Cancer cachexia

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Cancer Cachexia

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Cancer Cachexia

PROEFSCHRIFT

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aan de Universiteit Maastricht
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volgens het besluit van het College van Decanen,
in het openbaar te verdedigen
op vrijdag 12 december 1997 om 14.00 uur

door

Jean Paul François Henri Auguste Simons

geboren te Maastricht op 17 mei 1962

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Nihil homines mortem curant, non quia est, sed quia creditur longinqua
(Petrarca, *Epistulae de rebus familiaribus* 9. 5).

In memory of all the patients who participated in my studies.

To my parents

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ABBREVIATIONS

BCM	body-cell-mass
BCM-ix	body-cell-mass-index (BCM/height ²)
BIA	bioelectrical impedance analysis
BMI	body mass index
DEXA	dual-energy X-ray absorptiometry
ECW	extracellular water
EI	energy intake
FFM	fat-free mass
FM	fat mass
ht ² /R	height ² /resistance
ICW	intracellular water
IGF-I	insulin-like growth factor I
IL-1	interleukin-1
IL-6	interleukin-6
LBP	lipopolysaccharide-binding protein
LPL	lipoprotein lipase
MA	megestrol acetate
MPA	medroxyprogesterone acetate
QL	quality of life
QLQ	quality of life questionnaire
PIBW	percentage of ideal body weight
R	resistance
REE	resting energy expenditure
sTNF-R55	soluble TNF receptor 55
sTNF-R75	soluble TNF receptor 75
TBW	total body water
TBWdeu	total body water assessed by deuterium dilution
TNF	tumor necrosis factor
WT	weight

1

GENERAL INTRODUCTION

General introduction

1 INTRODUCTION

Involuntary weight loss is a well known complication of cancer (1). When weight loss is substantial, it eventually leads to cachexia, a state that is characterized by a low body weight, fatigue, lethargy, weakness and anaemia. Although cachexia (the word being derived from the Greek words *kakos* and *hexis*, meaning "bad condition") is mainly associated with terminal disseminated disease, it is also often present in the early stages of tumor growth, even before other signs and symptoms of malignancy appear.

1.1 Incidence

The incidence of weight loss at diagnosis is different for separate tumor types. It varies from 30% in patients with favorable non-hodgkin lymphoma and breast cancer, to 85% in patients with gastric and pancreatic cancer (2). In newly-diagnosed lung cancer, some degree of weight loss is found in 60% of the cases (2); severe weight loss of at least 10% of the pre-illness weight has been reported in 30% (3).

1.2 Consequences

The development of cancer-related cachexia has several negative consequences. In the first place, it may have an important negative impact on quality of life, not only in the sense of a decreased performance status (2), but certainly also in the sense of a worsened emotional well-being.

In the second place, cachexia is the single most documented cause of death in cancer. Already in 1932, Warren reported in an autopsy study that 22% of 500 patients investigated had died of cachexia, and that in many others cachexia was likely to have contributed to death from other causes, such as infection (4). The significance of weight loss as an independent predictor of survival has since been proven in several other studies (2,5,6).

In the third place, weight loss has been shown to be an independent negative predictor of treatment outcome. In breast cancer, the negative impact of weight loss on response to chemotherapy was shown to be approximately equal to the influence of tumor extent (7). In addition, in surgical patients preoperative depletion is associated with a significant increase in postoperative complications (8).

2 BODY COMPOSITION

One of the aspects of cancer-related weight loss that is highly relevant in relation to its pathophysiology, complications and treatment, is its composition.

Many methods of analysis are currently available to divide the human body into several compartments, varying from two to more than five (9). The simplest model is a 2-compartment model dividing the body into fat mass and fat-free mass. By using more advanced techniques, fat-free mass can be divided into body-cell-mass and extracellular mass (3-compartment model). Subsequently, the extracellular mass can be further subdivided into extracellular water and extracellular solids (4-compartment model). The body of a healthy male individual consists for approximately 20% of fat, 45% of body-cell-mass, 30% of extracellular water and 5% of extracellular solids (of which 85% are bone minerals) (9,10). Of these compartments, the body-cell-mass contains nearly all tissues that are vital for a normal functioning of the body, skeletal muscle ($\approx 75\%$ of body-cell-mass) and visceral organs in particular (10).

In simple starvation, more than 75% of weight is lost from body fat, and only a small amount from body-cell-mass. In cancer, however, weight loss is due to approximately equal losses of fat and body-cell-mass, particularly skeletal muscle (11-13). Visceral organ mass generally remains grossly unchanged (12,14), except for the spleen, which is often enlarged (14).

3 PATHOPHYSIOLOGY

One of the first theories concerning the pathophysiology of cancer cachexia was that of a competition between host and growing tumor for nutrients, amino acids in particular (15,16). During the past two decades, however, this theory gradually lost support, particularly as there seemed to be hardly any relationship between tumor size and the extent of the occurring weight loss (17).

Many new theories were however developed instead. To date, factors and mechanisms that are thought to play a major role are the presence of an increased systemic inflammatory state, the circulation of tumor-derived (non-cytokine) proteolytic and lipolytic factors, an increased Cori cycle activity, and a decreased food intake.

3.1 Systemic inflammation

Evidence exists that the development of cancer cachexia is for an important part related to

a chronic, low-grade, tumor-induced activation of the host immune system (18) that, though in a mitigated form, shares several characteristics with the so-called acute-phase response seen after major trauma and sepsis, a state characterized by an increased production of cytokines (19,20), elevated levels of catecholamines, cortisol and glucagon (19,21-23), increased peripheral amino acid mobilization and hepatic amino acid uptake (19,24), enhanced hepatic gluconeogenesis and acute-phase protein production (19,25,26), increased mobilization of free fatty acids (27) and hypermetabolism (25).

3.1.1 EXPERIMENTAL MODELS

Nearly all evidence that cancer cachexia is related to such an increased systemic inflammatory state has come from studies in animal models, showing an association between the development of weight loss and an increased production of cytokines like tumor-necrosis-factor (TNF), interleukin(IL)-1, IL-6, interferon- γ and leukemia inhibitory factor (28-39). The increased cytokine production has been assumed to be primarily the result of a tumor-induced activation of the host immune system (40-42), although there is presently evidence that several of these cytokines can also be produced by the tumor itself (33,34,43-52), and that this production can be further enhanced by local tumor-macrophage/cytokine interaction (44,47,53).

3.1.1.1 *Proteolysis and body-cell-mass-wasting*

It was shown that an increased production of particularly TNF and IL-6 was associated with significant wasting of muscle mass, by far the major determinant of body-cell-mass (32,54-56). In other studies it was observed that also exogenous TNF induced proteolysis and muscle-wasting (57-67). The exogenous administration of IL-6 revealed variable results. In a study in incubated rat muscle after *in vivo* pre-treatment with IL-6, a significant increase in total and myofibrillar protein degradation was found (68), and also in IL-6 transgenic mice significant muscle atrophy occurred that was reversible with anti-IL-6-receptor antibody (69). In another study in rats, however, muscle mass was not influenced by exogenous IL-6 administration (70).

Whether IL-1 can induce wasting of skeletal muscle protein is still a matter of debate. While in an ascites hepatoma model anti-TNF antibody treatment was shown to partially reverse protein loss (54), this was not the case for antibodies against the IL-1 receptor (71). Some authors nevertheless reported that exogenous IL-1 induced proteolysis and loss of muscle mass (59,66,72). Others, however, were not able to confirm these findings (65,60,73).

Several mechanisms by which inflammation may lead to muscle and body-cell-mass-wasting have been proposed.

In the first place there might be a direct effect of inflammatory mediators on myocytes. It was shown that the *in vitro* uptake of amino acids in skeletal muscle was reduced after incubation with plasma from septic rats (74,75), and that increased proteolysis occurred after incubation with plasma from septic humans (76) and partially purified supernatants from human macrophage cultures (77,78). Although studies in recombinant-TNF-incubated mouse diaphragms (79) and recombinant-IL-6-incubated cultured myotubes (80) seemed to confirm a direct proteolytic effect of these monokines, other studies did not (63,65,68,78,81-83).

In the second place, body-cell-mass-wasting may be related to a cytokine-induced decrease in food intake, which was reported particularly for TNF and IL-1 (28,29,55,61,84-87). A decreased food intake may be the result of a loss of appetite (anorexia) due to cytokine influences on peripheral (88) and hypothalamic (89,90) parts of the appetite regulation mechanism, and of cytokine-induced alterations in gastrointestinal motility (91,92) and food absorption (62). One of the mediators that might be involved in the development of cytokine-related anorexia is prostaglandin E2 (93).

In the third place, cancer-related body-cell-mass-wasting may be the consequence of an increased utilization of amino acids. This might be related to: [1] inflammation-induced changes in energy metabolism resulting in hypermetabolism (94,95) or diminished metabolic efficiency due to, for example, a relative increase in anaerobic glycolysis (96,97); [2] an inflammation-related increase in amino acid uptake by the liver (98-102) in association with enhanced gluconeogenesis (103) and acute-phase protein production (32,86,104-109); and [3] a cytokine-triggered rise of cortisol (26,62,72,98,100,102,110-113) which not only mediates the stimulatory effect of TNF, IL-1 and IL-6 on hepatic amino acid uptake (99-101) and acute-phase protein production (26,106,107), but also directly induces total and myofibrillar proteolysis (114-117).

In the fourth place, body-cell-mass-wasting may be related to a decreased muscle protein synthesis. TNF administration has been shown to attenuate skeletal muscle protein synthesis in rats (84). In addition, TNF and glucocorticoids were shown, *in vivo* and *in vitro*, to decrease the production of testosterone, an important mediator of muscle growth (118-120). Finally, another hormone with prominent anabolic effects on muscle that has been reported to be decreased in experimental cancer cachexia (121,122) is IGF-I (123). It has however not been investigated to date whether this decrease is inflammation-related and/or enhances muscle-wasting. What was shown *in vitro*, however, was a TNF-, IL-1- and IL-6-induced inhibition of IGF-I bioactivity (124).

3.1.1.2 Fat-wasting

In *in vitro* experiments in cultured mouse adipocytes, nearly all pro-inflammatory cytokines were shown to suppress the activity of the enzyme lipoprotein lipase (LPL), responsible for the uptake of very-low-density-lipoprotein-bound triglycerides from the circulation for storage (125-127). In the same models, it was shown that most cytokines were also able to inhibit the *de novo* adipocyte fatty acid synthesis and to stimulate adipocyte lipolysis (125-127). In a study using endotoxin-stimulated macrophage products, the latter was the result of an activation of the lipolytic enzyme hormone-sensitive lipase (128). Later studies with recombinant-TNF showed, however, that TNF inhibited -rather than stimulated- hormone-sensitive lipase gene expression, indicating that it was *not* TNF to be responsible for the observed hormone-sensitive lipase activation (129). Based on the above findings, it was postulated that inflammatory mediators, pro-inflammatory cytokines in particular, were able to induce fat-wasting through direct effects on the adipocyte. Other studies, however, were not able to reproduce these findings in comparable models using rat- or human adipocytes (83,130).

Whether or not TNF- and IL-1-related hypertriglyceridemia and fat-wasting are associated with inhibition of adipocyte LPL activity and fatty acid synthesis *in vivo*, is, after several years, still a matter of discussion (131,132). A study in fat-wasting rats bearing the Yoshida ascites hepatoma AH-130 did show a significantly decreased level of white adipose tissue LPL activity, reversible after treatment with anti-TNF α antibody (133). Several other *in vivo* studies observed a TNF- and IL-1-related increase in triglyceride synthesis by the liver as a result of an increased hepatic *de novo* fatty acid synthesis and enhanced reesterification of fatty acids present in the circulation as a result of increased peripheral lipolysis (134,135). It has been postulated that the latter mechanism might represent the main explanation for the hypertriglyceridemia seen after TNF administration (131).

3.1.2 HUMAN STUDIES

Only limited information is available on the role of systemic inflammation in the pathophysiology of body-cell-mass-wasting in *human* cancer.

An increased production of hepatic acute-phase proteins and IL-6 has been reported (136-141), and some authors (140,142-149), though not all (150-155), found evidence of an increased production of TNF. Discrepancies in the detection of an increased TNF production in human cancer might however in part be explained by differences in sensitivity (156) and specificity of the assays used, and the fact that the bioactivity of

TNF is probably much more determined by its local production and paracrine action (157), than by its presence in the systemic circulation where it is rapidly inactivated through binding with proteins like soluble TNF receptors (158). Serum IL-1, assessed by radioimmunoassay, was shown to be elevated in a study in patients with hepatocellular carcinoma, metastatic liver carcinoma and gastrointestinal carcinoma (140). However, other investigators were not able to detect serum/plasma IL-1 in cancer (151,159), and the capacity of peripheral blood monocytes of cancer patients to produce IL-1 *in vitro* has been reported to be normal (160) or even decreased (161-165).

In human cancer, the association between systemic inflammation and weight loss has presently been confirmed in two studies. Knapp *et al.* observed in stage IV breast cancer that the presence of severe weight loss ($\geq 20\%$) was accompanied by increased blood levels of TNF α , cortisol and glucagon (166), and Staal-van den Brekel *et al.* reported in newly-diagnosed lung cancer that both severe weight loss ($\geq 10\%$) and hypermetabolism were associated with increased plasma levels of soluble TNF-receptors and acute-phase proteins (167).

More indirect evidence of a relationship between systemic inflammation and weight loss and/or body-cell-mass-wasting came from several other studies. In a study in cachectic patients with pancreatic cancer it was shown that hypermetabolism and increased TNF- and IL-6 production by peripheral blood mononuclear cells were associated with an increased synthesis of hepatic acute-phase proteins (150). In a study in gastrointestinal cancer it was found that serum TNF levels were positively correlated with the degree of insulin resistance (168), a state potentially attenuating protein synthesis and enhancing protein breakdown, and shown to be present in weight-losing cancer patients (169). Another disturbance in carbohydrate metabolism reported in cachectic cancer patients, though in contrast with experimental studies not yet linked to systemic inflammation, is an up to 40% increased hepatic glucose production (170). In cancer patients participating in phase-I clinical trials, the use of TNF as a potential cytotoxic agent was associated with enhanced whole-body protein turnover with an increased efflux of alanine and glutamine from the forearm, in combination with significant hypermetabolism (171) and the development of anorexia (172). In a phase-I/II study investigating the treatment of post-chemotherapy thrombopenia, the administration of IL-6 was associated with a significant increase in hepatic acute-phase protein production (173). In a study in healthy individuals it was shown that exogenous TNF lowered circulating testosterone levels (174).

As far as fat-wasting is concerned, weight-losing cancer patients are characterized by an increased glycerol and fatty acid turnover (175). To date, however, few human studies specifically associated abnormalities in lipid metabolism with systemic inflammation. In a

phase-I trial in non-weight-losing cancer patients, the administration of high doses of TNF was associated with increased lipolysis and lipid utilization (171). In another study in cancer patients, an increased expression of adipose-tissue mRNA encoding for the lipolytic enzyme hormone-sensitive lipase was observed, while total adipose-tissue LPL-activity, as well as the relative levels of mRNA for LPL and fatty acid synthase, were not different in comparison with non-cancer controls (176). It is interesting to note that in earlier *in vitro* studies it was reported that hormone-sensitive lipase-related lipolysis was induced by inflammatory mediators (128).

3.2 Circulating tumor-derived catabolic factors

In addition to inflammatory mediators, the development of weight loss may be related to the circulation of so-called lipid- and protein mobilizing factors (LMF's and PMF's), tumor-derived catabolic factors that act directly on adipose tissue and skeletal muscle, without affecting food intake.

3.2.1 Lipid mobilizing factors

A lipolytic substance, called toxohormone L, was isolated from ascitic fluid of patients with hepatoma and sarcoma-bearing mice (177). Another LMF, isolated from mice bearing the cachexia-inducing murine carcinoma MAC16, was also identified in the serum of cachectic cancer patients (178). In these patients, the serum level was correlated with the degree of weight loss (189). A third LMF, isolated from a human melanoma cell line, was shown *-in vitro-* to induce lipolysis by stimulating the murine adipocyte enzyme triglyceride lipase, and *-in vivo-* to induce extensive fat-wasting in mice (180).

3.2.2 Protein mobilizing factors

Concerning the PMF's, particularly interesting is a recent report describing the isolation of a 24 KDa proteoglycan from the cachexia-inducing MAC16 colon adenocarcinoma. This proteoglycan was shown, *in vitro* and *in vivo*, to induce skeletal muscle protein degradation and skeletal muscle-wasting that could be attenuated by pre-treatment with a specific monoclonal antibody. Furthermore, the 24 KDa proteoglycan was isolated from the urine of weight-losing cancer patients, but not from the urine of weight-stable cancer patients and of weight-losing patients suffering from various non-malignant diseases (181).

3.3 Increased Cori cycle activity

In cancer cachexia, there is evidence of an increased production of lactate. Although this may in part be the result of an increased anaerobic glycolysis in the growing tumor, another mechanism that seems to be involved is an increased lactate formation in the peripheral tissues, skeletal muscle in particular. Both insulin-resistance (182) and cytokine-activity (96,97) have been postulated as possible causes of this peripheral lactate formation. This lactate is reconverted into glucose in the liver by the so-called Cori cycle. Holroyde *et al.* observed a strongly increased Cori cycle activity in weight-losing cancer patients in comparison with a weight-stable cancer control group, and showed that in the former group the Cori cycle accounted for 43% of the glucose turnover, as opposed to 18% (the expected normal value) in the weight-stable group (183). As the Cori cycle is a very energy-inefficient process, it was postulated that its increased activity may importantly add to the hypermetabolism often seen in cancer (183).

3.4 Decreased food intake

Few controlled studies have been performed in man to investigate the incidence of cancer-related reduction of food intake. Costa *et al.* found, in a large study in ambulatory patients with a variety of tumor types, a significantly decreased energy intake in the male, but not in the female, patients (6). From their data could be deduced that 71% of the male patients suffered from lung cancer. Unfortunately, no such information was available on the female subgroup. In this subset of male lung cancer patients, however, no correlation between food intake and weight-loss could be demonstrated. In another controlled study in weight losing cancer patients, Warnold *et al.* observed that energy intake, although varying strongly, was on average normal (184). In a recent study by Staal-van den Brekel *et al.* in newly-detected lung cancer patients (3), a significantly lower energy intake was observed in the patients who lost more than 10% of their pre-illness weight than in those who lost less than 10%.

Plausible explanations for the relative lack of studies investigating the presence of a decreased food intake in human cancer may be the methodological difficulties associated with human diet analysis, the need for relatively large patient- and control-groups, and the fact that (reliable) blinding is nearly impossible.

However, the fact that a decreased food intake is nevertheless regarded as an important pathophysiological mechanism of cancer cachexia seems to be justified by the results of many studies in animal models (185).

3.4.1 ANOREXIA

The main cause of a decreased food intake is loss of appetite, generally indicated as anorexia. Several mechanisms may be involved in its development: psychological factors (186), anti-neoplastic therapy (186,187), alterations in taste (188,189), delayed gastric emptying leading to early satiety and abdominal fullness (189-191), and learned food aversions (192). Taste changes and abdominal fullness were for example reported in approximately 60% of patients with non-small-cell lung cancer (189). The particular causes of delayed gastric emptying are not clear, but might be related to circulating cytokines (91,92) or the presence of autonomic neuropathy (193). Also learned food aversions have been associated with cytokine-activity (194).

3.4.1.1 Disturbed hypothalamic appetite regulation

In addition to the above, and probably most prominent, anorexia may be the result of abnormalities on the level of hypothalamic appetite regulation, a complex mechanism in which several interacting, locally released hormones play an important role. The most important are serotonin and corticotropin-releasing factor, both having a negative effect on appetite, and neuropeptide Y, having a strongly positive influence (195,196). In both animal- and human cancer, substantial evidence has been obtained that a direct relationship exists between anorexia and the presence of an increased hypothalamic activity of serotonin (90). In addition, hypothalamic neuropeptide Y was shown to be significantly lower in cachectic sarcoma-bearing rats than in their non-tumour-bearing, pair-fed counterparts (197). Furthermore, in experimental cancer anorexia also major alterations in neuropeptide Y receptor mechanisms were reported, such as the absence of compensatory up-regulation and the presence of a decreased receptor affinity (198).

To what extent, and by what mechanisms, tumor- or host-derived cytokines are responsible for these disturbances in hypothalamic appetite regulation has to be further determined. There are however ample reasons to believe that particularly IL-1 plays an important role. In a recent paper, most of the available evidence concerning the relationship between cancer anorexia and increased hypothalamic activity of serotonin has been reviewed, and the well-founded hypothesis was generated that, on the one side, an increased peripheral production of IL-1 stimulates hypothalamic serotonin synthesis by facilitating the supply of its precursor tryptophan, and that, on the other side, an increase in centrally acting IL-1 (due to an increased amount of peripheral IL-1 crossing the blood-brain barrier and/or an increased local IL-1 production) leads to an enhanced hypothalamic neuronal firing rate and serotonin release (90). In addition, other

investigators reported the potential of IL-1 to induce anorexia in rats by increasing hypothalamic production of corticotropin-releasing factor (89).

A recently discovered, peripherally produced hormone involved in the regulation of hypothalamic neuropeptide Y is the adipocyte *ob* gene product leptin (199-201). As there is evidence from studies in *in vitro* and *in vivo* animal models that *ob* gene expression may be up-regulated by substances like cortisol and pro-inflammatory cytokines such as TNF and IL-1 (202-205), particularly the possibility of high leptin levels playing a role in infection- and malignancy-related anorexia and cachexia has recently been hypothesized (204,205).

3.4.2 OTHER CAUSES OF A DECREASED FOOD INTAKE

Other factors than anorexia that might result in a decreased food intake are nausea and vomiting (186,187,206), and tumor- or treatment-related obstruction of the digestive tract (187). A decreased food absorption may be the result of tumor-, inflammation-, treatment- or malnutrition-induced intestinal malabsorption (62,187,207,208).

4 TREATMENT

At the time of the design (1990) of our medroxyprogesterone acetate studies, described in this thesis, few studies were published in literature dealing with the treatment of human cancer cachexia, and even fewer, if any, reported a therapeutical regimen that proved to be effective and feasible. Ever since, the pathophysiology of cancer cachexia has been more and more unravelled, and an increasing number of treatment-related papers has been published. What probably did not increase, however, was the number of effective treatments. The majority of therapies and drugs investigated in the past decades are reviewed below.

4.1 Enteral and parenteral nutrition

Dietary counselling, the administration of enteral tube feedings, and total parenteral nutrition (TPN) are three putative alternatives to ameliorate weight loss through an increase in food intake. However, studies investigating the effects of dietary counselling were reported to show no effect (209).

In patients with a functional gastrointestinal tract, the supplementation of nasogastric or gastrostomy tube feedings may be considered. In a study by Bennegard *et al.*, weight

losing cancer patients treated with nasogastric tube feedings for two weeks showed a significant increase in weight, nitrogen balance and (total-body-K⁴⁰-determined-) body-cell-mass, despite a significant decrease in spontaneous food intake (210). In a comparable study by Dresler *et al.*, although weight did not change significantly, a decrease in whole body protein breakdown, a reversal of negative nitrogen balance and a decrease in gluconeogenesis were observed (211). However, a drawback to enteral tube feeding remains the considerable distress to the patient in case of long-term treatment (212).

In case of TPN, efficacy has not been unequivocally proven, despite many studies (212,213). Its use is therefore not recommended, also in view of the practical disadvantages and the increased risk of complications. The only two exceptions probably are patients with operable gastrointestinal cancers to reduce surgical morbidity and mortality, and cachectic patients unable to eat or to use their gastrointestinal tract while still having a reasonable life expectancy (212).

4.2 Corticosteroids

More than 20 years ago, corticosteroids were the first drug hypothesized to be effective in the treatment of cancer cachexia. In the meantime their anti-inflammatory action has become well recognized, including the suppression of TNF production (214). Although in several randomized placebo-controlled studies it was shown that corticosteroids induce a -usually temporary- increase in appetite (215-218) and improve quality of life (217,218), in none of these studies a beneficial effect on weight was observed. As these agents also have well-established proteolytic properties (114-117) and their prolonged use is associated with considerable side effects, few arguments remain for their use in the treatment of cancer cachexia. Probably the only indication for (short-term) corticosteroid treatment is the attempt to improve quality of life in the end-stage phase of cancer.

4.3 Cyproheptadine

As there is evidence that cancer-induced anorexia is associated with increased serotonergic activity in the brain (90), it might in theory be improved by serotonergic blockade. One of the drugs that could be used in this regard is cyproheptadine, a histamine-antagonist with anti-serotonergic properties. However, in a placebo-controlled clinical trial in 293 cancer patients, cyproheptadine after a median treatment duration of 36 days only induced a slight improvement of appetite without a significant effect on body weight (219). Other anti-serotonergic drugs have not been investigated to date.

