 4.3% (acid) in cystic fibrosis patients (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 is still mainly based 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 at al. in 1949 (1).

Work by Kouri et al. has suggested that the titrimetric method largely overestimates nutritional fecal 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 Soudan stain provides a satisfactory screening method for steatorrhea, it is at best semiquantitative.

The steatocrit is 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 fecal acidification results in an enhanced sensitivity of the Sudan fecal 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 compared 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 fecal fat concentrations as measured by the reference chemical method of Van de Kamer et al. (1) to evaluate which of the two steatocrit methods gave the best estimate of fecal fat content.

Methods

Classic steatocrit method

Stool (at least 0.5 gr) was diluted (1/3) with deionized water and thouroughly 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 steatocrit results

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.

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 ± 0.4 and 3.8 ± 1% for classic and acid steatocrit, respectively. This difference was not statistically significant. Steatocrit means and SEM in cystic fibrosis patients were 5.4 ± 1.9 and 26.9 ± 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.

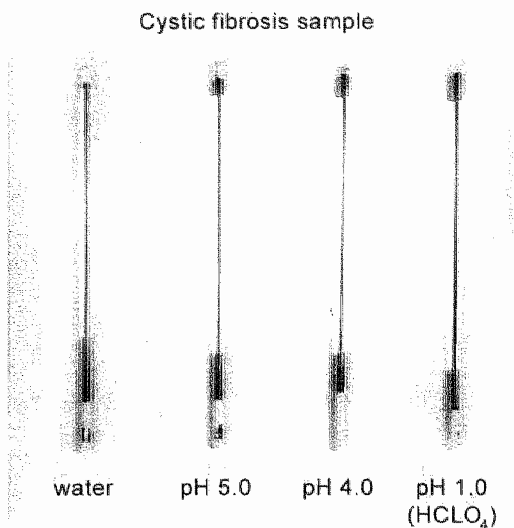


Figure 1. Effect of acidification with various concentrations of perchloric acid on the fat column length (upper part of picture) of a stool sample from a patient with cystic fibrosis.

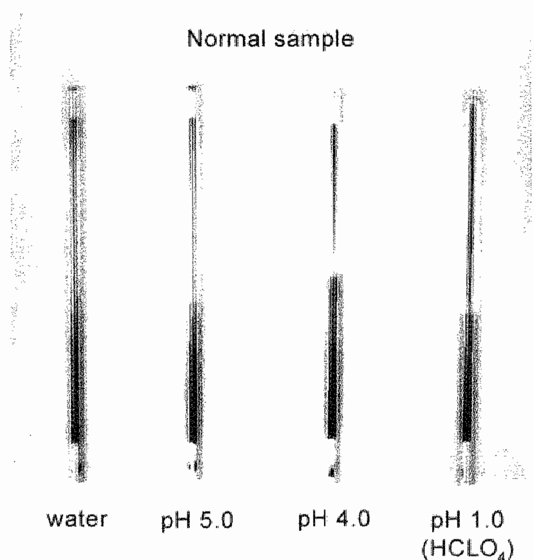


Figure 2. Effect of acidification with various concentrations of perchloric acid on fat extraction from a normal stool sample. Fat layer is absent at all pH values.

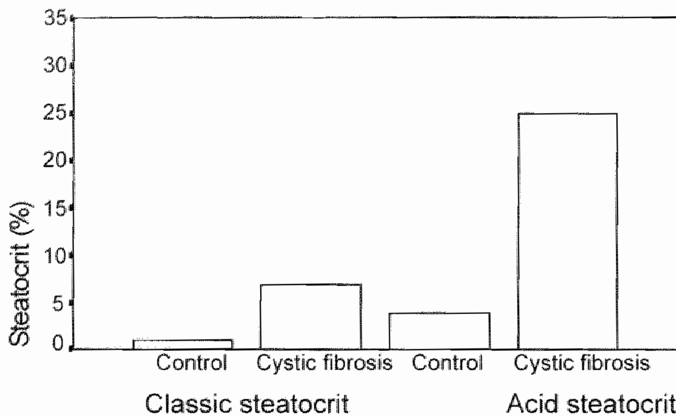


Figure 3. Classic and acid steatocrit results in six controls and nine patients with cystic fibrosis.

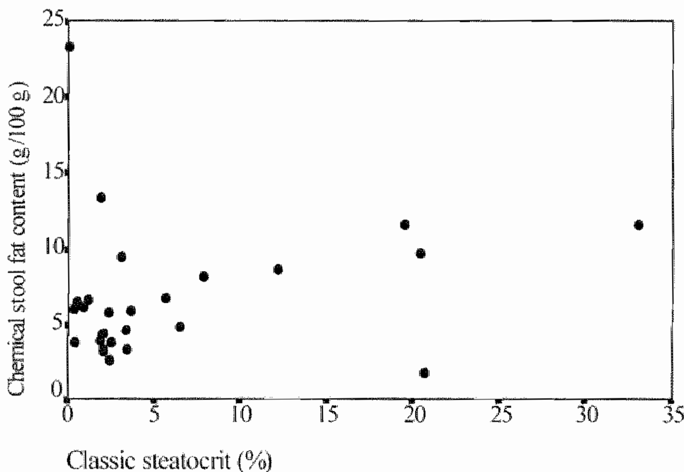


Figure 4. Relationship between classic fecal steatocrit and fecal fat content as measured by the method of van de
Kamer et al. In 27 fecal samples ($r = 0.18$; $p = 0.35$).

Correlation between steatocrit results and fecal 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 ($p < 0.0001$).

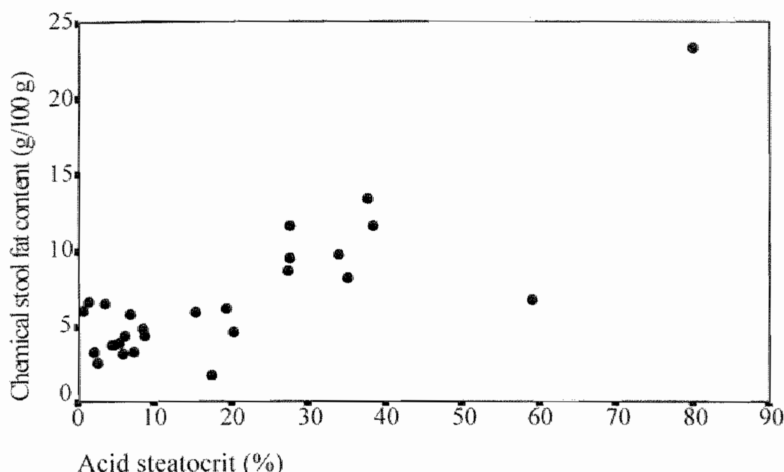


Figure 5. Relationship between acid fecal steatocrit and fat content as measured by the method of Van de Kamer et al. In 27 fecal samples ($r = 0.81$; $p < 0.0001$).

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, 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 Soudan stain, although staining does occur after acidification (2). By alkalinization 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 with 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.

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 litterature 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 (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 con-

tent 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 better prediction of fecal fat content as measured by chemical methods.

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: 421-7.
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: 725-7.
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: 926-30.
5. Iacono G, Carroccio A, Cavataio F et al. Steatocrit test: normal range and physiological variation in infants. J Pediatr Gastroenterol Nutr 1990; 11: 53-7.
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: 848-52.
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: G164-9.
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: 268-74.

CHAPTER 3

Comparison of near infrared reflectance analysis of faecal fat, nitrogen and water with conventional methods, and faecal energy content.

Anita Van den Neucker[1], Charles M.A. Bijleveld[2], Bert G. Wolthers[3],
Joost C.J.M. Swaaneburg[3], Arnold D.M. Kester[4], Bernard van Kreel[5], Pierre-Philippe Forget[1]
Departments of [1] Pediatrics and [5] Clinical Chemistry, University Hospital Maastricht,
Departments of [2] Pediatrics and [3] Central Laboratory, University Hospital Groningen,
Department of [4] Methodology and Statistics, Maastricht University, The Netherlands.
(submitted)

Abstract

Background: the Near-Infrared Analysis (NIRA) method for determining faecal fat, water and nitrogen is described as a new, easy and rapid method.
Aim of the study: to evaluate the Near-Infrared Analysis method.

Methods: the results of faecal fat, water and nitrogen by both NIRA and by standard techniques were compared. Standard techniques consisted of Van de Kamer and Acid Steatocrit (AS), the Dumas and the vacuum drying methods for fat, nitrogen and water respectively.

Results of fat methods were also compared with total faecal energy as obtained by bomb calorimeter.

Results: NIRA results correlated significantly ($p < 0.001$) with standard methods for nitrogen ($r=0.79$), fat ($r=0.84$ and $r=0.88$ for Van de Kamer and AS respectively) and water ($r=0.91$). The limits of agreement for nitrogen and fat results were too wide for the methods to be used interchangeably. The faecal fat results correlated significantly ($p < 0.001$) with faecal energy results.

Conclusion: NIRA may be valuable for monitoring malabsorption but the diagnostic value remains to be determined.

Abbreviations: NIRA: near-infrared analysis, AS: acid steatocrit, w w: wet weight.

Introduction

Stool composition may give important information for the diagnosis and monitoring of malabsorption. Various laboratory methods are used for the determination of faecal fat, faecal nitrogen, faecal energy and faecal water. Most of these methods are both time consuming and unpleasant to perform.

Near-Infrared Reflectance Analysis (NIRA) of faecal material is a new and rapid method for the determination of faecal fat, nitrogen and water content (1,2,3,4,5). Results obtained with new techniques should be compared with those of standard methods prior to introducing them as routine clinical methods.

The aim of the present study was to compare results of faecal fat, nitrogen and water as obtained by the NIRA, with those obtained by accepted techniques as routinely used in our laboratory: the titrimetric method of Van de Kamer (6), the acid steatocrit method (AS) (7) for the evaluation of faecal fat, the method of Dumas (Foss-Heraeus Macro- N) (8,9) for the determination of faecal nitrogen, vacuum-drying for the evaluation of faecal water content and bomb calorimetry (10) for obtaining the total amount of faecal energy.

Methods and material

60 stool samples (± 10 g faeces), immediately frozen at -18°C and taken from 11 different patients with known or suspected steatorrhoea were examined. All patients but one suffered from cystic fibrosis with pancreatic insufficiency and received enzyme substitution therapy. Faecal fat was determined (g/100 g faecal wet weight) by the NIRA (Fenir 8820-infrared- Analyser, Stimotron, Wendelstein, Germany) (1), by the titrimetric method of Van de Kamer (g/100 g faecal wet weight) (6) and by the Acid Steatocrit method (%) (7).

Faecal nitrogen was determined by the NIRA (g/100 g faecal wet weight) and by the method of Dumas (Foss Electric Benelux, Hoorn, The Netherlands) (mg/g faecal dry weight) (8).

Faecal water was determined by the NIRA (g/100 g faecal wet weight) and by weighing and vacuum-drying (Heraeus, Foss Electric Benelux, Hoorn, The Netherlands) (g/100 g faecal wet weight).

NIRA determinations and determinations of faecal fat according to the titrimetric method of Van de Kamer were performed in a separate laboratory and the investigators were blind for each others results.

The NIRA-apparatus was used with the calibration curve as delivered by the manufacturer.

Faecal energy (kJ/g faecal dry weight) was obtained using a bomb calorimeter (Ika-calorimeter system C700T, Heitersheim, Germany) (10).

Results were calculated in the same units for comparison.

Energy was calculated from different components by multiplying the amount of protein and lipid by 16.7 and 37.7 kJ/g respectively (Atwater and Benedict) (11).

All results were managed using the SPSS statistical program. Correlations between the results obtained by the different methods were evaluated making use of the Spearman correlation coefficient and the limits of agreement obtained by the method of Bland and Altman were indicated whenever applicable (12).

Results

Faecal nitrogen

Mean results of faecal nitrogen determinations expressed in g/100 g wet faecal weight for the Dumas and NIRA methods were: 1.18 (range: 0.73 - 1.70; SD: 0.32) and 1.24 (range: 0.73 - 1.99; SD: 0.29) respectively.

The correlation coefficient between results of the nitrogen concentration as determined by the Dumas (x) and results obtained by the NIRA (y) expressed in g per 100 g wet faeces was 0.79 ($y = 0.11 + 0.87\,x$; res. SD: 0.20). The limits of agreement were 0.45 g/100 g and -0.35 g/100 g faecal wet weight (fig 1.).

Faecal water

The faecal water content, measured with the vacuum drying method, reached a mean value of 72.57% (min.: 53%; max: 88%; SD: 6.27%), whereas the values obtained with the NIRA were (mean) 74.67% (min.: 58%; max.: 95%; SD: 7.25%).

The correlation coefficient between results of the faecal water content as determined by the vacuum-drying method (y) and the NIRA (x) was 0.91 ($y = 0.79\,x + 12.8$; res. SD: 2.59%) and the limits of agreement +8.15% and -3.65% (Fig.2).

