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Plasma concentration of total leptin and human lung-cancer-associated cachexia

Jean Paul F.H.A. SIMONS*, Annemie M.W.J. SCHOLS*, L. Arthur CAMPFIELD†, Emiel F.M. WOUTERS*, Wim H. SARIS‡

*Department of Pulmonology, University Hospital, Maastricht, P.O. Box 5800, 6202 AZ Maastricht, The Netherlands, †Department of Metabolic Diseases, Hoffmann-La Roche Inc., 340 Kingsland Street, Nutley 67110, NJ, USA, and ‡Department of Human Biology, Maastricht University, P.O. Box 616, 6200 MD, Maastricht, The Netherlands

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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, 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/m^2 compared with 19.4 kg/m^2, $P = 0.004$) and by a higher fat mass (21.4 kg compared 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 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 in 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

Key words: cancer-cachexia, fat-mass, leptin, pathophysiology.

Abbreviations: DEXA, dual-energy X-ray absorptiometry; FFM, fat-free mass; FM, fat mass; REE, resting energy expenditure.

Correspondence: Dr J.P.F.H.A. Simons.

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 animal models, *in vitro* and *in vivo*, 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], in particular 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.

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, WI, U.S.A.) 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 of the lower to higher energy (Rst) is inversely and linearly related to percentage fat, the latter can be derived directly from Rst. 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.

REE

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 min. 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, which has 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 h fast, blood was collected by venepuncture at 8.00 hours in evacuated EDTA blood-collection tubes (Sherwood Medical, Ballymoney, N. Ireland). The tubes were placed on ice-water and directly centrifuged for 5 min in a refrigerated (4°C) table-top centrifuge at a speed of 3000 *g*. Plasma samples were immediately stored at −70°C until analysis. Plasma levels of leptin were measured using a double-antibody 'sandwich' ELISA with a monoclonal antibody specific for human leptin, an assay that measures total (i.e. free plus bound) leptin. The measurement procedure is as follows. Microtitre 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 μl/well) and incubated at room temperature for 2 h. Plates are then washed and 100 μl/well of polyclonal rabbit-(anti-human leptin) antibody (R46) is added at 5 μg/ml in 0.1% BSA in PBS and incubated for 1 h at room temperature. Plates are then washed again and 100 μl/well of goat-(anti-rabbit IgG) conjugated to horseradish peroxidase at 1:20 000 dilution in 0.1% BSA in PBS and incu-

bated for 1 h at room temperature. Plates are washed again and 150 μl/well of peroxidase substrate solution (tetramethyl benzidine and hydrogen peroxide in citrate buffer) is added and incubated for 15 min. The reaction is stopped by adding 75 μl/well of 2N H_2SO_4 and the absorbance 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 variations were 9% and 12% respectively. The leptin levels of normal-weight subjects ranged from 1 to 12 ng/ml.

Statistical analysis

Results are expressed as median and range. Between-group differences were analysed by using the Mann–Whitney U-test. For all analyses, the statistical package SPSS/PC+ (version 6.0-Windows; SPSS Inc., Chicago, IL, U.S.A.) 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 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/m^2. In only six of these subjects was plasma leptin detectable, at a median level of 3.9 ng/ml.

Table 1. Patient characteristics

	Median (range) (n = 21)
Age (years)	69 (56–82)
Tumour type and stage, *n*	
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 to +1.5)
Body mass index (kg/m^2)	21.2 (15.9–24.9)
PIBW (%)†	94 (70–112)
FM (kg)	12.7 (2.1–22.9)
FM/weight (%)	19.8 (4.4–33.2)
FM index (kg/m^2)‡	4.5 (0.7–7.3)
FFM (kg)	49.7 (38.8–57.8)
FFM index (kg/m^2)‡	16.3 (14.4–18.0)
Total plasma leptin	
Detectable, *n* (ng/ml)	6: 3.9 (1.1–5.0)
Non-detectable, *n*	15
REE (kcal/day)§	1622 (1158–2024)‖
REE/FFM (kcal/day per kg)§‖¶	32.6 (22.7–40.0)‖

*Weight in relation to pre-illness weight.
†Body weight expressed as a percentage of ideal body weight.
‡FM and FFM adjusted for height^2.
§1 kcal = 4.184 kJ.
‖n = 19.
¶REE adjusted for FFM.

As can be derived from Table 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/m^2 compared with 19.4 kg/m^2, $P = 0.004$) 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 REE were lacking.

The relationship between total plasma leptin and the presence of weight loss is shown in Fig. 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/m^2) and, despite the severe amount of weight loss, a measurable leptin level of 4.5 ng/ml. Another exception was a patient at the other end 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/m^2) and, despite the lack of weight loss, a non-detectable leptin level.

Figure 2 shows a plot of total leptin and FM-index, illustrating a clear relationship between the presence of a low FM and the non-detectability of 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.

Table 2. Comparison of patients with and without detectable total plasma levels of leptin. Scores expressed as median (range).

	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 to +0.4)	−11.0 (−23.2 to +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
FM (kg)	21.4 (13.5–22.9)	9.7 (2.1–17.9)	0.001
FM/weight (%)	29.0 (21.4–33.2)	15.9 (4.4–24.0)	0.002
FM-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/day)¶	1683 (1369–2024)	1564 (1158–1846)**	0.14
REE/FFM (kcal/day per kg)¶††	33.8 (29.3–39.2)	31.3 (22.7–40.0)**	0.22

*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.
|| FM and FFM adjusted for height^2.
¶1 kcal = 4.184 kJ
**$n = 13$.
††REE adjusted for FFM.

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 decreased energy intake, increased energy expenditure (in combination with abnormalities in substrate metabolism), or both [15–17]. Particularly in lung cancer, the combination of 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 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 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

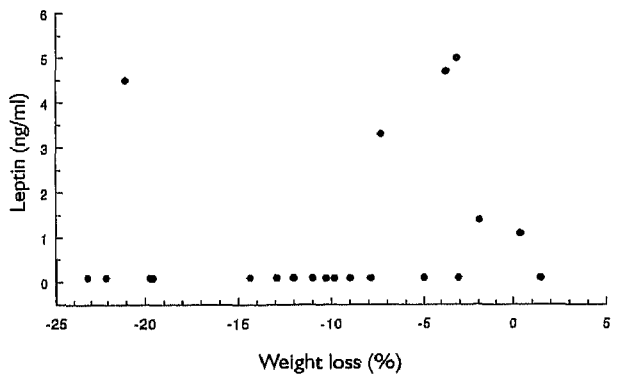

Fig. 1. Relationship between weight loss (expressed as percentage of pre-illness weight) and total plasma leptin.

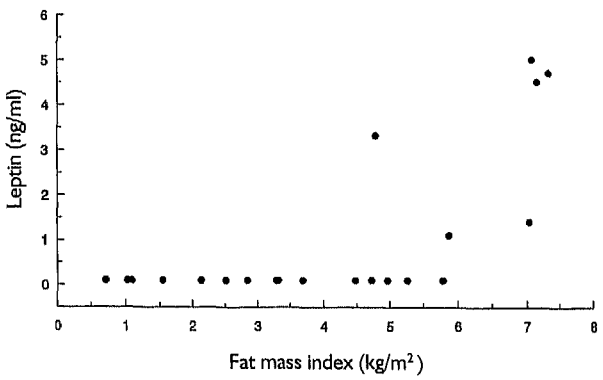

Fig. 2. Relationship between FM index and total plasma leptin.

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 suggested in the 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 over-ridden. 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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