0021-972X/96/$03.00/0
Journal of Clinical Endocrinology and Metabolism

Vol. 81, No. 11
Printed in U.S.A.

Response of Leptin to Short-Term and Prolonged Overfeeding in Humans*

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ABSTRACT

As one of the postulated roles of the *ob* gene product, leptin, is regulation of energy balance and preservation of normal body composition, we investigated the effect of acute and chronic calorie excess (weight gain) on serum leptin in humans. Two protocols were employed: 1) acute (12-h) massive (120 Cal/kg) voluntary overfeeding of eight healthy individuals; and 2) chronic overfeeding to attain 10% weight gain, with its subsequent maintenance for additional 2 weeks, involving six normal males. In the acute experiments (protocol 1), circulating leptin rose by 40% over baseline ($P < 0.01$) during the final hours of overfeeding; this increase persisted until the next morning. At the point of achievement and the 2-week maintenance of 10% weight gain (protocol 2), a more than 3-fold rise in the basal leptin concentration was observed ($P < 0.01$). A direct linear relationship was found between the magnitude of the leptin response to weight gain and the percent gain of body fat ($r = 0.88$; $P < 0.01$).

In summary, 1) in contrast to normal food intake (8), short term massive overfeeding is associated with a moderate elevation of circulating leptin levels that persists until next feeding cycle is initiated; and 2) a 10% weight gain causes different changes in the body composition, and the resulting rise in circulating leptin parallels the increase in the percentage of body fat. In conclusion, these studies document acute elevation of leptin in response to positive energy balance and suggest that developing resistance to leptin is associated with bigger fat deposition during weight gain in humans. (*J Clin Endocrinol Metab* **81:** 4162–4165, 1996)

THE DISCOVERY of the *ob* gene and its product, leptin (1, 2), is rapidly changing our understanding of the regulation of body fuels and opens a possibility for an entirely new approach for therapeutic interventions in obesity and its complications (3–7). Our previous experiments indicated that circulating levels of leptin remain unchanged in response to a normal balanced diet in humans (8). In contrast, a decline in circulating leptin is observed during weight loss achieved with the use of low calorie diet (8). Moreover, leptin undergoes a marked down-regulation when fasting exceeds 12 h, and it rapidly returns to normal values upon refeeding (9). Whether acute and chronic oral overfeeding in humans produces up-regulation in circulating leptin in humans is not known. During a massive parenteral overfeeding with glucose for 3 days, a 3-fold increase in circulating leptin was seen during the last day of the study (10), but the relevance of this finding to oral overfeeding of a mixed diet is difficult to ascertain. Consequently, we investigated the responses of leptin to acute and prolonged overfeeding in humans.

Subjects and Methods

Subjects and study design

Protocol 1: acute massive voluntary overfeeding. Eight healthy individuals (six men and two females), with no history of weight cycling (31.8 ± 3.2 yr of age; body mass index, 28.1 ± 1.9 kg/m^2; body fat, 25.8 ± 4.1%), volunteered to participate in the study. The study subjects were admitted to the clinical research center at 0730 h after an 8- to 10-h overnight fast. At 0800 h, they began consumption of food delivered from a fast-food restaurant consisting of 55% fat, 15% protein, and 35% carbohydrate, distributed in even portions as breakfast (0800–0900 h), lunch (1230–1330 h), and dinner (1700–1800 h), with additional snacking on cashew nuts and milk shakes in between the major meals to keep the satiety level constantly over 7 on a scale of 0–10 (where 7 is still comfortable, but approaching uncomfortable fullness, and 10 is absolutely stuffed, could not eat another bite). At 0800 h, the feeding was stopped, and water intake was only allowed thereafter. This pattern of overfeeding resulted in a relatively even rate of calorie delivery at about 10 Cal/kg·h; they all consumed a total of 120 Cal/kg.