The correlation between results of faecal water as determined by the vacuum-drying method (y), taken as reference method, and faecal fat (x) determined respectively by the method of Van de Kamer, the AS and the NIRA, were:

r = −0.54 (y = 80.1 − 0.89 x; res. SD: 5.35),
r = −0.70 (y = 80.6 − 0.28 x; res. SD: 4.55),
r = −0.85 (y = 0.85 − 1.63 x; res. SD: 3.33) respectively.

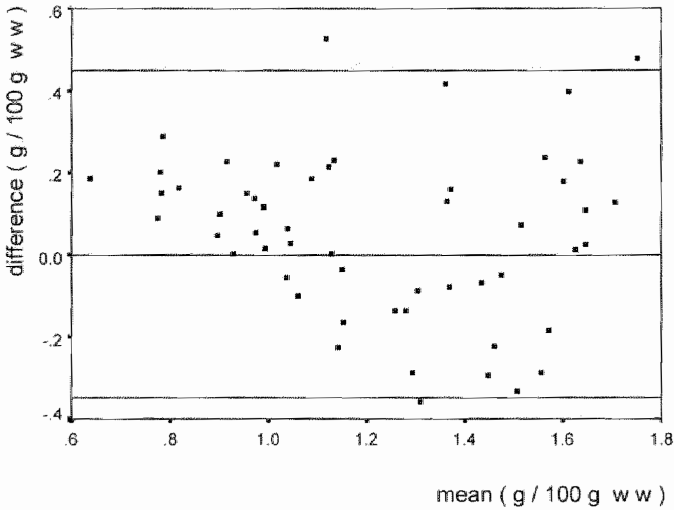


Figure 1. Limits of agreement between the nitrogen results as determined by the method of Dumas and NIRA.

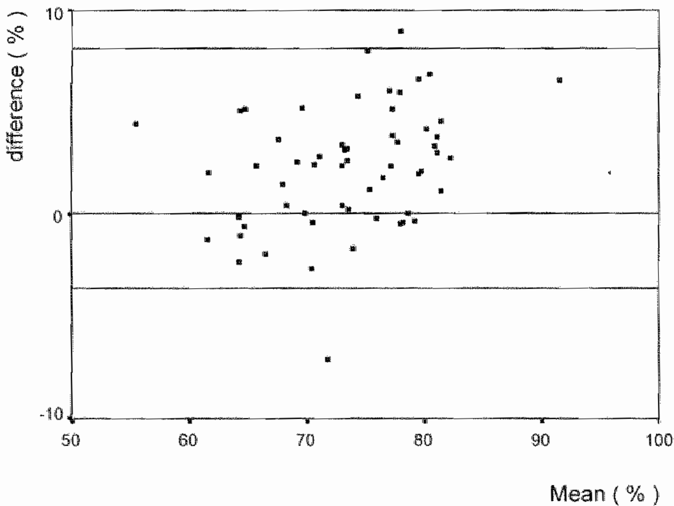


Figure 2. Limits of agreement between the water results as determined by the vacuum drying method and the NIRA.

Faecal fat

Mean value and SD for the AS results were 29.5 ± 16%. Mean results of the faecal fat determination according to the method of Van de Kamer and the NIRA were as follows: 8.88 and 8.05 g/100 g w w faeces with a standard deviation of 3.80 and 3.31 g/100 g w w faeces respectively.

Table 1. shows the regression analyses for results of the three faecal fat determination methods: AS, NIRA and Van de Kamer. The correlation coefficients for the three compared methods were almost equal (95% confidence interval of ρ for $n = 50$ and $r = 0.88$ is $0.78 < \rho < 0.92$).

Table 1. Results of regression analyses for results of faecal fat determination methods.

Method	Correlation coefficient	Equation	Residual SD
Vd Kamer *vs* AS	r = 0.81	y = 0.20 x + 2.96	2.24 g / 100 g
Vd Kamer *vs* NIRA	r = 0.84	y = 0.97 x + 0.93	2.09 g / 100 g
NIRA *vs* AS	r = 0.88	y = 0.18 x + 2.67	1.60 g / 100 g

As the results of the Van de Kamer method and the NIRA-method were expressed in the same units, the limits of agreement of these methods could be determined. These limits were between + 4.88 g/100 g w w faeces and -3.42 g/100 g w w faeces (Fig 3).

Faecal energy

Results of energy content (mean and SD) as measured by bomb calorimetry and expressed in kJ/g faecal dry weight were: 26.66 ± 2.32 and expressed in kJ/g w w faeces: 7.38 ± 2.11.

Table 2 shows the correlation between results obtained by the faecal fat determination methods and faecal energy as obtained by bomb calorimetry.

Table 2. Correlation between results of faecal fat and faecal energy.

Method	Correlation coefficient	Equation	Residual SD
Energy *vs* AS	0.78	y = 0.11 x + 4.28	1.33
Energy *vs* vd Kamer	0.66	y = 0.38 x + 4.15	1.60
Energy *vs* NIRA	0.91	y = 0.59 x + 2.68	0.90

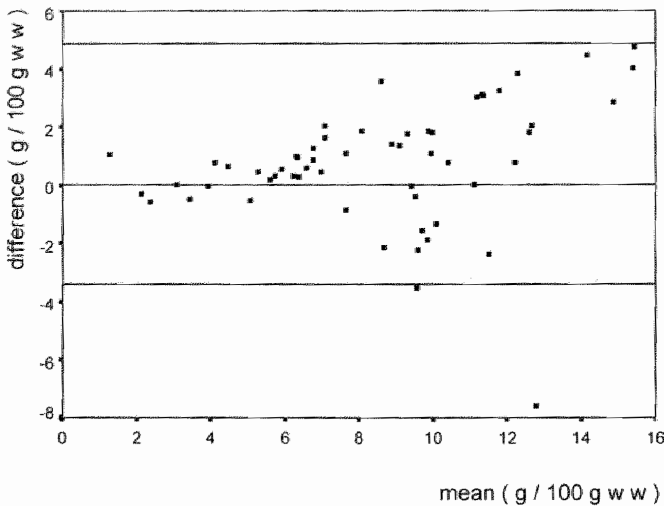


Figure 3. Limits of agreement between the fat results as obtained by the method of Van de Kamer and the NIRA.

Faecal energy content attributable to protein (nitrogen x 6.25) and fat was calculated and expressed as percentage of faecal energy. The remaining energy was assumed to be attributable to carbohydrates. The energy was calculated from the amount of protein and lipid. The mean energy percentage for protein was 16.8%. The mean energy percentage for fat varied between 44.1% and 42.6% of the total energy content depending on the method used for determining faecal fat, the titrimetric method of Van de Kamer and the NIRA respectively. The remaining energy amounted to 39.2% and 40.6% of the total energy. The mean faecal energy content was 7.38 kJ/g w w faeces (SEM: 0.27 kJ/g w w feces;SD: 2.11 kJ/g w w faeces).

The correlation between faecal energy (y) (kJ/g faecal wet weight) and faecal water (x) (%) as determined by the vacuum-drying method was r = -0.96 (y = 93.75 - 2.8 x; res. SD: 1.88).

Our results (mean ± SD) concerning faecal nitrogen, water, fat and energy are summarized in table 3.

Table 3. Results of faecal nitrogen, water, fat and energy determinations (mean and SD).

	Nitrogen (g /100 g w w)		Water (%)		Fat (mg / 100 gw w)		Energy (kJ / g w w)		
	Dumas	NIRA	Vacuum drying	NIRA	Vd Kamer	NIRA	Total	Fat (%)	Protein (%)
Mean	1.18	1.24	72.57	74.67	8.88	8.05	7.38	44.1 /42.6	16.8
SD	0.32	0.29	6.27	7.25	3.80	3.31	2.11	-	-

Discussion

We investigated 60 different samples from 11 different patients, assuming that these samples were all independent. Ideally, these samples should have been obtained from 60 different patients, especially when considering the limits of agreement. To assess the influence of using 60 samples from 11 patients, we applied a random effects model (DerSimonian and Laird, 1986) (13) to obtain separate values for the within-patient standard deviation and for the between-patient standard deviation. The limits of agreement calculated from the resultant total standard deviation were only slightly different from those reported; for the comparison of nitrogen concentration obtained by the method of Dumas and the NIRA on dry faeces the limits would be -12.85 and 16.06 mg/g instead of -12.81 and 16.55 mg/g. For comparing results of fat determinations according to the method of Van de Kamer and the NIRA, the limits would be -3.66 and 4.88 g/100 g w w faeces, instead of -3.42 and 4.88 g/100 g w w faeces. As these differences were very small, our results were further treated as if they came from 60 independent samples.

The near-infrared spectrometry technique on faecal material is shown to be reproducible, accurate and rapid for the determination of faecal fat, nitrogen, and water as previously reported (3). However, some difficulties have been reported concerning the calibration and the composition of the faecal material (4,5,15). Our NIRA instrument was previously calibrated by the manufacturer by making use of measurements obtained by the method of Van de Kamer for the determination of faecal fat and the Kjeldahl method for the determination of faecal nitrogen, *all performed in the same laboratory.*

In the present study NIRA-determinations and fat determinations according to the method of Van de Kamer were performed in a different laboratory than nitrogen determinations according to the Dumas method, water determination by vacuum drying, AS and calorimetry. And we correlated the results obtained by the NIRA with those obtained by our routinely used laboratory methods, which were not all the same as those used for calibration of the NIRA apparatus.

Faecal samples were used for NIRA-determinations as delivered by the patients without thorough homogenization, as recommended by the instructions of the manufacturer.

Faecal fat results were correlated with the AS results, since this method has been proven to be suitable for the evaluation of faecal fat (7,14).

The amount of nitrogen in the stools was determined by the NIRA method with the calibration by the Kjeldahl method. The Kjeldahl method is considered the standard method for nitrogen determination, but we used the combustion

method of Dumas for the nitrogen determination. This method is more suitable for handling a greater amount of samples at once and has proven to be of equal or even superior (as it recovers more nitrogen from the material) value for the determination of nitrogen in biological material (8,9).

The correlation coefficients between results obtained by the NIRA and those obtained by the reference methods for fat, nitrogen and water were lower than those reported by other authors (1,2,3,4,5) but still highly significant. Regarding the limits of agreement as calculated from our results we can conclude that, except for the water content the various methods can not be used interchangeably. We think that there are several explanations for these findings. First we compared NIRA results with results obtained by the methods currently used in our laboratory which were different from the methods used to calibrate the NIRA-apparatus. Secondly, our calibration of the analyser may have been insufficient, as different authors reported the difficulty of calibration as a problem associated with the spectrometric technique (2,4,5,15) and lastly the homogenization of the samples. Our samples were unhomogenized, as recommended by the manufacturers instructions, but different authors indicated this to be a problem due to inhomogeneous excretion and (consequently) distribution of fat/nitrogen in the faeces. Moreover, light reflection is influenced by inhomogeneous composition of the sample and particularly by the water content. Bekers et al (4) report that stools obtained from patients with diarrhea, when the water content exceeds 75%, are unsuitable for spectrometric analyses. As the mean water content value of our samples almost reached this value, a great part of our samples would accordingly not be suitable for spectrometry. All our samples except one were taken from cystic fibrosis patients with exocrine pancreas insufficiency receiving enzyme substitution therapy where monitoring of stool composition could be clinically useful.

Our results for water and energy are comparable with the results expressed per gram stool reported by Murphy et al. (16) for a group of patients with cystic fibrosis in whom stool lipid was reported to account for 41% of stool energy while we found 44.1% or 42.6% depending on the method used to determine faecal fat. The energy content per gram wet weight has been reported to be 8 kJ/g wet weight while we measured a mean of 7.38 kJ/g wet weight. Expressed in kJ/g faecal dry weight our mean result amount to 26.6 kJ/g while the reported mean result amounted 27.0 kJ/g faecal dry weight. Although reported results concerning energy were measured on 3 days stool collection and our results concern 'single stool' specimens, both were quite similar suggesting that single stool analysis can give useful clinical information in these patients with exocrine pancreatic insufficiency. From our own study we know that there is a significant correlation between the AS results from a single stool sample and results of a three days faecal fat

collection as determined by the method of Van de Kamer (14). Comparison with other components such as nitrogen and lipids is not possible as results in the literature are expressed in amounts per day, and not as a concentration.

Conclusion

In conclusion, our results show that NIRA results as obtained following the manufacturers instructions are not so accurate for the evaluation of fat, nitrogen and water in faecal material and that the results cannot be used interchangeably with the currently used methods. When taking the shortcomings into account this method might be valuable for the monitoring of malabsorption but, as our limits of agreement for fat and nitrogen were large, the diagnostic value of the method remains uncertain. Further experience with the NIRA method is needed before deciding whether or not NIRA can replace the presently used routine methods.

Comparison of our results with results of the literature suggests that measurements performed on stool collections and on single stool samples give rather similar information on stool composition.

