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Circulating Concentrations of "Free" Leptin in Relation to Fat Mass and Appetite in Gastrointestinal Cancer Patients

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Abstract: *Recent studies have suggested that circulating concentrations of leptin might play a role in cancer cachexia. In the first part of the study, we compared circulating concentrations of free and total leptin, percent fat mass, and the inflammatory markers C-reactive protein (CRP) and interleukin-6 (IL-6), together with appetite score, in age- and gender-matched healthy controls (*n = *11) and advanced gastrointestinal cancer patients (*n = *26). In the second part of the study, the same measurements were repeated before and after megestrol acetate treatment of weight-losing gastrointestinal cancer patients (*n = *10). Body mass index and percent fat mass were significantly lower (*$P < 0.05$*) and IL-6 and CRP were significantly higher (*$P < 0.05$*) in cancer patients than in controls. There was no difference in the percentage of leptin bound in the circulation between controls and cancer patients. Circulating "free" leptin concentrations correlated with percent fat mass in controls (*$r = 0.745$*, $P = 0.008$*) and cancer patients (*$r = 0.600$*, $P = 0.001$*). In cancer patients, circulating leptin concentrations, either free or total, were not correlated with IL-6 or CRP concentrations. When adjusted for fat mass, the circulating concentrations of free and total leptin were significantly lower in the cancer patients (*$P < 0.01$*). Megestrol acetate treatment significantly increased circulating free and total leptin concentrations in the cancer patients (*$P < 0.05$*). There was a significant positive correlation between the change in circulating concentrations of free and total leptin and the change in percent fat mass (*$r = 0.685$*, $P < 0.05$* and *$r = 0.661$*, $P < 0.05$*, respectively). The results of the present study indicate that the proportions of free and bound leptin in the circulation do not differ between normal subjects and patients with gastrointestinal cancer and in both groups are related to fat mass. Furthermore, the increase in circulating leptin concentrations after megestrol acetate treatment is not associated with any alteration in leptin binding.*

Introduction

Weight loss in advanced cancer patients is a major clinical problem that reduces the efficacy of anticancer treatment, results in loss of independence, and reduces the quality of life (1,2). Little is known about the mechanisms involved, but it is characterized by the disproportionate reduction of lean body tissue (3–5). Clearly, loss of appetite may contribute to such weight loss, and there is increasing evidence that the systemic inflammatory response also plays an important role (2,4).

Body weight homeostasis is maintained by a series of complex interactions between the brain (particularly, the hypothalamus) and the periphery involving the hormone leptin, which is synthesized in and secreted from adipose tissue (6–8). It has been suggested that increased circulating leptin concentration decreases appetite and food intake, increases energy expenditure, and is important for the regulation of metabolism and body weight in humans (9).

According to this paradigm, low circulating leptin concentrations increase appetite and decrease energy expenditure, resulting in gain of fat mass. This occurs in the rare condition of congenital leptin deficiency (10); in other pathological situations, the role of leptin is less clear. In healthy individuals, it has been shown that circulating leptin concentrations reflect fat mass (11). Furthermore, this relationship between fat mass and total leptin appears to be maintained in gastrointestinal cancer, where total leptin concentrations are low and correlate with the low fat mass (12–14).

It is known that specific binding components for leptin are present in the circulation (11,15–17), and one explanation for the apparent inability of a circulating low total leptin concentration to increase appetite in cancer patients might be a decrease in the leptin-binding component, resulting in high circulating concentrations of free leptin, which is the form of leptin in cerebrospinal fluid (18). In addition to measuring free leptin concentrations in a cross-sectional study, this hypothesis may be more rigorously tested in the context of a longitudinal study of treatment with an appetite stimulant such as megestrol acetate (19). To our knowledge, this approach has not been examined in cancer patients.

In the present study, we investigated the relationship between circulating concentrations of free leptin, appetite, fat mass, and the systemic inflammatory response in gastrointes-

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tinal cancer patients. In addition, we examined the effect of the appetite stimulant megestrol acetate on these relationships.