4.4 Hydrazine sulfate

In a study in rats, published in 1970, hydrazine sulfate was shown to inhibit hepatic gluconeogenesis by the inhibition of the enzyme phosphoenolpyruvate-carboxykinase (220). This led to the hypothesis that hydrazine sulfate might be effective in the treatment of cancer cachexia. Presently, however, several placebo-controlled clinical trials have failed to observe any beneficial effect on appetite or body weight (221-224).

4.5 Synthetic progestagens

Synthetic progestagens -megestrol acetate (MA, 17 α -acetoxy-6-methyl-4,6-pregnadione-3,20-dione) and medroxyprogesterone acetate (MPA, 17 α -acetoxy-6- α -methyl-4-pregnene-3,20-dione)- have been shown to improve appetite and weight, independent of tumor response, when used in the treatment of disseminated breast cancer (225,226). This led to the hypothesis that synthetic progestagens could also stimulate weight gain in patients with non-hormone-sensitive tumors. To date, our own studies not included, the results of nine double-blind placebo-controlled trials investigating the effects of synthetic progestagens on appetite and weight in non-hormone-sensitive cancer have been published: eight using MA (227-234) and one using MPA (235). As a lack of statistical power impedes a reliable interpretation of the results of one of the MA studies (231) and the only MPA study, only the remaining studies (all with MA) will be discussed below.

In 1990, Bruera *et al.* (227) reported the results of a cross-over study with MA 480 mg/day for one week. Forty patients were entered, 31 were evaluable. A statistically significant beneficial effect on appetite, caloric intake, weight, triceps skinfold thickness and subjective energy level was reported in the MA group. However, the clinical significance of these findings is unclear given the very short duration of the study.

Also in 1990, Loprinzi *et al.* (228) published the results of a parallel-design study in 133 patients (105 evaluable), treated with MA 800 mg/day for a median duration of 7 weeks. A significant, favorable effect on appetite was found (appetite increase in 64% of MPA patients versus 42% of placebo), as well as on weight (an increase of at least 15 lb [=6.8 kg] in 16% of the MA-treated patients versus 2% in the placebo-treated group). However, mean (rate of) weight change was not significantly different between the two groups. A beneficial effect of MA on nausea and vomiting was reported. The only side effect observed was the development of edema (29% MA, 13% placebo).

In 1992, Feliu *et al.* (229) reported the results of a parallel-design study in 150 patients (128 evaluable), treated with 240 mg MA per day for a duration of 8 weeks. Appetite improved significantly in the MA group, and an increase in weight of at least 2 kg was observed in 32% of the MA-treated patients versus 8% in the placebo group

($P < 0.01$). Average weight, however, did not improve in the MA group. No significant side effects were reported.

Also in 1992, Tchekmedyan *et al.* (230) published the results of a parallel-design study in 89 patients (67 evaluable), treated with 1600 mg MA per day for a duration of 4 weeks. Appetite improved significantly in the MA group, but the difference in weight change (MA +1.4 kg, placebo -0.2 kg) did not reach statistical significance. Also in this study, no significant side effects were reported.

In 1994, McMillan *et al.* (232) reported a study in 38 gastrointestinal cancer patients (21 evaluable) with MA 480 mg/day for 12 weeks. These patients were characterized by severe weight loss (median $\approx 20\%$ of the pre-illness weight). In the MA-treated patients, no beneficial effect on weight was observed. The effect on appetite was not reported.

In 1996, Rowland *et al.* (233) published a study with MA 800 mg/day for a maximum of two years in 243 chemotherapy-treated small-cell lung cancer patients. In this study, weight-gain $\geq 10\%$ was observed in 21% of patients in the MA group, versus 7% in the placebo group ($P = 0.004$). Average (rate of) weight change was not stated. There was also less anorexia, nausea and vomiting in the MA group. Reported MA side effects were edema (MA 30%, placebo 20%) and chemotherapy-associated thromboembolic complications (MA 9%, placebo 2%). Survival was not different between the two treatment groups.

In 1997, Beller *et al.* (234) reported the results of a randomized, placebo-controlled multicenter study investigating the effects of two different doses of MA (160 mg and 480 mg per day) on quality of life (investigated with six linear analogue self-assessment scales) and nutritional status (weight, triceps skinfold, mid-arm circumference). Two-hundred-forty patients were randomized, 148 were fully evaluable after 4 weeks, 83 after 12 weeks. After 4 weeks, in the high-dose group, but not the low-dose group, a beneficial effect of MA was observed on appetite, mood and "overall quality of life", and a trend was observed towards a beneficial effect on nausea and vomiting. Fifty percent of these patients were however concomitantly treated with chemotherapy, and the beneficial effect on quality of life was predominantly observed in this subgroup. In none of the MA groups an improvement of nutritional parameters was seen. One lung-embolism and four cases of edema were reported in the low-dose MA group.

In addition to the above placebo-controlled studies, an open, non-controlled study with MA in 12 weight-stable, non-anorectic breast cancer patients (11 females, 1 male) revealed that in those patients who gained weight ($n = 7$) this weight gain was predominantly the result of an increase in DEXA-derived fat mass (236).

As can be derived from the above studies, synthetic progestagen treatment is

generally well tolerated in advanced-stage cancer patients. The most frequent side effect is the development of peripheral edema, although in one of these studies, using a relatively high synthetic progestagen dose, also an increased incidence of thromboembolic complications was reported. Other side effects, although not observed in the studies reviewed above, might be related to the fact that synthetic progestagens, unlike progesterone, have a certain degree of glucocorticoid-like activity (237), which is also translated into decreased circulating levels of cortisol (238,239). However, the development of diabetes mellitus, Cushing's syndrome or a withdrawal response have only been reported incidentally (240-242), mostly after a treatment duration of several years (241,242). Other corticosteroid-related toxicities, such as myopathy, striae, opportunistic infections, femoral head necrosis or peptic ulcer disease, have not been described (239). Also hepatic toxicity has rarely been documented (243). As testosterone levels may decrease during synthetic progestagen treatment (244), a further potential side effect is impotence. Also this complication, however, has seldom been reported to date.

It is well known that the ovarian steroids estradiol and progesterone, and particularly their ratio and interaction, affect energy balance and fuel utilization in a wide range of mammals in the reproductive phase, including women (for a comprehensive review see reference [245]). Both hormones were shown to have direct effects on hypothalamus, liver and adipose tissue, although substantially more evidence exists on estradiol than on progesterone. In rats, for example, adipose tissue LPL has been shown to be inhibited by estradiol and stimulated by progesterone (246). When circulating estradiol levels are high, energy intake decreases, energy expenditure increases, and body fat stores are mobilized to make up the difference between intake and expenditure. When estradiol levels are low or the progesterone:estradiol ratio is elevated, energy intake increases, expenditure decreases, and fat accretion occurs (245). That appetite stimulation is not the only mechanism by which progestagens induce weight gain was illustrated by a study in progesterone-treated rats that, though pair-fed, gained significantly more fat than the non-treated control group (247). Importantly, however, it was also shown in several studies that the absence of estradiol largely prevents the metabolic effects of progesterone (245). In a study reported in 1967 by Hervey and Hervey, for example, female rats gained weight during progesterone treatment, while their male counterparts did not (248). Considering these findings, the mechanisms by which synthetic progestagens improve appetite and weight in males and post-menopausal females with cancer remains unclear. Probably the fact that in cancer cachexia relatively high supra-physiological doses of progestagens were used might form an explanation. As far as MA is concerned, it has been shown to stimulate the hypothalamic synthesis, transport and release of neuropeptide

Y in rats (249), and to directly stimulate adipocyte differentiation *in vitro* (250).

4.6 Metoclopramide

As many cancer patients have a delayed gastric emptying that might negatively influence food intake (189-191), it has been postulated that the prokinetic agent metoclopramide may have a place in the treatment of cancer cachexia. In a non-controlled study in 10 patients with non-obstructing upper-gastrointestinal cancer having complaints of delayed gastric emptying, three days of metoclopramide resulted in an objective increase in gastric emptying in 6 out of 7 patients with severe gastroparesis on gastric technetium passage (190). To date, however, there are no placebo-controlled studies that confirm its possible value in the treatment of cancer-related anorexia and cachexia.

4.7 Cannabinoids

More than two decades ago, dronabinol (delta-9-tetrahydrocannabinol, the active ingredient of marihuana), was shown to have a positive effect on appetite and chemotherapy-induced nausea (251,252). In a recent, large, randomized placebo-controlled study in AIDS, dronabinol was shown to improve appetite, but *not* weight. Furthermore, an important disadvantage was the relatively high frequency of side effects, such as euphoria, dizziness, thinking abnormalities and somnolence (253). In cancer, presently no placebo-controlled studies are available.

4.8 Pentoxifylline

Pentoxifylline, a methylxantine derivative approved for treatment of intermittent claudication, has been shown to inhibit TNF production *in vitro* and *in vivo* (214,254-256). However, a recent double-blind, placebo-controlled study was not able to show any beneficial effects on appetite or weight (257). Also in AIDS-cachexia, pentoxifylline proved to be non-effective in this regard (258).

4.9 Non-steroidal anti-inflammatory drugs (NSAID's)

The NSAID ibuprofen, an inhibitor of the enzyme cyclo-oxygenase, has been shown to attenuate hypermetabolism and hepatic acute-phase protein production in weight-losing patients suffering from pancreatic cancer (259). Its mechanism of action is not fully understood. It might be that ibuprofen reduces the production of the pro-inflammatory cytokines and/or interferes with their prostaglandin-mediated end-organ effects, such as the production of acute-phase proteins by the liver. In an *in vitro* study, in which rat

diaphragm muscles were incubated with an ultrafiltrated fraction of plasma or serum of cancer patients suffering from severe weight loss, proteolysis was significantly inhibited by indomethacin (260). Presently, in human cancer no studies are available that report the effects of NSAID's on body weight, body composition, quality of life or survival.

8.10 N-3 fatty acids

In healthy volunteers, the supplementation of n-3 polyunsaturated fatty acids, interfering with the cyclo-oxygenase and lipo-oxygenase pathways, was shown to inhibit TNF and IL-1 production by peripheral blood mononuclear cells (261). In a study in colorectal cancer patients, long-term treatment with a combination of eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and γ -linolenic acid resulted in a significant decrease in serum levels of IL-1, IL-6, TNF α and interferon- γ (262). In mice bearing the cachexia-inducing MAC16 tumor, known to produce (non-cytokine-) lipid- and protein mobilizing factors, treatment with EPA inhibited weight loss with evidence of direct anti-lipolytic and anti-proteolytic effects on respectively adipocytes and skeletal muscle (263). The latter was associated with a decrease in muscle prostaglandin E₂ concentration (263). In another animal study it was shown that n-3 fatty acids are able to attenuate IL-1-induced anorexia (264). Based on an *in vitro* experiment, it was hypothesized that the underlying mechanism of action was also in this case an attenuation of prostaglandin E₂ production (264).

Recently, two interesting papers were published by the same group, reporting the effects of n-3 fatty acid treatment in weight-losing patients with unresectable pancreatic cancer. In the first study, a phase-I study in 18 patients with a median weight loss rate of 2.9 kg/month, it was shown that oral supplementation with fish oil capsules (18% EPA, 12% DHA) for a median duration of 3 months led to a significant median weight gain of 0.3 kg/month. These changes were accompanied by a temporary, but significant, reduction in acute-phase protein production (265). In the second study, it was observed that four weeks of treatment with EPA resulted in a significantly decreased potential of lipopolysaccharide-stimulated peripheral blood mononuclear cells to produce IL-6, and in decreased hepatic production of acute-phase proteins, as measured by the serum levels of C-reactive protein (266).

8.11 Growth hormone and IGF-I

Growth hormone has been shown to have strong positive effects on nitrogen balance and protein mass in a variety of clinical and physiological situations (267). Most of its anabolic effects are mediated by IGF-I, produced by the liver (123,268). In a rat sarcoma

model, however, human growth hormone treatment failed to attenuate lean-body-mass-wasting (121). As in this study no rise in IGF-I levels was observed, it was hypothesized that the negative result of the study was due to hepatic growth hormone-resistance. In contrast, however, another group later reported in a study in doxorubicin-treated sarcoma-bearing mice an increase in IGF-I as well as a preservation of muscle mass as a result of growth hormone administration (269). In a pilot study in ten cancer patients, three days of growth hormone treatment significantly increased plasma IGF-I levels and attenuated urinary nitrogen losses. A significant improvement of nitrogen balance was however only observed in the patients who were not manifestly cachectic (weight >90% of ideal, $n=7$) (270). In a non-controlled study in 4 weight-losing AIDS patients treated with pharmacological growth hormone doses for a period of three months, significant increases in IGF-I levels, body weight and lean body mass were observed, as well as a decrease in fat mass. There were trends towards improvement in muscle strength and endurance (271). However, whether or not effective, a major drawback to the use of growth hormone in studies and clinical practice remains the high costs of the treatment.

As far as IGF-I is concerned, decreased levels were reported in several experimental models of cancer cachexia (121,122). In another study it was shown that IGF-I bioactivity was inhibited by TNF, IL-1 and IL-6 (124). Decreased concentrations of IGF-I, despite elevated levels of growth hormone, have also been reported in several studies in non-cancer-related malnutrition (272). In general, these low IGF-I concentrations have been assumed to be the result of a decreased protein intake (273). Several studies showed a beneficial effect of IGF-I treatment on (non-cancer) starvation-induced weight loss and protein catabolism (274-276). In an animal model of cancer cachexia, the continuous subcutaneous administration of IGF-I resulted in an attenuation of weight loss and muscle-wasting, without influencing tumor growth (277). In another model, however, no beneficial effect on weight loss was observed (122). It was hypothesized that in the latter model the lack of efficacy might have been due to high circulating levels of IL-6. In human cancer cachexia, the effects of IGF-I have not been tested to date. In a study in weight-losing AIDS patients, however, 10 days of iv administration of rIGF-I only resulted in a temporary increase in nitrogen retention (278).

8.12 Anabolic steroids

The anabolic effects of androgenic steroid hormones, particularly when combined with exercise programmes, are known for decades (279). Although in other wasting diseases beneficial effects of anabolic steroids on weight, muscle mass and/or performance status have been reported, few studies have been performed in cancer to date. In a study with

high-dose nandrolone propionate in weight-losing sarcoma-bearing rats, an increase in weight was observed that was entirely the result of fluid retention, while protein mass was not influenced (280). In a controlled study in 37 chemotherapy-treated non-small-cell lung cancer patients, 4 weeks of nandrolone decanoate failed to influence weight significantly (281). In chronic obstructive pulmonary disease, however, a placebo-controlled study in 217 patients showed a beneficial effect of 8 weeks nandrolone decanoate on fat-free mass and inspiratory mouth pressure (282). In a non-controlled study in 17 highly selected, weight-losing AIDS patients, a beneficial effect of nandrolone decanoate was observed on weight and fat-free mass (283).

5 AIM OF THE THESIS

The aim of the thesis was to clarify the following issues in the pathophysiology, diagnosis and treatment of cancer cachexia:

1. The relationship between both weight loss and body-cell-mass-wasting, and systemic inflammation, increased acute-phase response, anorexia, hypermetabolism and changes in circulating levels of catabolic (cortisol) and anabolic (testosterone, IGF-I) hormones in human lung cancer. *Chapter 2*
2. The relationship between total plasma leptin, weight loss, body composition, appetite and resting energy expenditure in lung cancer. *Chapter 3*
3. The applicability of single-frequency (50 kHz) bioelectrical impedance analysis to predict total body water in underweight cancer patients. *Chapter 4*
4. The applicability of single-frequency bioelectrical impedance analysis to predict *changes* in total body water in normal-weight and underweight cancer patients. *Chapter 5*
5. The effects of medroxyprogesterone acetate on appetite, weight and quality of life in patients with advanced-stage, non-hormone-sensitive cancer. *Chapter 6*
6. The effects of medroxyprogesterone acetate on food intake, body composition and resting energy expenditure in patients with advanced-stage, non-hormone-sensitive cancer. *Chapter 7*

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***WEIGHT LOSS AND BODY-CELL-MASS-WASTING IN HUMAN LUNG CANCER:
THE RELATIONSHIP WITH SYSTEMIC INFLAMMATION, ACUTE-PHASE
RESPONSE, RESTING ENERGY EXPENDITURE, SERUM TESTOSTERONE AND
INSULIN-LIKE GROWTH FACTOR I***

**Weight loss and body-cell-mass-wasting in human lung cancer:
the relationship with systemic inflammation, acute-phase response, resting energy
expenditure, serum testosterone and insulin-like growth factor I**

Submitted

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ABSTRACT

Purpose: To explore, in human lung cancer, the role of systemic inflammation, acute-phase response, anorexia, hypermetabolism and changes in anabolic and catabolic hormones in the pathophysiology of cachexia and, in particular, body-cell-mass(BCM)-wasting.

Patients and Methods: In twenty male lung cancer patients, pre-stratified by weight loss $\geq 10\%$ ($n=10$) or weight loss $<10\%$ ($n=10$), the following measurements were performed: BCM (DEXA/bromide-dilution), circulating levels of soluble TNF-receptors sTNF-R55 and sTNF-R75, interleukin-6, LPS-binding protein (LBP), albumin, appetite (0-10 scale), resting energy expenditure (REE, indirect calorimetry), and circulating levels of catabolic- (cortisol) and anabolic- (testosterone, insulin-like growth factor I [IGF-I]) hormones.

Results: Compared to the patients with weight loss $<10\%$, those with weight loss $\geq 10\%$ were characterized by higher sTNF-R55 levels (trend towards significance: $P=0.06$), and lower levels of albumin (27.4 vs 34.4 mmol/L, $P=0.02$), testosterone (13.2 vs 21.5 nmol/L, $P=0.01$) and IGF-I (119 vs 184 ng/mL, $P=0.004$). In the patient-group as a whole, the percentage of weight loss was significantly correlated with sTNF-R55 ($r=0.59$, $P=0.02$), albumin ($r=-0.63$, $P=0.006$) and IGF-I ($r=-0.50$, $P=0.02$). Height-adjusted BCM was significantly correlated with sTNF-R55 ($r=-0.57$, $P=0.03$), sTNF-R75 ($r=-0.50$, $P=0.04$), LBP ($r=-0.50$, $P=0.04$), albumin ($r=0.56$, $P=0.02$) and REE/BCM ($r=-0.54$, $P=0.03$), and there was a trend toward a correlation with IGF-I ($r=0.44$, $P=0.06$).

Conclusion: Human lung cancer-related weight loss and BCM-wasting are associated with systemic inflammation, increased acute-phase response and reduced levels of IGF-I. A decreased BCM was also related to hypermetabolism. Reduced testosterone levels were present in the patients with severe weight loss, although a significant direct association with a decreased BCM was lacking.

INTRODUCTION

Substantial involuntary weight loss, eventually leading to cachexia, is a frequent complication of cancer that is associated with an increased morbidity and mortality (1). Several studies have shown that both wasting of fat mass and body-cell-mass (BCM) contribute to this weight loss (2). As nearly all tissues that are vital for a normal

functioning of the body are part of the BCM (3), it is evident that particularly wasting of this body compartment can be expected to be responsible for the negative effects of weight loss on performance status and survival. Nevertheless, few studies in cancer specifically addressed this issue to date. In AIDS, however, the specific relationship between BCM-wasting and a shortening of survival has been clearly established (4).

If BCM-wasting is indeed an important determinant of performance status and survival in cancer, it obviously should become a key target for therapeutical intervention. To develop such specific treatment strategies, however, it is mandatory to have as much insight as possible in its underlying pathophysiology. Growing evidence exists that the development of cancer cachexia is for an important part related to a chronic, low-grade, tumor-induced activation of the host immune system (5) that, though in a mitigated form, shares several characteristics with the so-called acute-phase response seen after major trauma and sepsis, a state characterized by an increased production of cytokines (6), elevated levels of catecholamines, cortisol and glucagon (6-8), increased peripheral amino acid mobilization and hepatic amino acid uptake (6,9), enhanced hepatic gluconeogenesis and acute-phase protein production (6,10,11), increased mobilization of free fatty acids (12) and hypermetabolism (10).

Nearly all the evidence on which this view is based, however, has come from studies in experimental models (13-19). Relatively few confirmatory data came from studies in human cancer, and particularly the specific pathophysiology of cancer-related BCM-wasting has hardly been investigated in humans to date.

To address these issues, we investigated in a group of male lung cancer patients the relationship between both weight loss and BCM-wasting, and the putative presence of systemic inflammation, increased acute-phase response, anorexia, hypermetabolism and changes in circulating levels of several catabolic and anabolic hormones. In addition, to obtain more insight in the specific clinical relevance of cancer-related BCM-wasting, the relationship between height-adjusted BCM and Karnofsky performance status was assessed.

PATIENTS AND METHODS

Patients

Twenty newly-diagnosed male patients with cytologically or histologically proved untreated lung cancer were entered onto the study: ten patients with weight loss greater than 10% of the usual weight, and ten patients with weight loss less than 10% of the

usual weight. Patients were excluded if suffering from diabetes mellitus, hypo/hyperthyroidism, manifest infection or fever. The protocol was in accordance with the Declaration of Helsinki (1989), and informed consent was obtained from all patients.

Body composition

Bone mineral mass, fat mass, and lean soft tissue mass were measured in the morning, in the fasted state, by dual-energy x-ray absorptiometry (DEXA) with a total body scanner (model DPX-L; Lunar Corporation, Madison, Wisconsin, USA) that uses a constant potential x-ray source at 76 kVp and a K-edge filter (cerium) to achieve a congruent beam of stable dual-energy radiation with effective energies of 38 and 70 keV. The principles of dual-energy determination of body compartments, as well as of subject positioning and scan procedures for the scanner used, have been extensively described elsewhere (20,21). Scans were made in the fast speed mode. Baseline calibration of the scanner was performed daily against the standard calibration block supplied by the manufacturer. Based on the relative attenuations of the two energies through bone and soft tissue, bone mineral mass and soft tissue mass were calculated by the software provided by the manufacturer. As the ratio of beam attenuation at the lower to higher energy (R_{st}) is inversely and linearly related to percent fat, the latter can be derived directly from R_{st} . Fat mass was calculated by multiplying percent fat by the assessed soft tissue mass. The precision error (1 SD) of the DEXA measurement for fat mass has been reported to be 1.0 kg in healthy individuals (21). In a study in pigs, in which fat mass was assessed by DEXA as well as by chemical analysis after postmortem homogenization, bias was small ($r = 0.99$) (22).

Extracellular water (ECW) was estimated by bromide dilution. The following protocol was used. The evening before body composition assessment, at 10.00 p.m., all patients drank a weighted dose of sodiumbromide (60 mg Br/L-estimated-total-body-water) in 50 mL tap water. Then the bottle was rinsed once with ≈ 50 mL tap water, which was also drunk. Thereafter, the patients had to refrain from eating and drinking. Before and 10 hours after drinking the bromide water, a venous blood sample was taken. Bromide concentration in serum ultrafiltrate was determined with high-performance liquid chromatography according to the anion-exchange chromatographic method (23). ECW was estimated by the corrected bromide space (CBS) and calculated according to the following formula (23):

$$\text{CBS (L)} = (\text{Br dose (mmol)} / [\text{Br}_f] - [\text{Br}_b] \text{ (mmol/L)}) \times 0.90 \times 0.95$$

where $[\text{Br}_b]$ is the background bromide concentration in serum ultrafiltrate, $[\text{Br}_f]$ is the final bromide concentration in serum ultrafiltrate, 0.90 is the correction factor for the

distribution of bromide in the non-extracellular sites, and 0.95 is the correction factor for the Donnan equilibrium. The use of this test protocol with an overnight equilibration period of 10 hours has been extensively validated in our laboratory (24).

BCM was defined as the difference between DEXA-derived lean soft tissue mass and bromide dilution-derived ECW. In this model, BCM will be slightly over-estimated (≈ 0.5 kg) as no correction was made for non-bone extracellular solids (25). To correlate BCM with other variables, BCM was adjusted for height by calculating the BCM-index (BCM-ix, $\text{BCM}/\text{height}^2$).

Appetite, weight and performance status

Appetite was measured by self-assessment numerical rating scale ranging from 0 to 10: 0 indicating absolutely no appetite and 10 indicating an extremely good appetite. Weight was measured in the morning, in the fasted state, after voiding and without clothing and shoes, to the nearest 0.1 kg by using a beam scale (SECA, Hamburg, Germany). Performance status was assessed by determining the Karnofsky Performance Status score (26). This was performed in all patients by the same experienced physician.