Protocol 2: chronic overfeeding. Six lean healthy men (27.8 ± 0.8 yr of age; body mass index, 24.1 ± 0.9 kg/m^2; body fat, 15.8 ± 1.7%) volunteered to consume 22.5–27.5 Cal/kg over their normal calorie intake, given as liquid food supplement Sustacal Plus (Mead Johnson Co., Evansville, IN; 50% carbohydrate, 35% fat, and 15% protein), with the aim to gain 10% of their initial weight over a 5-week period, followed by weight maintenance for an additional 2 weeks.

None of the subjects was taking medications, consumed an unusual diet, or had any evidence of metabolic disease, except for obesity (based on BMI criteria) (11) in four of subjects in protocol 1. The nature and purpose of the studies were explained to all participants, and they signed an informed consent form approved by the institutional review board at Thomas Jefferson University (Philadelphia, PA).

Measurements

Physical parameters. The percentage of body fat was calculated with bioelectric impedance analysis (RJL Systems, Mt. Clemens, MI) (12) and skin-fold measurements. The results, all falling within ±4% variation in obtained values between the two methods, were averaged.

Blood samples. During the acute overfeeding (protocol 1), blood samples were obtained at baseline (0730 h), before lunch (1230 h), before diner

Received May 16, 1996. Revision received June 26, 1996. Accepted July 2, 1996.

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* This work was supported by NIH Grant RO1-DK-45592.

(1730 h), and on the following morning (0730 h). During the 10% weight gain study (protocol 2), blood samples were obtained at baseline (day 0) and in the fifth and seventh weeks. The separated serum was kept frozen at −80 C until further measurement. Serum leptin was measured by RIA, as previously described and validated (2). Serum insulin was quantitated by RIA (Linco Research, St. Charles, MO). Serum glucose was measured by oxidase method with a Beckman Glucose Analyzer II (Brea, CA).

Statistical analysis

The data were expressed as the mean ± SE and the coefficient of variation. The statistical analysis was performed with ANOVA and paired Student's *t* test, where applicable. The relationships between serum leptin and other variables measured were determined by linear regression analysis.

Results

Acute voluntary massive overfeeding

Figure 1 illustrates response of leptin to a 120 Cal/kg mixed diet consumed over 12 h. An acute, approximately 40% rise in serum leptin occurred between the fifth and tenth hours of the experiment and persisted until the morning of the following day ($P < 0.01$). As shown in Table 1, overfeeding resulted in a small, but significant, elevation in fasting glucose ($P < 0.005$); fasting serum insulin almost doubled on the following day ($P < 0.005$). No correlation between insulin and leptin responses was found.

10% weight gain

All study subjects achieved a 10% weight gain after 5 weeks of overfeeding and maintained it for another 2 weeks (72.2 ± 4.6 *vs.* 89.0 ± 4.6 *vs.* 90.5 ± 4.9 kg, respectively; $P < 0.001$; Fig. 2A). During this time, body fat increased from 15.8 ± 1.7% to 19.4 ± 1.4% ($P < 0.005$; Table 2). The differences in body fat were variable (coefficient of variation, 46%), ranging from 1.8–6.6%. As shown in Fig. 2B, the weight gain due to overfeeding resulted in a more than 3-fold elevation of basal serum leptin at both 5 and 7 weeks (2.0 ± 0.4 *vs.* 6.5 ± 1.2 *vs.* 6.07 ± 1.0 ng/mL; $P < 0.02$). The response of leptin to weight gain exhibited considerable variation (coefficient of variation, 56%), ranging from 1.3- to 7.9-fold. The magnitude of the leptin rise and the change in the percentage of body fat showed a linear correlation ($r = 0.88$; $P < 0.01$). No such correlation was found between changes in leptin and fasting insulin (Table 2) in response to the weight gain.