Materials and Methods

Subjects

Patients with histologically proven locally advanced or metastatic gastrointestinal cancer were included in the study. Cancer patients were weight stable (<5% gain or loss in body weight, $n = 13$) or weight losing (>5% body weight loss, $n = 13$) over the previous 6 mo. No patient complained of moderate or severe dysphagia, and none had an obvious functional obstruction to food intake.

After an overnight fast, height, weight, and percent fat mass were measured, and an appetite score was recorded. A venous blood sample was taken for the measurement of circulating concentrations of leptin, leptin binding, interleukin-6 (IL-6), and C-reactive protein (CRP). Similar measurements were performed in healthy weight-stable subjects for comparison. In addition, 10 weight-losing cancer patients (>5% body weight loss over the previous 6 mo) were studied before and after 6–12 wk of treatment with megestrol acetate BP (480 mg/day; Megace, Bristol-Myers Pharmaceuticals).

The study was approved by the local ethical committee. All patients were informed of the purpose of the study, and all gave written consent.

Methods

Total human leptin was measured by a validated "in-house" radioimmunoassay (11). Briefly, test serum or leptin standard was incubated with sheep antileptin antiserum (antibodies generated against recombinant human leptin) and radioiodinated (^{125}I) leptin at 4°C for 16 h. Sepharose-donkey anti-sheep globulin was added to the tubes after incubation, and the samples were reincubated for 1 h at ambient temperature. Free and bound fractions were then separated by centrifugation and washed three times. The bound fraction was counted on a gamma counter. The intra- and interassay coefficients of variation were <7% and <10%, respectively, over the concentration range, and limit of detection was 0.5 ng/ml.

Percent leptin binding in the circulation was determined after separation of bound and free fractions by a validated Sephadex G-100 gel filtration procedure (11). Briefly, serum and radioiodinated (^{125}I) leptin were incubated for 20–24 h at 4°C. Bound and free analyte were separated by gel filtration chromatography, also at 4°C, on a Sephadex G-100 column (1.5 × 40 cm). Eighty 1-ml fractions were collected, and each was counted on a gamma counter. The free fraction peak was eluted later and well separated from the bound fraction, and the concentration of free leptin was calculated as follows: free leptin (ng/ml) = total leptin (ng/ml, measured by radioimmunoassay) × %free leptin. The interassay coefficient of variation was 4.7% for multiple ($n = 18$) analyses of a serum sample in which bound fraction averaged 21.2% of total leptin.

Human IL-6 was measured by an enzyme-linked immunosorbent assay kit (Diaclone Research, Cedex, France, as supplied from IDS, Tyne and Weir, UK). A monoclonal antibody specific for IL-6 was provided coated onto the wells of microtiter strips. During the first incubation, standards, samples, and quality controls were incubated with the IL-6 antigen and a biotinylated monoclonal antibody specific for IL-6. After the samples were washed, the enzyme (streptavidin peroxidase) was added. After the samples were incubated and washed to remove unbound enzyme, a substrate solution that acts on the bound enzyme was added to produce a colored reaction product. The intra- and interassay coefficients of variation were <5% and <7%, respectively, over the sample concentration range. The limit of detection of the assay was 2 pg/ml.

CRP was measured on an Olympus analyzer (model AU5200, Olympus Diagnostic Systems, Eastleigh, UK) by turbimetry after binding to a specific antibody. The limit of sensitivity was 5 mg/l, and the intra- and interassay coefficients of variation were 5% and 7%, respectively, over the sample concentration range.

Appetite was measured using a 10-cm linear analog scale, ranging from poor to good appetite (20).

Total body water was determined by bioelectrical impedance (model 4000B, Xitron Technologies, San Diego, CA). The error of the method is ~10% (20). Percent body fat was calculated as follows: fat-free mass (kg) = total body water ÷ 0.73 and body fat (%) = [(weight – fat-free mass)/weight] × 100.