References

1. Benini L, Caliari S, Guidi GC, Vaona B, Talamini G, Vantini I, Scuro LA. Near infrared spectrometry for faecal fat measurement: comparison with conventional gravimetric and titrimetric methods. Gut 1989;30:1344-7.
2. Stein J, Purschian B, Bieniek U, Caspary WF, Lembcke B. Near-infrared reflectance analysis: a new dimension in the investigation of malabsorption syndromes. Eur J Gastro Hepatol 1994;6:889-94.
3. Picarelli A, Greco M, Giovambattista F, Ramazzotti A, Cedrone C, Corazziari E, Torsoli A. Quantitative determination of faecal fat, nitrogen and water by means of a spectrophotometric technique: near infrared reflectance analysis (NIRA). Assessment of its accuracy and reproducibility compared with chemical methods. Clin Chim Acta 1995;234:147-56.
4. Bekers O, Postma C, Lombarts AJPF. Determination of faecal fat by near- infrared spectroscopy. Eur J Chem Clin Biochem 1995;33:83-6.
5. Bekers O, Postma C, Fischer JC, Franck PFH, Lombarts JPF. Faecal nitrogen determination by near-infrared specrtroscopy. Eur J Clin Chem Clin Biochem 1996;34:561-3.
6. Van de Kamer JH, Huinink HTB, Weijers HA. Rapid method for determination of fat in faeces. J Biol hem 1949;177:349-55.
7. 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.
8. Wilson PR. A new instrument concept for nitrogen / protein analysis. A challenge to the Kjeldahl method. Aspects of Applied Biology 25, Cereal Quality II 1990;443-6.

9. Smith ID. Evaluation of the Foss-Heraeus Macro N for the determination of nitrogen in a wide range of foodstuffs, ingredients and biological materials and comparison with the Kjel-foss. Analytical proceedings 1991;28:320-4.
10. Miller DS, Payne PR. A ballistic bomb calorimeter. Br J Nutr 1959;13:501-8.
11. Atwater WO, Benedict FG. Experiments on the metabolism of matter and energy in the human body. In: Bull Off Exp St Washington, D.C.:U.S. Dept Agr 1903:136.
12. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. Lancet 1986;8:307-10.
13. DerSimonian R, Laird N. Meta-analysis in clinical trials. Control Clin Trials 1986;7:177-88.
14. Van den Neucker A, Pestel N, Tran TMD, Forget P-Ph, Veeze HJ, Bouquet J, Sinaasappel M. Clinical use of acid steatocrit. Acta Pædiatr 1997;86:466-9.
15. Grimble GK. Near- infrared reflectance analysis as an investigative tool in gastroenterology. Eur J Gastroen Hepat 1994;6:885-8.
16. Murphy JL, Wootton SA, Bond SA, Jackson AA. Energy content of stools in normal healthy controls and patients with cystic fibrosis. Arch Dis Child 1991;66:495-500.

CHAPTER 4

Lipid, nitrogen, water and energy content of a single stool sample in healthy children and children with cystic fibrosis.

Anita Maria Van den Neucker[1*], MD, Pierre-Philippe Forget[1], PhD, Bernard van Kreel[2], PhD.

[1] *Department of Pediatrics and* [2] *Department of Clinical Chemistry*

University Hospital Maastricht, The Netherlands.

(submitted)

Abstract

Objective: evaluate the usefulness of 'single stool sample' analysis in the investigation of steatorrhoea.

Methods: we examined 57 stool samples of cystic fibrosis (CF) patients while on pancreatic enzyme therapy and 29 stool samples of healthy children.

We compared results of fat, nitrogen, water and energy content. Fat was determined by the method of Van de Kamer and the Acid steatocrit (AS) method, water by vacuum drying, nitrogen by the Dumas method and energy was obtained using a bomb calorimeter.

Results: results (median,10th and 90th percentile) of fat were: 8.90 (4.68 - 13.90) and 4.75 (2.27 - 6.52) g / 100 g w w stool by the method of Van de Kamer and 28.6 (5.5 - 49.8) and 7.2 (3.0 - 15.0)% by the AS method; energy results were: 742.96 (525.59 - 997.24) and 549.32 (451.48 - 710.72) kJ / 100 g w w stool all for CF patients and healthy controls respectively ($p \leq 0.0001$ for both fat and energy).

The Mann-Witney U-test was used for comparing means.

Conclusions: results of single stool sample water and nitrogen were similar in both groups. Results were comparable to those reported from samples taken from stool collections as far as fat and energy concentrations are concerned and yield clinically useful information.

Introduction

Most investigators and clinicians still make use of the 72-hour fecal collections for determining fecal losses of fat, water, nitrogen and energy for clinical investigations (1,2) and for monitoring patients with pancreatic insufficiency. However, different attempts have been made in order to develop alternative rapid and simple techniques (3,4). In pediatrics, stool collections are mostly used for the monitoring of steatorrhoea in patients with cystic fibrosis. It is most often assumed that 'single stool' analysis does not give useful diagnostic information in these patients.

The present study was designed in order to compare results of fat, nitrogen, water and energy of single stool samples in cystic fibrosis patients and in normal children in order to establish whether or not a single stool sample analysis can yield useful diagnostic information.

Methods and material

57 Stool samples of cystic fibrosis patients receiving enzyme substitution therapy, aged between 1.5 years and 11.6 years (mean: 7 years) and 29 samples of normal children, aged between 0.6 year and 16 years (mean: 7.6 years) were collected and immediately stored at -18°C.

Fecal fat was determined by both the titrimetric method of Van de Kamer (5), expressed in g / 100 g fecal wet weight (w w), and the Acid Steatocrit (AS) method (3), expressed in %. Fecal water was determined by weighing and vacuum drying (apparatus: Heraeus, Foss Electric Benelux, Hoorn, The Netherlands) and expressed in %.

Fecal nitrogen was determined by the method of Dumas (6) (Foss Electric, Hoorn, The Netherlands) and expressed in g / 100 g fecal wet weight. Fecal energy, expressed in kJ / 100 g fecal wet weight, was obtained using a bomb calorimeter (Ika-calorimeter system C700, Heitersheim, Germany) (7).

Results were managed using the SPSS statistical program. The Mann-Whitney U-test was used for comparing means.

Results

Results are shown in table 1.

Table 1. Comparison of median, 10th and 90th percentile and p-values for fecal fat, nitrogen, water and energy of single stool samples from healthy children and cystic fibrosis patients.

Method	Groups	n	Median	P10	P90	p-value
Van de Kamer	normal	28	4.75	2.27	6.52	< 0.0001
(g / 100 g w w)	CF	55	8.90	4.68	13.90	
AS	normal	28	7.2	3.0	15.0	< 0.0001
(%)	CF	57	28.6	5.5	49.8	
Nitrogen	normal	27	1.322	1.078	1.871	= 0.037
(g / 100 g w w)	CF	55	1.150	0.736	1.629	
Water	normal	27	73.9	67.9	78.6	= 0.650
(%)	CF	58	73.2	64.2	79.3	
Energy	normal	27	549.31	451.475	710.72	< 0.0001
(kJ / 100 g w w)	CF	57	742.96	525.59	997.24	

Median, 10th and 90th percentile for fecal fat as determined by the method of Van de Kamer and expressed in g / 100 g w w stool were 8.90 (4.68 - 13.90) and 4.75 (2.27 - 6.52) for CF patients and normal children respectively (fig 1). This difference is significant ($p < 0.0001$).

Median, 10th and 90th percentile of Acid Steatocrit results were 28.6% (5.5% - 49.8%) for CF patients and 7.2% (3.0% -15.0%) for healthy children. This difference is highly significant ($p < 0.0001$) (fig. 2).

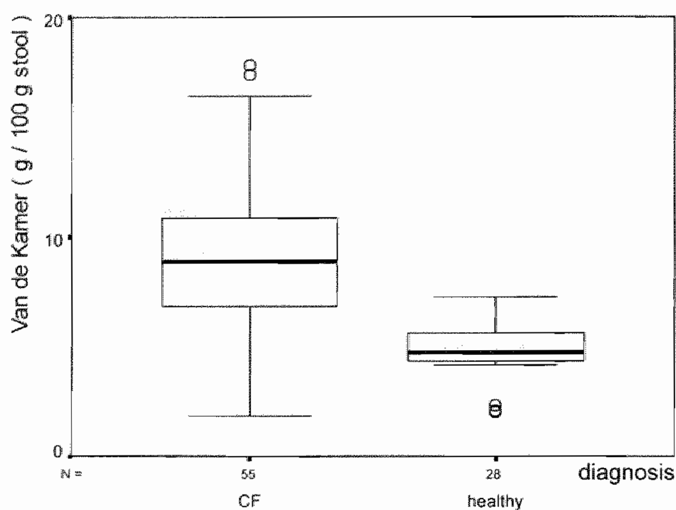


Figure 1. Boxplot of fecal fat results as measured by the method of Van de Kamer.

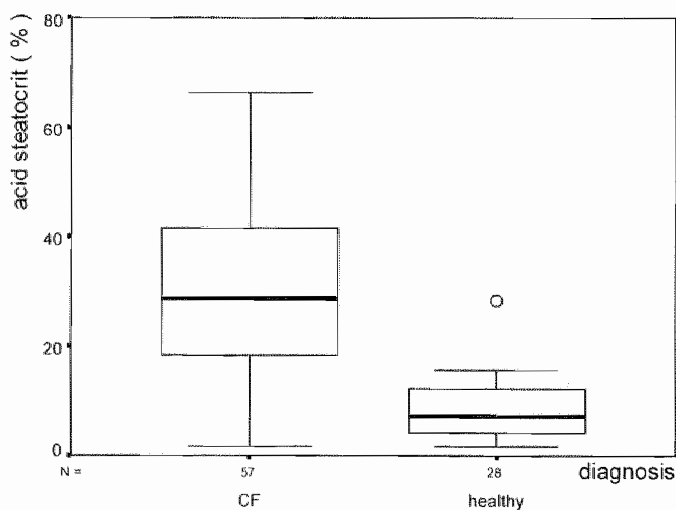


Figure 2. Boxplot of fecal fat results as measured by the AS method.

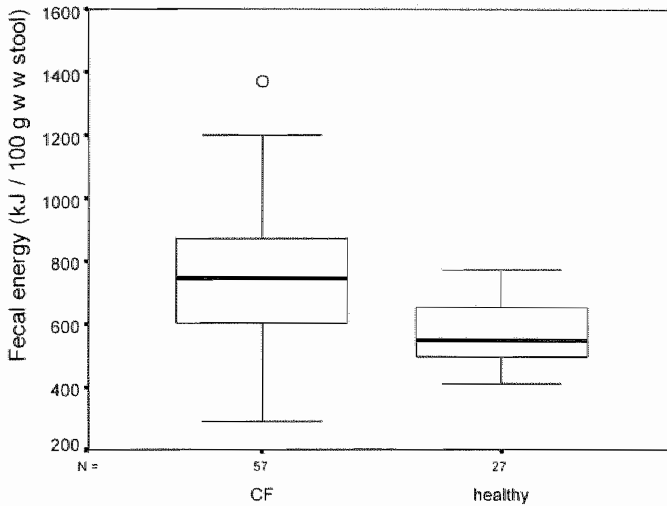


Figure 3. Boxplot of fecal energy results as measured by bomb calorimeter.

Single stool sample energy results (median; 10th and 90th percentile) expressed in kJ / 100 g w w stool were: 742.96 (525.59 - 997.24) for CF patients and: 549.32 (451.48 - 710.72) for normal children (fig 3). The differences found were significant ($p < 0.0001$).

Median, 10th and 90th percentile of nitrogen determinations were 1.150 g / 100 g w w (0.736 g - 1.629 g / 100 g) for CF children and 1.322 g / 100 g w w (1.078 g - 1.871g / 100 g) for healthy children ($p=0.037$).

Median, 10th and 90th percentile of fecal water contents were: 73.2% (64.2% - 79.3%) for CF patients and 73.9% (67.9% - 78.6%) for healthy children ($p=0.650$).

The correlation coefficient between fecal fat results by the method of Van de Kamer and by the AS method was 0.80 ($y = 3.35 + 0.66\,x$; SEE = 2.19; $p \leq 0.0001$) for CF patients and 0.14 ($y = 4.60 + 0.45\,x$; SEE = 1.28; $p=0.481$) for healthy children.

The correlation coefficient between fecal energy results and fecal fat results by the method of Van de Kamer was 0.62 ($y = 42.77 + 3.7\,x$; SEE =16.2; $p \leq 0.0001$) and 0.48 ($y = 38.78 + 3.7\,x$; SEE = 8.9; $p=0.011$) for CF patients and normal children respectively.

The correlation coefficient between fecal energy results and the AS results was: 0.77 ($y = 44.03 + 1.05\,x$; SEE = 13.4; $p \leq 0.0001$) for CF patients and 0.25 ($y = 53.10 + 4.33\,x$; SEE = 9.79; $p=0.214$) for healthy controls.

