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EFFECT OF FASTING ON SERUM LEPTIN IN NORMAL HUMAN SUBJECTS

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ABSTRACT We have studied the effect of fasting on serum leptin levels in normal volunteers. Five normal-weight (BMI < 28, 2 males/3 females) and five obese subjects (BMI > 28, 2 males/3 females) were fasted (0 Kcal) for 52 h. Mean plasma glucose decreased from 88 ± 3 to 63 ± 5 mg/dl, serum insulin from 16 ± 1 to 10 ± 1 μU/ml, plasma β-hydroxybutyrate increased from 0.2 ± 0.1 to 1.8 ± 0.4 μmol/ml. Serum leptin levels were higher in the obese than in the normal-weight volunteers (31 ± 12 vs 11 ± 3 ng/ml, $p < 0.01$). In the obese, serum leptin decreased from 31 ± 10 to 12 ± 5 ng/ml after 52 h (-72%, $p < 0.001$); in the normal-weight it decreased from 11 ± 3 to 4 ± 0.5 ng/ml (-64%, $p < 0.001$). Serum leptin correlated positively with serum insulin ($r = 0.51$, $p < 0.001$) and with plasma glucose ($r = 0.61$, $p < 0.001$). To determine effects of fasting induced decreases in plasma glucose and insulin on serum leptin, four normal subjects (3 males/1 female) were fasted for 72 h while their plasma glucose was clamped at basal levels with a variable rate glucose infusion. In these volunteers, serum leptin and insulin concentrations remained unchanged. In summary, the rapid decrease in serum leptin levels during fasting indicated that leptin release was regulated by factors other than changes in body fat mass. The lack of leptin changes during fasting, when basal insulin and glucose levels were maintained at basal levels, suggested that insulin and/or glucose may play a role in the regulation of leptin release.

INTRODUCTION

An obesity gene has recently been cloned in rodents and humans (1). The obesity gene product, a 16 kD protein called leptin (Greek for thin) is synthesized and released exclusively from adipose tissue (1,2). Leptin, when administered to leptin deficient ob/ob mice, resulted in decreased food intake , weight loss and increased caloric expenditure (2-4). These animal data support the concept that accumulation of body fat increases leptin synthesis and release from the adipose tissue. Leptin binds then to specific receptors in the hypothalamus and eventually leads to reduced appetite, and increased caloric expenditure (5). There are presently few human data on leptin. It is, therefore, not clear whether leptin plays a role in human appetite and weight control. It is known, however, that leptin mRNA and plasma leptin levels correlate with bodyweight, i.e. they are increased in obesity and decrease with weight loss (6). In view of the postulated role of leptin as a satiety factor, we felt it would be of interest to determine plasma leptin levels during a 52 h total fast in healthy normal weight and overweight subjects.

METHODS AND PROCEDURES

Table 1 shows the clinical characteristics of the 14 study subjects. All studies were performed at the General Clinical Research Center at Temple University Hospital. Flexible IV catheters with heparin locks were placed into antecubital veins and blood samples were collected at 2 h intervals beginning ~ 6 h after the last meal.

STUDY SUBJECTS

	Study 1		Study 2†
	Obese*	Normal-weight	
Sex (m/f)	2/3	2/3	3/1
Age (yrs)	35.6 ± 6.7	42.2 ± 5.8	35.0 ± 6.0
Height (cm)	170.2 ± 4.3	173.0 ± 3.5	172.5 ± 5.4
Weight (kg)	100.5 ± 13.1	61.4 ± 4.6	81.1 ± 8.0
BMI	34.1 ± 2.8	20.7 ± 2.0	27.2 ± 2.0

* BMI > 28
† One male and 1 female were obese (BMI 28.2 and 31.6 respectively).

Study 1: Ten volunteers (4 males/6 females) underwent a 52 h fast. They had free access to water, but did not receive any calories by mouth or parenterally.

Study 2: Another four volunteers (3 males/1 female) also did not receive any calories by mouth, but their blood glucose was prevented from decreasing by a variable rate intravenous infusion of 5% glucose. All volunteers were confined to their room, where they spent the time sitting in a chair or lying in bed.

ANALYTICAL PROCEDURES

Plasma glucose was measured with a glucose analyzer (Beckman Instruments, Palo Alto, CA), serum insulin with an antibody with minimal crossreactivity with proinsulin (0.2%), leptin by radio-immunoassay with a kit (both from Linco Research Inc., St. Charles, MO). β-hydroxy-butyrate was determined enzymatically.