Statistics

Data are presented as the median and range; where approriate, control and group differences were examined using the Mann-Whitney U test. The Wilcoxon signed rank test was used to investigate changes after megestrol acetate treatment. Measures of association were performed using Spearman's rank correlation test (Minitab, State College, PA).

Results

The characteristics of healthy controls ($n = 11$) and gastrointestinal cancer patients [$n = 26$ (20 colorectal, 3 gastric, 2 gastroesophageal, 1 pancreatic)] are shown in Table 1. Body mass index and percent fat mass were significantly lower ($P < 0.05$) and IL-6 and CRP were significantly higher ($P < 0.05$) in the cancer patients than in the controls. The circulating concentrations of free and total leptin were significantly lower in the cancer patients ($P < 0.01$). When free and total leptin concentrations were adjusted for percent fat mass, they remained significantly lower in the cancer patients ($P < 0.05$). There was no significant difference in percent leptin binding between cancer patients and controls.

In the controls and cancer patients, the free and total leptin concentration correlated with the measured percent fat mass

Table 1. Characteristics of Healthy Controls and Gastrointestinal Cancer Patients[a,b]

	Healthy Controls	Cancer Patients	*P*
Gender, M/F	7/4	18/8	
Age, yr	66 (46–74)	62 (47–88)	NS
BMI, kg/m^2	25.2 (22.0–28.0)	23.4 (16.6–29.2)	0.01
% Fat mass	35 (14.5–47.1)	31.1 (15.2–41.2)	<0.05
Free leptin, ng/ml	8.9 (0.9–31.6)	2.5 (0.9–6.6)	<0.01
Total leptin, ng/ml	12.9 (0.9–42.3)	3.9 (0.9–9.3)	<0.01
Leptin-to-% fat mass ratio	0.40 (0.1–0.26)	0.14 (0.04–0.9)	<0.05
% Bound leptin	36.3 (21.1–41.9)	34.3 (9.2–47)	NS
IL-6, pg/ml	<2 (<2 to <2)	2 (<2 to 178)	<0.05
CRP, mg/l	<5 (<5 to 30)	21 (<5 to 127)	<0.01

a: Values are medians, with range in parentheses.
b: Abbreviations are as follows: M, male; F, female; BMI, body mass index; IL-6, interleukin-6; CRP, C-reactive protein; NS, not significant.

($r = 0.745$, $P = 0.008$ [controls] and $r = 0.600$, $P = 0.001$ [patients] for free leptin concentration and $r = 0.745$, $P = 0.008$ [controls] and $r = 0.558$, $P = 0.003$ [patients] for total leptin; Figs. 1 and 2).

In the cancer patients, there was a positive correlation between the circulating free and total leptin concentrations and appetite scores ($r = 0.575$, $P = 0.003$ and $r = 0.605$, $P = 0.001$, respectively). When free and total leptin concentrations were adjusted by dividing by percent fat mass, there remained a significant positive correlation with appetite scores ($r = 0.720$, $P < 0.0001$ and $r = 0.720$, $P < 0.0001$, respectively). In the cancer patients, circulating leptin concentrations, either free or total, were not correlated with IL-6 or CRP concentrations.

Treatment with megestrol acetate significantly increased circulating free and total leptin concentrations ($P < 0.05$; Table 2). There was a significant positive correlation between the change in circulating concentrations of free and total leptin and the change in percent fat mass ($r = 0.685$, $P < 0.05$ and $r = 0.661$, $P < 0.05$, respectively; Fig. 3). However, there was no correlation between the change in free and total leptin concentrations and appetite when adjusted for percent fat mass.