Resting energy expenditure

Resting energy expenditure (REE) was assessed by indirect calorimetry by using a ventilated hood system (Oxycon β , Mijnhardt, Bunnik, The Netherlands). After an overnight fast, CO_2 production and O_2 consumption were measured at complete rest during a period of 20 minutes. REE was calculated by using the abbreviated Weir formula (27). The equipment was calibrated at the start of each experiment. The precision of the system was checked monthly by burning methanol with a theoretical respiratory quotient of 0.667 after complete combustion. Further details on the technique used in our laboratory have been described elsewhere (28). To assess between-group differences in REE and to correlate REE with other variables, REE was adjusted for BCM (REE/BCM), as BCM is the body compartment mainly determining the level of metabolic activity.

Plasma and serum samples

After an overnight fast, blood was collected by venepuncture at 8.00 a.m. in evacuated EDTA blood collection tubes (Sherwood Medical, Ballymoney, N. Ireland) and evacuated integrated serum separator tubes (Sherwood Medical, St. Louis, USA). The EDTA tubes were placed on ice-water and directly centrifuged for 5 minutes in a refrigerated (4°C) table top centrifuge at 3000 g. Plasma samples were immediately stored at -70°C until

analysis. After full coagulation, the serum separator tubes were also centrifuged for 5 minutes at 3000 g. Serum samples were immediately stored at -20°C until analysis.

Soluble tumor-necrosis-factor(TNF)-receptors and Interleukin(IL)-6

To assess systemic inflammation, plasma levels of soluble TNF receptors R55 and R75 (29) and IL-6 were measured by sandwich enzyme-linked immunosorbent assay (ELISA), as described elsewhere (30,31). In short, for measurement of sTNF-R55 and sTNF-R75, monoclonal antibodies (Mab) MR1-1 and MR2-2 were used for coating, respectively. Specific biotin-labelled polyclonal rabbit anti-human-sTNF-R immunoglobulin(Ig)G's were used as detector reagents. The standards used were recombinant human sTNF-R55 and sTNF-R75. For both assays the limit of detection was 100 pg/mL. In the IL-6 ELISA, Mab 5E1 was used as coating, polyclonal rabbit antihuman IL-6 antibodies as detecting reagents. The standard used was recombinant human IL-6. IL-6 could be detected with a lower detection limit of 0.02 ng/mL.

LPS-binding protein (LBP) and albumin

To assess hepatic acute-phase response, LBP (32,33) and albumin were measured in plasma and serum respectively. LBP was measured by sandwich ELISA, as described elsewhere (34). In short, polyclonal rabbit anti-human recombinant LBP IgG was used as coating, and biotin-labelled polyclonal rabbit antihuman recombinant LBP IgG was used for detection. The standard used was recombinant LBP. Washing and dilution was performed in buffer that contained 40 mmol/L magnesium chloride to prevent disturbance by LPS of LBP recovery in the ELISA. The limit of detection was 200 pg/mL. Albumin was measured by using the Bromocresol purple (BCP) method on the Synchron CX-7 (Beckman, Mijdrecht, The Netherlands).

Catabolic and anabolic hormones

Cortisol, testosterone, insulin-like growth factor-I (IGF-I) were measured in serum. Cortisol was measured by solid-phase chemiluminescent immunoassay (Immulite® cortisol, Diagnostic Products Corporation, Los Angeles, CA). The limit of detection was 5.5 nmol/L. Testosterone was measured by solid-phase ¹²⁵I radioimmunoassay (Coat-A-Count® total testosterone, Diagnostic Products Corporation). The limit of detection was 0.14 nmol/L. Total IGF-I was measured by immunoradiometric assay (Nichols Institute Diagnostics, San Juan Capistrano, CA). For this assay, the limit of detection was 6 ng/mL.

Statistical analysis

Results are given as score mean $\pm$ SD. Between group means were compared by using one-way analysis of variance (ANOVA). Differences in categoric variables were analyzed by χ^2 test with Yates' continuity correction or Fisher's exact test, where appropriate. Pearson's product-moment correlation coefficients were used to evaluate linear relationships between variables. As sTNF-R levels are linearly related with serum creatinine (35,36), between group differences in sTNF-R levels were analyzed in a covariance model (ANCOVA) using serum creatinine as covariable. Also in the analysis of linear relationships between sTNF-R's and other variables, the assessed correlation coefficients were partial correlation coefficients after adjustment for serum creatinine as covariable. As BCM decreases with age (3), also in the analysis of linear relationships between BCM-ix and other variables the assessed correlation coefficients were partial correlation coefficients after adjustment for age as covariable. For all analyses the statistical package SPSS/PC+ (version 6.0-Windows; SPSS Inc., Chicago, IL) was used. Statistical significance was determined at the 5% level, two-sided.

RESULTS

In **Table 2.1**, the patients with weight loss $\geq 10\%$ are compared with the patients with weight loss $< 10\%$. Between the two groups, there were no significant differences in age, tumor stage, tumor histology, smoking behaviour, prevalence and severity of chronic obstructive pulmonary disease, use of anti-inflammatory medication, or body temperature. Although both fat mass and BCM were lower in the group with severe weight loss, the between-group differences did not reach statistical significance. The patients with weight loss $\geq 10\%$ were characterized by higher sTNF-R55 levels (trend towards significance: $P=0.06$), and lower levels of albumin (27.4 vs 34.4 mmol/L, $P=0.02$), testosterone (13.2 vs 21.5 nmol/L, $P=0.01$) and IGF-I (119 vs 184 ng/mL, $P=0.004$).

To further clarify the relationships between the development of weight loss, and the presence of systemic inflammation (sTNF-R55, sTNF-R75 and IL-6), acute-phase response (LBP and albumin), hypermetabolism, anorexia, changes in cortisol (as catabolic hormone), and changes in testosterone and IGF-I (as anabolic hormones), correlation coefficients for these parameters and the percentage of weight loss were assessed in the patient group as a whole. As can be derived from the **upper panel of Figure 2.1**, the development of weight loss was associated with high circulating levels of both soluble TNF receptors and LBP, high BCM-adjusted REE, high levels of cortisol, and low levels

TABLE 2.1

Patient characteristics stratified by weight loss

	Weight loss $\geq 10\%$ ($n=10$)	Weight loss $< 10\%$ ($n=10$)	Between-group <i>P</i>
Age (y)	67 $\pm$ 7 ^a	67 $\pm$ 9	1.00
Tumor stage (<i>n</i>)			
non-small-cell (I-II/III/IV)	2/1/6	0/4/5	0.27
small-cell (limited/extensive)	1/0	1/0	
Histology (<i>n</i>)			
ad/sq/un/sc ^b	2/4/3/1	2/4/3/1	1.00
Active smokers (<i>n</i>)	4	8	0.17
COPD (<i>n</i>) ^c	5	2	0.35
FEV1 (%-pred) ^d	65 $\pm$ 12	73 $\pm$ 17	0.30
Anti-inflammatory drugs (<i>n</i>)			
corticosteroids/nsaids	2/1	1/3	0.49
Body temperature (°C)	35.9 $\pm$ 0.8	36.4 $\pm$ 0.5	0.13
Pre-study weight loss (%)	16.6 $\pm$ 5.0	4.5 $\pm$ 3.9	<0.001
Height (m)	174 $\pm$ 6	175 $\pm$ 5	0.53
Weight (kg)	59.6 $\pm$ 9.4	67.6 $\pm$ 8.9	0.07
Body mass index (kg/m ²)	19.7 $\pm$ 2.6	22.0 $\pm$ 2.7	0.07
Fat mass (kg)	10.2 $\pm$ 6.1	13.9 $\pm$ 6.6	0.21
Bone mineral mass (kg)	2.57 $\pm$ 0.21	2.83 $\pm$ 0.44	0.11
Extracellular water (L)	17.8 $\pm$ 2.3	18.6 $\pm$ 2.2	0.43
Body-cell-mass (kg)	27.5 $\pm$ 4.0	30.0 $\pm$ 2.4	0.11
Appetite (0-10)	5.4 $\pm$ 3.0	6.6 $\pm$ 1.5	0.27
REE/BCM (kcal/d/kg)	58.0 $\pm$ 7.9	53.1 $\pm$ 6.2	0.16
Soluble TNF-receptor 55 (ng/mL)	1.05 $\pm$ 0.41	1.39 $\pm$ 1.06	0.06 ^e
Soluble TNF-receptor 75 (ng/mL)	1.53 $\pm$ 0.64	1.59 $\pm$ 0.97	0.13 ^e
Interleukin-6			
detectability (%)	56	78	0.62
plasma level (ng/mL)	0.15 $\pm$ 0.09	0.15 $\pm$ 0.19	0.67
LPS-binding protein (μ g/mL)	26.5 $\pm$ 15.2	17.5 $\pm$ 7.2	0.14
Albumin (mmol/L)	27.4 $\pm$ 5.7	34.4 $\pm$ 5.4	0.02
Hemoglobin (mmol/L)	8.0 $\pm$ 1.2	8.1 $\pm$ 0.5	0.75
Cortisol (nmol/L)	621 $\pm$ 235	571 $\pm$ 179	0.60
Testosterone (nmol/L)	13.2 $\pm$ 7.5	21.5 $\pm$ 5.6	0.01
Insulin-like growth factor I (ng/mL)	119 $\pm$ 31	184 $\pm$ 55	0.004
Blood urea nitrogen (mmol/L)	6.0 $\pm$ 2.4	7.6 $\pm$ 3.4	0.25
Serum creatinine (μ mol/L)	82 $\pm$ 11	113 $\pm$ 44	0.06
Karnofsky Performance Status (%)	72 $\pm$ 15	82 $\pm$ 12	0.12

^a Mean $\pm$ SD; ^b ad=adeno, sq=squamous-cell, un=undifferentiated large-cell, sc=small-cell; ^c chronic obstructive pulmonary disease; ^d forced expiratory volume in 1 sec.; ^e higher in patients with weight loss $\geq 10\%$ after adjustment for serum creatinine.

FIGURE 2.1 Diagram of the Pearson correlation coefficients for the correlations between both weight loss (upper panel) and body-cell-mass-index (BCM-ix, lower panel) and their putative determinants.

of testosterone, IGF-I and the "negative" acute-phase protein albumin. However, the only correlations reaching statistical significance were those between weight loss and sTNF-R55 ($r=0.59$, $P=0.02$), IGF-I ($r=-0.50$, $P=0.02$) and albumin ($r=-0.63$, $P=0.006$).

As we, in addition, explicitly aimed at identifying factors associated with wasting of BCM, the parameters that were assessed with respect to weight loss were also investigated in relation to BCM-ix. As shown in the **lower panel of Figure 2.1**, the presence of a low BCM-ix was significantly correlated with high levels of sTNF-R55 ($r=-0.57$, $P=0.03$) and sTNF-R75 ($r=-0.50$, $P=0.04$), high BCM-adjusted REE ($r=-0.54$, $P=0.03$), and increased hepatic acute-phase protein production reflected in high levels of LBP ($r=-0.50$, $P=0.04$) and low levels of albumin ($r=0.56$, $P=0.02$). Though having a positive direction, the correlations between BCM-ix and appetite ($r=0.34$), testosterone ($r=0.36$) and IGF-I ($r=0.44$) did not reach statistical significance. In the latter case, however, significance was closely approached ($P=0.06$). BCM-ix was not correlated with IL-6 or cortisol.

As there is evidence from earlier studies that increased hepatic acute-phase protein production, hypermetabolism, anorexia and changes in cortisol and testosterone levels might be the result of systemic inflammation, also the direct correlations between plasma sTNF-R55 and these factors were investigated. In addition, also the correlation with IGF-I was assessed. It is shown in **Figure 2.2** that sTNF-R55 was significantly positively correlated with REE/BCM ($r=0.54$, $P=0.03$) and negatively correlated with albumin ($r=-0.57$, $P=0.03$). Furthermore, there were trends toward a positive correlation with LBP ($r=0.48$, $P=0.06$) and a negative correlation with appetite ($r=-0.45$, $P=0.08$). Although the direction of the correlation with cortisol was positive ($r=0.36$), it did not reach statistical significance. The correlation coefficients for the correlation with testosterone ($r=-0.19$) and IGF-I ($r=-0.22$) were low.

Karnofsky performance score was not significantly correlated with weight loss ($r=0.32$, $P=0.17$), body-mass-index ($r=0.35$, $P=0.13$) or fat-mass-index ($r=0.13$, $P=0.59$). However, the correlation with BCM-ix was clearly significant ($r=0.53$, $P=0.02$), and is visualized in **Figure 2.3**.

DISCUSSION

Substantial involuntary weight loss, eventually leading to cachexia, is a frequent complication of cancer (1). Although this weight loss can be the result of both fat loss and

FIGURE 2.2 Diagram of the Pearson correlation coefficients for the correlations between soluble TNF-receptor sTNF-R55 levels and other determinants of weight loss and body-cell-mass-(BCM)-wasting.

FIGURE 2.3 Plot of the correlation between body-cell-mass-ix and Karnofsky performance status ($r=0.53$, $P=0.02$).

wasting of BCM (2), the present study shows that it is specifically the presence of a low BCM that is associated with a deterioration of performance status. Although the existence of this association has been hypothesized already in 1981 by DeWys *et al.* (37), it has, to our knowledge, not been explicitly confirmed since. In AIDS, however, BCM-wasting has been specifically linked to a decreased survival (4).

In animal models of cancer cachexia, many investigators found evidence of a relationship between the development of weight loss and the presence of an increased inflammatory state, characterized by an increased production of cytokines like TNF, IL-6, interferon- γ and leukemia inhibitory factor (14,16-19). This increased cytokine production has been assumed to be primarily the result of a tumor-induced activation of the host immune system (38,39), although there is evidence that these cytokines can also be produced by the tumor itself (19,40,41). It was also shown that an increased production of particularly TNF and IL-6 was associated with significant wasting of muscle mass, which is the major determinant of BCM (18,42-44). In other studies it was observed that also the exogenous administration of TNF, IL-1 and IL-6 led to proteolysis and muscle wasting (45-49).

Several mechanisms have been proposed by which inflammation may lead to muscle- and BCM-wasting. Firstly, it has been postulated that inflammatory mediators have direct proteolytic effects on myocytes. This was for example suggested by studies in recombinant-TNF-incubated mouse diaphragms (50) and recombinant-IL-6-incubated cultured myotubes (51). Other investigators, however, did not confirm this view (48,52). Secondly, weight-loss and BCM wasting may be related to a cytokine-induced decrease in food intake, which has been reported particularly for TNF and IL-1 (13,14,43,45,53,54). Thirdly, cancer-related BCM-wasting may be the consequence of an increased utilization of amino acids due to [1] inflammation-induced hypermetabolism (55), [2] increased amino acid uptake by the liver (56-58) associated with enhanced gluconeogenesis (59) and acute-phase protein production (18,53,60), and [3] a cytokine-triggered rise of circulating cortisol (11,56,61) inducing proteolysis (62). Fourthly, BCM-wasting might be related to an inflammation-related decrease in muscle protein synthesis. TNF administration has in this regard been shown to attenuate skeletal muscle protein synthesis in rats (54). In addition, TNF and glucocorticoids were shown, *in vivo* and *in vitro*, to decrease the production of testosterone, an important mediator of muscle growth (63-65). Finally, another hormone with prominent anabolic effects on muscle that has been reported to be decreased in experimental cancer cachexia (66,67) is IGF-I (68). It has, to our knowledge, not been investigated to date whether this decrease is inflammation-related and/or enhances muscle-wasting.

The results of the present study confirm that not only in experimental cancer, but also in human cancer weight loss is associated with the presence of an increased systemic inflammatory state. Two other studies reported this relationship before. Knapp *et al.* observed that in stage IV breast cancer patients the presence of severe weight loss ($\geq 20\%$) was accompanied by increased blood levels of $\text{TNF}\alpha$, cortisol and glucagon (69), and Staal-van den Brekel *et al.* reported in newly-diagnosed lung cancer that severe weight loss ($\geq 10\%$) was associated with increased plasma levels of soluble TNF-receptors and the acute-phase proteins LBP and C-reactive protein (70).

For the first time, the present study also specifically documents the presence of an association between systemic inflammation and wasting of BCM in human cancer, an association that, to our knowledge, formerly has been described in experimental models only (18,43-49). Particularly the assessment of plasma soluble TNF-receptors revealed this association, whereas no correlation was found between BCM-wasting and plasma levels of IL-6. The latter was in accordance with results from other studies, for example in systemic lupus erythematosus in which a significant correlation was found between disease activity and circulating levels of sTNF-R55 and sTNF-R75, but not IL-6 (71). In addition, other investigators reported a lack of correlation between changes in serum IL-6 -as opposed to changes in peripheral-blood-mononuclear-cell IL-6 release, and changes in acute-phase protein production, and postulated that particularly local -rather than systemic- levels of IL-6 may determine its biological effect (72).

In addition to the relationship between BCM-wasting and systemic inflammation, we also observed that BCM-wasting was associated with both increased hepatic acute-phase protein production, represented by increased levels of LBP and decreased levels of the "negative" acute-phase protein albumin, and hypermetabolism. The increased hepatic acute-phase response and hypermetabolism were in their turn associated with elevated levels of sTNF-R55. This was in accordance with the results of Staal-van den Brekel *et al.*, who reported the association between elevated plasma levels of sTNF-R55 and hypermetabolism in lung cancer (70), and with those of Falconer *et al.*, who found a relationship between enhanced production of TNF- and IL-6 by peripheral blood mononuclear cells and increased hepatic acute-phase protein production in patients with pancreatic cancer (73).

Although there was a trend toward a negative correlation between circulating levels of sTNF-R55 and appetite, which is in close agreement with the anorectic effects of inflammation observed in many experimental studies (13,14,43,45,53,54), we were not able to link anorexia with weight loss or BCM-wasting directly. This might have been due to the relatively small sample size, but also a poor correlation between subjective appetite

loss and objective food intake might have played a role.

We found evidence that also decreased IGF-I levels might be involved in the pathophysiology of cancer cachexia. There was, however, no obvious relationship with systemic inflammation. Also in AIDS-wasting and other non-cancer-related forms of malnutrition decreased blood concentrations of IGF-I have been reported, often in combination with elevated levels of growth hormone (74,75). These low IGF-I concentrations largely have been assumed to be the result of a decreased protein intake (76), which might have been also the case in the present study. In accordance with earlier reports in human cancer (77), we also observed decreased testosterone levels in patients with severe weight loss. Although in a recent study in AIDS a significant correlation between low testosterone levels and loss of BCM was reported (75), in the present study, probably in part due to sample size, the correlation between testosterone and BCM-ix did not reach statistical significance. We were not able to confirm a relationship between low testosterone levels and increased systemic inflammation, a relationship that was suggested by studies reporting a decreased testosterone production after exogenous TNF administration *in vivo* (63,78).

In conclusion, the present study in human lung cancer confirms that the development of weight loss is associated with the presence of an increased systemic inflammatory state. More importantly, however, also the specific association between systemic inflammation and BCM-wasting was documented. Furthermore it was demonstrated that also decreased circulating IGF-I levels may be involved. As it was shown that BCM-wasting is associated with a deterioration of performance status, and as it is known from studies in other diseases that BCM-wasting significantly decreases survival, our results should further encourage the search for specific therapies that, in addition to treatments that primarily aim at increasing nutrient intake, are based on a direct interference with BCM-wasting and its underlying pathophysiology. Some agents deserving further research in this regard are the non-steroidal anti-inflammatory drugs (79), N-3 fatty acids (80), IGF-I (81), branched-chain amino acids (82), and β_2 -adrenergic agonists (83).

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3

***TOTAL PLASMA CONCENTRATION OF LEPTIN
AND HUMAN LUNG CANCER-ASSOCIATED CACHEXIA***

**Total plasma concentration of leptin
and human lung cancer-associated cachexia**

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ABSTRACT

1. Adipocyte-derived leptin is postulated to represent the afferent hormonal signal to the hypothalamus in a feedback mechanism that regulates fat mass. In this proposed feedback mechanism, an increased fat mass leads to an elevated plasma leptin level that eventually induces a decrease in appetite and an increase in energy expenditure, and *vice versa*.

2. As anorexia and hypermetabolism play a role in the development of cancer cachexia, we investigated the hypothesis that underlying abnormalities in the leptin feedback mechanism (in particular relatively high plasma leptin levels or, on the other hand, a hypothalamic insensitivity to a fall in leptin levels) might be involved. For this purpose, total plasma leptin, body composition (fat mass and fat-free mass), appetite and resting energy expenditure were assessed in 21 male lung-cancer patients.

3. Total leptin was detectable in six patients and non-detectable in 15. In comparison with the latter, the patients with detectable leptin were characterized by a trend towards less weight loss (3.4% compared with 11.0%, $P=0.07$), as being less underweight (body mass index 23.8 kg/m² compared with 19.4 kg/m², $P=0.004$) and by a higher fat mass (21.4 kg compare with 9.7 kg, $P=0.001$). Significant between-group differences in appetite and resting energy expenditure were lacking.

4. Based on these findings, we conclude that in cancer the afferent part of the leptin feedback mechanism functions normally and that, in particular, elevated leptin levels are not involved in the development of cachexia. Since the absence of plasma leptin was not associated with an increased appetite and a decreased energy expenditure, disturbances in the hypothalamic part of the feedback mechanism are hypothesized.

INTRODUCTION

Leptin, the product of the adipocyte *ob* gene, is a recently identified hormone that is thought to represent the afferent signal in a feedback mechanism regulating fat mass (FM) (1). After release by the adipocyte, leptin is assumed to bind to a specific receptor in the hypothalamus (2-4), the brain nucleus that plays a central role in the regulation of feeding behaviour and energy balance (5,6). In animal models, the result of this interaction is a decrease in food intake and an increase in energy expenditure (3,4,7,8). These effects seem to be mediated by a leptin-induced decrease of the hypothalamic biosynthesis and release of neuropeptide Y (4,9), a hormone that potently stimulates appetite and food intake, induces insulin release, enhances lipoprotein lipase activity and reduces energy

expenditure (10,11). The end-result of these effects is the accretion of fat. As in many patients with obesity, blood leptin levels are high and correlated with the percentage of FM, it is suggested that in obesity a central insensitivity ("resistance") to this hormone might play a pathogenetic role (12,13).

In advanced-stage cancer, including lung cancer, substantial weight loss, predominantly consisting of fat and muscle (14), is frequently observed (15). The pathophysiology of this weight loss, eventually leading to cachexia, is complex and only partly understood. A decreased food intake and a range of metabolic disturbances, in part attributed to the circulation of tumour-derived and host-derived substances, including various cytokines, are considered as important contributing factors (15-19).

In view of the fact that anorexia and hypermetabolism frequently play a role in the development of cancer cachexia, underlying abnormalities in the leptin feedback mechanism might, hypothetically, be present. In particular, elevated levels of circulating leptin or, on the other hand, a hypothalamic insensitivity to a fall in leptin levels, might be involved. As there is evidence from studies in *in vitro* and *in vivo* animal models that *ob* gene expression may be up-regulated by substances like cortisol and pro-inflammatory cytokines such as tumour necrosis factor (TNF) and interleukin-1 (20-23), substances that also seem to be involved in the pathophysiology of cancer cachexia (19,24), particularly the possibility of high leptin levels playing a role in infection- and malignancy-related cachexia has recently been suggested (22,23). To shed some light on this issue, we investigated the relationship between total plasma leptin, weight loss, body composition, appetite and resting energy expenditure (REE) in a group of male lung-cancer patients.

PATIENTS AND METHODS

Patients

The protocol was in accordance with the Declaration of Helsinki (1989), and informed consent was obtained from all subjects. Twenty-one, newly-diagnosed, male lung-cancer patients were studied. In all patients, diagnosis was histologically or cytologically proved.

Appetite and weight

Appetite was assessed by a numerical rating scale ranging from 0 to 10: 0 indicating absolutely no appetite and 10 indicating an extremely good appetite. Weight was measured in the morning, in the fasted state after voiding and without clothing and shoes, to the nearest 0.1 kg by using a beam scale (SECA, Hamburg, Germany).