The correlation coefficient between fecal nitrogen and fecal energy results was 0.59 ($y = 0.483 + 9.264E\text{-}04\ x$; SEE =0.268; $p \le 0.001$) for CF patients and 0.77 ($y = 0.197 + 2.065E\text{-}03\ x$; SEE = 0.174; $p \le 0.0001$) for healthy children.

Discussion

This study shows that single stool sample results of fecal fat determinations (method of Van de Kamer and AS) and fecal energy in cystic fibrosis children are significantly different from those obtained in healthy control children. Fecal fat and fecal energy results are, as expected, significantly higher in CF patients when compared to healthy controls. There is a good correlation between fecal fat results determined by the method of Van de Kamer and by the AS method. This correlation coefficient concerning the whole group is comparable to our previously reported data, where we correlated AS results with fecal fat as measured by the titrimetric method of Van de Kamer on a single stool sample taken from a homogenized 72 hours stool collection (8).

The poorer correlation between results of the AS determination and the Van de Kamer method in healthy controls can probably be explained by the small range of values obtained in healthy controls.

As reported by Murphy et al., fecal energy percentage attributable to the colonic bacterial flora is comparable in healthy children and CF patients while the daily bacterial mass is three times greater in CF patients than in healthy controls (9). We can consequently expect that fecal fat in CF patients contributes most to fecal energy as demonstrated by the good correlation that we obtained between fecal fat and fecal energy.

The stool energy concentration of CF patients is significantly higher than that of healthy controls. Our results concerning stool energy and fat content are comparable to those reported by Murphy et al. (9) and Thomson et al. (10) for 72 hours stool collections.

Concerning fecal nitrogen, results show slightly lower values in CF patients when compared to healthy controls. Previous studies have shown the daily loss of fecal nitrogen to be twice as high in CF patients when compared to healthy children, with a similar proportional increase in daily stool weight (9). In this situation similar nitrogen concentrations should be expected.

Our findings show slightly higher nitrogen concentration in healthy children when compared to CF patients.

In agreement with literature data (9), fecal water concentration of CF patients and healthy children was similar.

Our results show that single stool sample fecal lipid and energy concentrations were significantly higher in CF patients when compared to healthy controls, while no clear differences were found for nitrogen and water concentrations. This is in agreement with literature data (9). Different studies indicate steatorrhoea to be mainly responsible for the loss of energy in exocrine pancreatic insufficiency. This information can be obtained from a single stool sample. Concerning the nitrogen loss, the daily loss rises with the daily stool weight, which consequently has to be measured. The latter information is not obtainable from a single stool sample analysis.

Conclusion

Our study shows that in CF patients the fecal lipid, water, nitrogen and energy concentrations obtained from single stool samples are similar to those reported from three day stool collections. Increased fat and energy losses are clearly apparent from single stool sample results, where as nitrogen losses are not. For the latter evaluation and for detailed quantitative evaluation of fecal losses the fecal balance technique remains necessary. For the frequent monitoring of fecal fat and energy, single stool sample analysis can be useful.

References

1. Lowdon J, Goodchild MC, Reyley HC, Doull IJM. Maintenance of growth in cystic fibrosis despite reduction in pancreatic enzyme supplementation. Arch Dis Child 1998; 78:377-78.
2. Regele S, Henker J, Munch R, BarbierY, Stern M. Indirect parameters of pancreatic function in cystic fibrosis (CF) during a controlled double- blind trial of pancreatic supplementation. J Pediatr Gastroenterol Nutr 1996; 22:68-72.
3. 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.
4. Benini L, Caliari S, Guidi GC, Vaona B, Talamini G,Vantini I, Scuro LA. Near infrared spectrometry for faecal fat measurement: comparison with conventional gravimetric and titrimetric methods. Gut 1989; 30: 1344-7.
5. Van de Kamer JH, Huinink HTB, Weijers HA. Rapid method for determination of fat in feces. J Biol Chem 1949; 177: 349-55.
6. Wilson PR. A new instrument concept for nitrogen? protein analysis. A challenge to the Kjeldahl method. Aspects of Applied Biology 25, Cereal Quality II 1990; 443-6.
7. Miller DS, Payne PR. A ballistic bomb calorimeter. Br J Nutr 1995; 13: 501-8.
8. Van den Neucker A, Pestel N, Tran TMD, Forget P-Ph, Veeze HJ, Bouquet J, Sinaasappel M. Clinical use of Acid Steatocrit. Acta Pædiatr 1997; 86: 466-9.

9. Murphy JL, Wootton SA, Bond SA, Jackson AA.Energy content of stools in normal healthy controls and patients with cystic fibrosis. Arch Dis Child 1991; 66: 495-500.
10. Thomson M, Clague A, Cleghorn GJ and Shepherd RW. Comparative in vitro and in vivo studies of enteric-coated pancrelipase preparetions for pancreatic insufficiency. J Pediatr Gastroenterol Nutr 1993; 17: 407-13.

CHAPTER 5

Acid steatocrit during infancy.

A Van den Neucker, P Forget, J A Veneberg, B J Schutten, B van Kreel
Department of Pediatrics and Chemistry,
University Hospital, Maastricht, The Netherlands
Acta Paediatr 1996; 85: 1153-5.

Abstract

The fecal acid steatocrit is an improved steatocrit method for the evaluation of fecal fat. The present study was set up in order to establish reference values during the first year of life.

Fecal acid steatocrit values were determined in 58 healthy full term and in 16 healthy prematurely born infants.

Very high acid steatocrit results (>60%) were found in all premature and many formula-fed term infants during the first six months of age, with thereafter values <10%. In 40- to 120-day-old infants acid steatocrit results of human milk-fed infants were significantly lower than those of formula-fed infants ($p < 0.01$).

High acid steatocrit results after the age of six months are indicative of fat malabsorption while, before this age high values can be due to physiological steatorrhea. The acid steatocrit should be useful for the evaluation of milkfat absorption in infants.

Introduction

Adequate food intake and absorption is of great importance for the development of children especially during the first year of life. Fat malabsorption has to be considered as an important cause of poor growth in infancy and childhood, in both term and premature babies. The 72-hours balance study is considered to be the gold standard for the diagnosis of fat malabsorption in children and adults (1).This test is not only unpleasant and time consuming, but even very difficult to perform in infants with stools often loose and watery.

In 1981 Phuapradit introduced the steatocrit as a simple and rapid test for the evaluation of fat malabsorption (2-3). Acidification of fecal samples was shown to result in a major improvement of the method (4). This latter improvement is due to a better extraction of fecal fat, resulting in a better correlation between acid steatocrit results and chemically measured fecal fat (4).

Before this test can be widely used clinically, normal values for the acid steatocrit should be obtained especially during the first year of life, when a higher amount of fecal fat can be physiologic (5).

The present study was designed to establish the normal range of acid steatocrit values during the first year of life in term and premature babies, and to evaluate the possible influence of formula versus human milk feeding on fecal fat content as determined by the acid steatocrit.

Patients

For the present study 58 healthy full-term infants (0-1 year), 31 girls and 27 boys were recruited at the well baby health control center.

16 healthy prematurely born infants (mean gestational age: 204 days, range: 183-225 days), 6 girls and 10 boys were studied one week after they were on full enteral nutrition, this occurred at a mean postnatal age of 27 days (range: 15-52 days).

In order to evaluate the influence of feeding regimens, acid steatocrit results from 18 term infants of this group, 9 boys and 9 girls, aged between 40 and 120 days and exclusively milk-fed (formula or human milk), were compared. Human milk-fed infants all received their own mother's fresh milk, while formula-fed infants were given Nutrilon Premium (Nutricia,The Netherlands), a humanized cow milk formula with a fatty acid composition similar to that of human milk (palmitic acid 18.1 g/100g fat and stearic acid 3.9g/100g fat versus respectively 22.1g/100g fat and 6.7g/100g fat for human milk (7).

Materials and Methods

Fecal single stool samples were collected and frozen at -18°Celsius. Acid steatocrit measurements were performed using the method of Tran et al. (4). In short this method was as follows: stool (0.5g) was diluted (1/4) with deionized water and thorougly homogenised 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 use of a standard Vortex mixer. The homogenate was aspirated into a 75µl plain glass haematocrit capillary. The capillary was subsequently centrifuged horizontally (13000 rpm. for 15 min.) in a standard haematocrit 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 the percentage of the fatty layer length over the sum of both the fatty and solid layers lengths. The variation coefficient of the acid steatocrit based on duplicate determinations in our laboratory was 5.1% (4).

The differences in acid steatocrit values between infants fed human milk and those fed formula were evaluated using the Wilcoxon rank sum test.

Results

The acid steatocrit results of term and premature born children in the first year of life showed wide variability within the first 200 days (± 6 months) of life, and decreased to values lower than 10% after the age of 6 months.

Fig.1 shows the individual acid steatocrit results according to age and type of feeding. The acid steatocrit results in the first month of life showed very high values, with no differences between feeding regimens. All premature infants results were obtained before day 44 as indicated by the reference line on fig.1. The figure shows that similar steatocrit results were found in premature and formula-fed infants under the age of 100 days. Later on acid steatocrit values decline to values below 10% in practically all infants after the age of 6 months. Values of human milk-fed babies dropped earlier than those of formula-fed babies.

Regarding the fatty layer and solid layer columns length (fig. 3) we noticed that high acid steatocrit values always corresponded with a large fatty layer length with often a small solid layer length and on the contrary that low acid steatocrit values always corresponded to a small or absent fatty layer length and a large solid layer length.

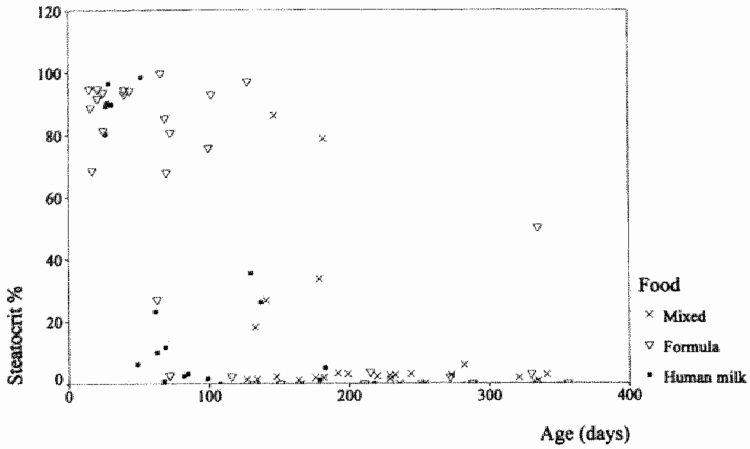


Figure 1. Individual values of acid steatocrit during the first year of life with indication of the food regimen, showing normalisation after the age of six months. Results of the premature babies are shown at the left of the reference line.

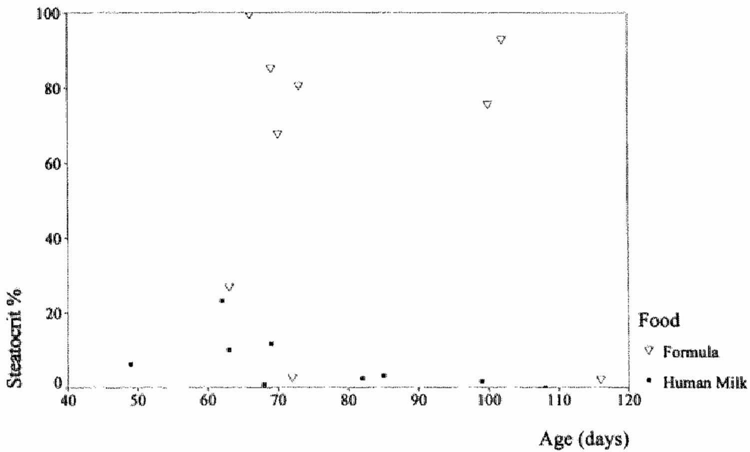


Figure 2. Acid steatocrit results in exclusively human milk or formula-fed infants aged between 40 and 120 days.

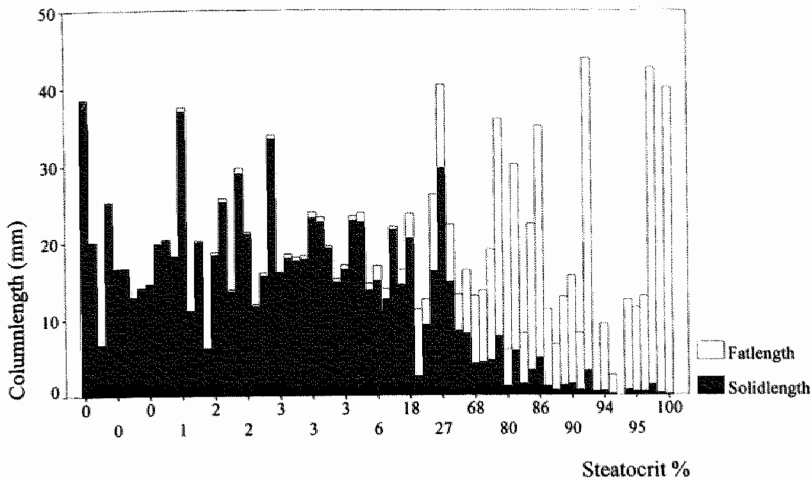


Figure 3. Fatlayer and solidlayer lengths related to acid steatocrit results.

