Dysregulation of Leptin in Response to Fasting in Insulin-Resistant *Psammomys Obesus* (Israeli sand rats)

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Leptin is thought to play a significant role in energy balance as an afferent signal to the hypothalamus that reflects body fat content. In addition, leptin may also act as an acute sensor of energy balance independent of body fat mass, since *ob* gene expression and plasma leptin concentrations are decreased in lean animals and humans in response to short-term caloric deprivation. However, in obese animals and humans, the acute response of leptin to fasting is less clear. We investigated the effects of a 24-hour fast on circulating plasma leptin concentrations in lean and obese *Psammomys obesus* (Israeli sand rats). In the lean, insulin-sensitive group (n = 25) a 24-hour fast caused a 44% decrease in plasma leptin, whereas in the obese, insulin-resistant group (n = 24) plasma leptin increased by 18% after fasting ($P < .003$). There was no difference between the two groups regarding the effect of a 24-hour fast on body weight, blood glucose, or plasma insulin. Within the insulin-resistant group, there was no difference in the response of leptin to fasting between hyperglycemic and normoglycemic animals. We conclude that there is a dysregulation of leptin in response to acute caloric deprivation in obese, insulin-resistant but not in lean, insulin-sensitive *P obesus*.

LEPTIN, the product of the *ob* gene, is secreted exclusively from adipocytes and is thought to play an important role in the regulation of energy metabolism and body weight.[1] The hyperphagia, obesity, and insulin resistance that characterize the *ob/ob* mouse phenotype were all shown to be rapidly reversed by leptin administration, which also caused significant weight loss in wild-type mice.[2-4]

Numerous studies in both humans[5-8] and animal models of obesity other than *ob/ob* mice[2-4,9,10] have demonstrated that *ob* gene expression and plasma leptin concentrations reflect body fat mass, and suggest that leptin may act as an afferent signal to the brain influencing energy balance.

The proposal that leptin plays an important role in the control of energy balance and utilization has led to interest in the short-term regulation of *ob* gene expression and the circulating plasma leptin concentration. In wild-type mice, fasting for 48 to 72 hours decreased plasma leptin by 60% to 70%,[11,12] while *ob* gene expression decreased by 85% after a 16-hour fast[13] and 90% after a 48-hour fast.[14] In lean control rats, fasts of 16 to 72 hours' duration decreased plasma leptin by 50% to 80%.[13,15-17] Similarly, in lean humans, fasting for 24 to 60 hours caused a 60% to 70% reduction in plasma leptin.[18-20] It appears that fasting significantly reduces *ob* gene expression and plasma leptin in lean, healthy animals and humans.

In obesity, the response of leptin to fasting is less clear. Fasting did not significantly reduce *ob* gene expression in *ob/ob* mice after 16 hours, in diet-induced obese mice after 48 hours, or in *fa/fa* rats after 72 hours.[13,14,16] However, several small studies in obese humans have shown that fasting for 24 to 60 hours reduced circulating plasma leptin by 42% to 88%.[18-20] To further clarify this, we investigated the response of leptin to a 24-hour fast in a polygenic, heterogeneous model of obesity and non–insulin-dependent diabetes mellitus (NIDDM), *Psammomys obesus* (the Israeli sand rat).

P obesus is a unique animal model of obesity and NIDDM. *P obesus* remains lean and normoglycemic in the wild on its natural low-energy diet.[21] However, when taken into the laboratory and fed ad libitum chow, a relatively energy-dense food, the animals exhibit a range of pathophysiological responses, with approximately half becoming obese and about one third developing NIDDM.[22,23] *P obesus* exhibits a range of body weight and blood glucose and insulin concentrations that form a continuous curve, closely resembling the pattern found in human population.[22,24] It is the heterogeneity of the phenotypic response of *P obesus* that makes it a useful model to study the etiology and pathophysiology of obesity and NIDDM.

The aim of this study was to investigate the response of leptin to a 24-hour fast in *P obesus* with a wide range of body weight and circulating glucose and insulin concentrations.

MATERIALS AND METHODS

Breeding of the Colony

A *P. obesus* colony is maintained at Deakin University, with the breeding pairs fed ad libitum a diet of alfalfa and standard laboratory chow. Experimental animals were weaned at 4 weeks of age and sustained on a diet of standard laboratory chow from which 12% of the energy was derived from fat, 63% from carbohydrate, and 25% from protein (Barastoc, Pakenham, Australia). The animals were housed in a humidity- and temperature-controlled room (22° ± 1°C) with a 12-hour light-dark cycle. The animals used in the study were 12 to 14 weeks of age when fasted.

Experimental Protocol

The animals (N = 49) were weighed and blood was collected from the tail vein in the fed state. They were then fasted for 24 hours before being weighed again, and blood was collected again in the fasted state. The animals were evaluated for a further 28 days with ad libitum access to food and water before being weighed and bled again. All blood was collected into heparinized tubes. The experiments were performed following the Australian National Health and Medical Research Council principles of laboratory animal care, and were approved by the Deakin University Animal Ethics Committee.