Body composition

FM and fat-free mass (FFM) were assessed by dual-energy x-ray absorptiometry (DEXA) with a total body scanner (model DPX-L; Lunar Corporation, Madison, Wisconsin, USA) that uses a constant potential x-ray source at 76 kVp and a K-edge filter (cerium) to achieve a congruent beam of stable dual-energy radiation with effective energies of 38 and 70 keV. The principles of dual-energy determination of body compartments, as well as of subject positioning, and scan procedures for the scanner used, have been extensively described elsewhere (25-27). Scans were made in the fast-speed mode. Baseline calibration of the scanner was performed daily against the standard calibration block supplied by the manufacturer. Based on the relative attenuations of the two energies through bone and soft tissue, bone mass and soft-tissue mass were calculated by the software provided by the manufacturer. As the ratio of beam attenuation at the lower to higher energy (R_{st}) is inversely and linearly related to percentage fat, the latter can be derived directly from R_{st} . FM is calculated by multiplying the percentage fat by the assessed soft tissue mass, FFM by adding non-fat soft-tissue mass and bone mass. The precision error (1 SD) of the DEXA measurement for FM has been reported to be 1.0 kg in healthy individuals (27). In a study in pigs, in which FM was assessed by DEXA as well as by chemical analysis after postmortem homogenization, bias was small ($r = 0.99$) (28). To adjust for height, FM-index and FFM-index were calculated by dividing respectively FM and FFM by the square of the height.

Resting energy expenditure

REE was assessed by indirect calorimetry by using a ventilated hood system (Oxycon β , Mijnhardt, Bunnik, The Netherlands). After an overnight fast, CO_2 production and O_2 consumption were measured at complete rest over a period of 20 minutes. REE was calculated by using the abbreviated Weir formula (29). The equipment was calibrated at the start of each experiment. The precision of the system was checked monthly by burning methanol, with a theoretical respiratory quotient of 0.667 after complete combustion. Further details on the technique used in our laboratory have been described elsewhere (30). To assess between-group differences in REE, an adjustment was made for FFM, as FFM is the body compartment mainly determining the level of metabolic activity.

Leptin

After an overnight 10 hours fast, blood was collected by venepuncture at 8.00 a.m. in

evacuated EDTA blood collection tubes (Sherwood Medical, Ballymoney, N. Ireland). The tubes were placed on ice-water and directly centrifuged for 5 minutes in a refrigerated (4°C) table top centrifuge at 3000 g. Plasma samples were immediately stored at -70°C until analysis. Plasma levels of leptin were measured using a double-antibody "sandwich" ELISA using a monoclonal antibody specific for human leptin, an assay that measures total (i.e. free plus bound) leptin. The measurement procedure is as follows. Microtiter plates are coated overnight with monoclonal antibody for human leptin (2A5). Plates are then blocked with 1 % BSA in PBS, washed, and standards (recombinant human leptin diluted [5:100] in leptin-free human plasma), quality control serum pools and unknowns are added (100 μ L/well) and incubated at room temperature for 2 hours. Plates are then washed and 100 μ L/well polyclonal rabbit-anti-human leptin antibody (R46) is added at 5 μ g/mL in 0.1 % BSA in PBS and incubated for 1 hour at room temperature. Plates are then washed again and 100 μ L/well of goat-anti-rabbit IgG conjugated to HRP at 1:20,000 dilution in 0.1 % BSA in PBS and incubated for 1 hour at room temperature. Plates are washed again and 150 μ L/well of peroxidase substrate solution (tetramethyl benzidine and hydrogen peroxide in citrate buffer) is added and incubated for 15 minutes. The reaction is stopped by adding 75 μ L/well of 2NH₂SO₄ and the optical density is measured at 450 nm in a plate reader. The lower level of detection is 0.5 ng/mL and the upper limit 50 ng/mL. The intra- and inter-assay variation were 9% and 12%, respectively. The leptin levels of normal weight subjects ranged from 1 - 12 ng/mL.

Statistical analysis

Results are expressed as median and range. Between-group differences were analyzed by using the Mann-Whitney-U-test. For all analyses, the statistical package SPSS/PC+ (version 6.0-Windows; SPSS Inc., Chicago, USA) was used. Statistical significance was determined at the 5% level, two-sided.

RESULTS

The characteristics of the 21 male lung-cancer patients investigated in the study are given in **Table 3.1**. On average, the patients were characterized by a weight loss of approximately 10% of the pre-illness weight and a body weight of 94% of ideal (31), corresponding to a body-mass-index of 21.2 kg/m². In only six of these subjects was plasma leptin detectable, at a median level of 3.9 ng/mL.

TABLE 3.1
Patient characteristics

	Median (range) (<i>n</i> = 21)
Age (y)	69 (56 - 82)
Tumour type and stage, <i>n</i>	
non-small-cell (I/III/IV)	3/5/11
small-cell (limited/extensive)	2/0
Karnofsky Performance Status (%)	80 (50 - 100)
Height (m)	1.75 (1.64 - 1.82)
Weight (kg)	62.8 (47.5 - 79.3)
Appetite (0-10)	6 (1 - 10)
Weight change (%)*	-9.8 (-23.2 - +1.5)
Body mass index (kg/m ²)	21.2 (15.9 - 24.9)
PIBW (%)†	94 (70 - 112)
Fat mass (kg)	12.7 (2.1 - 22.9)
Fat mass / weight (%)	19.8 (4.4 - 33.2)
Fat mass index (kg/m ²)‡	4.5 (0.7 - 7.3)
Fat-free mass (kg)	49.7 (38.8 - 57.8)
Fat-free mass index (kg/m ²)§	16.3 (14.4 - 18.0)
Total plasma leptin	
detectable, <i>n</i> (ng/mL)	6: 3.9 (1.1 - 5.0)
non-detectable, <i>n</i>	15
REE (kcal/d)	1622 (1158 - 2024)¶
REE/FFM (kcal/d/kg)**	32.6 (22.7 - 40.0)¶

* Weight in relation to pre-illness weight; † body weight expressed as percentage of ideal body weight; ‡ fat mass adjusted for height²; § fat-free mass adjusted for height²; || resting energy expenditure; ¶ *n* = 19; ** REE adjusted for fat-free mass.

As can be derived from Table 3.2, the patients with detectable total leptin levels were characterized by a trend towards less weight loss (3.4% compared with 11.0%, $P=0.07$), as being less underweight (body mass index 23.8 kg/m² compared with 19.4 kg/m², $P=0.004$) and by a higher FM (21.4 kg compared with 9.7 kg, $P=0.001$) than the subjects with non-detectable leptin levels. Significant between-group differences in appetite and resting energy expenditure were lacking.

TABLE 3.2

Comparison of patients with and without detectable total plasma levels of leptin*

	Leptin detectable† (n = 6)	Leptin non-detectable (n = 15)	P ‡
Height (m)	1.76 (1.68 - 1.80)	1.75 (1.64 - 1.82)	0.91
Weight (kg)	75.3 (63.0 - 79.3)	59.1 (47.5 - 76.0)	0.005
Appetite (0-10)	5.5 (3 - 8)	7.0 (1 - 10)	0.50
Weight change (%)§	-3.4 (-21.1 - +0.4)	-11.0 (-23.2 - +1.5)	0.07
Body mass index (kg/m ²)	23.8 (22.1 - 24.9)	19.4 (15.9 - 24.5)	0.004
PIBW (%)	107 (97 - 112)	85 (70 - 110)	0.004
Fat mass (kg)	21.4 (13.5 - 22.9)	9.7 (2.1 - 17.9)	0.001
Fat mass / weight (%)	29.0 (21.4 - 33.2)	15.9 (4.4 - 24.0)	0.002
Fat mass index (kg/m ²)¶	7.1 (4.8 - 7.3)	3.3 (0.7 - 5.8)	0.001
Fat-free mass (kg)	50.2 (43.6 - 57.8)	48.7 (38.8 - 56.7)	0.61
Fat-free mass index (kg/m ²)**	16.6 (14.9 - 18.0)	16.2 (14.4 - 17.9)	0.70
REE (kcal/d)††	1683 (1369 - 2024)	1564 (1158 - 1846)‡‡	0.14
REE/FFM (kcal/d/kg)§§	33.8 (29.3 - 39.2)	31.3 (22.7 - 40.0)‡‡	0.22

* Scores expressed as median and range; † limit of detection 0.5 ng/mL; ‡ between-group difference P (Mann-Whitney-U-test); § weight in relation to pre-illness weight; || body weight expressed as percentage of ideal body weight; ¶ fat mass adjusted for height²; ** fat-free mass adjusted for height²; †† resting energy expenditure; ‡‡ n = 13; §§ REE adjusted for fat-free mass.

The relationship between total plasma leptin and the presence of weight loss is shown in **Figure 3.1**. In general, leptin levels were undetectably low in patients who were characterized by substantial weight loss. One exception was a patient who was initially obese (pre-illness body weight 135% of ideal) and subsequently lost 21% of this weight, resulting in a still normal weight of 107% of ideal, a normal FM of 29.9% (FM-index 7.2 kg/m²) and, despite the severe amount of weight loss, a measurable leptin level of 4.5 ng/mL. Another exception was a patient on the other side of the spectrum, who was characterized by a low pre-illness body weight of 93% of ideal and subsequently remained weight-stable (weight-change +1.5%), at the time of the study resulting in a relatively low FM of 17.7% (FM-index 3.7 kg/m²) and, "despite" the lack of weight loss, a non-detectable leptin level.

FIGURE 3.1 Relationship between weight loss (expressed as percentage of the pre-illness weight) and total plasma leptin.

Figure 3.2 shows a plot of total leptin and FM-index, illustrating a clear relationship between the presence of a low fat mass and the non-detectability of leptin.

FIGURE 3.2 Relationship between fat mass index and total plasma leptin.

DISCUSSION

The aim of the present study was to investigate whether abnormalities in the leptin-dependent feedback mechanism regulating FM might be involved in the pathophysiology of cancer cachexia. As fasting total plasma leptin levels were undetectably low in those patients with substantial weight loss and a low FM, and were in the normal range in those without weight loss and a normal FM, we conclude that in cancer patients, in analogy to normal and obese individuals (12,13), leptin levels are directly related to the amount of body fat, which is compatible with normal functioning of the afferent loop of the leptin feedback mechanism. Based on fasting plasma leptin levels, we were therefore unable to confirm the hypothesis (22,23) that elevated leptin levels may be involved in the pathophysiology of cancer cachexia. Whether in cachectic cancer patients leptin secretion is altered in the fed state is presently unknown. As low total leptin levels were not reflected in decreased energy expenditure and an increased appetite, we hypothesize that

in cancer cachexia the leptin feedback mechanism is probably dysfunctional at the hypothalamic level.

Severe weight loss, leading to cachexia, is a common problem in several types of malignancy, including lung cancer (15). It is characterized by depletion of FM and muscle mass (14), has a major impact on morbidity, quality of life and mortality (32,33), and is difficult to treat. The cause of cancer-induced weight loss is a negative energy balance due to a decreased energy intake, increased energy expenditure (in combination with abnormalities in substrate metabolism), or both (15-17). Particularly in lung cancer the combination of a decreased energy intake and hypermetabolism is frequently seen (18). The pathophysiology of cancer-related anorexia and hypermetabolism is complex and, for the most part, unexplained. A range of circulating tumour-derived and host-derived substances, including various cytokines, are thought to be involved (15-17). As appetite as well as energy expenditure are assumed to be regulated to a large extent by the hypothalamus (5,6), abnormalities in the regulation at that level might well be involved in the disturbed energy balance in cancer cachexia.

Recently, the discovery of leptin, an adipocyte derived hormone, has led to the unravelling of a feedback mechanism that is probably of major importance in the regulation of FM (1). In this proposed feedback mechanism, a starvation-induced decrease in FM, accompanied by low circulating insulin levels and high intracellular non-esterified (free) fatty acids, leads to a decreased adipocyte production and release of leptin (12,34,35), resulting in an increased hypothalamic production and release of neuropeptide Y, the production of which is normally suppressed by leptin after binding to a specific hypothalamic receptor (2-4,9). Neuropeptide Y subsequently induces an increase in appetite and food intake, an increased release of insulin, an increase in lipoprotein lipase activity, and a decrease in energy expenditure (10,11), leading to fat accretion and restoration of FM. To close the circle, leptin levels rise as a result of increased insulin levels and the increasing FM (12,34,35).

If this leptin feedback mechanism does indeed exist and function as is suggested in literature, one would expect the unmeasurably low leptin levels in the underweight cancer patients, in analogy to the *ob/ob* mice which become extremely obese as a result of the genetic inability of adipocytes to synthesize leptin (3,7,8), to induce an increase in appetite and a decrease in energy expenditure in an attempt to restore FM. In view of the fact that clear differences in appetite and REE between the patients with and without detectable leptin in the present study were lacking, this was obviously not the case. This suggests that in cancer cachexia the hypothalamus is insensitive to the low levels of leptin, which fits with the hypothesis of Dryden *et al.* (10) that in cancer cachexia the

normal homeostatic mechanisms defending body weight against losses have been overridden. This hypothesis was based on their observation that, in cachectic sarcoma-bearing rats, hypothalamic neuropeptide Y was significantly lower than in non-tumour-bearing controls, whose food intake was restricted to the same degree (36). In addition, other investigators recently found in experimental cancer anorexia, evidence of major alterations in neuropeptide Y receptor mechanisms, such as the absence of compensatory up-regulation and the presence of decreased receptor affinity (37). Whether tumour- or host-derived cytokines or other tumour- or host-derived substances are responsible for the over-ruling of normal weight homeostasis in cancer anorexia and cachexia still remains to be further clarified.

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4

***THE USE OF BIOELECTRICAL IMPEDANCE ANALYSIS
TO PREDICT TOTAL BODY WATER IN PATIENTS WITH CANCER CACHEXIA***

**The use of bioelectrical impedance analysis
to predict total body water in patients with cancer cachexia**

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ABSTRACT

The applicability of bioelectrical impedance analysis (BIA) to predict total body water (TBW) was assessed in 16 underweight (<95% of ideal body weight [IBW]) and 25 normal-weight (>95% of IBW) cancer patients. Although height²/resistance (ht²/R) proved to be a strong single predictor of TBW measured by deuterium dilution in both groups (normal-weight patients: $r^2 = 0.85$, SEE 2.16 L; underweight patients: $r^2 = 0.86$, SEE 2.24 L), TBW would be significantly overestimated in the underweight group if the prediction formula developed in the normal-weight group was used (bias 1.67 L [5%], 95% CI 0.20-3.15 L). A systematic overestimation of TBW in the underweight patients was also found when TBW was predicted in our two patient groups by several previously published BIA formulas developed in normal-weight individuals. We conclude therefore, that although a similar relationship is found between ht²/R and TBW in normal-weight and underweight cancer patients, single frequency BIA overestimates TBW in underweight patients when prediction formulas are used that have been developed in normal-weight subjects.

INTRODUCTION

Body-composition measurement is important to assess nutritional depletion. Based on a two-compartment-model (fat mass and fat-free mass), two methods are available in clinical practice: skinfold anthropometry and, more recently introduced, bioelectrical impedance analysis (BIA). BIA is based on the principle that a fixed, low-voltage, high-frequency current introduced into the human body, is conducted almost completely through the fluid compartment of the fat-free mass (FFM) which is an equivalent of total body water (TBW) (1,2). The opposition to the flow of this current, the resistance (R), is measured. The assumption that R can be used to predict TBW has been proved by many studies in healthy individuals (3-12). Especially the so-called "impedance-index", which is defined as body height squared divided by resistance (ht²/R), proved to be an especially strong predictor (13). To calculate FFM, TBW is divided by the hydration coefficient of the FFM, which is ≈ 0.73 in normal individuals (14,15).

In contrast with established techniques such as hydrodensitometry (16), isotope dilution (17) and total-body K⁴⁰ determination (18), BIA is inexpensive, can be performed without specific operator skills, and is no burden on the measured subject (2). This makes BIA a promising, broadly applicable technique to measure body composition.

Very few data are available however on the applicability of BIA in nonhealthy individuals, especially in those with an abnormal state of hydration, obesity or cachexia.

The aim of our study was to investigate the applicability of BIA to determine TBW by using deuterium dilution as a reference method in end-stage cancer patients with and without cachexia.

PATIENTS AND METHODS

Patients

The protocol was approved by the institutional review board for human research of the University Hospital Maastricht and informed consent was obtained from all subjects.

Forty-one ambulatory patients were consecutively admitted to the study. All patients suffered from a histologically diagnosed, incurable form of cancer: non-small-cell carcinoma of the lung ($n=24$), adenocarcinoma of the gastrointestinal tract ($n=7$), adenocarcinoma of the pancreas ($n=2$), disseminated adenocarcinoma of unknown origin ($n=6$), mesothelioma of the pleura ($n=1$) and carcinoma of the ureter ($n=1$).

Sixteen patients had body weights below 95% of ideal body weight (IBW; 19) and were considered underweight. Characteristics of the normal-weight ($>95\%$ of IBW) and underweight patients are given in **Table 4.1**. Average weight loss in the underweight group amounted to 15.4% of their preillness weight, vs 6.0% in the normal-weight subjects. Seven of the total group of patients were characterized by an excess of extracellular fluid; one underweight and three normal-weight patients had a slight to moderate amount of unilateral pleural fluid, one underweight and one normal-weight patient had a slight amount of bilateral edema of the lower extremities, and one normal-weight patient suffered from extensive edema and ascites.

Height and weight

Height (ht) was measured, without shoes, to the nearest 0.1 cm by using a wall-mounted stadiometer. Weight (wt) was measured in the morning, in the fasted state, after voiding and without clothing and shoes, to the nearest 0.1 kg by using a beam scale (SECA, Hamburg, Germany).

Bioelectrical impedance analysis

R was also measured in the morning, in the fasted state, and after voiding, by using a

TABLE 4.1

Patient characteristics

	NORMAL-WEIGHT GROUP (n = 25)	UNDERWEIGHT GROUP (n = 16)	
Sex (m/f)	17/8	14/2	ns
Age (y)	64 ± 9 ¹	65 ± 9	ns
Height (cm)	168 ± 7	171 ± 11	ns
Weight (kg)	70.7 ± 7.2	57.1 ± 7.8	†
PIBW (%) ²	110 ± 10	86 ± 7	†
BMI (kg/m ²) ³	25.0 ± 2.3	19.6 ± 1.7	†
Weight loss (%) ⁴	6.0 ± 6.5	15.4 ± 8.5	†
TBWdeu (L)	38.2 ± 5.5	33.2 ± 5.8	‡
TBWdeu/Wt (%)	54.1 ± 7.1	58.2 ± 7.4	ns
Ht ² /R (cm ² /Ohm)	55.3 ± 9.8	49.0 ± 10.2	§
R (Ohm)	525 ± 85	611 ± 83	
Albumin (g/L)	37.3 ± 4.5	35.6 ± 5.7	ns
BUN (mmol/L) ⁵	5.4 ± 1.9	6.0 ± 1.8	ns
Creatinin (μmol/L)	79 ± 20	76 ± 20	ns

¹ Mean ± SD; ² weight expressed as percentage of ideal body weight; ³ body mass index; ⁴ weight loss as percentage of pre-illness weight; ⁵ blood urea nitrogen; † $P < 0.001$, || $P < 0.005$, ‡ $P < 0.01$, § $P = 0.054$, ns not significant: compared with normal-weight patients.

800-μA, 50-kHz tetrapolar impedance plethysmograph (BIA 101; RJL systems Inc, Detroit). The measurements were performed in the supine position at the right side of the body. As described by Lukaski et al (9), two disposable pregelled electrodes were affixed to the dorsum of the right hand and two to the dorsum of the right foot.

Total body water

The evening before visiting the hospital, at ≈ 23.00 h, all patients drank a weighted dose of ≈ 4.00 g deuterium (99.84 atom percent excess) in 50 mL water. Then the bottle was rinsed once with ≈ 50 mL tap water, which was also drunk. Thereafter, the patients had to refrain from eating and drinking. They were asked to void at least once at home before attending the hospital. Before and 10 h after drinking the deuterium water, a urine sample was taken. Deuterium was measured in duplicate in these two samples using an isotope ratio-mass spectrometer (Aqua Sira, VG Isogas, Cheshire, UK) (20). Comparisons of the duplicate measurements showed a precision $> 99\%$. TBW (TBW_{deu}) was calculated by dividing the measured volume of distribution of deuterium by 1.04, thereby correcting for the exchange of deuterium with nonaqueous hydrogen (17). In healthy individuals it was shown that TBW measurement after an overnight equilibration period of 10 h corresponds very well (bias 1%) with TBW derived from hydrodensitometry (21).

TBW prediction formulas

To compare, in our normal-weight and underweight patients, the prediction of TBW by previously published formulas developed in normal-weight adults, the following prediction formulas were used:

- RJL BIA-101 (12): (m) $TBW = 0.3963(Ht^2/R) + 0.143(Wt) + 8.4$,
 (f) $TBW = 0.3821(Ht^2/R) + 0.1052(Wt) + 8.3148$
- Deurenberg (4): $FFM = 0.671(Ht^2/R) + 3.1(\text{Gender}; m=1, f=0) + 3.9$
- Deurenberg (5): $FFM = 0.340(Ht^2/R) + 15.34(Ht) + 0.273(Wt) - 0.127(A) + 4.56(\text{Gender}; m=1, f=0) - 12.44$
- Heitmann (7): $TBW = 0.266(Ht^2/R) + 0.186(Wt) + 4.702(\text{Gender}; m=1, f=0) - 0.081(A) + 11.03$
- Kushner (13): $TBW = 0.59(Ht^2/R) + 0.065(Wt) + 0.04$
- Lohman 1988,
 rep. by Graves (6): (m) $FFM = 0.485(Ht^2/R) + 0.338(Wt) + 5.32$,
 (f) $FFM = 0.475(Ht^2/R) + 0.295(Wt) + 5.49$
- Lukaski (9): $FFM = 0.810(Ht^2/R) + 6.39$
- Lukaski (10): $TBW = 0.377(Ht^2/R) + 0.14(Wt) - 0.08(A) + 2.9(\text{Gender}; m=1, f=0) + 4.65$
- Segal (11): (m) $FFM = 0.00132(Ht^2) - 0.0439(R) + 0.305(Wt) - 0.1676(A) + 22.67$,
 (f) $FFM = 0.00108(Ht^2) - 0.0209(R) + 0.232(Wt) - 0.0678(A) + 14.59$
- Van Loan (12): $TBW = 0.000724(Ht^2) + 0.2822(Wt) - 0.0153(R) - 2.3313(\text{Gender}; m=0, f=1) - 0.1319(A) + 9.9868$

To allow comparison between the formulas, FFM was converted into TBW by the equation $TBW = FFM \times 0.72$ (16), except for the Lukaski et al formula (9) which was corrected using 0.736 as hydration coefficient because in the development of this formula (hydrodensitometric) FFM was calculated by using the formula of Brozek et al (22).

Statistical analysis

Results are expressed as mean $\pm$ SD. Group means were compared by using unpaired Student's *t* tests. For all statistics, significance was determined at the 5% level (two-sided). The relevant predictors of TBW_{deu} were selected in the underweight and normal-weight patients by stepwise-linear-regression analysis by using the statistical package SPSS/PC+ (version 4.0; SPSS Inc, Chicago). Slopes and intercepts of different regression lines were statistically compared by using a small sample *t* test as described by Kleinbaum et al (23). The residuals relative to TBW predicted from the regression equation for the total study group were compared in the underweight and normal-weight patients by using a chi-square statistic with Yates continuity correction.

RESULTS

Ht²/R, wt, sex and age were considered as possible predictors of TBW_{deu} in both the normal-weight and underweight patients. In both groups only ht²/R was identified as significant predictor of TBW_{deu} by stepwise-linear-regression analysis when these variables were used. The regression lines in the two patient groups are shown with their characteristics in Figure 4.1.

FIGURE 4.1 Linear regression of TBW_{deu} on Ht²/R in the normal-weight (○,A) and underweight (●,B) patients. Characteristics of the regression lines: normal-weight group (n=25): $y=9.64+0.516x$, SEE 2.16 L, $r^2=0.85$, $p<0.0001$; underweight group (n=16): $y=7.33+0.528x$, SEE 2.24 L, $r^2=0.86$, $p<0.0001$.

To assess whether there was a significant difference in the prediction of TBW_{deu} by ht^2/R between the underweight and normal-weight individuals, slopes and intercepts of the respective regression lines were compared statistically (Table 4.2). The slopes of the two lines were identical, but the intercept of the regression line of the underweight patients was significantly lower than the intercept of the normal-weight patients (estimated vertical distance 1.67 L, 95% CI 0.20-3.15 L).

TABLE 4.2

Comparison of regression lines (TBW_{deu} on Ht^2/R): normal-weight (A) versus underweight (B) patients

	Intercept (L)	Slope
(A) NORMAL-WEIGHT GROUP	9.64	0.516
(B) UNDERWEIGHT GROUP	7.33	0.528
difference A-B	1.67 ¹	-0.012
95%-CI ² difference A-B	0.20 - 3.15	-0.129 - 0.154

¹ Estimated difference (adjusted for difference between slopes); ² 95% Confidence Interval.