STATISTICAL ANALYSIS

All results are shown as means ± SE. Analysis of variance with repeated measures was used to determine changes in substrate and hormone concentrations over time and groups. Student's t-test was used to analyze differences between groups at specific time points and linear regression equations and correlation coefficients were determined by least square analysis.

RESULTS

Figure 1 shows glucose, insulin, β-hydroxy-butyrate (β-OH butyrate) and leptin concentrations in obese (n=5) and normal weight (n=5) volunteers undergoing a 52 h period of total starvation. Plasma glucose concentrations in the normal weight subjects decreased from 83 ± 4 mg/dl at the beginning of the study to 76 ± 4 mg/dl after 24 h and to 68 ± 5 mg/dl after 52 h. In the obese subjects, glucose decreased from 94 ± 3 mg/dl to 85 ± 4 after 24 h and to 69 ± 7 mg/dl after 52 h.

Serum insulin concentrations in the normal weight subjects decreased from 15 ± 1 μU/ml at the beginning of the study to 12 ± 2 and to 12 ± 1 μU/ml 24 and 52 h later. In the obese subjects, insulin decreased from 20 ± 4 to 12 ± 2 μU/ml after 24 h and to 8 ± 2 μU/ml after 52 h.

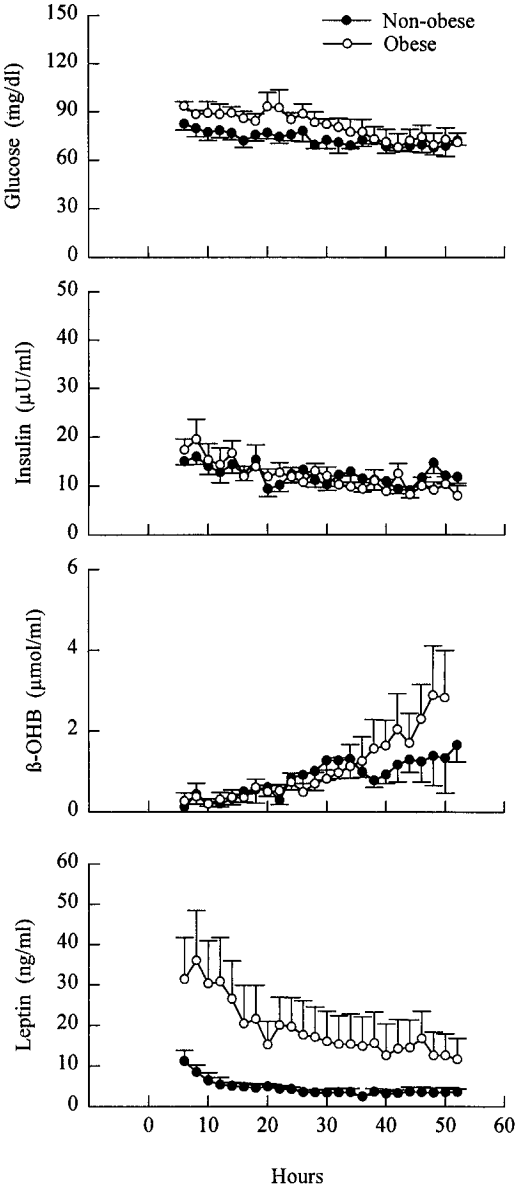

Figure 1. Plasma glucose, insulin, β-hydroxy-butyrate (β-OHB) and leptin levels in healthy non-obese (n=5) and obese (n=5) subjects during a 52 h total fast. Shown are means ± SE.

Plasma β-OH butyrate concentrations in the normal weight subjects increased from 0.1 ± 0.0 μmol/ml at the beginning of the study to 0.85 ± 0.3 and 1.65 ± 0.4 μmol/ml after 24 and 52 h.

In the obese subjects, β-OH butyrate increased from 0.26 ± 0.21 to 0.73 ± 0.22 μmol/ml after 24 h and to 1.96 ± 0.89 μmol/ml after 52 h.

Basal serum leptin concentrations were significantly higher in the obese than in the normal weight subjects ($p < 0.01$). In the obese, leptin decreased from 31 ± 10 ng/ml at the beginning of the study to 20 ± 7 ng/ml after 24 h and to 12 ± 5 after 52 h (-72%, $p < 0.001$). In the normal-weight subjects, serum leptin decreased from 11 ± 3 to 4 ± 0.5 ng/ml after 24 h and to 4 ± 0.8 ng/ml after 52 h (-64%, $p < 0.001$).