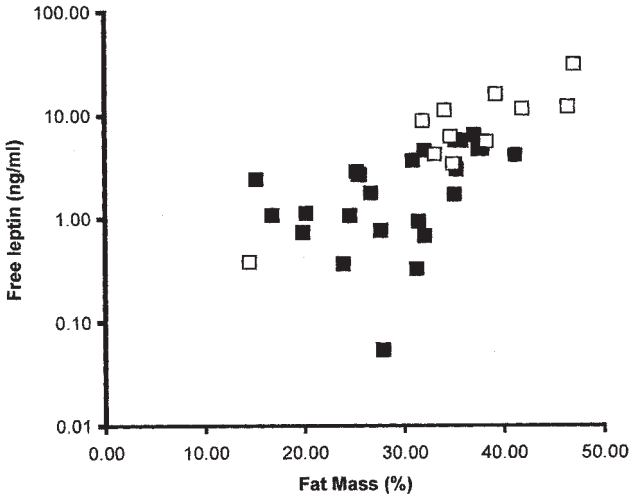

Figure 1. Relationship between percent fat mass and circulating free leptin concentration in controls (*open squares*) and gastrointestinal cancer patients (*filled squares*).

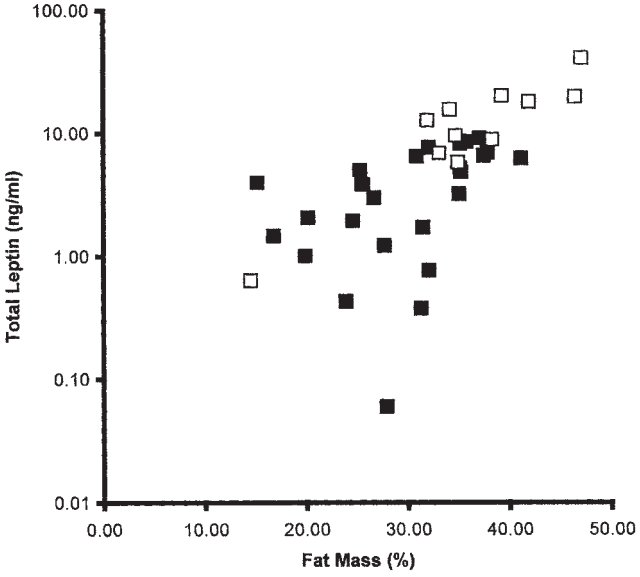

Figure 2. Relationship between percent fat mass and circulating total leptin concentration in controls (*open squares*) and gastrointestinal cancer patients (*filled squares*).

Discussion

Body weight homeostasis appears to be maintained by a normal, dynamic equilibrium between anabolism and catabolism controlled by orexigenic and anorexigenic neuropeptides. In cancer cachexia, this normal mechanism is disrupted, and it is not surprising that leptin has been implicated, because it has been reported to control the balance between these neuropeptides (9). Furthermore, recent research indicates that advanced cancer is associated with the systemic inflammatory response and increased concentrations of relevant cytokines such as IL-6 (4,22,23). In addition, circulating leptin has been shown to increase as part of the acute-phase

Table 2. Characteristics of Weight-Losing Gastrointestinal Cancer Patients Before and After Treatment With Megestrol Acetate[a]

	Before Treatment	After Treatment	*P*
BMI, kg/m^2	17.9 (15.9–21.4)	18.0 (15.4–22)	NS
Appetite score	1.3 (0–10)	6.9 (0–10)	NS (<0.10)
% Fat mass	21.5 (10.4–34.7)	21.7 (11–34.8)	NS
Total leptin, ng/ml	1.8 (0.8–7.7)	2.3 (0.9–9.6)	<0.05
Free leptin, ng/ml	1.1 (0.6–5.1)	1.4 (0.6–6.8)	<0.05
% Bound leptin	35.5 (26–49)	35.4 (29–51)	NS
IL-6, pg/ml	<2 (<2 to 37.4)	7.6 (<2 to 52.3)	NS (<0.10)
CRP, mg/l	6 (<5 to 160)	10 (<5 to 165)	NS

a: Values are medians, with range in parentheses; $n = 10$.