The acid steatocrit values in the 18 exclusively milk-fed (formula or human milk) infants aged between 40 and 120 days, were significantly ($p < 0.01$) lower in human milk versus formula fed babies (fig 2). This difference was due to significantly ($p < 0.01$) smaller fatty layer lengths in the human milk fed infants than in the formula fed infants, with similar solid layer lengths ($p > 0.1$) in both groups.

Discussion

The diagnosis of fat malabsorption is often very important in young infants when growth velocity is maximal. The method of Van de Kamer (1) is generally accepted for the diagnosis of fat malabsorption but, this method is difficult to use, especially in infants when stools are often loose and watery and difficult to separate from urine. Therefore a simpler test such as the steatocrit micromethod (2) would be very welcome. Unfortunately, the method was considered unreliable by some authors (7). We could show that fat extraction was improved by acidification of fecal samples and that acid steatocrit results correlate much better with chemically measured fecal fat (4).

In the present study the acid steatocrit values in healthy term and premature born infants were measured.

When we consider our results, we notice a large variability of values in the first 200 days of life. Several factors could be responsible for these findings. First, fat malabsorption in the first months of life is a well known previously described phenomenon (5). This fat malabsorption is considered physiologic and has been attributed to several possible factors such as reduced duodenal lipase activity (8), a reduced concentration of luminal bile salts (9), or the composition of the feeding fat (10).

The acid steatocrit values in premature infants are very high. This is in agreement with literature data on balance studies in premature infants (11).

These high values are most probably due to both the presence of physiological higher amounts of fecal fat (high fatty layers lengths) but also to the small lengths of the solid layers measured by the acid steatocrit determination of these premature and term born young infants receiving a fiber-free diet in the first postnatal months.These latter findings could explain the difference with previously published results of the steatocrit method without acidification (12) whereby fat remains as soaps in the solid layer (4) resulting in lower steatocrit results wich normalize at 45 days of age.In the present study acid steatocrit values only become normal after the age of 200 days. Concerning the significantly lower acid steatocrit values in human milk-fed babies than in formula-fed babies,we noticed that the difference was due to significantly smaller fatty layer lengths in human-milk-fed babies, while solid layer lengths were similar in the two feeding groups.As suggestions have been made in literature (13) that the total daily fecal output is comparable in infants who are fed either fresh human milk or formula milk, we conclude that fat in fresh human milk-fed babies is better absorbed than formula fat. This finding is supported by previous findings from several authors who showed absorption of human milk fat to be much better than formula fat in young infants (14-15). We therefore consider the acid steatocrit a good tool for the evaluation of dietary fatabsorption.

Conclusion

Due to the presence of 'physiologic steatorrhea' high steatocrit levels can be found in normal infants during the first six months of life. Low results at this age point to normal fat absorption. After this age normal infants show low (below 10%) steatocrit results. High values are then indicative of fat malabsorption, lower acid steatocrit values were found in human milk-fed versus formula-fed infants, acid steatocrit should be useful for the evaluation of milk fat absorption in infants.

References

1. Van de Kamer JH, Huinink HTB, Weijers HA. Rapid method for determination of fat in feces. J Biol Chem 1949; 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. 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.
5. Weijers HA. Fat absorption in normal infants and children. Thesis 1950 Utrecht, The Netherlands.
6. Walthers MP, Kelleher J, Gilbert J, Littlewood JM. Clinical monitoring of steatorrhoea in cystic fibrosis. Arch Dis Child 1990; 65:99-120.
7. Koletzko B, Mrotzek M, Eng B, Bremer HJ. Fatty acid composition of mature human milk in Germany. Am J Clin Nutr 1988; 47: 954-9.
8. Lebenthal E, Lee PC. Development of functional response in human exocrine pancreas. Pediatrics 1980; 66: 556-60.
9. Watkins JB, Szczepanik P, Gould JB, Klein P, Lester R. Bile salt metabolism in the human premature infant. Gastroenterology 1975; 69: 706-13.
10. Hanna FM, Navarrete DA, Husu FA.Calcium-fatty acid absorption in term infants fed human milk and prepared formulas simulating human milk. Pediatrics 1970; 45: 216-24.
11. Shaw JCL. Evidence for defective skeletal mineralization in low-birthweight infants: the absorption of calcium and fat.Pediatrics 1976; 57: 16-25.
12. Iacono G, Carroccio A, Cavataio F et al. Steatocrit test: normal range and physiological variation in infants. J Pediatric Gastroenterol Nutr 1990; 11: 53-7.
13. Atkinson SA, Bryan MH, Anderson GH. Human milk feeding in premature infants: protein, fat and corbohydrate balances in the first two weeks of life. J Pediatr 1981; 99: 617-24.
14. De Curtis M, Senterre J, Rigo J, Putet G. Carbohydrate derived energy and gross energy absorption in premature infants fed human milk or formula. Arch Dis Child 1986; 61:867-70.
15. Williamson S, Finucane E, Ellis H, Gamsu HR. Effect of heat treatment of human milk on absorption of nitrogen, fat, sodium, calcium and phosphorus by premature infants. Arch Dis Child 1978; 53: 555-63.

CHAPTER 6

Clinical use of acid steatocrit.

A. Van den Neucker[1], N. Pestel[1], T. My Dung Tran[1], P.Ph. Forget[1],
H.J. Veeze[2], J. Bouquet[2], M. Sinaasappel[2].
[1] *Department of Paediatrics, University Hospital Maastricht and*
[2] *Sophia Children's Hospital Rotterdam, The Netherlands*
Acta Paediatr 1997; 86: 466-9.

Abstract

Malabsorption of fat is an important gastrointestinal cause of malnutrition and growth retardation in childhood. The gold standard for the evaluation of fat malabsorption is the faecal fat balance method. The acid steatocrit method has recently been introduced as a simple method to evaluate faecal fat. The present study was aimed at evaluating the acid steatocrit in clinical practice.

Faecal 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 faecal fat excretion ($p < 0.01$) and faecal 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 steatorrhoea.

Introduction

Malabsorption of fat is the most important gastrointestinal cause of malnutrition and growth retardation in childhood. The detection of steatorrhoea 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 faecal 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 faecal 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 faecal samples, acid steatocrit (AS) (5).

The present study was designed to compare the faecal fat excretion with the acid steatocrit for the diagnosis and monitoring 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 of these children were suspected of fat malabsorption, on the basis of anamnestic and clinical parameters. The different diagnoses of our patients are given in Table 1.

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

Diagnosis	N
Cystic fibrosis	20
Mental retardation	2
Recurrent diarrhoea	5
Failure to thrive	5
Coeliac disease	2
Inflammatory bowel disease	1
Short bowel	1
Choledochus cyste	1
Sucrase-isomaltase deficiency	1
Recurrent abdominal pain	1
Unknown	3

All children with cystic fibrosis (CF) had a normal daily fat intake according to the recommendations of the 'Dutch Nutritional Council'. The clinical and laboratory data concerning these children are shown in Table 2.

Table 2. Clinical and laboratory data concerning the 20 CF patients.

Age (years)	AS (%)	Absorption Coefficient (%)	Pancreatic Enzym Substitution	Pancreatic Insufficientcy
4.6	31	48	yes	yes
2.35	16.7	-	yes	yes
7.16	18.7	-	yes	yes
14.3	27.3	88	yes	yes
5.91	5.75	98	no	no
1.76	40.15	78	yes	yes
1.29	17.35	91	-	yes
7.02	27.65	78	yes	yes
8.7	25.1	77	yes	yes
4.85	6.1	94	yes	yes
7.05	2.9	-	-	no
11.58	21.45	79	yes	yes
13.68	20.35	77	yes	yes
12.47	9.9	93	yes	yes
2.95	39.35	85	yes	yes
18.73	31.95	-	no	yes
5.95	18.6	85	yes	yes
6.33	31.45	80	yes	yes
0.55	83.3	-	yes	yes
12.31	3.95	-	-	yes

Methods and Material

Stools from 3 consecutive days from each patient were collected in separate containers, one container for each day. The stools were immediately frozen at -18° Celsius. Fat content in each 72 hours 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). Faeces (0.5 g) was diluted (1/4) with deionized water and thoroughly homogenized making use of a 5 ml. 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 using a standard Vortex mixer. The homogenate was aspirated into a 75 µl 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:

$$\frac{\text{length of fat layer (mm)}}{\text{length of fat layer (mm)} + \text{length of solid layer (mm)}} \times 100$$

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

The investigators of the AS and of the faecal fat determinations following the method of Van de Kamer were unaware of both the diagnosis and each others results.

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 faecal fat excretion and the faecal fat concentration are shown in Table 3.

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 faecal fat concentration, for the diagnosis of steatorrhoea are shown in Table 4.

Table 3. Correlation between the results of the AS from either a single stool sample and a sample from the homogenised stool collection and the results of both fat excretion and faecal fat concentration in 42 children suspected of malabsorption.

	Fat excretion	Fat concentration
AS single stool	r= 0.4 ($p < 0.01$)	r= 0.82 ($p < 0.001$)
AS collection	r= 0.68 ($p < 0.001$)	r= 0.82 ($p < 0.001$)

Table 4. Sensitivity and specificity of the AS determination from a single stool sample and from a homogenised stool collection sample and of the faecal fat concentration, for the diagnosis of steatorrhoea.

	Sensitivity (%)	Specificity (%)
AS single stool	75	84
AS collection	90	100
Fat concentration	100	76

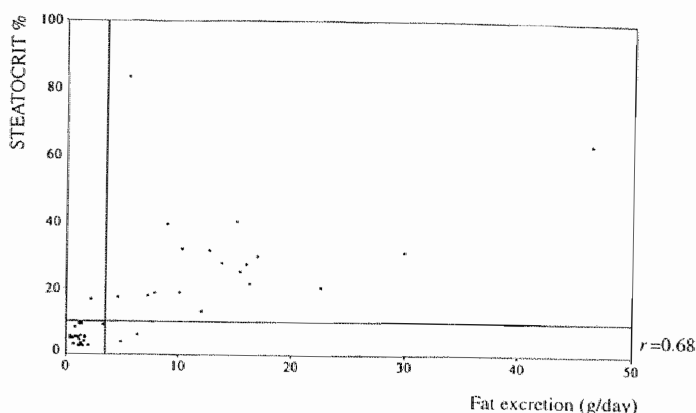


Figure 1. Relationship between AS results and fat excretion. Reference lines for AS at 10% and for fat excretion at 3 g/day.

The correlation coefficient between the AS results and the fatty layer lenghts in the present study was 0.83 whereas the correlation coefficient between the AS results and the solid layer lengths was 0.36.

Figure 1 shows our AS results from the homogenized faecal collection sample related to the faecal 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. Examining 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 faecal fat excretion corresponding to the false negative steatocrit results were 4.9; 6.4 and 7.7 g/day, and concern children aged 12, 6 and 13 years respectively.

Discussion

Since the faecal fat balance method 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 faecal 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 faecal sample, whereby fat extraction is improved, and steatocrit results correlate much better with chemically measured faecal fat (5).

Our AS results correlate satisfactorily with chemically measured faecal fat concentrations and somewhat less, but still significantly, with faecal 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 faecal 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: faecal fat excretion, which is expressed as faecal fat in grams per day and acid steatocrit which measures the ratio of the fat layer 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 chemical method also measures the endogenous fat from bacteria and shed cells.

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

Our unpublished data show that in our laboratory the day-to-day variability of the AS results from three stool samples taken on three consecutive days of adults (who have a more constant daily fat intake than children) is rather small. The standard deviation of the differences ranged between 13.6% and 0.23% with a mean of 5.2%.

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 fact that there are no reference values for the normal faecal fat excretion according to age. Faecal fat excretion higher than 4.5 g/24 hours is considered pathologic (6,8) for children and adolescents, whereas other authors consider 7 g/day the upper limit of normal faecal fat excretion in adults (9). The reference line for normal daily faecal 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 2/3 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.

Conclusion

Acid steatocrit results are highly correlated with the chemically measured faecal fat concentration and significantly correlated with the faecal 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 homogenized collection compare favourably with the faecal fat concentration. From this point of view we consider that the acid steatocrit method could be useful for the screening and monitoring of patients with pancreatic steatorrhoea, using the result of a stool sample from an homogenised 3-day collection or the mean of the results of single stool samples from three different days. The faecal fat balance method is needed whenever one wishes to evaluate the fat absorption coefficient.

References

1. Van de Kamer JH, Huinink HTB, Weyers HA. Rapid method for determination of fat in faeces. 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 steatorrhoea 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 Médicine 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 Nutr 1943; 25, 379.
9. Bai JC, Andrüsh A, Matelo G, Martinez C, Vazquez H, Boerr L, Sambuelli A. Faecal fat concentration in the differential diagnosis of steatorrhoea. Am. J. Gastroenterol. 1989; 27-30.