Plasma glucose and serum insulin concentrations correlated positively with serum leptin concentrations ($r = 0.61$ and $r = 0.51$, $p < 0.0001$ respectively). There was no significant correlation between leptin and β-OH butyrate concentrations ($r = -0.02$, NS).

Figure 2 depicts glucose, insulin and leptin levels in four volunteers (3 normal weight/1 obese) undergoing a 72 h fast during which plasma glucose and serum insulin concentrations were prevented from falling by a various rate infusion of 5% glucose. The amount of glucose needed to maintain euglycemia was 68.8 ± 35.9, 82.7 ± 26.1 and 111.8 ± 24.5 g/24 h or 289 ± 150, 347 ± 109 and 470 ± 103 Kcal/24 h for Days 1, 2 and 3 respectively. In contrast to Study 1 (decreasing glucose and insulin levels), serum leptin levels remained unchanged in Study 2 (stable glucose and insulin concentrations).

DISCUSSION

Caloric deprivation and loss of fat cell mass have been shown to decrease, while increased food intake and fat accumulation have been shown to increase ob gene expression (6-14). These observations have led to the hypothesis that leptin serves as a signal from the adipose tissue to the brain where it acts as a regulator of satiety. This concept was strongly supported by the demonstration that administration of the ob gene product, leptin, decreased food intake and fat cell mass in ob/ob mice and increased their energy expenditure (2-4).

The results of the present study indicated that the regulation of leptin release in humans may be more complex. For instance, it can be estimated

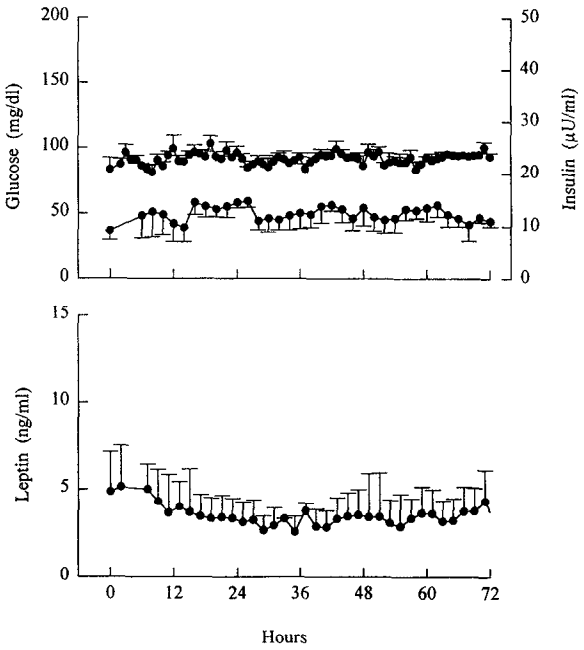

Figure 2. Plasma glucose, insulin and leptin levels in 4 healthy subjects during a 72 h euglycemic clamp and fasting (no calories by mouth).

that during the first 24 h of fasting, the subjects in this study lost maximally 160 grams of fat i.e. ~ 0.5% of their total body fat. (Their total energy expenditure was ~ 1500 Kcal/24 h, their body fat mass was ~ 24.5 kg). During the same time, however, their serum leptin concentrations fell by ~ 50%. It appears highly unlikely that the small change in fat mass could have been responsible for the profound decrease in serum leptin concentrations. Our results suggested, therefore, that release of leptin was regulated by factors other than changes in body fat mass. (These considerations are based on the assumption that changes in serum leptin levels reflected changes in leptin secretion rather than changes in leptin clearance which cannot presently be assessed).

To our knowledge, there are currently no other published data on the effect of short term fasting on circulating leptin levels in humans or in animals. Our results are, however, compatible with several reports demonstrating reduced ob gene expression in adipose tissue of rodents

during starvation. For instance, Trayhurn et al. and McDougald et al. found leptin mRNA reduced by 75% and by 80%, respectively, in 24 h and 16 h fasted mice (13,15), while Cusin et al. and Saladin et al. reported decreases of similar magnitude in adipose tissue leptin mRNA in 2 and 3 day fasted rats (12).