Another way of visualizing the difference in prediction of TBW between the two study groups is by plotting the residuals of all patients ($n=41$) relative to TBW predicted by the regression equation for the total study group as a function of the percentage of IBW (Figure 4.2). Seventeen of 25 normal-weight patients (68%) had a positive residual, whereas 11 of 16 underweight patients (69%) had a negative residual (χ^2 3.92, $P < 0.05$). Visual inspection of the plotted residuals in the relatively small group of underweight patients did not reveal clear evidence for a graded error in the prediction of TBW related to the degree of underweight.

To investigate whether there was also a difference in the prediction of TBW between the normal-weight and underweight patients when other prediction formulas were used, TBW was calculated in both groups by using 10 different previously published formulas developed in normal-weight adults. The results are given in Table 4.3. There was a large variation in the prediction of TBW by the respective formulas, ranging from 31.0 L to 39.6 L in the normal-weight group and from 28.4 L to 35.6 L in the underweight group.

FIGURE 4.2 Plot of the residuals of all patients ($n = 41$) relative to TBW predicted by the total-study-group regression equation ($TBW = 7.44 + 0.545(Ht^2/R)$, SEE 2.28 L, $r^2 = 0.86$) as a function of the percentage of IBW.

TABLE 4.3

Prediction of TBW by previously published formulas

Formula ¹	NORMAL-WEIGHT GROUP		UNDERWEIGHT GROUP		
	TBWpr ² (L)	Dif1 ³ (%)	TBWpr (L)	Dif2 ⁴ (%)	Dif2 - Dif1 (%)
RILBIA-101 (12)	39.3	+2.9	35.6	+7.2	+4.3
Deurenberg (4)	31.0	-18.8	28.4	-14.5	+4.3
Deurenberg (5)	33.4	-12.6	30.1	-9.3	+3.3
Heitmann (7)	36.9	-3.4	33.5	+0.9	+4.3
Kushner (13)	37.3	-2.4	32.7	-1.5	+0.9
Lohman (6)	39.6	+3.7	34.6	+4.2	+0.5
Lukaski (9)	37.7	-1.3	33.9	+2.1	+3.4
Lukaski (10)	32.2	-15.7	28.5	-14.2	+1.5
Segal (11)	34.0	-11.0	29.6	-10.8	+0.2
Van Loan (12)	33.2	-13.1	29.1	-12.3	+0.8

¹ Exact formulas are given in the methods section; ² TBW predicted by prediction formula;

³ relative difference between measured TBWdeu (38.2 L) and TBWpr in the normal-weight group: $((TBWpr - 38.2)/38.2) \times 100\%$; ⁴ relative difference between measured TBWdeu (33.2 L) and TBWpr in the underweight group: $((TBWpr - 33.2)/33.2) \times 100\%$.

When for the respective formulas the relative difference between measured TBW_{deu} and predicted TBW in the normal-weight patients was subtracted from the difference in the underweight patients, for each formula a positive result (ranging from +0.2 to +4.3%) was obtained, indicating an overestimation of TBW in the underweight patients relative to the normal-weight patients.

DISCUSSION

The aim of our study was to investigate the applicability of BIA to predict TBW (as a measure of fat-free mass) in end-stage cancer patients with and without cachexia. Our data indicate a systematic difference in the prediction of TBW between the normal-weight and underweight patients. In the underweight patients (body weight 87% of ideal, body mass index 19.6 kg/m²) TBW (assessed by deuterium dilution) would be overestimated by 5.0% (1.67 L) when the prediction formula was used, which applied to the normal-weight group (body weight 110% of ideal, BMI 25.0 kg/m²). We did not find a clear linear relationship between the magnitude of the overestimation of TBW and the degree of underweight, but this was probably due to the relatively small number of underweight patients. Our results were confirmed when TBW was predicted in both the normal-weight and underweight patients by 10 previously published formulas developed in normal-weight adults. Relative to the normal-weight group, TBW was systematically overestimated by all formulas in the underweight patients.

Recently Royall et al (24) also reported in 19 underweight patients (body mass index 20.3 kg/m²) suffering from active Crohn's disease that TBW was overestimated when a 'normal-weight' prediction formula was used. As reference method H₂¹⁸O-dilution was used and TBW was predicted by the sex-specific BIA equations of Kushner and Schoeller (8), corrected for the difference between deuterium space and TBW. In their study it was concluded that BIA overestimated TBW by 5.9%. A drawback of this study, however, is the fact that no control group of normal-weight patients suffering from Crohn's disease was measured, because the outcome of the prediction of TBW by BIA largely depends on the chosen prediction formula. For instance, in our group normal-weight patients the average predicted TBW varied, depending on the formula used, from 31.0 L to 39.6 L. This particular problem is well known and was recently discussed by Fuller (25).

The reason why, per unit body water, a relatively higher ht^2/R was measured in our underweight patients than in the normal-weight patients is unclear. Probably the phenomenon can be explained by the fact that in cachexia body-cell-mass and intracellular

water (ICW) may decrease, whereas the extracellular water (ECW) remains relatively unchanged (26-28). Because a 50-kHz electrical current is more easily conducted through ECW than through ICW (29), a decrease of the ICW-ECW ratio would result in a relatively lower R per unit body water and thus a relatively higher value of ht^2/R . This would lead to the observed shift of the regression line to the right with a corresponding decrease of the intercept. Other factors that could influence the ICW-ECW ratio are dehydration, edema and probably also sex (15). The observed difference in intercept between the underweight and normal-weight patients in our study could however not be attributed to differences in these factors.

In summary, we conclude that in both normal-weight and underweight cancer patients the single-frequency BIA parameter ht^2/R is highly correlated with TBW. An important problem, however, is the fact that BIA systematically overestimates TBW in underweight patients when a prediction formula is used that was developed in normal-weight subjects. Further studies are needed in larger groups of underweight patients to investigate whether a direct relationship exists between the error of the prediction of TBW by BIA and the degree of underweight. If so, possibly a correction factor could be developed to improve prediction.

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5

***BIOELECTRICAL IMPEDANCE ANALYSIS TO ASSESS
CHANGES IN TOTAL BODY WATER IN PATIENTS WITH CANCER***

**Bioelectrical impedance analysis to assess
changes in total body water in patients with cancer**

Submitted

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ABSTRACT

Background: Bioelectrical impedance analysis (BIA) is a body composition analysis technique to measure total body water (TBW) and fat-free mass. Although already widely used in prospective clinical trials, the applicability of BIA to measure changes in TBW and fat-free mass has been questioned. As this scepticism has been based predominantly on studies in obese individuals, the aim of the present study was to investigate BIA for its ability to predict changes in TBW in patients with cancer.

Methods: In cancer patients participating in a clinical trial, changes in total-body resistance (Δ -R) were measured by BIA (800 μ A, 50-kHz) and compared with changes in TBW assessed by deuterium dilution (Δ -TBWdeu).

Results: Thirty-three patients (mean body mass index 23.2 ± 3.5 kg/m²) were studied after an average follow-up of 11 weeks. Changes in TBWdeu occurred in both directions (mean $+0.2 \pm 1.6$ L, range -3.3 to +3.1 L). BIA-derived Δ -R proved to be a significant predictor of Δ -TBWdeu (r^2 0.48, $P < 0.0001$, SEE 1.17 L). There were in this regard no significant differences between patients with and without underweight.

Conclusions: In underweight and normal-weight cancer patients, BIA-derived Δ -R significantly predicts changes in TBW assessed by deuterium dilution.

INTRODUCTION

Bioelectrical impedance analysis (BIA) is a body composition analysis technique to measure fat mass and fat-free mass (FFM) according to a two-compartment model. Single-frequency BIA is based on the principle that a fixed, low-voltage, high-frequency current, introduced into the human body, is conducted almost completely through the fluid compartment of the FFM, which is an equivalent of total body water (TBW) (1,2). The opposition to the flow of this current, the resistance (R), is measured. The assumption that R can be used to predict TBW and FFM has been proven by many studies in healthy individuals (3-14).

Although already widely used in prospective clinical trials (15-19), the applicability of BIA to measure *changes* in TBW and FFM is far from clear. Several studies, most of which were performed in obese individuals on a weight loss program, showed that the reliability of BIA to measure changes in TBW or FFM was disappointing (20-24). To expand our knowledge on the ability of BIA to predict changes in TBW, we measured changes in BIA-derived R in advanced-stage cancer patients who participated in a

randomized placebo-controlled trial investigating the effects of a synthetic progestagen on food intake and body composition (25). In this study, TBW was assessed by deuterium dilution, which was thus used as a reference method for the validation of BIA.

PATIENTS AND METHODS

Patients

Patients with the following histologically or cytologically diagnosed, incurable malignancies were considered eligible: non-small-cell carcinoma of the lung; mesothelioma; carcinoma of the digestive tract or pancreas; carcinoma of the kidney, bladder or ureter; and disseminated carcinoma of unknown origin. All patients were required to have a Karnofsky performance status score of at least 60%. The protocol was in accordance with the Helsinki Declaration of 1975, as revised in 1983, and was approved by the institutional review board for human research of the University Hospital Maastricht. Written informed consent was obtained from all patients.

Study design

The study duration was 12 weeks. At baseline and after 6 and 12 weeks, body weight, BIA-derived R and deuterium dilution-derived TBW (TBW_{deu}) were assessed.

Height and weight

Height (ht) was measured, without shoes, to the nearest 0.1 cm by using a wall-mounted stadiometer. Body weight (wt) was measured in the morning, in the fasted state, after voiding and without clothing and shoes, to the nearest 0.1 kg by using a beam scale (SECA, Hamburg, Germany).

Bioelectrical impedance analysis

R was also measured in the morning, in the fasted state and after voiding, by using a 800 μ A, 50-kHz tetrapolar impedance plethysmograph (BIA 101, RJL systems Inc, Detroit, MI). The measurements were performed in the supine position on the right side of the body. As described by Lukaski et al (9), two disposable pregelled electrodes were affixed to the dorsum of the right hand and two to the dorsum of the right foot.

Total body water

The evening before each visit to the hospital, at ≈ 23.00 h, all patients drank a weighted dose of ≈ 4.00 g deuterium (99.84 atom percent excess) in 50 mL water. Then the bottle was rinsed once with ≈ 50 mL tap water, which was also drunk. Thereafter, the patients had to refrain from eating and drinking. They were asked to void at least once at home before attending the hospital. Before and 10 hours after drinking the deuterium water, a urine sample was taken. Deuterium was measured in duplicate in these two samples by using an isotope ratio-mass spectrometer (Aqua Sira, VG Isogas, Cheshire, UK) (26). Comparison of the duplicate measurements showed a precision $>99\%$. TBW_{deu} was calculated by dividing the measured volume of distribution of deuterium by 1.04, thereby correcting for the exchange of deuterium with non-aqueous hydrogen (27). The use of this test protocol with an overnight equilibration period of 10 hours has been extensively validated in our laboratory (28). It was shown that TBW assessed by using this protocol corresponds very well (bias 1%) with TBW derived from hydrodensitometry (28).

Statistical Analysis

Results are expressed as mean $\pm$ SD. For all statistics, significance was determined at the 5% level (two-sided). The prediction of Δ -TBW_{deu} by Δ -wt and Δ -R was evaluated by linear-regression analysis. For all analyses, the statistical package SPSS/PC+ (version 6.0-Windows; SPSS Inc., Chicago, USA) was used. Slopes and intercepts of different regression lines were statistically compared by using a small-sample *t* test as described by Kleinbaum et al (29). Subjects were considered non-evaluable in case of the development of peripheral edema, as it could not be excluded that such large fluid shifts would substantially bias the reliability of the BIA-prediction of TBW change.

RESULTS

Fifty-four advanced-stage cancer patients participated in the clinical trial that formed the base for the present study (25). In 38 patients at least one follow-up measurement of both BIA and deuterium dilution was available. The other patients dropped-out before reaching the first evaluation (at 6 weeks) due to death or physical deterioration ($n=6$), side effects ($n=4$), refusal to continue ($n=2$), protocol violation ($n=2$), mechanical obstruction of the digestive tract ($n=1$) and hospitalization due to sigmoid perforation and peritonitis ($n=1$). As five of the patients with available follow-up measurements developed peripheral edema, the total number of patients used in the analysis was 33. Of these patients, the

baseline measurements and those made on the last follow-up visit (week 6 in seven patients), were analyzed. In Table 5.1, the baseline characteristics of these patients are given.

TABLE 5.1
Baseline characteristics¹

	(<i>n</i> = 28 males, 5 females)
Age (y)	66 ± 8
Height (cm)	169 ± 7
Weight (kg)	66.3 ± 10.0
PIBW (%) ²	101 ± 15
Body mass index (kg/m ²)	23.2 ± 3.5
Weight loss (%) ³	8.4 ± 9.0
Resistance (Ω)	535 ± 85
TBWdeu (L) ⁴	37.2 ± 5.3
FFMdeu/wt (%) ⁵	
males	79.3 ± 6.1
females	66.1 ± 7.9
Blood urea nitrogen (mmol/L)	6.1 ± 1.8
Creatinine (μmol/L)	83 ± 18

¹ Mean ± SD; ² weight expressed as percentage of ideal body weight; ³ weight loss as percentage of pre-illness weight; ⁴ total body water by deuterium dilution; ⁵ fat-free mass by deuterium dilution (TBWdeu / 0.73) as percentage of body weight.

During the study, changes in body weight as well as TBWdeu occurred in both directions: mean weight change was $+0.1 \pm 3.7$ kg, range -7.6 to +13.7 kg; mean TBWdeu change amounted to $+0.2 \pm 1.6$ L, range -3.3 to +3.1 L.

Δ -Wt and Δ -R were significantly correlated with Δ -TBWdeu (respective r 0.43 [$P=0.01$] and 0.69 [$P<0.0001$]). On stepwise regression analysis, however, only Δ -R

was identified as significant independent predictor of Δ -TBWdeu ($y = 0.07 - 0.0387x$, SEE 1.17 L, $r^2=0.48$, $P<0.0001$). In Figure 5.1, the regression line of Δ -TBWdeu on Δ -R is shown.

FIGURE 5.1 Linear regression of changes in total body water by deuterium dilution (TBWdeu) on changes in resistance (R) by bioelectrical impedance analysis. Characteristics of the regression line: $y = 0.07 - 0.0387x$, SEE 1.17 L, $r^2 = 0.48$, $P < 0.0001$.

To investigate whether there was a difference in the prediction of Δ -TBWdeu by Δ -R in underweight patients (defined as having a body weight below 95 percent of the ideal body weight [PIBW] [30]) and normal-weight patients, a separate linear-regression-analysis of Δ -TBWdeu on Δ -R was performed in each subgroup. Mean PIBW was $85 \pm 7\%$ in the underweight patients ($n=11$) and $110 \pm 11\%$ in the normal-weight subjects ($n=22$). The characteristics of the regression line of the underweight patients ($y = 0.09 - 0.0306x$, SEE 1.06 L, $r^2=0.52$, $P=0.01$) were not significantly different from those of the regression line of the normal-weight patients ($y = 0.02 - 0.0460x$, SEE 1.24 L, $r^2=0.48$, $P<0.001$); the 95% confidence interval for the difference between slopes was -0.0143 to 0.0450.

DISCUSSION

The results of the present study indicate that in cancer patients, with or without underweight, the BIA-parameter Δ -R is a significant predictor of Δ -TBW, and that the use of Δ -wt as an additional factor does not further improve the prediction.

These results are in contrast with those of several other investigators, who overtly raised doubts about the ability and reliability of BIA to detect changes in TBW and FFM in longitudinal studies. Some even suggested that Δ -wt is a better predictor of Δ -TBW than Δ -R (20,31). In 1989, Deurenberg *et al.* (21) reported a study in 13 obese women losing weight on a 8-week diet. In this study, mean Δ -FFM measured by densitometry was significantly under-estimated by BIA (respective mean Δ -FFM: -2.3 kg and -0.6 kg). No correlation data were reported. In 1992, Forbes *et al.* (20) reported in a review of six studies in adults who incurred a change in body weight and FFM as a result of diet or diet plus exercise, that in four studies no significant correlation was found between Δ -R and Δ -FFM. Also in 1992, van der Kooy *et al.* (22) reported the results of a study in 84 obese patients losing weight on a 13-week diet. In this study, no correlation was found between Δ -R and Δ -FFM measured by densitometry. BIA-derived Δ -FFM was calculated by using three different previously published prediction equations. In contrast with the Deurenberg study, all equations, in a varying degree, significantly *over*-estimated mean Δ -FFM. In 1995, a study was reported by Beshyah *et al.* (23) investigating the changes in FFM assessed by three methods (BIA, dual-energy X-ray absorptiometry [DXA] and total body potassium [TBK]) in a group of 14 hypopituitary adults during growth hormone treatment. No significant correlation was found between Δ -FFM_{BIA} and Δ -FFM assessed by DXA and TBK. BIA strongly over-estimated Δ -FFM measured by TBK, but accurately predicted mean Δ -FFM measured by DXA. In contrast with the previous studies, Valtueña *et al.* (32) reported in 1995 in 9 obese women, losing weight on a 4-week low-energy diet, a significant correlation between densitometric Δ -FFM and Δ -FFM_{BIA} ($r=0.69$, $P<0.05$, SEE not stated). Accuracy was relatively good: mean densitometric Δ -FFM amounted to -3.4 kg, versus -2.9 kg for Δ -FFM_{BIA}. In a recent study by Abrahamsen *et al.* in 18 hemodialysis patients before and after ultrafiltration (24), no significant correlation was observed between Δ -R measured by BIA and Δ -TBW measured as weight reduction by dialysis.

Why did the present study show a significant correlation between Δ -R and Δ -TBW, while most of the longitudinal BIA studies, reviewed above, did not? Apart from differences in group size, rapidity, magnitude and range of TBW changes, and reference methods used, an additional explanation could be the fact that in most studies the validity

of BIA to measure changes in FFM was investigated in obese individuals on a weight-loss program. Several previous studies revealed that obesity, on a body compositional level, is characterized by an absolute elevation of ICW and an absolute *and* relative increase of ECW, resulting in an increased hydration coefficient of the FFM and an increased ECW/ICW ratio (33,34). It was also shown that weight loss in these patients is accompanied by a decrease in ICW, while ECW remains stable or decreases only slightly (33,35). If the 50-kHz BIA-current is more easily conducted through the ECW than through the ICW, which is suggested in literature (36), over-time changes in ECW/ICW ratio, and inter-individual variation in these changes, might importantly contribute to the poor BIA reliability observed in most follow-up obesity studies. In addition, the results might have been further confounded by concomitant changes in the hydration coefficient of the FFM, for example due to ICW losses mainly consisting of (glycogen-bound) pure water. Finally, in some of the reviewed studies the reliability of BIA might have been also influenced by the use of TBW prediction equations that, in addition to R, contain variables (for example weight) of which changes are not necessarily proportionally related to changes in TBW.

Although in the present study it was shown that changes in BIA-derived R significantly predict changes in TBW in normal-weight and underweight individuals, it should be stressed that the use of different prediction equations (as presently available in literature) to calculate TBW from R not only leads to different absolute values of TBW (37,38), but also to different values of TBW-*change*. The larger the relative importance of R (or ht^2/R) in the formula is, the larger the assessed Δ -TBW for a given Δ -R will be. When for example R changes from 540 ohm to 500 ohm and weight from 66 kg to 71 kg in a 65 y old male with a height of 170 cm, TBW_{bia} will change from 33.2 L to 35.3 L when the formula developed by Deurenberg *et al.* ($TBW = 0.72 * (0.340(ht^2/R) + 0.1534(ht) + 0.273(wt) - 0.127(A) + 4.56(\text{sex}; m=1, f=0) - 12.44)$ [5]) is used, versus 35.9 L to 38.8 L in case of the formula by Kushner *et al.* ($TBW = 0.59(ht^2/R) + 0.065(wt) + 0.04$ [13]). The latter increase in TBW_{bia} is $\approx 40\%$ higher than the increase assessed by using the first formula. This inherent problem of quantification can only be circumvented by exclusively analyzing and reporting within- and between-group changes in R. When quantification is nevertheless desired, which probably often will be the case, it is obviously important to report in addition to (the analysis of) the "crude" R values, the exact formula by which TBW or FFM is calculated. Unfortunately, however, this is not at all common practice in prospective clinical studies to date.

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6

***THE EFFECTS OF MEDROXYPROGESTERONE ACETATE ON
APPETITE, WEIGHT AND QUALITY OF LIFE
IN ADVANCED-STAGE NON-HORMONE-SENSITIVE CANCER
A PLACEBO-CONTROLLED MULTICENTER STUDY***

**The effects of medroxyprogesterone acetate on
appetite, weight and quality of life
in advanced-stage non-hormone-sensitive cancer**

A placebo-controlled multicenter study

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ABSTRACT

Purpose: To investigate the effects of medroxyprogesterone acetate (MPA) on appetite, weight and quality of life (QL) in patients with advanced stage, incurable, non-hormone-sensitive cancer.

Patients and Methods: Two hundred six eligible patients were randomized between double-blind MPA 500 mg twice daily or placebo. Appetite (0 to 10 numerical rating scale), weight, and QL (European Organization for Research and Treatment of Cancer Quality of Life Questionnaire [EORTC-QLQ-C30]) were assessed before the start of treatment (t=0), and 6 weeks (t=6) and 12 weeks (t=12) thereafter.

Results: One hundred thirty-four patients (68 MPA and 66 placebo) were assessable at t=6 and 99 patients (53 MPA and 46 placebo) at t=12. A beneficial effect of MPA on appetite was observed after both 6 weeks ($P=0.008$) and 12 weeks ($P=0.01$) of treatment. After 12 weeks, a mean weight gain of 0.6 ± 4.4 kg was seen in the MPA group, versus an ongoing mean weight loss of 1.4 ± 4.6 kg in the placebo group. This difference of 2.0 kg was statistically significant ($P=0.04$). During the study, several areas of QL deteriorated in the total group of patients. With the exception of an improvement in appetite and possible also a reduction in nausea and vomiting, no measurable beneficial effects of MPA on QL could be demonstrated. The side effects profile of MPA was favorable: only a trend toward an increase in (usually mild) peripheral edema was observed.

Conclusion: In weight losing, advanced-stage non-hormone-sensitive cancer patients, MPA exhibits a mild side effects profile, has a beneficial effect on appetite, and may prevent further weight loss. However, general QL in the present study was not measurably influenced by MPA treatment.

INTRODUCTION

The development of substantial weight loss is a well known sequela in the majority of patients with advanced-stage cancer (1,2). The etiology of this weight loss, which may ultimately lead to cachexia, is thought to be complex. Particularly, anorexia and metabolic disturbances are regarded as important contributing factors (3). As cachexia negatively influences prognosis (4-6), several agents have been tested for their ability to reverse weight loss in these patients. To date, corticosteroids, cyproheptadine and hydrazine sulfate have failed to influence weight loss in controlled human studies (7-10).

Synthetic progestagens -megestrol acetate (MA) and medroxyprogesterone acetate (MPA)- have been shown to improve appetite and weight, independent of tumor response, when used in the treatment of disseminated breast cancer (11,12). This has led to the hypothesis that synthetic progestagens could also stimulate weight gain in patients with non-hormone-sensitive tumors. Several double-blind, placebo-controlled trials that tested this hypothesis have been published. In one study, using a cross-over design with a treatment duration of one week, a statistically significant beneficial effect of MA on appetite, caloric intake, weight, triceps skinfold thickness and subjective energy level was reported (13). Three other studies have shown a significant positive effect of MA on appetite (14-16), while two of these studies also report a favorable effect on weight in a subgroup of patients (14,16). However, a statistically significant beneficial influence on average weight was not demonstrated. The effects of MA on quality of life were not investigated in these placebo-controlled studies, at least not with the use of standard, well-validated QL instruments.

We conducted a multicenter, double-blind, placebo-controlled study of MPA in patients with advanced-stage, incurable, non-hormone-sensitive cancer. The objectives of the study were to generate further evidence regarding the usefulness of synthetic progestagens in the treatment of anorexia and weight loss, and to investigate the possible effects of these agents on patients' QL.