CHAPTER 7

Acid Steatocrit:
A reliable screening tool for steatorrhoea.

AM Van den Neucker [1]*, EM Kerkvliet [2], PMVM Theunissen [3], P-Ph Forget [1]

[1] *Department of Pediatrics, University Hospital Maastricht,*

[2] *St. Jans Gasthuis, Weert,* [3] *Atrium Medisch Centrum, Heerlen*

The Netherlands.

Acta Paediatr 2001; 90: 873-5.

Abstract

This study compared the acid steatocrit (AS) results of healthy children with those of sick children with and without gastrointestinal involvement. Stool samples of 166 children were investigated, comprising 50 healthy children, 26 asthma patients, 90 patients with gastrointestinal problems divided into: 34 treated cystic fibrosis (CF) patients with exocrine pancreatic insufficiency, 16 untreated coeliac disease (CD) patients and 40 patients with various gastrointestinal problems.

The median values (5th - 95th percentile) of AS results were 3.3% (0.0 - 21%) for healthy children, 4.5% (1.8 - 22.5%) for asthma patients, 24.7% (2.6 - 68.2%) for treated CF patients with exocrine pancreatic insufficiency, 19.8% (3 - 77.7%) for untreated CD patients and 5.5% (1.8 - 29%) for patients with various gastrointestinal diseases.

The AS results of treated CF patients with exocrine pancreatic insufficiency and untreated CD patients are similar and significantly higher than those of healthy children and asthma patients. AS can be considered to be a reliable tool in the screening for steatorrhoea in pediatric patients.

Introduction

Fat malabsorption is an important cause of poor growth in infancy and childhood. The 72-hours faecal fat balance according to the method of Van de Kamer is still considered to be the gold standard for the diagnosis of fat malabsorption (1). In 1981 Phuapradit described the steatocrit method as a simple procedure for estimating stool fat content in newborn infants (2). Acidification of the faecal homogenate has been shown to improve the reliability of steatocrit results (3, 4). Several clinical studies have shown the acid steatocrit (AS) to yield a clinically useful sensitivity and specificity for the detection of steatorrhoea (5, 6, 7).

Reported studies using the AS method concerned mainly healthy infants, cystic fibrosis patients with pancreatic insufficiency and adult patients (5, 6, 7). The present study was undertaken to compare the AS results of healthy children with those of sick children without (patients with asthma) and with (CD, CF, various) gastrointestinal involvement.

Material and Methods

Stool samples from 166 children were taken and immediately frozen at - 18°C. AS was performed using the method of Tran et al. (3).

The children were divided into five groups:

1. 50 healthy children (25M, 25F) with a mean age of 3.9 y (range 0.5 - 3.8 y);
2. 26 patients with asthma (17M, 9F) with a mean age of 5.9 y (range 0.9 - 17.6 y);
3. 34 CF patients (20M, 14F) with a mean age of 7.5 y (range 0.5 - 18.7 y). These patients have exocrine pancreatic insufficiency and receive pancreatic enzyme substitution therapy;
4. 16 patients with symptomatic CD (6M, 10F) with a mean age of 4.5 y (range 0.9 - 16.6 y). These patients were examined before treatment with a gluten free diet and had positive antigliadin and antiendomysium antibodies and subtotal villous atrophy at duodenal biopsy. They presented with failure to thrive and improved after gluten-free diet;
5. 40 patients (22M, 18F) with a mean age of 4.6 y (range 0.5 - 14.6 y), with various gastrointestinal problems (Table 1).

All data were managed with the SPSS statistical program. The Mann-Whitney U-test for independent samples was used for comparing means.

Results

The median AS values of healthy children, astma patients and patients with gastrointestinal diseases, other than CF or symptomatic CD before treatment, were lower than 10% (Table 2). The mean AS values of CF patients with enzyme substitution therapy and symptomatic coeliac patients before treatment were significantly higher than those of healthy children and astma patients ($p < 0.0001$).

Compared with healthy children, patients with 'various gastrointestinal disease' showed only a slightly increased mean AS result ($p = 0.04$). In this group there were three outliers: one patient with biliary atresia and two patients with short bowel disease. The individual AS results of these outliers were 18.7%, 29% and 36.1%, respectively. After exclusion of these outliers, the mean AS results did not differ from that of the healthy controls ($p = 0.11$).

The mean AS results of treated CF patients and of untreated coeliac patients were not significantly different ($p = 0.5$). Comparing the individual AS results of treated CF patients and untreated coeliac patients, 76% and 75% of these patients respectively, showed a value above 10%.

Regarding individual AS results within the different groups (Fig. 1), one child in the group of healthy children showed an unexplained high value of 44.8%.

Table 1. Diagnoses of sick control patients (n = 40) with various gastrointestinal problems.

Diagnosis	N
Disaccharidase deficiency	1
Short bowel syndrome	2
Diarrhoea (infectious, toddler's diarrhoea)	12
Failure to thrive (endocrine, nutritional deficiency)	13
Biliary atresia	1
Abdominal pain	3
Inflammatory bowel disease	1
Parasitosis	2
Eating disorders	2
Cow's milk protein intolerance	2
Coeliac disease, on diet	1

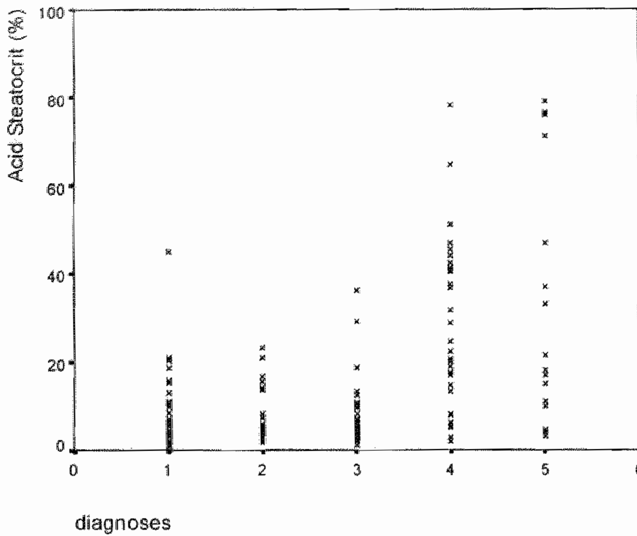


Figure 1. Acid steatocrit results of 1: healthy children; 2: asthma patients; 3: various gastrointestinal disease; 4: cystic fibrosis patients; and 5: coeliac disease patients.

Table 2

Subjects	AS (%) median	AS (%) 5th percentile	AS (%) 90th percentile
Healthy children	3.3	0.0	21
Asthma patients	4.5	1.8	22.5
CF patients	24.7	2.6	68.2
CD patients	19.8	3	77.7
Patients with various G.I-disease	5.5	1.8	29

AS: acid steatocrit; CF: cystic fibrosis; CD: coeliac disease; GI: gastrointestinal.

Discussion

The results show that the mean AS results of children with treated exocrine pancreatic insufficiency due to CF and of children with CD with gastrointestinal symptoms before treatment with a gluten-free diet, were both significantly increased compared to the mean AS results of healthy children. The mean AS results of asthma patients and healthy children were comparable.

This confirms that patients with exocrine pancreatic insufficiency due to CF while on enzyme substitution therapy keep some degree of fat malabsorption as reported in the literature (8,9), and coeliac patients with the typical gastrointesti-

nal symptoms such as weight loss, diarrhoea, abdominal distention and growth retardation often show steatorrhoea. In his work on CD, Dicke suggested steatorrhoea in these patients to be due to malabsorption associated with mucosal atrophy but also mentioned the difficulty at that time in excluding pancreatic fibrosis in these patients (10).

Other authors such as Bo-Linn and Fordtran (11), and Bai et al. (12) similarly consider steatorrhoea in coeliac patients to be due to mucosal disease and not to pancreatic disease. Recently reported studies (13,14, 15, 16, 17) however show that pancreatic insufficiency contributes to steatorrhoea in CD patients in 24% (18) to 33% (19) of the cases.

The present results show that the mean and median values of faecal fat content as measured by the AS in CF patients while on substitution therapy and in symptomatic coeliac patients before treatment are very similar, which could be explained by pancreatic involvement in both diseases. If one accepts 10% as cut-off value for the AS, as suggested by Tran et al. (3) three quarter of both groups appear to show steatorrhoea.These results are in agreement with literature data (8,9,17,18,20,21).

Although the steatocrit test may be a good screening test for detecting CD, it is well known that steatorrhoea is not always present in CD (22, 23), and that serological methods are the best screening methods available (24, 25). Furthermore, it remains unclear whether steatorrhoea in CD is mainly of intestinal or pancreatic origin, and whether the pancreatic involvement is primary or secondary to poor enterohormon secretion linked to mucosal atrophy.(26,27)

In the group of patients with various gastrointestinal problems the higher values of AS were due to results in two patients suffering from short bowel disease and one patient with biliary atresia. Faecal fat balance had shown these patients to have steatorrhoea.

Most of the other patients in this group had either diarrhoea (infectious or toddler's diarrhoea) or failure to thrive not due to gastrointestinal disease and showed normal steatocrit results.

Conclusion

Acid Steatocrit results are similarly low in healthy children and in patients with asthma. Only patients presenting with gastrointestinal disease known to be associated with steatorrhoea show abnormally high faecal fat content as measured by the acid steatocrit. The Acid Steatocrit can be considered to be a reliable tool in the screening for steatorrhoea in pediatric patients.

References

1. Van de Kamer JH, Huinink HTB, Weijers HA. Rapid method for determination of fat in faeces. J Biol Chem 1949: 349-55.
2. Phuapradit P, Narang A, Mendonca P, Haris DA, Baum JD. The steatocrit: a simple method for monitoring fat malabsorption in patients with cystic fibrosis. Arch Dis Child 1981; 56: 725-7.
3. 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.
4. Tran M, Forget P, Van den Neucker A, Van Kreel B. Improved steatocrit results obtained by acidification of faecal homogenate are due to improved fat extraction. J Pediatr Gastroenterol Nutr 1996; 22: 157-60.
5. Amann ST, Josephson SA, Toskes PP. Acid steatocrit: a simple, rapid gravimetric method to determine steatorrhoea. Am J Gastroenterol 1997; 92: 2280-4.
6. Van den Neucker A, Pestel N, Tran MD, Forget PPh, Veeze HJ, Bouquet J, Sinaasappel M. Clinical use of acid steatocrit. Acta Paediatr 1997; 86: 466-9.
7. Van den Neucker A, Forget P, Veneberg JA, Schutten BJ, Van Kreel B. Acid steatocrit during infancy. Acta Paediatr 1996; 85: 1153-5.
8. Kalivianakis M, Minich DM, Bijleveld CMA, Van Aalderen WMC, Stellaard F, Laseur M, et al. Fat malabsorption in cystic fibrosis patients receiving enzyme replacement therapy is due to impaired intestinal uptake of long-chain fatty acids. Am J Clin Nutr 1999; 69: 127-34.
9. Di Magno EP, Go VLW, Summerskill WHJ. Impaired cholecystokinin-pancreozymin secretion, intraluminal dilution, and maldigestion of fat in sprue. Gastroenterology 1972; 63: 25-32.
10. Dicke W K. Coeliac disease, investigation of the harmful effects of certain types of cereal on patients with coeliac disease; Utrecht 1950.
11. Bo-Linn, Fordtran JS. Faecal fat concentration in patients with steatorrhoea. Gastroenterol 1984; 87:319-22.
12. Bai JC, Andrüsh A, Matelo G, Martinez C, Vazquez H, Boerr L, Sambuelli A. Faecal fat concentration in the differential diagnosis of steatorrhoea. Am J gastroenterol 1989;84: 27-30.
13. Robinson P, Sly P. High dose pancreatic enzymes in cystic fibrosis. Arch Dis Child 1990; 65: 311-2.
14. Regan PT, Di Magno EP. Exocrine pancreatic insufficiency in celiac sprue: a cause of treatment failure. Gastroenterology 1980; 78: 484-7.
15. Ansaldi N, Oderda G. Exocrine pancreatic insufficiency in celiac sprue. Gastroenterology 1981; 80: 883.
16. Delco F, El-Serag HB, Sonnenberg A. Celiac sprue among US military veterans: associated disorders and clinical manifestations. Dig Dis Sci 1999; 44: 966-72.
17. Nousia-Arvanitakis S, Karagiozoglou-Lamboudes T, Aggouridaki C, Malaka-Lambrellis E, Galli-Tsinopoulou A, Xefteri M. Influence of jejunal morphology changes on exocrine pancreatic function in celiac disease. J Pediartr Gastroenterol Nutr 1999; 29: 81-5.
18. Perri F, Pastore M, Festa V, Clemente R, Quitadamo M, D'altilia MR, et al.. Intra duodenal lipase activity in celiac disease assessed by means of ^{13}C mixed-triglyceride breath test. J Pediatr Gastroenterol 1998; 27: 407-10.
19. Carroccio A, Iacono G, Ipolito S, Verghi F, Cavataio F, Soresi M, et al. Usefulness of faecal elastase-1 assay in monitoring pancreatic function in childhood coeliac disease. Ital J Gastroenterol Hepatol 998;30:500-4.