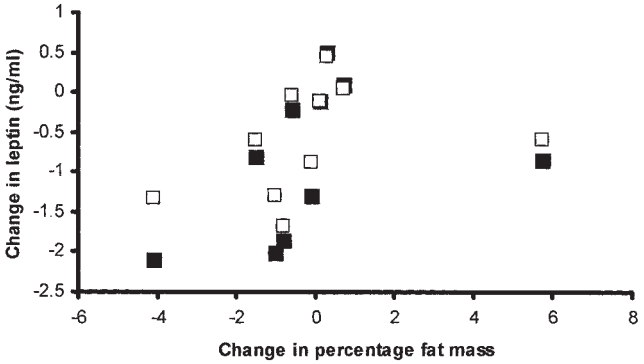

Figure 3. Relationship between change in percent fat mass and free (*open squares*) and total (*filled squares*) leptin concentrations in gastrointestinal cancer patients.

response during surgical stress (24,25) and acute sepsis (26) and after cytokine administration (27,28).

In the present study, we confirm earlier findings of low total leptin concentrations in gastrointestinal cancer patients (12,14) and the correlation between these low concentrations and percent fat mass. We have extended these findings to show that circulating free leptin concentrations parallel total leptin concentrations. This observation is of considerable significance, because the presumed bioavailable component of total leptin is the free fraction, which is the only form found in cerebrospinal fluid (18). In addition, we have shown that there is no difference in the percentage of leptin bound between cancer patients and controls, suggesting that there is no alteration in the overall concentration of leptin-binding proteins in the circulation. These novel findings in cancer patients are consistent with accumulating evidence that, in healthy subjects, circulating free leptin parallels the total leptin concentration (11,16,29) and also that circulating leptin-binding proteins are directly related to the amount of bound leptin (30).

A significant inverse relationship between circulating IL-6 and total leptin concentrations in advanced cancer has previously been reported (13). In contrast, after adjustment for fat mass, Moses and co-workers (14) reported a significant positive correlation between total leptin and IL-6 concentrations. In the present study, despite higher circulating IL-6 and CRP concentrations in the cancer patients, there were no correlations with free or total leptin concentrations with or without adjustment for percent fat mass. The basis of the different relationship between leptin and IL-6 reported in these three studies remains unclear, and such relationships merit further investigation in a larger group of cancer patients to clarify this issue. Nevertheless, the low leptin concentrations in the cancer patients do not suggest a systemic role for increased leptin in the anorexia/cachexia of cancer patients. In addition, even when corrected for percent fat mass, the cancer patients produce significantly lower circulating leptin concentrations than controls. The reason for this suppression is not known, but it is known that leptin-to-fat mass ratios are reduced in periods of extended fasting or starvation (31), and it is possible that a similar mechanism might occur here. It could also be that a leptin-suppressing substance is produced in advanced cancer. Obviously, further research is required to investigate this in more detail.

In the present study, there was a positive, rather than, as might be expected, a negative, relationship between free leptin and appetite and total leptin and appetite in cancer patients. Although results from self-reporting appetite scores have their limitations (32), we believe that this is a reliable approach in a study of this type. Our results may simply reflect the fact that the circulating leptin concentration is an indicator of fat mass and that decreasing fat mass is a consequence of poor appetite, rather than a direct association between leptin and appetite. Indeed, this is supported by our finding that, on megestrol acetate treatment, the change in free and total leptin concentrations was correlated with the change in percent fat mass, but not appetite.

It was also of interest that in the cancer patients the improvement of appetite with megestrol acetate treatment was associated with a significant increase in circulating free and total leptin. There was, however, no overall change in body mass index or fat mass. The small, but significant, increase in leptin may suggest that it is more sensitive to an improvement in energy balance than either weight or fat mass measurements. Indeed, a similar situation has recently been described in growth hormone-deficient adults treated with growth hormone, where a decrease in the circulating leptin concentration occurred before a reduction in fat mass (33).

The results of the present study indicate that the proportions of free and bound leptin in the circulation do not differ between controls and cancer patients and are related to fat mass in both groups. Furthermore, the increase in circulating leptin concentrations after megestrol acetate treatment is not associated with any alteration in leptin binding.

Acknowledgments and Notes

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