What could have been responsible for the rapid decrease in serum leptin? A definitive answer to this question is presently not available. The observation in this study, that the fall in serum leptin during fasting was prevented by infusion of a small amount of glucose, however, offered several clues. First, it suggested that leptin release might have been influenced by serum insulin. Both decreased during the complete fast and there was a significant correlation between plasma insulin and leptin levels ($r = 0.51$, $p < 0.0001$) (Study 1). In contrast, leptin levels remained unchanged during Study 2, when insulin remained stable. Moreover, there are several reports in the literature, demonstrating a strong influence of insulin on in vivo and in vitro ob gene expression. In vivo, Cusin et al. have reported marked increases in leptin mRNA in adipose tissue of rats after 2 days of euglycemic-hyperinsulinemia (12). McDougald et al. reported downregulation of leptin mRNA in the adipose tissue of streptozotocin diabetic rats, which was rapidly reversed by injection of insulin (13). In vitro, Leroy et al. reported a 5-10 fold increase in leptin mRNA in 3T3-F442A adipocytes incubated with 1 nM insulin for 24 h (16). Second, the decrease in plasma leptin levels during fasting could have been mediated by changes in plasma glucose which also correlated positively with serum leptin levels. Alternatively, it seems possible that fasting produced changes in autonomic nervous impulses to the adipose tissue. In this respect it is noteworthy that stimulation of beta 3 adrenergic receptors has been shown to decrease ob gene expression in A/J mice (10).

In summary, we have shown that fasting reduced serum leptin concentrations by 60-70% within 52 hours in normal-weight and obese subjects and that the fall in serum leptin was prevented when basal plasma glucose and insulin concentrations were maintained by IV infusion of a small amount of glucose. These data suggested that leptin release was regulated by factors other than changes in fat cell mass.

REFERENCES

1. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. 1994 Positional cloning of the mouse obese gene and its human homologue. Nature. 372:425-432.
2. Pelleymounter MA, Cullen MJ, Baker MB, et al. 1995 Effects of the obese gene product on body weight regulation in ob/ob mice. Science. 269:540-543.
3. Halaas JL, Gajiwala KS, Maffei M, et al. 1995 Weight-reducing effects of the plasma protein encoded by the obese gene. Science. 269:543-546.
4. Campfield LA, Smith FJ, Guisez Y, Devos R, Burn P. 1995 Recombinant mouse OB protein: evidence for a peripheral signal linking adiposity and central neural networks. Science. 269:546-549.
5. Stephens TW, Basinski M, Bristow PK, et al. 1995 The role of neuropeptide Y in the anti-obesity action of the obese gene product. Nature. 377:530-532.
6. Considine RV, Sinha MK, Heiman ML, et al.1996 Serum immunoreactive-leptin concentrations in normal-weight and obese humans. N Engl J Med. 334:292-295.
7. Masuzaki H, Ogawa Y, Isse N, et al. 1995 Human obese gene expression: adipocyte-specific expression and regional differences in the adipose tissue. Diabetes. 44:855-858.
8. Lonnqvist F, Arner P, Nordfors L, Schalling M. 1995 Overexpression of the obese (ob) gene in adipose tissue of human obese subjects. Nat Med. 1:950-953.
9. Hamilton BS, Paglia D, Kwan AYM, Deitel M. 1995 Increased obese mRNA expression in omental fat cells from massively obese humans. Nat Med. 1:953-956.
10. Collins S, Surwit RS. 1996 Pharmacologic manipulation of ob expression in a dietary model of obesity. J Biol Chem. 271:9437-9440.

11. Maffei M, Halaas J, Ravussin E, et al. 1995 Leptin levels in human and rodent: measurement of plasma leptin and ob RNA in obese and weight-reduced subjects. Nat Med. 1:1155-1161.
12. Cusin I, Sainsbury A, Doyle P, Rohner-Jeanrenaud F, Jeanrenaud B. 1995 The ob gene and insulin. A relationship leading to clues to the understanding of obesity. Diabetes. 44:1467-1470.
13. MacDougald OA, Hwang CS, Fan H, Lane MD. 1995 Regulated expression of the obese gene product (leptin) in white adipose tissue and 3T3-L1 adipocytes. Proc Natl Acad Sci. 92:9034-9037.
14. Saladin R, De Vos P, Guerre-Millo M, et al. 1995 Transient increase in obese gene expression after food intake or insulin administration. Nature. 377:527-529.
15. Trayhurn P, Thomas MEA, Duncan JS, Rayner DV. 1995 Effects of fasting and refeeding on ob gene expression in white adipose tissue of lean and obese mice. FEBS Letters. 368:488-490.
16. Leroy P, Dessolin S, Villageois P, et al. 1996 Expression of ob gene in adipose cells. Regulation by insulin. J Biol Chem 271: 2365-2368.

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