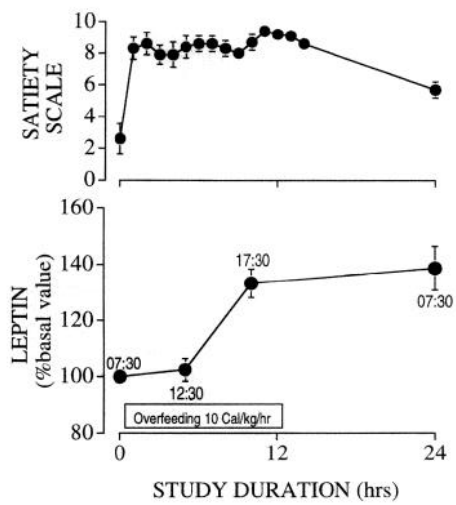

FIG. 1. Acute massive (120 Cal/kg over 12 h) overfeeding in humans. A, Satiety scale; see *Materials and Methods* for a description of the values. B, Leptin response. The numbers at *data points* represent the clock time.

Discussion

In our previous reports, we have documented that daytime circulating levels of leptin remained remarkably stable in response to a 75.0-g oral glucose challenge and a balanced diet (8, 13). Moreover, leptin levels are highly reproducible within individuals whose body mass is stable (14) (Ohannesian, J., unpublished data). On the other hand, when total fasting extends over 12 h, a rapid decline in leptin occurs, with a rapid return to baseline levels upon refeeding (9). Moreover, a decline in leptin is observed during weight loss achieved by a low calorie diet in humans (8). These data prompted us to study the responses of leptin under the reverse scenario, *i.e.* under conditions creating acute and chronic positive energy balance. Although there are no comparable data in humans, in one report from experiments in FVB mice, both normal and with transgene-induced ablation of brown adipose tissue, voluntary overfeeding of a high fat Western diet was associated with the development of obesity and the inability of the appropriately rising serum leptin level to control food intake and body fat (15).

From the results of the presented experiments in humans, the following picture emerges. First, in response to a massive oral overfeeding with 10 Cal/kg·h for 12 h (which equals almost 10,000 Cal for an 80-kg individual, as were the majority of participants), leptin deviates from the daytime plateau (8, 13) to exhibit a moderate sustained rise that persists even after an overnight fast. Second, in response to chronic overfeeding for 5 weeks, which resulted in 10% weight gain, an average rise in leptin of more than 3-fold is higher than

TABLE 1. Changes in fasting glucose, insulin, fatty acids, triglycerides, and leptin before and after 1 day of massive (120 Cal/kg) overfeeding

Parameter	Before	After	Significance (*P*)
Fasting glucose (mg/dL)	91.0 ± 1.6	96.8 ± 2.9	<0.005
Fasting insulin (μU/mL)	18.6 ± 3.5	33.0 ± 6.3	<0.005
Leptin (ng/mL)	12.9 ± 4.6	18.4 ± 6.6	<0.05

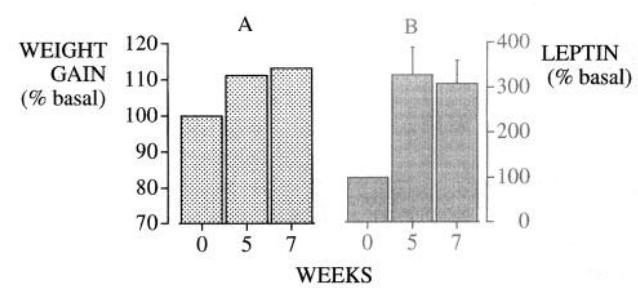

FIG. 2. Chronic overfeeding in humans. Weight gain (A) and response of leptin (B) after 5 weeks of active weight gain and a 2-week period of weight maintenance.