20. Carrocio A, Iacono G, Lerro P, Cavatio F, Malorgio E, Soresi M et al. Role of pancreatic impairement in growth recovery during gluten-free diet in childhood celiac disease. Gastroenterology 1997; 112: 1839-44.
21. Iacono G, Carroccio A, Montalto G, Cavataio F, Balsamo V, Notarbartolo A. Steatocrit test after a standard fatty meal: a new simple and sensitive test to detect malabsorption. J Pediatr Gastroenterol Nutr 1991; 13: 161-7.
22. Anderson CM, Gracey M, Burke V. Coeliac Disease. Arch Dis Child 1972; 47: 292-298.
23. Pournelle ND, Ciclitira PJ. Review article: coeliac disease and its management. Aliment Pharmacol Ther 1999; 13 (1):1-13.
24. Russo PA, Chartrand LJ, Seidman E. Comparative analysis of serologicscreening tests for the initialdiagnosis of celiac disease. Pediatrics 1999; 104 (1):75-8.
25. Vitoria JC, Arrieta A, Arranz C, Ayesta A, Sojo A, Maruri N, Garcia-Masdevall MD. Antibodies to gliadin, endomysium, and tissue transglutaminase for the diagnosis of coeliac disease. J Pediatr Gastroenterol Nutr 1999; 29 (5): 571-4.
26. Gomez JC. Exocrine pancreatic insufficiency in celiac disease. Gastroenterol 1998; 114: 621-22.
27. Carroccio A, Iacono G, Montalto G, Cavataio F, Di Marco C, Balsamo V, Notar bar tolo A. Exocrine pancreatic function in children with coeliac disaese before and after glutenfree diet. Gut 1991; 32: 796-9.

CHAPTER 8

Discussion and conclusion

Growth and development are the most important features of childhood (1). Energy is needed for this purpose and this energy is provided by our food. Per gram of food constituents, fat provides the highest amount of calories (lipid: 9 Kcal/g compared to protein and carbohydrates: 4 Kcal/g), and therefore fat malabsorption interferes strongly with growth and development in childhood (1, 2).

To prevent interference with growth it is important for the paediatrician to be able to diagnose fat malabsorption early and, as it concerns children, in a non-invasive way. If dietary fat absorption or digestion is disturbed and fat intake remains unchanged, fat excretion will increase. Determination of faecal fat seems to be the most appropriate investigation to perform. Until now determination of the fat absorption coefficient by the 72-hour faecal fat balance and faecal fat determination by the chemical titrimetric method of Van de Kamer has been considered the gold standard for diagnosing fat malabsorption (3).

However, this method has many disadvantages: the variability of the daily fat intake, the difficulty to collect stools, the toxicity of the solvents used for the technique itself and incomplete faecal fat extraction are well recognized problems (4, 5, 6, 7).

Due to these inconveniences, faecal fat balance in clinical practice is often reduced to the determination of the daily faecal fat excretion as a measurement of steatorrhoea: excessive loss of fat in the stools. In adults, 6-8g daily faecal fat is considered the upper limit of normal (8). In children the limit of normal daily faecal fat excretion varies with age; more than 3g of daily faecal fat is considered to be abnormal, but in young infants higher amounts of faecal fat are often found due to immaturity of the pancreas, to low duodenal concentration of intra-luminal bile salts, or to the variable composition of dietary fat. Faecal fat excretion in adolescents is more comparable to that found in adults (5, 9, 10, 11, 12).

It is obvious that this gold standard is far from ideal. Many other tests for diagnosing fat malabsorption have consequently been described and are used clinically. These different tests are summarized and discussed in *chapter 1*.

These tests can be divided into two groups:

1-tests evaluating the presence of faecal fat, such as the fat balance technique, the microscopic examination of fat droplets in stool specimens, the near-infrared reflectance analysis, the NMR measurement of faecal fat, and the steatocrit method,

2-tests evaluating lipid absorption such as fat loading test, the vitamin A loading test, the determination of plasma beta-carotene and radioactive or isotope labelled fat absorption tests (3, 8, 13, 14, 15).

As all these tests have their shortcomings, it is necessary for the clinician to make a good choice for routine clinical use. Such a test should be accurate, easy to perform, repeatable, non-invasive, and with a good specificity and sensitivity.

The steatocrit method as described in 1981 by Phuapradit et al. seemed to satisfy these needs (16). We introduced this method in our laboratory, but despite the promising results of some authors this method was reported not to be reliable by Walters et al. (17, 18, 19). The delineation and subsequent measurement of the three different layers in the haematocrit capillary, obtained after centrifugation of the faecal homogenate seemed to be the main problem. Different authors tried to improve the method by adding sand to the homogenate or by using sophisticated meters (20, 21). In our laboratory we ameliorated the test by acidification and demonstrated that 'acid steatocrit' results correlated better than the original test with results of the chemical titrimetric method of Van de Kamer and that its variation coefficient was lower *chapter 2*.

It is understandable that acidification ameliorates fat extraction, as non-absorbed dietary fats mainly occur as soaps in the faeces. This has been clearly demonstrated by Tran et al. (22, 23). Other authors confirmed this improvement (24, 25).

To demonstrate that this improved 'acid steatocrit' was at least as reliable as the other tests routinely used for evaluation of faecal fat, we compared the results of the AS with the results of faecal fat determination by the chemical titrimetric method of Van de Kamer and by NIRA. We compared the results of these three tests with that of faecal energy as measured by the bomb-calorimeter (*chapter 3*). We did not compare our AS results with results of the microscopic examination of the stool specimen for fat droplets. This test is considered a reliable test for evaluating faecal fat as reported in the last edition of Nelson's textbook of Pediatrics, but in 1948 Van de Kamer found this test already unreliable after comparison with the results of his method (2, 3).

We demonstrated that the results of the AS method were comparable to those of these other tests estimating faecal fat. The advantage of the AS in comparison with these other tests is its cheapness and simplicity. The NIRA apparatus is indeed very expensive and the method of Van de Kamer is laborious and dangerous for the laboratory personnel through inhalation of the toxic solvents. By its sim-

plicity and cheapness the AS test is also suitable for countries with restricted public health budgets.

As the gold standard for the diagnosis of fat malabsorption is the faecal fat balance technique with 72-hour stool collection, we had to demonstrate that a single stool sample could give similar information to a stool collection. For this purpose we examined stool samples from cystic fibrosis patients with steatorrhoea and from healthy children. We determined faecal lipid, water, nitrogen and energy concentration, and compared our results with those obtained from three-day stool collections as reported in literature (*chapter 4*). Why we compared our results to those reported in the literature was because most of our patients and healthy children, or their parents, were unwilling to collect stools for three days. Most of them had no problem with the delivering of different stool samples for AS determination. In his thesis in 1950 Weijers already mentioned the difficulty to collect stools, especially in young children and he also described a pot specially made for this purpose by the 'Société Céramique Maestricht' (5). Furthermore, we know from our own experience and from colleagues that many stool collections performed at home are incomplete and therefore give inaccurate information.

Our study shows that, concerning concentrations, single stool sample results for lipid, water, nitrogen and energy are comparable with those obtained by stool collections and that increased fat and energy losses are clearly apparent from data provided by single stool samples.

The value of faecal fat concentration instead of the total amount of daily faecal fat excretion for the diagnosis of steatorrhoea is discussed in the literature. Some authors believe that high faecal fat concentration suggests the presence of pancreatic or biliary steatorrhoea, but that faecal fat concentration might not be sensitive enough to detect steatorrhoea of intestinal origin. The diluting effects of coexisting fluid malabsorption could diminish the faecal fat concentration (8). This suggest the AS method might be a better alternative than the faecal fat concentration as in the AS method the water component is not included in the equation; the results being calculated from the amount of faecal fat and of faecal solid omitting faecal water. Some literature data support our opinion (25). Comparison of AS results with results of more specific tests for the investigation of intestinal lipid absorption, such as isotope labelled fat absorption tests, can possibly answer this question.

Steatorrhoea can be normal in neonates and young infants (5). We had to determine the duration of this neonatal steatorrhoea, because discrimination between normal and pathologic steatorrhoea is impossible during this period. For this purpose we examined stool samples from healthy infants during the first year of life (*chapter 5*). Our AS results were low (below 10%) in healthy children from the age of 6 months. Before this period results showed wide variability. After the

age of 6 months increased values are indicative of fat malabsorption, whereas before the age of 6 months this can also be due to the normal neonatal steatorrhoea. Low results before the age of 6 months on the other hand suggest that fat absorption is normal providing that fat intake is sufficient. Furthermore, our results showed significantly lower results in the group of infants fed human milk than in the formula-fed group, suggesting that the AS method could be useful for investigation of dietary fat absorption in order to improve the composition of formula fat.

In *chapter 6* we compared results of a three-day faecal fat balance with AS results obtained from a single stool sample and from a sample taken from the homogenized stool collection in a group of patients with and without steatorrhoea. The AS results were highly correlated with the chemically measured faecal fat concentration and significantly with the daily faecal fat excretion. Single stool sample AS results are slightly less sensitive and specific than AS results from samples taken from the homogenized stool collections. But, single stool sample AS results compare favourably with the chemically measured fat concentration from these collections. The difference between the single sample results and the results from the homogenized collections can be explained by the variability of the daily fat intake in children. We can try to prevent this problem by keeping the amount of ingested fat stable, but especially in small children this will be difficult, unless tube feeding is used.

The AS method can be useful for the monitoring of steatorrhoea in cystic fibrosis patients on enzyme substitution therapy. Clinicians dealing with these patients know that during the stool collection period parents and patients try to do their best to follow the physician's instructions and their usual diet is often much more irregular than during the collection period. As stool samples can be taken frequently at different moments in time, we may have a more realistic idea of their fat malabsorption than we would have by performing an annual faecal fat balance. This can be advantageous for monitoring enzyme substitution therapy. Whether or not this method of monitoring steatorrhoea will result in a better nutritional status of our cystic fibrosis patients is difficult to evaluate due to the confounding effects of respiratory infections and the natural evolution of the disease. We have shown that the AS method is useful in demonstrating the effect of therapeutic interventions on fat malabsorption in CF patients (26, 27).

We thus demonstrated that the AS method is clinically useful for the diagnosis of fat malabsorption and the monitoring of steatorrhoea in cystic fibrosis patients.

In *chapter 7* we evaluated the screening value of the AS method. We determined the AS in different groups of children: healthy children, asthma patients and patients with gastrointestinal diseases. This last group was subdivided in cystic fibrosis patients with exocrine pancreatic insufficiency while on substitution therapy, coeliac disease patients before treatment with gluten-free diet, and pa-

tients with various gastrointestinal diseases. The AS results were similarly low in healthy children and asthma patients and only elevated in patients suffering from gastrointestinal diseases known to be associated with steatorrhoea: cystic fibrosis patients with exocrine pancreatic insufficiency on enzyme substitution therapy, coeliac disease patients before treatment, and patients with biliary steatorrhoea.

The similarity of the results found in cystic fibrosis and coeliac disease patients is remarkable. Steatorrhoea in cystic fibrosis patients is caused by pancreatic insufficiency and intestinal malabsorption while in coeliac disease fat malabsorption was believed to be mainly caused by intestinal malabsorption. Our results suggest the same origin of their fat malabsorption and this is in agreement with recent data in the literature (28, 29).

In conclusion we believe AS method to be a clinically useful test to screen for steatorrhoea in childhood from the age of 6 months up to adulthood. As in all tests dealing with faecal fat for the diagnosis of steatorrhoea, an adequate intake of dietary fat is the only precaution. Because of its cheapness and simplicity the test is also useful in countries with a restricted public health budget. Its repeatability makes it useful for the monitoring of steatorrhoea and for the evaluation of the effects of new therapeutic interventions. The faecal fat balance and more specific and invasive investigations are available for the quantitative evaluation of fat malabsorption.

References

1. Behrman RE, Kliegman RM, Jenson H B (2000). Nelson Textbook of Pediatrics; 16th Edition. W.B. Saunders Company, Philadelphia USA.
2. Nederlandse voedingsmiddelentabel. Voorlichtingsbureau voor voeding.1999.
3. Van de Kamer JH, Huinink H TB, Weyers HA. Rapid method for determination of fat in feces. J Biol Chem 1949; 177: 349-55.
4. Sinaasappel M, Houwen RHJ, Van der Laag J, Gysbers CFM, Taminiau JAMJ, Van den Berg JWO. The faecal fat balance and the ^{13}C-mixed triglyceride breath test; diagnostic precision as determined in repeated studies in patients with cystic fibrosis.Abstracct 22th European CF Conference, Berlin 1998.
5. Weijers HA. Fat absorption in normal and abnormal infants and children. Thesis 1950 Utrecht The Netherlands.
6. Bekers O, Postma C, Lombarts AJPF. Determination of faecal fat by near-infrared spectroscopy. Eur J Clin Chem Clin Biochem 1995; 33: 83-6.
7. Jeejeebhoy KN, Ahmad S, Kozak C. Determination of fecal fats containing both medium and long chain triglycerides and fatty acids. Clin Biochem 1970; 3: 157-63.
8. Fine KD, Schiller LR. AGA technical review on the evaluation and management of chronic diarrhea. Gastroenterology 1999; 116: 1464-86.