PATIENTS AND METHODS

Patients

Patients with the following histologically or cytologically diagnosed, non-hormone-sensitive, incurable malignancies were considered eligible for the present study: non-small-cell carcinoma of the lung; mesothelioma; carcinoma of the digestive tract or pancreas; carcinoma of the kidney, bladder or ureter; and disseminated carcinoma of unknown origin. All patients were required to have a Karnofsky Performance Status score $\geq 60\%$. Exclusion criteria were as follows: brain metastases; thromboembolic events during the last year; poorly controlled hypertension or heart failure; diabetes mellitus or thyroid disease; the use of oral or parenteral corticosteroids, androgens, progestagens, anticonvulsants, or rifampicin; jaundice; serum creatinine level $>200 \mu\text{mol/L}$; enteral or parenteral tube feedings; and known malabsorption or mechanical obstruction of the digestive tract. The protocol was approved by the institutional review boards for human research of the participating centers, and written informed consent was obtained from all

patients.

Study design

Eligible patients were randomized (block randomization in permutation blocks of four) between MPA (Farlutal; Pharmacia Inc, Woerden, The Netherlands) 500 mg twice daily or placebo. Stratification was applied for the participating center (University Hospital, Maastricht; University Hospital, Leuven; de Wever Hospital, Heerlen; St Catharina Hospital, Eindhoven; and Maasland Hospital, Sittard) and for the concurrent use of chemotherapy. The design of the study was double-blind with a treatment duration of 12 weeks. All patients were instructed to take one tablet before breakfast and one tablet before the evening meal. Appetite, weight, and QL were assessed before the start of treatment ($t=0$), and 6 weeks ($t=6$) and 12 weeks ($t=12$) thereafter. Subjects were considered assessable when at least $t=6$ was reached.

Appetite and weight

Appetite was assessed by a numerical rating scale that ranged from 0 to 10: in which 0 indicated absolutely no appetite and 10 indicated an extremely good appetite. For each patient, consecutive weights were measured on the same scale, in light indoor clothing without shoes.

Quality of life

QL was assessed by the European Organization for Research and Treatment of Cancer Quality of Life Questionnaire (EORTC-QLQ-C30), a 30-item questionnaire developed specifically for use in cancer patients (17). The QLQ-C30 incorporates nine scales: five functioning scales (physical, role, cognitive, emotional, and social), three symptom scales (fatigue, pain, and nausea/vomiting), and one global QL scale. Additionally, it contains five single items that address other symptoms often experienced by cancer patients (dyspnea, sleep disturbance, loss of appetite, constipation, and diarrhea) and one item concerning possible financial problems brought about by the disease or treatment. The reliability and validity of the QLQ-C30 have been confirmed in a number of international studies of patients with a range of cancer diagnoses (17-21).

Side effects

The following possible side effects were monitored explicitly at each visit by the patients' physician: hypertension, edema, heart failure, jaundice, vaginal spotting, and

thromboembolic events. Additionally, restlessness, abdominal fullness, muscle cramps, tremors, vertigo, headache, and sweating were considered as possible side effects of synthetic progestagens. These symptoms were monitored with the use of a separate, patient self-report questionnaire, which consisted of descriptive 4-point scales (not at all, a little, quite a bit, and very much), which was appended to the EORTC-QLQ-C30. If a symptom score at $t=6$ or $t=12$ was higher than at $t=0$, the symptom was considered to be a side effect. Finally, the patients were asked at the end of the study, by means of an open-ended question, what side effects, if any, they had experienced during the treatment period.

Survival

Although it was not a primary end-point of this study, survival analysis was performed. Two types of survival analysis were performed, as follows: (1) an analysis based on the entire patient sample (according to the intention-to-treat principle); (2) an analysis based on the subset of patients who completed the study.

Statistical analysis

Results are given as the score means $\pm$ SD. Group means were compared using one-way analysis of variance (ANOVA). Differences in categoric variables were analyzed by means of the chi-square test with Yates' continuity correction or Fisher's exact test, where appropriate. Longitudinal changes were compared between the two groups by means of repeated-measures ANOVA. For ease of presentation, the data are reported in tabular form as change scores. Survival is expressed as median months $\pm$ SE and was compared between groups by means of the log-rank test. For all analyses, the statistical package SPSS/PC+ (version 5.0 or 6.0-Windows; SPSS Inc., Chicago, IL) was used. As weight changes are difficult to interpret in the presence of edema, patients with edema at the start of the study or who developed this condition during the study were excluded from weight analyses. Statistical significance was determined at the 5% level and all values are two-sided.

RESULTS

Two hundred six eligible patients were randomized to the MPA group ($n=103$) or the placebo group ($n=103$). Of these patients, 134 (68 MPA, 66 placebo) were assessable at $t=6$, and 99 (53 MPA, 46 placebo) at $t=12$. The reasons for sample attrition were the

TABLE 6.1 Baseline characteristics of the eligible and assessable patients¹

	ELIGIBLE		EVALUABLE ²	
	MPA (n = 103)	PLACEBO (n = 103)	MPA (n = 68)	PLACEBO (n = 66)
Gender (%)				
male	75	75	78	85
female	25	25	22	15
Age (y)	65 ± 10	63 ± 10	64 ± 10	63 ± 9
Tumor type (%)				
non-small-cell lung carcinoma	59	52	62	64
digestive tract carcinoma	23	23	25	21
other	17	24	13	15
Histology (%)				
adeno	47	56	44	52
squamous-cell	25	29	25	31
undifferentiated large-cell	22	12	24	12
other	6	3	7	5
Metastases (%)				
yes	70	80	66	77
no	30	20	34	23
Karnofsky Performance Status (%)	76 ± 11	75 ± 11	78 ± 11	78 ± 9
Chemotherapy (%)				
yes	8	10	12	12
no	92	90	88	88
Appetite (0-10)	5.8 ± 2.9	6.0 ± 2.9	6.3 ± 2.8	6.7 ± 2.7
Weight loss (kg) ³	6.4 ± 6.1	8.0 ± 7.8	5.7 ± 5.5	6.4 ± 6.0
Weight loss (%) ³	8.8 ± 8.3	10.7 ± 9.0	8.1 ± 7.6	8.6 ± 8.0
Weight (kg)	65.0 ± 11.0	64.4 ± 10.6	65.2 ± 11.7	66.4 ± 10.0
BMI (kg/m ²) ⁴	22.8 ± 3.4	22.7 ± 3.4	22.8 ± 3.5	23.0 ± 3.5
PIBW (%) ⁵	100 ± 15	100 ± 15	100 ± 15	101 ± 15
PIBW < 95% (%)				
yes	37	38	36	35
no	63	62	64	65
Albumin (g/L)	36.2 ± 5.5	35.5 ± 6.1	36.7 ± 5.3	37.1 ± 5.1
Hemoglobin (mmol/L)	7.9 ± 1.1	7.6 ± 1.2	7.8 ± 1.0	7.8 ± 1.2

¹ Scores expressed as mean ± SD or percentage; ² at least one follow-up analysis performed; ³ weight loss in relation to pre-illness weight; ⁴ body mass index; ⁵ weight expressed as percentage of ideal body weight.

TABLE 6.2

Baseline characteristics of the eligible and assessable patients: QL

	ELIGIBLE		EVALUABLE ¹	
	MPA (n = 103) Mean $\pm$ SD	PLACEBO (n = 103) Mean $\pm$ SD	MPA (n = 68) Mean $\pm$ SD	PLACEBO (n = 66) Mean $\pm$ SD
<i>Functioning scales²</i>				
Physical	56.6 $\pm$ 28.4	51.0 $\pm$ 26.8	61.5 $\pm$ 26.1	60.0 $\pm$ 25.0
Role	55.1 $\pm$ 36.1	55.4 $\pm$ 36.5	62.3 $\pm$ 33.1	63.3 $\pm$ 35.5
Cognitive	82.8 $\pm$ 20.3	82.2 $\pm$ 21.8	86.2 $\pm$ 18.5	86.4 $\pm$ 18.3
Emotional	62.1 $\pm$ 26.4	67.0 $\pm$ 24.6	65.3 $\pm$ 24.6	65.8 $\pm$ 24.9
Social	71.9 $\pm$ 30.5	70.9 $\pm$ 27.7	79.0 $\pm$ 28.3	75.0 $\pm$ 26.1
Global QL	51.1 $\pm$ 26.0	49.2 $\pm$ 22.4	57.6 $\pm$ 22.0	52.8 $\pm$ 20.2
<i>Symptom scales and/or items³</i>				
Fatigue	52.6 $\pm$ 29.3	49.5 $\pm$ 28.5	45.5 $\pm$ 26.9	40.7 $\pm$ 27.4
Nausea and vomiting	15.5 $\pm$ 26.9	14.1 $\pm$ 21.1	11.8 $\pm$ 23.5	8.9 $\pm$ 14.5
Pain	36.0 $\pm$ 34.4	39.2 $\pm$ 31.9	31.0 $\pm$ 30.2	36.7 $\pm$ 32.7
Dyspnea	39.1 $\pm$ 29.0	38.2 $\pm$ 30.5	34.9 $\pm$ 27.3	36.1 $\pm$ 29.0
Sleep disturbance	34.7 $\pm$ 34.3	35.3 $\pm$ 33.4	30.3 $\pm$ 32.7	37.2 $\pm$ 31.3
Appetite loss	35.7 $\pm$ 37.9	29.1 $\pm$ 37.1	27.7 $\pm$ 32.8	22.8 $\pm$ 32.8
Constipation	15.8 $\pm$ 29.1	11.8 $\pm$ 22.8	13.8 $\pm$ 26.3	8.9 $\pm$ 18.3
Diarrhea	11.8 $\pm$ 27.5	7.2 $\pm$ 19.1	8.2 $\pm$ 22.8	4.4 $\pm$ 14.3
Financial impact	8.1 $\pm$ 21.3	19.6 $\pm$ 30.2 ⁴	8.2 $\pm$ 21.3	19.4 $\pm$ 29.6 ⁵

¹ At least one follow-up analysis performed; ² scores ranging from 0-100, a higher score representing a higher level of functioning; ³ scores ranging from 0-100, a higher score representing a greater degree of symptoms; ⁴ MPA vs placebo: $P < 0.005$; ⁵ MPA vs placebo: $P < 0.02$.

following: death or physical deterioration (28 MPA, 39 placebo), side effects (six MPA, eight placebo), refusal to continue (five MPA, eight placebo), protocol violation (five MPA, two placebo), mechanical obstruction of the digestive tract (four MPA), sigmoid perforation and peritonitis (one MPA), and loss to follow-up (one MPA).

General baseline characteristics of the eligible, as well as of the assessable, patients are listed in **Table 6.1**. The treatment groups were comparable on all variables. The majority of patients were male, the predominant malignancy was non-small-cell carcinoma of the lung, the majority of patients suffered from distant metastases, and only a small subgroup was concurrently treated by chemotherapy. On average, the patients were characterized by a substantial involuntary loss of weight. The magnitude of prestudy weight loss can also be expressed as percentage of ideal body weight (PIBW) (22), which was more than 10% lower than expected for a healthy Dutch elderly population (23). However, the majority of patients did not suffer from manifest cachexia (defined as a PIBW <95%). Baseline QL scores are listed in **Table 6.2**. Except for the financial impact item, the two treatment groups were comparable on all QL parameters assessed.

Observed changes in appetite and weight are listed in **Table 6.3**. After both 6 and 12 weeks of treatment, a significant beneficial effect of MPA was found on appetite. At $t=12$, a slight weight gain was seen in the MPA group, versus an ongoing weight loss in the placebo group. The between group difference of 2.0 kg was statistically significant ($P=0.04$).

TABLE 6.3

Changes (Δ) in appetite and weight after 6 weeks and 12 weeks of treatment

	MPA		PLACEBO		MPA vs PLACEBO
	<i>N</i>	Mean $\pm$ SD	<i>N</i>	Mean $\pm$ SD	<i>P</i>
<u>6 Weeks</u>					
Δ -Appetite (0-10)	65	+0.4 $\pm$ 2.7	59	-0.8 $\pm$ 2.2	0.008
Δ -Weight (kg)	59	-0.1 $\pm$ 2.5	58	-0.9 $\pm$ 3.1	0.15
<u>12 Weeks</u>					
Δ -Appetite (0-10)	53	+0.8 $\pm$ 2.7	45	-0.6 $\pm$ 2.4	0.01
Δ -Weight (kg)	42	+0.6 $\pm$ 4.4	42	-1.4 $\pm$ 4.6	0.04

Table 6.4 lists weight changes for both treatment groups, stratified by the presence of cachexia. In the placebo group, progressive weight loss was observed during the study period in patients who were noncachectic, while in the manifestly cachectic subjects no further weight loss occurred. In the MPA treated patients, these subgroup differences were not found; weight remained relatively stable in both cachectic and noncachectic patients. While this would suggest that weight losing but not yet cachectic patients may benefit more from MPA treatment than those who are already manifestly cachectic, formal statistical tests (i.e., treatment by subgroup interaction) could not confirm this hypothesis. In part, this could be due to the relatively small numbers of patients in the respective subgroups.

TABLE 6.4

Comparison of weight changes (kg) in cachectic (PIBW <95%) and noncachectic (PIBW ≥95%) patients in both study groups at t=6 and t=12

	MPA				PLACEBO				<i>P</i> ¹
	CACHECTIC		NON-CACHECTIC		CACHECTIC		NON-CACHECTIC		
	<i>N</i>	Mean ±SD	<i>N</i>	Mean ±SD	<i>N</i>	Mean ±SD	<i>N</i>	Mean ±SD	
t=6	21	- 0.2 ±1.6	38	- 0.2 ±2.9	21	+0.3 ±1.8	37	-1.6 ±3.5 ²	0.07
t=12	11	+0.7 ±2.7	31	+0.6 ±4.9	14	+0.3 ±3.2	28	-2.3 ±5.0 ³	0.23

¹ Based on 2x2 repeated measures analysis of variance: statistical test for group (MPA vs placebo) by nutritional status (cachectic vs non-cachectic) by time interaction; ² *P*=0.009, main effect of cachectic vs non-cachectic status in the placebo group at t=6; ³ *P*=0.04, main effect of cachectic vs non-cachectic status in the placebo group at t=12.

The results of the QL analysis are listed in **Tables 6.5 and 6.6**. Among assessable patients, all QL questionnaires were available at t=0 and t=12. At t=6, 7% were missing. After a study duration of 6 weeks, a significant worsening of physical functioning and an increase in fatigue, dyspnea, and constipation were observed for the total sample. Comparison of the two treatment groups showed a trend toward a beneficial effect of MPA on appetite loss (*P*=0.06) and nausea and vomiting (*P*=0.10). No other differences between the two treatment groups were observed on any scale or item. At t=12, for the total sample, a significant deterioration in physical, role and cognitive functioning, as well as an increase in dyspnea and constipation, was observed. Again,

TABLE 6.5

Changes (Δ) in QL after 6 weeks of treatment

6 WEEKS	MPA (n = 65)	PLACEBO (n = 60)	TOTAL GROUP (n = 125)	MPA vs PLACEBO
	Mean $\pm$ SD	Mean $\pm$ SD	P ¹	P
<i>Functioning scales²</i>				
Δ -Physical	- 6.5 $\pm$ 18.4	- 4.0 $\pm$ 28.0	0.01	0.56
Δ -Role	- 1.5 $\pm$ 36.4	- 5.0 $\pm$ 37.6	0.33	0.60
Δ -Cognitive	- 2.3 $\pm$ 20.6	- 1.4 $\pm$ 15.1	0.26	0.78
Δ -Emotional	+1.4 $\pm$ 17.7	- 3.2 $\pm$ 20.0	0.60	0.18
Δ -Social	- 3.6 $\pm$ 26.1	+1.9 $\pm$ 33.8	0.76	0.31
Δ -Global QL	- 1.8 $\pm$ 20.0	- 4.0 $\pm$ 21.0	0.12	0.54
<i>Symptom scales and/or items³</i>				
Δ -Fatigue	+3.6 $\pm$ 19.6	+7.0 $\pm$ 25.1	0.009	0.39
Δ -Nausea and vomiting	- 1.8 $\pm$ 27.7	+5.3 $\pm$ 18.8	0.42	0.10
Δ -Pain	+3.6 $\pm$ 29.2	+4.2 $\pm$ 28.4	0.14	0.91
Δ -Dyspnea	+8.7 $\pm$ 23.8	+7.2 $\pm$ 26.8	0.001	0.74
Δ -Sleep disturbance	+2.1 $\pm$ 29.4	0.0 $\pm$ 30.7	0.70	0.70
Δ -Appetite loss	- 6.2 $\pm$ 39.0	+6.1 $\pm$ 33.9	1.00	0.06
Δ -Constipation	+7.2 $\pm$ 28.5	+11.7 $\pm$ 32.3	0.001	0.41
Δ -Diarrhea	- 1.5 $\pm$ 25.3	+2.8 $\pm$ 17.7	0.75	0.28
Δ -Financial impact	+4.6 $\pm$ 19.4	- 2.8 $\pm$ 31.5	0.69	0.11

¹ Scores at t=6 compared with t=0 within the total group of patients (MPA and placebo); ² a positive score represents an improved level of functioning; ³ a positive score represents a worsening of symptoms.

TABLE 6.6

Changes (Δ) in QL after 12 weeks of treatment

12 WEEKS	MPA (n = 53)	PLACEBO (n = 46)	TOTAL GROUP (n = 99)	MPA vs PLACEBO
	Mean $\pm$ SD	Mean $\pm$ SD	P^1	P
<i>Functioning scales²</i>				
Δ -Physical	- 4.9 $\pm$ 20.0	- 6.1 $\pm$ 26.0	0.02	0.80
Δ -Role	- 2.9 $\pm$ 30.1	-14.2 $\pm$ 45.5	0.03	0.14
Δ -Cognitive	- 3.8 $\pm$ 20.8	- 6.8 $\pm$ 23.2	0.02	0.48
Δ -Emotional	+ 3.6 $\pm$ 19.3	+ 1.2 $\pm$ 21.8	0.24	0.57
Δ -Social	- 3.5 $\pm$ 24.5	+ 1.8 $\pm$ 33.0	0.78	0.37
Δ -Global QL	+ 0.1 $\pm$ 19.6	+ 2.7 $\pm$ 21.6	0.49	0.54
<i>Symptom scales and/or items³</i>				
Δ -Fatigue	+ 1.5 $\pm$ 26.1	+ 2.4 $\pm$ 27.4	0.47	0.86
Δ -Nausea and vomiting	- 1.9 $\pm$ 28.4	+ 6.1 $\pm$ 19.4	0.39	0.11
Δ -Pain	- 0.4 $\pm$ 30.9	+ 5.5 $\pm$ 28.6	0.40	0.34
Δ -Dyspnea	+ 16.1 $\pm$ 29.7	+ 8.0 $\pm$ 29.1	<0.001	0.13
Δ -Sleep disturbance	+ 4.4 $\pm$ 30.0	+ 0.7 $\pm$ 25.8	0.37	0.52
Δ -Appetite loss	- 5.7 $\pm$ 32.5	+ 7.2 $\pm$ 33.6	0.81	0.05
Δ -Constipation	+ 3.1 $\pm$ 31.5	+ 13.1 $\pm$ 28.5	0.009	0.11
Δ -Diarrhea	- 3.2 $\pm$ 18.8	- 0.7 $\pm$ 16.5	0.28	0.50
Δ -Financial impact	+ 1.9 $\pm$ 16.6	- 5.1 $\pm$ 33.7	0.54	0.19

¹ Scores at $t=12$ compared with $t=0$ within the total group of patients (MPA and placebo); ² a positive score represents an improved level of functioning; ³ a positive score represents a worsening of symptoms.

apart from a trend toward a MPA-induced reduction in nausea and vomiting ($P=0.11$) and a statistically significant MPA-related amelioration of self-reported appetite loss, no further differences in QL were observed between the two treatment groups.

As noted previously, side effects were the reason for sample attrition for six MPA and eight placebo patients. Among MPA patients, these side effects included painful breasts ($n=1$, male), headache ($n=1$), heart failure ($n=1$), abdominal discomfort/nausea/vomiting ($n=2$) and autopsy-proven fatal lung embolism ($n=1$). The side effects observed in the placebo patients were abdominal discomfort/nausea/vomiting ($n=6$), deep venous thrombosis of the leg ($n=1$), and newly developed diabetes mellitus ($n=1$). At $t=6$, newly developed edema was encountered in 5% of the MPA patients and in 6% of the placebo patients. At $t=12$, this condition was seen in 17% and 4% of MPA and placebo patients, respectively ($P=0.09$). However, this edema was generally mild and well tolerated, and was never a reason for study drug discontinuation. No other side effects of MPA treatment were observed during the study.

Based on analysis of all patients entered onto the study, the median survival time was 10.8 ± 1.1 months in the MPA group versus 10.0 ± 1.1 months in the placebo group ($P=0.97$). For those patients who completed the study, these figures were 15.9 ± 1.7 months and 12.5 ± 1.7 months for the MPA and placebo groups, respectively ($P=0.24$).

DISCUSSION

The aim of this study was to investigate the effects of MPA on appetite, weight and quality of life in patients with advanced-stage, incurable, non-hormone-sensitive cancer. The results indicate that in these patients, characterized by substantial prestudy loss of weight, MPA has a beneficial effect on appetite and may prevent further weight loss. The results of a subgroup analysis suggested that patients who are weight-losing but not yet cachectic may benefit most from MPA treatment. However, the small number of patients available per subgroup precluded a rigorous test of this hypothesis. If future studies are able to confirm this observation, it would imply that MPA treatment may be most effective when it is initiated early in the course of the disease, before manifest cachexia has developed. Over the course of the study, several areas of self-reported QL deteriorated in the total group of patients. However, apart from a significant MPA-related amelioration of appetite loss and a trend toward a MPA-induced reduction in nausea and vomiting, no differences in QL were observed between the two treatment groups over

time. It should be noted that the sample size used in our study was sufficiently large to detect a difference of one-half standard deviation (i.e., an effect size of 0.50) between groups in QL scores with a significance level of 0.05 and a power of 0.80. An effect size of 0.50 is generally considered to be of a moderate magnitude (24). The effect sizes observed in the current study were well-below this 0.50 level, indicating not only a paucity of statistically significant differences between the MPA and the placebo groups in QL outcomes, but also of substantively meaningful differences. Except for a trend toward a higher incidence of (generally mild) peripheral edema in the MPA-treated patients, no significant differences in treatment-related side effects were observed between the MPA and placebo groups. No MPA-related increase in thromboembolic events was seen: one lung embolism was recorded in the MPA group versus one deep venous thrombosis of the leg in the placebo group. Based on the entire patient sample, no differences in median survival time were observed between the MPA and placebo groups. The results of a second analysis, based on the subset of patients that completed the 12 week study, indicated a median survival advantage of 3.4 months for patients who were treated with MPA. However, these latter results did not reach statistical significance.

To date, the results of six double-blind placebo-controlled trials that investigated the effects of synthetic progestagens on appetite and weight in non-hormone-sensitive cancer have been published; five using MA (13-16,25) and one using MPA (26). A lack of statistical power unfortunately impedes the interpretation of the results of one of the MA studies (25) and the only MPA study. Of the four remaining MA studies, Bruera *et al.* (13), who used a cross-over design with a treatment duration of 1 week, found a statistically significant beneficial effect on appetite, caloric intake, weight, triceps skinfold thickness, and subjective energy level. However, the clinical significance of these findings is unclear given the short study duration. The other three studies (14-16) showed a significant beneficial effect of MA on appetite. In addition, Loprinzi *et al.* (14) found a significant, favorable effect on weight (defined as an increase of at least 15 lb [=6.8 kg]) in 16% of MA-treated patients versus 2% of placebo-treated patients. Feliu *et al.* (16) observed a significant increase in weight of at least 2 kg in 32% of MA-treated patients versus 8% of placebo patients. However, in these studies, and in the study reported by Tchekmedyan *et al.* (15), average weight was not influenced significantly.

The results of the current MPA trial are in agreement with the aforementioned studies with respect to the beneficial effects of synthetic progestagens on appetite. Additionally, our findings enhance the evidence that these agents also have a favorable influence on weight loss. In contrast to previous studies, we were able to document specifically a significant effect on average weight. This can probably be explained by the

fact that our patients were generally less cachectic and therefore, as suggested in this study, were more responsive to treatment than the subjects investigated in earlier studies. An additional contributing factor might be the longer average follow-up period in combination with sufficient statistical power to detect differences between the two study groups after 12 weeks of treatment. Also in agreement with previous MA studies (14,15) are our findings that MPA can have a beneficial effect on nausea and vomiting and that its side effects profile is relatively benign.

Although few data are available on this matter, it would seem plausible that anorexia and weight loss have a negative influence on QL. However, the most important negative effects on both physical and mental well-being are likely to be expected when weight loss is so far advanced that muscle and organ dysfunction occurs as a result of the wasting of ample amounts of protein mass. The majority of patients in the current study, though losing weight, were not manifestly cachectic. In such cases, QL is likely to be affected more by factors related to tumor extension and progression *per se*, than by anorexia and weight loss. This could possibly explain the observed deterioration of QL in the total group of patients without any demonstrable beneficial effect on QL of MPA patients. However, if synthetic progestagens are able to prevent (further) weight loss and the treatment is initiated in an early phase (i.e., from the moment a patient experiences involuntary weight loss), one could hypothesize that, in the long run, a significant improvement in QL can be achieved.