TABLE 2. Changes in body composition and fasting glucose, insulin, and leptin before and after a 10% weight gain followed by weight maintenance for 2 additional weeks

Parameter	Baseline	At 10% wt gain	After 2 weeks of wt maintenance	Significance (*P*, by ANOVA)
BMI (kg/m^2)	24.1 ± 0.9	26.7 ± 1.0	27.1 ± 1.0	<0.001
Body fat (%)	15.8 ± 1.7		19.4 ± 1.4	<0.005
Fasting glucose (mg/dL)	83.8 ± 2.9	87.7 ± 2.13	78.2 ± 4.8	NS
Fasting insulin (U/mL)	10.6 ± 1.4	40.8 ± 15.6	40.5 ± 19.3	<0.02
Fasting leptin (ng/mL)	2.0 ± 0.4	6.5 ± 1.2	6.1 ± 1.0	<0.02

would be expected for the achieved rise in BMI and percentage of body fat (the expected difference is an ~2-fold rise, as calculated from the data presented in Ref. 8). Third, the increases in fasting insulin and leptin in response to both acute and chronic overfeeding, in contrast to studies in rodents (16, 17), do not appear to follow each other. Moreover, consistent with the previous human studies (18, 19), the 10% weight gain resulted in variable increases in lean body mass and percentage of body fat. Finally, in chronic overfeeding experiments, the subjects who gained more fat exhibited higher elevations in leptin levels.

Our acute overfeeding experiments (protocol 1) complement the picture that has developed from our fasting experiments (9); that is, the system regulating the leptin level responds to the extremes of energy balance with up- and down-regulation of the hormone, respectively. In addition, the percent increase in leptin concentration during acute overfeeding is virtually the same in lean and obese subjects. Interestingly, in our fasting experiments (9), the percentage of leptin decline was not related to the degree of adiposity. On the other hand, it is hard to conceive that the observed acute changes in serum leptin levels in response to the extremes of energy balance are due to similar in magnitude loss or deposition of fat. Rather, these oscillations further support our concept of the dual role of the leptin system in human physiology (9). Specifically, under conditions of normal food intake, leptin serves as a static index of fat deposition. As such, leptin interacts with the hypothetical "lipostat" in the brain that controls body composition. This role of leptin is overridden by a sensor of energy balance, a hypothetical "energostat" that up- or down-regulates leptin during drastic departures from the normal equilibrium between energy intake and expenditure.

Consistent with the above concept are previously reported parabiosis experiments in rodents (20) and recent data documenting the effects of recombinant leptin administration in the *ob/ob* mouse (3–6) that all document a unique role of leptin in the regulation of energy expenditure and body composition. Specifically, the parabiosis of ventromedial hypothalamus-lesioned rats (a model resembling the *fa/fa* rat and *db/db* mouse) with normal littermates resulted in almost selective loss of body fat in the nonlesioned animal (20). Similarly, weight loss after the administration of leptin to the *ob/ob* mouse, with a mutation in the *ob* gene causing total leptin deficiency, appears to spare lean body mass (3).

Can recombinant human leptin be expected to work in a similar way in humans (*i.e.* be a single factor restoring normal body composition)? If this single factor theory is correct, then the chronic overfeeding experiments should confirm recent cross-sectional data (2, 8, 21–23) showing that the rise in leptin in obesity is a reflection of resistance to leptin action. Specifically, we document a somewhat disturbing picture that among previously lean individuals who maintain positive energy balance, those predestined to gain fat (*i.e.* change body composition to a more obese phenotype) do so despite an exaggerated leptin response. Whether the defect lies in the leptin signaling pathway or is a reflection of leptin action being overridden by another component of the complex system controlling energy balance in humans is presently unknown.

In conclusion, our data illustrate acute and chronic up-regulation of leptin in response to overfeeding and suggest that resistance to leptin favors greater accumulation of fat during weight gain. Thus, as hypothesized by us previously (24), the development of obesity in response to calorie excess is a feature of the hyperleptinemic "thrifty phenotype."

Acknowledgments

The secretarial help of Ms. Rolma Mancinow and the technical assistance of Mrs. Irina Opentanova are greatly appreciated.

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