9. Williams HH, Endicott EN, Shepherd ML, Galbraith H, Macy I G. Fat excretion by normal children. J of Nutrition 1943; 25: 379-87.
10. Lebenthal E, Lee PC, Development of functional response in human exocrine pancreas. Paediatrics 1980; 66:556-60.
11. Watkins JB, Szczepanik P, Gould JB, Klein P, Lester R. Bile salt metabolism in the human premature infant. Gastroenterology 1975; 69: 706-13.
12. Hanna FM, Navarrete DA, Husu FA. Calcium-fatty acid absorption in term infants fed human milk and prepared formulas simulating human milk. Pediatrics 1970; 45: 216-24.
13. Burtis CA, Ashwood ER (1999). Tietz Textbook of Clinical Chemistry, 3th ed. Saunders, New York.
14. Lembke B, Braden B, Stein J. Diagnosis of Steatorrhea. Z Gastroenterol 1994; 32(5): 256-61.
15. Leus J, Van Biervliet S, Robberecht E. Detection and follow up of exocrine pancreatic insufficiency in cystic fibrosis: a review. Eur J Pediatr 2000; 159: 563-8.
16. 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.
17. 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.
18. 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: 268-74.
19. Walters MP, Kelleher J, Gilbert J, Littlewood JM. Clinical monitoring of steatorrhea in cystic fibrosis. Arch Dis Child 1990; 65: 99-102.
20. Lloyd DR, Rowashdeh MO, Booth IW, Brown GA. The steatocrit: an improved procedure. Ann Clin Biochem 1992; 29(Pt5):535-40.
21. Rawashdeh MO, Lloyd DR, Puntis JWL, Brown GA, Booth IW. Using the steatocrit to determine optimal fat content in modular feeds. Arch Dis Child 1992; 67: 608-12.
22. Hill E, Bloor WR. Fat excretion. J Biol Chem 1922; 53: 171-8.
23. Tran M, Forget P, Van den Neucker A, Van Kreel B. Improved steatocrit results obtained by acidification of fecal homogenates are due to improved fat extraction. J Pediatr Gastroenterol Nutr 1996; 22: 157-60.
24. Bettinardi N, Colombo C, Corbetta C. In vitro influence of different kinds of fats on results and imprecision of classic and acid steatocrits. Clin Chem 1999; 45: 406-7.
25. Amann ST, Josephon SA, Toskes PP. Acid steatocrit: a simple, rapid gravimetric method to determine steatorrhea. Am J Gastroenterol 1997; 92: 2280-4.
26. Tran TMD, Van den Neucker A, Hendriks JJE, Forget P, Forget P. Role of lansoprazole in children with cystic fibrosis: evidence for improved fat absorption and nutritional status.
27. Hendriks JJ, Kester AD, Donckerwolcke R, Forget PP, Wouters EF. Changes in pulmonary hyperinflation and bronchial hyperresponsiveness following treatment with lansoprazole in children with cystic fibrosis. Pediatr Pulmonol 2001; 31: 59-66.
28. Kalivianakis M, Minich DM, Bijleveld CMA, Aalderen van WMC, Stellaard F, Laseur M, Vonk R, Verkade H J. Fat malabsorption in cystic fibrosis patients receiving enzyme replacement therapy is due to impaired intestinal uptake of long-chain fatty acids. Am J Clin Nutr 1999; 69: 127-34.
29. Nousia-Arvanitakis S, Karagiozoglou-Lamboudes T, Aggouridaki C, Malaka-Lambrellis E, Galli-Tsinopoulou A, Xefteri M. Influence of jejunal morphology changes on exocrine pancreatic function in celiac disease. J Pediatr Gastroenterol Nutr 1999; 29: 81-5.

Summary

Excessive dietary fat consumption is considered to be the most important cause of vascular diseases and these are associated with a high mortality rate. However fat is the most important source of energy in our diet. Fat is essential for the energy supply of the growing child, acts as a vehicle for fat-soluble vitamins and is a source of essential fatty acids. Fats are responsible for various important physiological processes.

Children fail to thrive as the intake, the digestion and/or absorption of fats are insufficient.

The *introduction* gives an overview of the fat digestion and absorption by the enterocyte so for as it is known today. Different causes of increased faecal fat loss are listed. Usable methods for determining faecal fat are described: the gravimetric method, the chemical titrimetric method, the method of Jeejeebhoy, the microscopic method, the reflectance method, the magnetic resonance, the steatocrit method. Furthermore currently used fat absorption tests are described: determination of serum triglycerides and the fat loading test, determination of serum ß-carotene, the vitamin-A-test, fat loading tests with radioactive or isotope marked fats.

In *chapter 2* the amelioration of the steatocrit method by acidification is described. A better extraction of fats is achieved by acidifying of the faecal homogenate, what results in a better delineation of the different layers obtained after centrifugation of the homogenate in a haematocrit capillary. This results in a better accuracy.

Chapter 3 shows the comparison of the results of the acid steatocrit method with those obtained by currently used methods as the NIRA and the chemical titrimetric method of Van de Kamer, and these results correlated with the faecal energy obtained by bomb calorimeter.

In *chapter 4* is demonstrated that similar information is obtained by a faecal sample as by a three days stool collection.

Chapter 5 indicates that the acid steatocrit method is usable from the age of 6 months and before this age faecal fat is elevated by 'physiological' reasons.

In *chapter 6* the clinical usefulness of the AS method for the diagnosis of steatorrhoea is proven by comparison of the AS results with the daily faecal fat excretion and the faecal fat concentration.

In *chapter 7* AS results in different groups of children are studied: healthy children, asthma patients, children suffering from various gastrointestinal diseases and children with exocrine pancreatic insufficiency. AS results are only raised in

this children presenting gastrointestinal diseases known to be associated with steatorrhoea.

Chapter 8 concludes, after the general discussion, that the AS is a reliable and clinical useful method for screening and monitoring steatorrhoea in children.

Samenvatting

Voedingsvetten worden de dag van vandaag beschouwd als de oorzaak van vasculaire aandoeningen, de meest frequente doodsoorzaak. Nochtans is vet de meest belangrijke bron van energie in onze voeding. Vet is essentieel voor het groeiende kind, als energiebron en als vehikel van vetoplosbare vitamines en bron van essentiële vetzuren. In het lichaam zijn vetten verantwoordelijk voor verschillende belangrijke fysiologische processen.

Kinderen groeien slecht als de inname, de digestie of de absorptie van vetten insufficiënt is.

De *introductie* geeft een overzicht van de vetvertering en de vetopname in de enterocyt, voor zover dit nu bekend is. Verschillende oorzaken van vet malabsorptie worden vermeld. De verschillende gangbare methoden om fecaal vet te bepalen worden beschreven: de gravimetrische methode, de chemische titreer methode, de methode van Jeejeebhoy, de microscopische methode, de reflectie methode, de magnetische resonantie, de steatocriet methode. Bovendien worden de meest courante vetabsorptie testen beschreven: de bepaling van serum triglycerides and de vetbelasting test, de bepaling van serum ß-caroteen in het serum, vitamine A test, vetbelasting tests met radioactief of met stabiele isotopen gemerkte vetten.

In *hoofdstuk 2* wordt de verbetering van de steatocriet door aanzuren beschreven. Door aanzuren wordt een betere vetextractie bekomen in een feceshomogenaat wat resulteert in een beter afgrenzen van de verschillende lagen die worden bekomen door centrifugeren van het feceshomogenaat in een haematocriet capillair; waardoor de accuraatheid van de test verbetert.

Hoofdstuk 3 vergelijkt de resultaten van de zure steatocriet met deze van de momenteel gangbare methoden: de NIRA en de chemische titreer methode van Van de Kamer. Al de bekomen resultaten worden bovendien gecorreleerd met de fecale energie bekomen door bomcalorimetrisch onderzoek.

In *hoofdstuk 4* wordt aangetoond dat een fecesmonster vergelijkbare informatie geeft als een ontlastingsverzameling van 3 dagen.

In *hoofdstuk 5* wordt aangetoond dat de zure steatocriet methode bruikbaar is vanaf de leeftijd van 6 maand. Vóór deze leeftijd bestaat er een fysiologische vetverhoging in de feces.

Hoofdstuk 6 toont ons de klinische bruikbaarheid van de methode voor de diagnose van steatorrhoea door vergelijk met de dagelijkse feces vetexcretie en de vetconcentratie.

In *hoofdstuk 7* worden de zure steatocriet resultaten bekeken in verschillende groepen kinderen: gezonde kinderen, astma patiënten, kinderen met diverse gastro-enterologische aandoeningen en kinderen met exocriene pancreasinsufficiëntie. De zure steatocriet waarden zijn enkel verhoogd bij deze kinderen met aandoeningen waarbij we steatorrhoe verwachten.

In *hoofdstuk 8* komen we na een algemene discussie tot de conclusie dat de zure steatocriet een betrouwbare en klinisch bruikbare methode is voor de diagnose en het vervolgen van vetmalabsorptie bij kinderen.

Dankwoord

Een thesis is zelden het werk van een enkeling en daarom is het gepast iedereen die heeft bijgedragen tot het tot stand komen van deze thesis te bedanken.

Vooreerst wil ik mijn dank richten tot mijn leermeesters die mij in navolging van Hippocrates de geneeskunde en de kindergeneeskunde hebben onderwezen.

Mijn dank gaat naar Prof. Dr. R. Donckerwolcke, mijn promotor, voor de mogelijkheid om deze thesis te verdedigen.

Mijn dank gaat uit naar mijn co-promotores Dr. P.-Ph. Forget en Dr. B. van Kreel voor hun kennis, steun, geduld en toewijding waarmee ze mij hebben begeleid doorheen de jaren dat dit werk heeft gekost.

Mijn dank gaat ook naar Dr. T.M.D. Tran voor haar vrienschap en morele steun en voor de gezellige congressen die we samen doorbrachten ter verdediging van de 'Zure Steatocriet'.

Dank aan het personeel van het laboratorium van de klinische chemie dat steeds klaar stond om mij met woord en daad te helpen.

Dank aan de ouders van onze patiënten en de ouders van de gezonde kinderen, dank aan de kinderen, voor hun bereidwilligheid om materiaal voor het onderzoek op te vangen en in te leveren.

Dank aan mijn collega's van de capaciteitsgroep Kindergeneeskunde die mij daadwerkelijk en moreel gesteund hebben.

Dank aan de collega's gastro-enterologen voor hun steun, sympathie en interesse.

Dank aan de collega's en verpleegkundigen, paramedisch personeel, onderhoudspersoneel betrokken bij de Kindergeneeskunde voor de jarenlange samenwerking.

Dank aan het administratief personeel betrokken bij de Kindergeneeskunde en vooral aan mijn secretaresse mevrouw Lea Lapré voor haar sympathie en ondersteuning.

Een speciale dank aan mijn vrienden en familie voor hun onvoorwaardelijke steun en vriendschap.

Curriculum vitae

- Anita Maria Van den Neucker
- Geboren te Aalst (Oost-Vlaanderen, België) op 19 december 1954
- Middelbare school (Latijn-Wiskunde Humaniora) aan het Instituut Dames van Maria te Aalst tot 1972
- Geneeskunde studie aan de Rijks Universiteit te Gent 1972 – 1979
- Agnio voor Interne Geneeskunde in het Onze Lieve Vrouw Ziekenhuis te Aalst (Hoofd: Dr. E. Bougeois en Dr. P Harth) 1979 - 1980
- Kinderarts in opleiding in de Kinderkliniek C. Hooft van het Universitair Ziekenhuis te Gent 1980 – 1985 (opleiders: Prof. Dr. M.-J. Delbeke en Prof. Dr. J. Leroy)
- Fellow kinder-gastro-enterologie in het Sophia Kinderziekenhuis van het Universitair Ziekenhuis te Rotterdam (Dr. M. Sinaasappel – Dr. J. Bouquet) 1985 -1986
- Kinderarts in het St. Vincentius Ziekenhuis te Antwerpen en St. Anna Ziekenhuis te Beveren-Waas (België) 1987 – 1988
- Kinderarts in het centrum voor kindermishandeling: 'Kind in Nood' te Gent 1989
- Kinderarts / Kinder-gastro-enteroloog in het Academisch Ziekenhuis Maastricht sinds 1990 (Hoofd: Prof. Dr. R. Kuijten tot 1995; Prof. Dr. C. Blanco a.i. tot 1997; Prof. Dr. R. Donckerwolcke)