Additional studies are needed to address this issue, as well as to investigate the potential impact of MPA treatment on QL of family members of cancer patients who may be distressed by the consequences of cancer-induced anorexia and cachexia. Further studies are also needed to clarify the following: (1) whether the best MPA response can be achieved in weight losing patients who are still not manifestly cachectic, which implies that prevention or postponement of the development of cachexia is more likely to succeed than treating it once it has developed; and (2) whether MPA has a significant beneficial effect on survival.

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7

***THE EFFECTS OF MEDROXYPROGESTERONE ACETATE ON
FOOD INTAKE, BODY COMPOSITION AND RESTING ENERGY EXPENDITURE
IN ADVANCED-STAGE NON-HORMONE-SENSITIVE CANCER***

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**The effects of medroxyprogesterone acetate on
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A randomized placebo-controlled trial

Cancer, in press

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ABSTRACT

Background: Anorexia and cachexia are well known sequela of cancer, having a negative influence on morbidity and mortality. In several studies in patients with non-hormone-sensitive tumors synthetic progestagens were shown to exert beneficial effects on appetite and weight loss. The aim of the present study was to investigate the effects of medroxyprogesterone acetate (MPA) on food intake, body composition and resting energy expenditure.

Methods: Fifty-four patients with non-hormone-sensitive cancer, on average characterized by substantial weight loss and hypermetabolism, were randomized between MPA 500 mg or placebo, twice daily, for 12 weeks (double-blind). Food intake was measured by dietary history; body composition was assessed by deuterium dilution (fat mass, fat-free mass); resting energy expenditure was obtained by indirect calorimetry.

Results: In contrast with placebo treatment, 12 weeks of MPA led to an increase in energy intake (between-group difference 426 kcal/d, $P=0.01$) that was significantly associated ($r=0.68$, $P=0.003$) with an increase in fat mass (between-group difference 2.5 kg, $P=0.009$). Fat-free mass was *not* significantly influenced. Resting energy expenditure increased during MPA-treatment: at 6 weeks the between-group difference in change was 135 kcal/d ($P=0.009$); after 12 weeks this difference amounted to 93 kcal/d ($P=0.07$).

Conclusions: We conclude that MPA is able to significantly increase food intake and to concomitantly reverse fat loss in patients with non-hormone-sensitive cancer.

INTRODUCTION

Substantial weight loss, eventually leading to cachexia, is a well known complication of advanced stage cancer (1). Particularly metabolic disturbances (including hypermetabolism) and a food intake that is too low to meet the metabolic needs are considered to be important contributing factors in its complex pathophysiology (2-4). As cachexia is associated with an unfavorable effect on morbidity and mortality (5-7), several agents have been tested for their ability to improve cancer-related weight loss. Agents like corticosteroids (8,9), cyproheptadine (10) and hydrazine sulfate (11), however, failed to show such properties in controlled human studies.

Synthetic progestagens -megestrol acetate and medroxyprogesterone acetate (MPA)- have been shown to improve appetite and weight, independent of tumor response, when used in the treatment of disseminated breast cancer (12,13). Subsequently, several

placebo-controlled studies with these agents have been performed in patients with *non*-hormone-sensitive cancer (14-19). Also in these studies, a beneficial influence was observed, with minimal side effects, on appetite and, at least in a subgroup of patients, on weight gain.

Very few evidence from controlled studies exists, however, whether an improved appetite in these patients also results in an increase in food intake and whether the observed weight gain represents tissue mass and not just fluid retention, which is a reported side effect of synthetic progestagens (15,18,20). To clarify these issues, we conducted a double-blind placebo-controlled study in patients suffering from advanced stage, non-hormone-sensitive cancer to investigate the effects of MPA on food intake and body composition. In addition, also the effects of MPA on resting energy expenditure (REE) were assessed.

PATIENTS AND METHODS

Patients

The present study was performed in a subgroup of patients participating in a multicenter study investigating the effects of MPA on appetite, weight and quality of life in advanced stage, non-hormone-sensitive cancer (19). The subgroup was formed by the contingent of patients recruited at the University Hospital Maastricht, The Netherlands. Patients with the following histologically or cytologically diagnosed, non-hormone-sensitive, incurable malignancies were considered eligible: non-small-cell carcinoma of the lung; mesothelioma; carcinoma of the digestive tract or pancreas; carcinoma of the kidney, bladder or ureter; and disseminated carcinoma of unknown origin. All patients were required to have a Karnofsky performance status score $\geq 60\%$. Exclusion criteria were as follows: brain metastases; thrombo-embolic events during the last year; poorly controlled hypertension or heart failure; diabetes mellitus or thyroid disease; the use of oral or parenteral corticosteroids, androgens, progestagens, anticonvulsants, or rifampicin; jaundice; serum creatinine $> 200\mu\text{mol/L}$; enteral or parenteral tube feedings; and known malabsorption or mechanical obstruction of the digestive tract. The protocol was in accordance with the Helsinki Declaration of 1975, as revised in 1983, and was approved by the institutional review board for human research of the University Hospital Maastricht. Written informed consent was obtained from all patients.

Study design

Eligible patients were randomized (block randomization in permutation blocks of four) between MPA (Farlutal; Pharmacia Inc, Woerden, The Netherlands) 500 mg twice daily or placebo twice daily. Stratification was applied for primary disease site (lung vs other). The design of the study was double-blind with a treatment duration of 12 weeks. All patients were instructed to use one tablet before breakfast and one tablet before the evening meal. Food intake, body composition and REE were assessed before the start of treatment ($t=0$), and 6 weeks ($t=6$) and 12 weeks ($t=12$) thereafter.

Food intake

Energy, protein, fat and carbohydrate intake were obtained by using the dietary history method with a reference period of 30 days (21). All interviews were performed by the same, trained dietician. For the calculation of energy intake (EI) and macronutrient intake the Dutch food composition table was used (22).

Body composition

According to a two-compartment body composition model, the human body can be divided into fat mass (FM) and fat-free mass (FFM). In the present study, FFM was assessed by deuterium dilution (23,24). The following protocol was used. The evening before each visit to the hospital, at ≈ 23.00 h, all patients drank a weighted dose of ≈ 4.00 g deuterium (99.84 atom percent excess) in 50 mL water. Then the bottle was rinsed once with ≈ 50 mL tap water, which was also drunk. Thereafter, the patients had to refrain from eating and drinking. They were asked to void at least once at home before attending the hospital. Before and 10 hours after drinking the deuterium water, a urine sample was taken. Deuterium was measured in duplicate in these two samples by using an isotope ratio-mass spectrometer (Aqua Sira, VG Isogas, Cheshire, UK) (25). Comparison of the duplicate measurements showed a precision $>99\%$. Total body water (TBW) was calculated by dividing the measured volume of distribution of deuterium by 1.04, thereby correcting for the exchange of deuterium with non-aqueous hydrogen (23). The use of this test protocol with an overnight equilibration period of 10 hours has been extensively validated in our laboratory (26). It was shown that TBW assessed by using this protocol corresponds very well (bias 1%) with TBW derived from hydrodensitometry (26). FFM was calculated by dividing the measured TBW by 0.73, the assumed hydration coefficient of the FFM in normal-weight healthy individuals (27). FM was calculated by subtracting FFM from body weight.

Resting energy expenditure

REE was assessed by indirect calorimetry by using a ventilated hood system (Oxycon β , Mijnhardt, Bunnik, The Netherlands). After an overnight fast, CO_2 production and O_2 consumption were measured at complete rest during a period of 20 minutes. REE was calculated by using the abbreviated Weir formula (28). The equipment was calibrated at the start of each experiment. The precision of the system was checked monthly by burning methanol with a theoretical respiratory quotient of 0.667 after complete combustion. Further details on the technique used in our laboratory have been described elsewhere (29). To document hypermetabolism, REE was compared (after adjustment for differences in sex and FFM) with REE measured in a group of healthy individuals (mean age 70 ± 5 y). This reference group, recently assessed in our laboratory, was characterized by a REE of 1623 ± 192 kcal/d in males ($n=16$), and of 1271 ± 108 kcal/d in females ($n=9$).

Statistical analysis

All patients finishing the 12-week study period were analyzed. Group means were compared by Student's *t*-test. Differences in categorical variables were analyzed by χ^2 test with Yates' continuity correction or Fisher's exact test, where appropriate. Longitudinal changes were analyzed within and between groups by means of a repeated measures analysis capable of dealing with randomly missing values (30,31). This analysis yields estimated values per group for each measurement time, adjusted for missing values, and tests of changes from $t=0$ to $t=6$ and $t=0$ to $t=12$. As FFM is the body compartment mainly determining the level of metabolic activity, in all REE analyses FFM was used as a covariable. Pearson's product-moment correlation coefficients were used to evaluate linear relationships between variables. The repeated measures analysis was performed by using BMDP 5V (31). For all other analyses SPSS/PC+ (version 6.0-Windows; SPSS Inc., Chicago, USA) was used. Statistical significance was determined at the 5% level, two-sided. Unless otherwise stated, results are given as score mean $\pm$ SD.

RESULTS

Fifty-four eligible patients were randomized. Twenty-seven patients were assigned to each treatment group. Thirty-three patients (18 MPA, 15 placebo) completed the 12-week study period. The reasons for sample attrition were the following: death or physical deterioration (3 MPA, 4 placebo), side effects (4 placebo: gastro-intestinal discomfort),

TABLE 7.1

Baseline characteristics of the eligible and assessable patients, stratified by treatment group^a

	ELIGIBLE		ASSESSABLE ^b	
	MPA (n = 27)	PLACEBO (n = 27)	MPA (n = 18)	PLACEBO (n = 15)
Sex (%)				
male	74	78	78	87
female	26	22	22	13
Age (y)	66 ± 10	65 ± 8	68 ± 8	67 ± 7
Tumor type (%)				
non-small-cell lung carcinoma	63	59	61	73
digestive tract carcinoma	19	7	22	7
other	19	34	17	20
Histology (%)				
adeno	37	52	33	47
squamous-cell	22	37	22	47
undifferentiated large-cell	37	11	39	7
other	4	0	6	0
Metastases (%)	89	93	89	93
Karnofsky Performance Status (%)	76 ± 9	74 ± 9	77 ± 9	77 ± 10
Chemotherapy (%)	0	0	0	0
Edema (%)	4	11	0	0
Appetite (0-10) ^c	6.2 ± 2.8	6.9 ± 2.5	7.1 ± 2.5	6.9 ± 2.8
Weight loss (kg) ^d	5.6 ± 7.2	7.1 ± 5.7	5.0 ± 6.4	4.8 ± 5.1
Weight loss (%) ^d	7.4 ± 9.6	9.9 ± 7.9	6.9 ± 8.6	6.5 ± 6.8
Weight (kg)	65.3 ± 8.3	65.2 ± 10.6	65.1 ± 9.2	69.4 ± 10.2
Height (m)	1.68 ± 0.09	1.69 ± 0.08	1.68 ± 0.08	1.70 ± 0.07
Body mass index (kg/m ²)	23.1 ± 2.3	22.9 ± 4.1	23.0 ± 2.3	24.2 ± 4.0
WT/IBW (%) ^e	101 ± 11	100 ± 17	101 ± 11	106 ± 17
Fat-free mass (kg)	49.8 ± 9.4	49.0 ± 6.5	49.4 ± 9.4	52.0 ± 6.0
Fat mass (kg)	15.5 ± 6.3	16.2 ± 8.3	15.8 ± 5.1	17.3 ± 7.2
Energy intake (kcal/d)	2267 ± 754	2151 ± 610	2320 ± 756	2025 ± 529
REE (kcal/d) ^f	1639 ± 276	1661 ± 234	1659 ± 285	1699 ± 235

^a Scores expressed as mean ± SD or percentage; ^b 12-week study completed; ^c appetite expressed on a 0-10 numerical rating scale, 0 indicating absolutely no appetite and 10 indicating an extremely good appetite;

^d weight in relation to pre-illness weight; ^e body weight expressed as percentage of ideal body weight;

^f resting energy expenditure.

refusal to continue (3 placebo), protocol violation (2 MPA, 1 placebo), mechanical obstruction of the digestive tract (3 MPA), and sigmoid perforation and peritonitis (1 MPA).

In **Table 7.1**, both the baseline characteristics of the patients who entered the study and those of the patients who completed the study are given, stratified by study arm. There were no significant differences between the treatment groups. The majority of patients were male, the predominant malignancy was non-small-cell carcinoma of the lung, the vast majority suffered from distant metastases, and none of the patients was concurrently treated by chemotherapy. The majority of patients was characterized by substantial involuntary weight loss. Sixty-five percent of the eligible patients had lost more than 5% of the pre-illness weight, and 20% of the subjects had lost 0 - 5%. This pre-study weight loss was also demonstrated by the fact that the average body weight was approximately 100% of ideal (32), while in a healthy Dutch elderly population body weight is normally over 110% of ideal in males and over 120% of ideal in females (33). In comparison with the healthy reference group, the patients entered onto the study were significantly hypermetabolic ($P = 0.02$).

In **Figure 7.1**, the observed changes in food intake are visualized for both treatment arms. As food intake data were not available in one placebo patient, 14 placebo patients and 18 MPA patients were used in the analysis. At $t=12$, a mean increase in EI of 418 kcal/d was found in the MPA group, versus no change in the placebo group (between-group difference 425 kcal/d, $P=0.01$). This increase was the result of a significantly improved intake of both protein, fat and carbohydrates.

In **Figure 7.2**, changes in body composition are shown. All subjects reaching $t=12$ were used in the analysis. During the study, peripheral edema developed in four MPA patients and one placebo patient. At $t=12$, a mean gain in FM of 1.0 kg was observed in the MPA treated patients, versus a fat loss of 1.5 kg in the placebo group (between-group difference $P=0.009$). An increase in FM was seen in 71% of the MPA patients, while in the placebo group a FM-increase was observed in 20% of the cases (between-group difference $P=0.01$). FFM increased by 1.0 kg in the MPA group ($P=0.04$), and by 0.4 kg in the placebo group. Half of the average FFM-increase in the MPA group was however accounted for by the subjects developing edema. When these patients were excluded from FFM-analysis, the remaining mean within-group change (+0.5 kg) lost statistical significance.

FIGURE 7.1 Intake of energy, protein, fat and carbohydrates (mean values and SEM) at baseline and after 6 weeks and 12 weeks of treatment in the MPA group (solid lines) and placebo group (dashed lines). *P*-values for changes from baseline: (within-group) ###, $P < 0.001$; #, $P < 0.05$; &, $P < 0.1$; (between-group) **, $P < 0.01$; *, $P < 0.05$; \$, $P < 0.1$.

FIGURE 7.2 Fat mass and fat-free mass (mean values and SEM) at baseline and after 6 weeks and 12 weeks of treatment in the MPA group (solid lines) and placebo group (dashed lines). *P*-values for changes from baseline: (within-group) #, $P < 0.05$; (between-group) **, $P < 0.01$.

FIGURE 7.3 Correlation between changes in energy intake and changes in fat mass in the MPA patients (●, solid line, $r=0.68$, $P=0.003$) and placebo patients (○, dashed line, $r=0.09$, $P=0.77$) after 12 weeks of treatment.

FIGURE 7.4 Resting energy expenditure (mean values and SEM) at baseline and after 6 weeks and 12 weeks of treatment in the MPA group (solid line) and placebo group (dashed line). P -values for changes from baseline: (within-group) &, $P<0.1$; (between-group) **, $P<0.01$; \$, $P<0.1$.

In **Figure 7.3** is shown that the MPA-related changes in FM were significantly correlated with changes in EI ($r=0.68$, $P=0.003$).

In **Figure 7.4**, the observed changes in REE are visualized for both treatment groups. As REE data were not available in two MPA patients (technical reasons) and in one placebo patient (claustrophobia), 14 placebo patients and 16 MPA patients were used in the analysis. At $t=6$, a mean increase in REE of 65 kcal/d was observed in the MPA group, versus a decrease of 70 kcal/d in the placebo group (between-group difference 135 kcal/d, $P=0.009$). At $t=12$, the between-group difference in change was 93 kcal/d ($P=0.07$).

DISCUSSION

Substantial weight loss, eventually leading to cachexia, is thought to play an important role in cancer-related morbidity and mortality (5-7). Previous studies showed that this weight loss is the result of a combined wasting of FM and body cell mass (3,34), the latter being the body compartment containing most of the tissues (muscle, organs, etc.) that are vital to a normal functioning of the body (27). Several placebo-controlled studies in patients with non-hormone-sensitive cancer reported a beneficial influence of synthetic progestagens on appetite and, at least in a subgroup of patients, on weight (14-19). In an open, non-controlled study with megestrol acetate in 12 weight-stable, non-anorectic breast cancer patients (11 females, 1 male) Loprinzi *et al.* reported that, in those patients who gained weight ($n=7$), this weight gain was predominantly the result of an increase in dual-energy X-ray absorptiometry (DEXA)-derived fat mass (35). Bruera *et al.* observed, in a placebo-controlled cross-over study with a treatment duration of one week, a statistically significant beneficial effect of megestrol acetate on EI (14). To our knowledge, in cancer no other studies, and in particular no placebo-controlled studies with a relatively long treatment duration, were to date performed to study the effects of synthetic progestagens on energy balance and body composition. In two placebo-controlled studies in AIDS cachexia, megestrol acetate was reported to increase caloric intake (36,37). With respect to (bioelectrical impedance analysis-derived) body composition, one of these studies showed a beneficial effect of megestrol acetate on FM, while the other found also a beneficial effect on lean body mass. The latter finding was however contradicted by the fact that there was no significant change in TBW. It should be noted that in both studies a relatively high daily dose of 800 mg megestrol acetate was used (160 mg megestrol acetate is assumed to be equivalent to 1000 mg MPA [38]).

The present study was designed to investigate, in non-hormone-sensitive cancer, the effects of a 12-week treatment with MPA on food intake, body composition and energy expenditure. The study was carried out, in a randomized placebo-controlled fashion, in a subgroup of patients participating in a multicenter study investigating the effects of MPA on appetite, weight and quality of life. In the latter study, a significant MPA-related beneficial effect on both appetite and weight was observed (19). The results of the present study indicate that in these patients, on average characterized by weight loss and hypermetabolism, the increase in appetite actually leads to a significant increase in energy, protein, fat and carbohydrate intake, and that concomitantly a reversal of ongoing fat loss can be established. Although in some MPA-treated patients an edema-related increase in FFM was observed, FFM-changes seemed to play in general a minor, if any, role in MPA-induced weight change. Although TBW-derived changes in FFM not by definition represent changes in body cell mass, this finding might nevertheless indicate that a 12-week treatment with MPA fails to improve this functionally important body compartment. A factor that in theory might form an explanation for this negative finding in our predominantly male study population might be a testosterone-lowering effect of MPA (39). However, the small number of assessable MPA-treated female patients (three edema-free subjects) precluded further elaboration of this hypothesis. Finally, in addition to its effects on food intake and FM, the use of MPA was associated with a (further) increase in REE. Although the explanation for this finding remains speculative (a direct metabolic effect of the agent?), the induced improvement of EI was obviously amply sufficient to achieve a positive energy balance. A possible limitation of the present study was the fact that 40% of the eligible patients were inassessable due to premature drop-out. This is however a common and unavoidable problem in clinical trials in advanced-stage cancer, particularly in those with a relatively long treatment duration.

Considering the present study, some methodological aspects deserve further discussion. Firstly, body composition was derived from TBW, measured by deuterium dilution. When using this method, FFM is calculated by dividing the measured TBW by 0.73, the assumed hydration coefficient of the FFM in normal-weight healthy individuals (27). FM is subsequently calculated by subtracting FFM from body weight. To our knowledge, limited data are available in literature on the hydration coefficient of the FFM in normal-weight or underweight cancer patients. However, in a group of 20 male lung cancer patients with a mean body mass index of $20.8 \pm 2.8 \text{ kg/m}^2$ (range 15.9 - 24.9 kg/m^2), recently analyzed in our laboratory by DEXA-scanning and deuterium dilution, the mean hydration coefficient was 0.73 ± 0.03 (range 0.68 - 0.79) and was not correlated with body mass index ($r=0.16$, $P=0.51$) (unpublished observations).

Nevertheless, since fluid retention occurred in some patients, changes in hydration coefficient during the follow-up period could have introduced some bias in the MPA group towards an over-estimation of FFM-changes and an under-estimation of FM-changes. If this phenomenon indeed played a role, it would however rather strengthen than weaken the conclusions drawn from the results of the study. Secondly, food intake was assessed by the dietary history technique, a method that has recently been validated against measurements of total energy expenditure using whole-body indirect calorimetry (40). In this study in elderly women, an average under-estimation of EI of 12% was reported, a finding that also applies to most other self-report food intake assessment methods (41). For the present study, which aimed at comparing within-group changes between two groups in a randomized placebo-controlled fashion, it was however more important that several earlier studies reported a very good reproducibility of the dietary history method (see review by Block [21]).

The mechanism of action of synthetic progestagens in the treatment of cancer-related weight loss is still obscure. They obviously interfere with the complex regulation of food intake (42,43), but on what level (hypothalamic, neuro-endocrine, gut) is unclear. Furthermore, it is not known whether these agents also have specific *direct* effects on metabolism. In a study in rats, megestrol acetate has been shown to stimulate the hypothalamic synthesis, transport and release of neuropeptide Y (44), a potent stimulator of appetite (45). In another *-in vitro-* study with megestrol acetate, a direct stimulation of adipocyte differentiation was reported (46).

In conclusion, the present study demonstrates that the synthetic progestagen MPA has the potential to increase food intake and to concomitantly reverse fat wasting in weight-losing cancer patients. It remains to be determined, however, whether also protein wasting can be influenced by MPA, as on the longer term particularly a preservation of protein mass can be expected to interfere beneficially with morbidity and mortality.

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8

SUMMARY AND GENERAL DISCUSSION

Summary and general discussion

(Chapter 1) Involuntary weight loss, eventually leading to cachexia, is a clinically highly relevant complication of cancer (1) that negatively influences quality of life, survival (2-5), and treatment response (6,7). Although it might occur in any type of cancer, its incidence is particularly high in malignancies of the lung, pancreas and gastrointestinal tract (2,8).

The pathophysiology of this weight loss is complex and only partly understood. Predominantly based on studies in experimental models, several mechanisms are thought to play a major role: an increased systemic inflammatory state (9), the circulation of tumor-derived (non-cytokine) lipolytic and proteolytic factors (10,11), increased Cori cycle activity (12), and a decreased food intake (8).

Despite the fact that in the past decade many agents have been tested for their ability to reverse cancer-related weight loss, the number of therapies presently recommendable for use in daily clinical practice is still disappointingly limited.

(Chapter 2) As nearly all tissues that are vital for a normal functioning of the body are part of the body-cell-mass (BCM) (13), it is evident that particularly wasting of this body compartment can be expected to be responsible for the negative effects of weight loss on performance status and survival. If so, BCM-wasting should become a specific target for therapeutical intervention. However, to develop such specific treatment strategies it is mandatory to have a maximum of insight in its underlying pathophysiology.

Particularly studies in experimental models revealed that the development of cancer cachexia is for an important part related to a chronic, low-grade, tumor-induced activation of the host immune system (9). Until the start of our studies, however, confirmatory data were scarce in *human* cancer (14,15), and particularly the specific pathophysiology of cancer-related BCM-wasting had not been investigated.

To explore these issues, we investigated in 20 male lung cancer patients the relationship between both weight loss and BCM-wasting, and the putative presence of systemic inflammation (sTNF-R55, sTNF-R75, IL-6), increased acute-phase response (LBP, albumin), anorexia, hypermetabolism and changes in circulating levels of catabolic (cortisol) and anabolic (testosterone, IGF-I) hormones. In addition, to obtain more insight in the specific clinical relevance of cancer-related BCM-wasting, the relationship between height-adjusted BCM (BCM-ix) and Karnofsky performance status was assessed.

Karnofsky performance score was shown to be explicitly correlated with BCM-ix. Severe weight loss was significantly correlated with high sTNF-R55 levels, low levels of the "negative" acute-phase protein albumin, and low IGF-I. In addition, BCM-wasting was specifically correlated with high circulating levels of sTNF-R55 and sTNF-R75, high

plasma levels of LBP, low serum albumin and high BCM-adjusted resting energy expenditure (REE/BCM). Furthermore, a trend towards a (positive) correlation between BCM-ix and IGF-I was observed. sTNF-R55 levels correlated negatively with albumin and positively with REE/BCM, and there were trends towards a positive correlation with LBP and a negative correlation with appetite.

Based on these results, we concluded that human lung cancer-related weight loss and BCM-wasting are associated with systemic inflammation, increased hepatic acute-phase protein production and, in case of BCM-wasting, hypermetabolism. In addition, we were able to show that, although not evidently related to systemic inflammation, also decreased IGF-I levels might be involved.

(Chapter 3) Leptin, the product of the adipocyte *ob* gene, is a recently identified hormone that is thought to represent the afferent signal in a feedback mechanism regulating fat mass (16). After release by the adipocyte, leptin is assumed to bind to a specific receptor in the hypothalamus (17-19), the brain nucleus that plays a central role in the regulation of feeding behaviour and energy balance (20,21). In animal models, the result of this interaction is a decrease in food intake and an increase in energy expenditure (18,19,22,23). In view of the fact that anorexia and hypermetabolism frequently play a role in the development of cancer cachexia, underlying abnormalities in the leptin feedback mechanism might, in hypothesis, be present. In particular, elevated levels of circulating leptin or, on the contrary, a hypothalamic insensitivity to a fall in leptin levels might be involved. As there is evidence from studies in *in vitro* and *in vivo* experimental models that *ob* gene expression may be up-regulated by substances like cortisol and pro-inflammatory cytokines such as TNF and IL-1 (24-27), substances that also seem to be involved in the pathophysiology of cancer cachexia (15,28), particularly the possibility of high leptin levels playing a role in infection- and malignancy-related cachexia has been suggested in literature (26,27).

To clarify this issue, we investigated the relationship between total plasma leptin, weight loss, body composition, appetite and REE in lung cancer.

Twenty-one male patients were studied. These subjects were on average characterized by a weight loss of approximately 10% and a body weight of 94% of ideal. In only 6 of these patients plasma leptin was detectable, with levels within the normal range. These subjects were characterized by less weight loss, by less underweight, and by a higher fat mass than the patients with non-detectable leptin levels, and significant between-group differences in appetite and REE were lacking.

Based on these results, we concluded that in cancer patients, in analogy to normal

and obese individuals (29,30), leptin levels are directly related to the amount of body fat, being compatible with a normal function of the afferent loop of the leptin feedback mechanism. We were therefore not able to confirm the hypothesis (26,27) that elevated leptin levels may be involved in the pathophysiology of cancer cachexia. As low total leptin levels were not reflected in decreased energy expenditure and increased appetite, it was hypothesized that in cancer cachexia the leptin feedback mechanism dysfunctions at the hypothalamic level. Whether tumor- or host-derived cytokines or other tumor- or host-derived substances are responsible for this overruling of normal weight homeostasis still has to be further clarified.

(Chapter 4) As wasting of fat-free mass (FFM) and BCM may have substantively more impact on performance status, quality of life and prognosis of cancer patients than wasting of fat, a far more differentiated diagnosis of cancer cachexia can be made when not only the amount of weight loss and the degree of underweight are taken into account, but also body composition. Furthermore, body composition data can be used in the decision to start a specifically tailored therapeutical intervention, and to evaluate its results. Also in clinical trials, body composition analysis is indispensable for a reliable interpretation of the efficacy of anti-cachectic interventions.

Apart from skinfold anthropometry with its known limitations, bioelectrical impedance analysis (BIA) is probably the only body composition analysis technique that is inexpensive, can be performed without specific operator skills, and forms no burden on the measured subject. This makes BIA potentially suitable for body composition analysis in "daily" clinical practice, as well as in large-scale clinical trials, including multicenter studies.

Whereas the reliability of BIA (in particular the parameter ht^2/R) to predict total body water (TBW) and its derivative FFM has been proven in many studies in healthy individuals (31-35), few studies investigated the applicability of BIA to assess TBW and FFM in non-healthy individuals, especially those with cachexia.

To clarify this issue, the applicability of single-frequency 50-kHz BIA to predict deuterium dilution-derived TBW was assessed in 16 underweight and 25 normal-weight cancer patients.

Although ht^2/R proved to be a strong single predictor of TBW in both groups, it was shown that TBW would be significantly over-estimated in the underweight group when the prediction formula developed in the normal-weight group was used (bias 1.67 L [5%]). A systematic over-estimation of TBW in the underweight patients was also found when TBW was predicted by several previously published BIA formulas developed in normal-

weight individuals. In addition, also some other drawbacks to the use of BIA were documented. First of all, it was illustrated that the outcome of the prediction of TBW by BIA largely depends on which prediction formula is chosen from the long list of "validated" equations published to date. In our group of normal-weight patients, for example, the average predicted TBW varied, depending on the formula used, from 31.0 L to 39.6 L. Secondly, the SEE of the TBW-prediction was large (≈ 2.2 L, equivalent to 3 kg FFM), hampering the use of BIA in individual cases.

(Chapter 5) Although already widely used in prospective clinical studies (36-40), the applicability of BIA to measure *changes* in TBW and FFM is far from clear. Predominantly in obese individuals on a weight loss program, several investigators observed that its reliability was straight out disappointing (41-45).

To expand our knowledge on this issue, we measured changes in BIA-derived R in the patients participating in the longitudinal study described in chapter 7. In this study, TBW was assessed by deuterium dilution, which was thus used as a reference method for the validation of BIA.

Thirty-three patients were analyzed after an average follow-up of 11 weeks. Changes in TBW_{deu} occurred in both directions (mean $+0.2 \pm 1.6$ L, range -3.3 to +3.1 L). BIA-derived Δ -R proved to be a significant predictor of Δ -TBW_{deu} (r^2 0.48, $P < 0.0001$, SEE 1.17 L). There were in this regard no significant differences between the patients with and without underweight.

We concluded therefore that in underweight and normal-weight cancer patients, BIA-derived Δ -R can be used to predict changes in TBW.

Nevertheless, there are also some pitfalls in the use of BIA to measure *changes* in body composition that need to be further discussed. First of all, on an individual level the reliability is poor in view of the relatively high SEE in relation to the TBW-changes observed. Secondly, based on literature data presently available, it seems likely that BIA is substantially less suitable to measure changes in body composition in situations where weight changes coincide with substantial fluid shifts. Thirdly, it is important to realize that the use of different prediction equations to calculate TBW from R not only leads to different absolute values of TBW, but also to different values of TBW-*change*. The larger the relative importance of R (or ht^2/R) in the formula, the larger the assessed Δ -TBW for a given Δ -R will be. This inherent problem of quantification can only be circumvented by exclusively analyzing and reporting within- and between-group changes in R. When quantification is nevertheless desired, which probably often will be the case, we suggest to report in addition to (the analysis of) "crude" R values, the exact formula by which

TBW or FFM is calculated. Unfortunately, however, this is not at all common practice in prospective clinical studies to date.

(Chapter 6) In view of the negative effects of weight loss on quality of life, survival and treatment response, several agents have been tested for their ability to reverse the process of cancer cachexia. While agents like corticosteroids, cyproheptadine and hydrazine sulfate failed to influence weight loss in controlled human studies, synthetic progestagens -MA and MPA- were shown to improve appetite and weight, independent of tumor response, when used in the treatment of disseminated breast cancer (46,47). Subsequently, also several double-blind, placebo-controlled trials in non-hormone-sensitive cancer observed a favorable effect of MA on appetite (48-52). Some of these studies, though not all, also found a significant beneficial effect on weight (48,49,51). This weight effect was however only seen in a subgroup of patients, while a significant beneficial influence on average weight was not demonstrated. In addition, the effects of MA on quality of life were hardly investigated, at least not with the use of standard, well-validated quality of life instruments.

To generate further evidence regarding the usefulness of synthetic progestagens in the treatment of cancer cachexia, we conducted a multicenter, double-blind, placebo-controlled study in 206 patients suffering from advanced-stage, incurable, non-hormone-sensitive cancer. In this study, we investigated the effects of MPA (1000 mg/day) on appetite, weight and quality of life.

A beneficial effect of MPA on appetite was observed after both 6 weeks and 12 weeks of treatment. After 12 weeks, a mean weight gain of 0.6 kg was seen in the MPA group, versus an ongoing, mean weight loss of 1.4 kg in the placebo group. This difference was statistically significant. The results of a subgroup analysis suggested that patients who are weight losing but not yet cachectic may benefit most from MPA treatment. During the study, several areas of quality of life deteriorated in the total group of patients. With the exception of an improvement of appetite and possibly also a reduction in nausea and vomiting, no measurable beneficial effects of MPA on quality of life could however be demonstrated. The side effects profile of MPA was favorable: only a trend toward an increase in (usually mild) peripheral edema was observed. Survival analysis, based on the subset of patients that fully completed the 12 week study, indicated a median survival advantage of 3.4 months for patients who were treated with MPA. However, this finding did not reach statistical significance in the relatively small group of patients investigated.

Based on these results, we concluded that in advanced-stage, non-hormone-sensitive

cancer MPA exhibits a mild side effects profile, has a beneficial effect on appetite, and prevents further weight loss. General quality of life was however not clearly influenced. Several explanations for this negative finding might nevertheless be given. First, the magnitude of weight changes might have been too small to influence general quality of life, especially when the major part was fat (see chapter 7). Second, in many cancer patients overall quality of life might be affected more by factors related to tumor extension and progression *per se*, than by anorexia and weight loss. Third, the daily dose of 1000 mg MPA might have been too low to have an effect on mood. In a recent study by Beller *et al.*, MPA in a daily dose of 480 mg significantly improved mood and "overall quality of life" independent of weight change, while this (glucocorticoid-like?) effect was not observed when a daily dose of 160 mg (equivalent to MPA 1000 mg) was used (52).

Further studies are needed to investigate whether progestagens may prevent further weight loss and improve quality of life when the treatment is initiated earlier (i.e., from the moment a patient experiences involuntary weight loss), and continued longer. This design would also be appropriate to further clarify the effects of MPA on survival.

(Chapter 7) To investigate whether an MPA-induced improvement of appetite also results in an increase in food intake and whether the observed weight gain represents tissue mass and not just fluid retention, which is a reported side effect of synthetic progestagens (48,51,53), we conducted an additional study in 54 patients to investigate the effects of MPA on food intake and body composition. In addition, also the effects of MPA on REE were assessed.

In contrast with placebo treatment, 12 weeks of MPA led to an increase in energy intake that was significantly associated with an increase in fat mass. The increase in energy intake was the result of a significantly improved intake of both protein, fat and carbohydrates. FFM was not significantly influenced. In addition, MPA-treatment was associated with an increase in REE.

Based on these results, we concluded that MPA is able to significantly increase food intake and to concomitantly reverse fat loss in weight-losing patients with non-hormone-sensitive cancer. However, we were not able to show a beneficial effect on FFM.

Epilogue

When we overlook the whole spectrum of treatment modalities that have been investigated in cancer cachexia to date, we have to conclude that the synthetic progestagens are the only agents that have been shown to improve appetite and weight in double-blind,

placebo-controlled clinical trials (*chapter 6*). A disadvantage, however, remains the fact that these agents probably only affect fat mass (*chapter 7*), while particularly wasting of BCM is associated with a deterioration of performance status (*chapter 2*) and survival (54). There are, however, several promising agents that in future might prove to be able to specifically attenuate BCM-wasting, as their aim is to interfere directly with the underlying pathophysiology (*chapter 2*). Examples of such challenging compounds, all having specific anti-inflammatory-, anti-proteolytic- or protein-synthetic effects, are the n-3 fatty acids (55,56), non-steroidal anti-inflammatory drugs (NSAID's, [57,58]), insulin-like growth factor I (59), branched-chain amino acids (60-62), β_2 -adrenergic agonists (63,64) and melatonin (65). It might be hypothesized that a combination of one or more of these agents with synthetic progestagens and nutrition might not only be distinctly rational, but also highly effective.

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NEDERLANDSTALIGE SAMENVATTING

Onvrijwillig gewichtsverlies is een veel voorkomende complicatie van kanker. Wanneer dit gewichtsverlies ernstige vormen aanneemt, wordt gesproken van cachexie. Aangezien het optreden van cachexie een negatieve invloed heeft op kwaliteit van leven, overlevingsduur en succeskans van behandeling, is deze complicatie van evident klinisch belang. De frequentie van voorkomen van cachexie is niet voor alle tumorvormen hetzelfde. Tumoren waarbij ernstig gewichtsverlies met name wordt gezien zijn longtumoren en tumoren van alveesklier, maag en darmen.

De ontstaanswijze van kanker-gerelateerd gewichtsverlies is slechts ten dele opgehelderd. Voornamelijk vastgesteld in dierproeven lijkt een complex van factoren een rol te spelen. Tot deze factoren behoren een algemene toename van ontstekingsactiviteit in het lichaam, het door de tumor in omloop brengen van stoffen die leiden tot vet- en eiwit afbraak, een toegenomen productie van glucose uit lactaat door de lever (een zeer energie-inefficiënt proces), en een verminderde voedsel-inname.

Bij kanker cachexie is niet alleen de mate van gewichtsverlies van belang, maar ook de samenstelling hiervan. Volgens een zogenaamd "4-compartimenten model" bestaat het (mannelijk) lichaam bij benadering uit 20% vet, 45% celmassa, 30% extracellulair water en 5% extracellulaire solide componenten (voornamelijk botmineraal). Waar gewichtverlies als gevolg van hongeren voor het overgrote deel bestaat uit vet, bestaat kanker-gerelateerd gewichtsverlies voor de helft uit vet en voor de helft uit lichaams-celmassa. Aangezien nagenoeg alle weefsels die onmisbaar zijn voor een normaal functioneren van het lichaam onderdeel uitmaken van die lichaams-celmassa (spieren, organen), mag worden aangenomen dat met name het celmassa-verlies bij kanker in belangrijke mate verantwoordelijk is voor de negatieve effecten van gewichtsverlies op kwaliteit van leven en overleving.

Ondanks het feit dat het afgelopen decennium veel onderzoek is gedaan naar geneesmiddelen die het optredende gewichtsverlies bij kanker kunnen stoppen of voorkomen, zijn er voor de klinische praktijk nog weinig bruikbare middelen beschikbaar.

Dit proefschrift is gebaseerd op zes studies op het gebied van kanker cachexie: twee studies onderzochten vraagstellingen op het gebied van de ontstaanswijze van kanker cachexie (hoofdstuk 2 en 3), twee studies bestudeerden de betrouwbaarheid van een bepaalde meetmethode om bij cachectische kanker-patiënten de lichaamssamenstelling te

meten (hoofdstuk 4 en 5), en twee studies onderzochten het mogelijke therapeutische effect van het geneesmiddel medroxyprogesteron acetaat (hoofdstuk 6 en 7).

In **Hoofdstuk 2** wordt het onderzoek beschreven naar factoren die mogelijk specifiek geassocieerd zouden kunnen zijn met het optreden van lichaams-celmasa-verlies bij kanker. Het belang van dit onderzoek was enerzijds het feit dat vergelijkbare studies tot op dat moment wel in dierproeven, maar nog niet bij de mens waren uitgevoerd, en anderzijds dat het verder ophelderen van de ontstaanswijze van kanker-gerelateerd celmasa-verlies het zoeken naar meer specifieke therapeutische interventies belangrijk zou kunnen stimuleren. In een groep van 20 mannelijke longkanker patiënten werd aangetoond dat verlies van lichaams-celmasa samenhangt met het bestaan van een toegenomen algemene ontstekingsactiviteit in het lichaam (gemeten via de bloed-concentratie van receptoren voor het cytokine "tumor-necrosis-factor"), een toegenomen productie van acute-fase eiwitten door de lever (gemeten via de bloed-concentratie van het acute-fase eiwit "LPS-binding protein"), het bestaan van een verhoogde ruststofwisseling, en verlaagde bloed-spiegels van het spier-opbouwende hormoon "insulin-like growth factor I". Tevens waren er aanwijzingen dat de toegenomen productie van acute-fase eiwitten (waarvoor als grondstof spier-eiwitten worden gebruikt) en de verhoogde ruststofwisseling op hun beurt samenhangen met de toegenomen gegeneraliseerde ontstekingsactiviteit.

In **Hoofdstuk 3** wordt de studie beschreven die onderzocht of stoornissen in het "leptin-feedback-mechanisme" een rol zouden kunnen spelen bij het ontstaan van kanker cachexie. Leptin is een hormoon dat geproduceerd wordt door vetcellen. Een toename van vetweefsel leidt tot een toename van leptin productie, hetgeen via beïnvloeding van het eetcentrum in de hersenen, gelegen in de hypothalamus, leidt (in diermodellen) tot een verminderde eetlust/voedsel-inname en een toegenomen ruststofwisseling. Door dit feedback mechanisme zal vervolgens de vetmasa weer afnemen. Aangezien de productie van leptin gestimuleerd kan worden door verschillende ontstekingsmediatoren zoals deze ook bij kanker cachexie een rol lijken te spelen, werd in de literatuur de hypothese naar voren gebracht dat relatief verhoogde leptin-spiegels (leidend tot eetlust-gebrek en hypermetabolisme) mogelijk een rol zouden kunnen spelen in het ontstaan van kanker cachexie. Wij onderzochten deze hypothese in 21 mannelijke longkanker patiënten. De hypothese werd echter niet bevestigd: de patiënten met veel gewichtsverlies en een lage vetmasa bleken onmeetbaar lage leptin spiegels te hebben, hetgeen aangeeft dat het afferente deel van het feedback mechanisme normaal functioneerde. Het feit dat onmeetbare leptin spiegels niet gepaard gingen met een toename van eetlust en een daling van het rustmetabolisme deed veeleer vermoeden dat het leptin feedback-mechanisme

gestoord was op hypothalamus-niveau.

In **Hoofdstuk 4 en 5** worden de onderzoeken beschreven die de betrouwbaarheid onderzochten van bioelectrische impedantie analyse (BIA) voor het meten van (veranderingen in-) de lichaamssamenstelling bij kanker. BIA is in principe één van de weinige meettechnieken die aantrekkelijk zijn voor gebruik in de "dagelijkse praktijk" aangezien zij goedkoop is, zonder veel specifieke expertise kan worden uitgevoerd, en nauwelijks een belasting vormt voor de patiënt. Het principe van BIA komt erop neer dat wanneer een zwakke stroom door het lichaam loopt, deze geheel door het lichaamswater zal worden voortgeleid, en dat de gemeten weerstand gerelateerd is aan de omvang van het totale lichaamswater (TBW). Wanneer wordt aangenomen dat TBW een vast percentage (73%) vormt van de vet-vrije massa, kan via het meten van de weerstand niet alleen TBW (via een regressie-vergelijking met hierin de weerstand als voorspellende variabele), maar ook de vet-vrije massa en (uitgaande van een 2-compartimenten model) de vetmassa (gewicht minus vet-vrije massa) worden bepaald. Dat deze redenering klopt werd voor gezonde vrijwilligers in een groot aantal studies aangetoond. Er was tot voor kort echter nauwelijks iets bekend of een en ander ook zou gelden voor individuen met ondergewicht. Hiernaast werd door verschillende onderzoekers openlijk betwijfeld, grotendeels op basis van teleurstellende resultaten van studies in dieet-houdende patiënten met overgewicht, of BIA in staat is om *veranderingen* in de grootte van de verschillende lichaams-compartimenten te meten. Ondanks dit laatste wordt BIA momenteel op grote schaal toegepast in klinische studies waarin veranderingen in de lichaamssamenstelling centraal staan. De conclusie van onze onderzoeken was dat de gemeten weerstand, en veranderingen hierin, op groepsniveau op betrouwbare wijze (veranderingen in-) TBW voorspellen. Er werden echter ook een aantal nadelen van de techniek geconstateerd. Op de eerste plaats bleek dat wanneer in patiënten met ondergewicht voorspellingsvergelijkingen gebruikt worden die ontwikkeld zijn bij individuen met een normaal gewicht, TBW systematisch wordt overschat. Op de tweede plaats werd vastgesteld dat wanneer een bepaalde (verandering in-) weerstand wordt gemeten, de berekende (verandering in-) TBW of vet-vrije massa sterk varieert afhankelijk van de gekozen voorspellingsformule, waarvan er inmiddels vele in de literatuur zijn gepubliceerd. Op de derde plaats bleek BIA onbetrouwbaar voor het meten van (veranderingen in-) TBW op individueel niveau.

In **Hoofdstuk 6 en 7** worden de onderzoeken beschreven die de effecten van het synthetische progestageen medroxyprogesteron acetaat (MPA) onderzochten op de eetlust, de voedsel-inname, het gewicht, de lichaamssamenstelling, het rustmetabolisme en de kwaliteit van leven bij patiënten met een vergevorderd stadium van een niet-hormoon-

gevoelige vorm van kanker. Beide onderzoeken hadden een gerandomiseerd, dubbel-blind, placebo-gecontroleerd karakter. De effecten van MPA (2 dd 500 mg) op eetlust, gewicht en kwaliteit van leven (EORTC-QLQ-C30: gevalideerde kanker-specifieke vragenlijst) werden gemeten in 206 patiënten in multicenter verband; de effecten van MPA op voedsel-inname (dieet-analyse via "diet-history methode"), lichaamssamenstelling (vetmassa en vet-vrije massa via "deuterium-dilutie") en ruststofwisseling werden onderzocht in een subgroep van 54 patiënten. De totale studieduur bedroeg 12 weken. De studies werden verricht omdat uit eerdere onderzoeken gebleken was dat synthetische progestagenen bij de behandeling van gemetastaseerde borstkanker, onafhankelijk van het anti-tumor effect, een toename van de eetlust en het gewicht bleken te veroorzaken. In vergelijking met placebo bleek MPA een significant gunstig effect te hebben op de eetlust, de voedsel-inname en het gewicht. Het onderzoek van de lichaamssamenstelling leverde op dat de gewichtstoename nagenoeg geheel was toe te schrijven aan een toename van de vetmassa. De vet-vrije massa verbeterde niet. Het rustmetabolisme vertoonde een stijging onder MPA. Het bijwerkingen-profiel van MPA was gunstig: er was alleen een trend tot het ontwikkelen van (merendeels mild) oedeem. Er werd, met uitzondering van een verbeterde eetlust en een trend tot minder misselijkheid en braken, geen duidelijk effect van MPA waargenomen op de algemene kwaliteit van leven gemeten met de EORTC-vragenlijst.

Epiloog

Wanneer het hele spectrum van behandelingsmogelijkheden voor het kanker-cachexie-syndroom wordt overzien, dan moet worden geconcludeerd dat synthetische progestagenen tot op heden de enige middelen zijn waarvan in dubbel-blind, placebo-gecontroleerd onderzoek is aangetoond dat ze de eetlust en het gewicht in gunstige zin beïnvloeden. Een nadeel blijft vooralsnog het feit dat ze alleen de vetmassa lijken te verbeteren, terwijl juist het verlies van lichaams-celmassa geassocieerd is met een achteruitgang van het fysiek functioneren en de overleving. Er zijn echter verschillende veelbelovende stoffen waarvan in de toekomst wellicht kan worden aangetoond dat ze het lichaams-celmassa-verlies kunnen remmen, aangezien ze direct aangrijpen op pathofysiologische mechanismen die aan dit celmassa-verlies ten grondslag liggen. Voorbeelden van dergelijke middelen, alle met een specifieke ontstekingsremmende-, eiwit-afbraak-remmende- of eiwit-opbouwende werking, zijn n-3 vetzuren, NSAID's, insulin-like growth factor I, vertakte-keten aminozuren, β_2 -adrenerge agonisten en melatonine. Een combinatie van een of meer van deze middelen met synthetische progestagenen en voeding zou in de toekomst wel eens

niet alleen een zeer rationele behandeling kunnen blijken, maar ook een bijzonder effectieve.

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CURRICULUM VITAE

Jean Paul Simons was born on May 17th, 1962 in Maastricht, The Netherlands. He attended secondary school (Gymnasium B) at the 'Henric van Veldeke College' in Maastricht from 1974 to 1980. Subsequently, he studied medicine at Maastricht University, and obtained his medical degree "Cum Laude" in 1986. From 1986 to 1991, he was a resident at the Department of Internal Medicine, University Hospital Maastricht (Head: Prof. Dr. J.A. Flendrig), and from 1991 to 1995 at the Department of Pulmonology, University Hospital Maastricht (Head: Prof. Dr. E.F.M. Wouters). In 1995, he was registered as a pulmonologist and worked, until May 1997, as a staff member at the Department of Pulmonology, University Hospital Maastricht. Presently, he is a member of the pulmonology staff of the Pulmonary Rehabilitation Centre Hornerheide, Horn, The Netherlands.