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Submitted May 8, 1997; accepted June 25, 1997.

Supported in part by the National Health and Medical Research Council of Australia and Deakin University Internal Research Grants.

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0026-0495/98/4701-0024$03.00/0

Table 1. Effects of a 24-Hour Fast on Body Weight and Blood Glucose, Plasma Insulin, and Leptin Concentrations in Insulin-Sensitive (n = 25) and Insulin-Resistant (n = 24) *P obesus* (mean ± SEM)

Group	Body Weight (g)	Glucose (mmol/L)	Insulin (μU/mL)	Leptin (ng/mL)
Insulin-sensitive (n = 25)				
Fed	167.6 ± 7.1	4.01 ± 0.14	55.3 ± 15.4	24.9 ± 4.1
Fasted	164.5 ± 7.5	3.54 ± 0.12	15.1 ± 1.2	13.8 ± 3.0
P	.002	.005	.015	.001
Insulin-resistant (n = 24)				
Fed	214.2 ± 5.7	7.44 ± 0.81	305.4 ± 55.4	64.3 ± 6.3
Fasted	210.3 ± 6.0	5.43 ± 0.83	143.3 ± 34.3	75.6 ± 11.8
P	.005	.018	<.001	.120

Analytical Methods

The whole-blood glucose level was measured immediately using an enzymatic glucose analyzer (model 27; Yellow Springs Instruments, Yellow Springs, OH). Plasma insulin concentrations were determined using a double-antibody solid-phase radioimmunoassay (Phadeseph; Kabi Pharmacia Diagnostics, Uppsala, Sweden). Plasma leptin levels were measured in a solid-phase double-enzyme immunoassay (EIA) with affinity-purified polyvalent antibodies. The concentrations were calculated from standard curves generated with recombinant murine leptin. The limits of detection for the leptin EIA are 20 pg/mL serum or plasma. The interassay coefficient of variation is 7.7% for the high standard and 10.5% for the low standard.

Statistical Analysis

The data are expressed as the mean ± SEM. A one-way ANOVA in combination with Tukey's multiple-comparison test was used to compare means between and within groups, and a paired *t* test was used where appropriate. In all instances, *P* values less than .05 were considered significant.

RESULTS

Significant correlations were found between the fasting plasma leptin concentration and fasting body weight ($r = .502$, $P < .001$) and the fasting leptin and insulin concentrations ($r = .737$, $P < .001$). Corresponding correlations in the fed state were also statistically significant; (fed leptin and body weight, $r = .545$, $P < .001$; fed leptin and insulin, $r = .665$, $P < .001$).

The animals were separated into two groups based on the fasting plasma insulin as a surrogate measure of insulin sensitivity. The insulin-resistant group (n = 24) had fasting plasma insulin greater than 30 μU/mL (range, ≤755 μU/mL). Corresponding values in the insulin-sensitive group (n = 25) ranged from 5 to 25 μU/mL.

The response to a 24-hour fast in the two groups is detailed in Table 1, and percentage changes in the variables are summarized in Fig 1. The fast caused small but significant decreases in body weight in both groups (−1.8% in both groups; the change in body weight induced by fasting was not significantly different between the two groups, $P = .621$). Blood glucose also decreased significantly in both groups, 12% in insulin-sensitive and 27% in insulin-resistant animals (Table 1). Once again, the change in blood glucose was not significantly different between the two groups ($P = .057$).

Plasma insulin decreased significantly in both groups, 73% and 53%, respectively. The absolute difference between the two groups was significant ($P = .001$); however, the grouping of the animals ensured that the insulin-resistant group would have a far greater mean fed plasma insulin concentration. When the percentage changes in plasma insulin were analyzed, there was no significant difference between the two groups.

Plasma leptin concentrations decreased significantly after a 24-hour fast in the insulin-sensitive group. The 44% decrease was highly significant ($P < .001$), with all of the animals showing a decrease in plasma leptin after the fast. Conversely, plasma leptin did not decrease in the insulin-resistant group; in fact, they showed a tendency for increased plasma leptin after a 24-hour fast. Overall, the insulin-resistant group had an 18% increase in circulating leptin, significantly different versus the insulin-sensitive group ($P = .003$).

The animals were also investigated after 4 weeks of ad libitum access to food and water following the fast. No significant differences were found between the two groups in terms of body weight, glucose, insulin, and leptin changes over this period. Additionally, there were no significant correlations evident between the leptin response to fasting and any of the changes in these parameters over the ensuing period.

The effect of hyperglycemia was also investigated within the insulin-resistant group. Of these animals, 11 had a fasting blood glucose greater than 8 mmol/L and were considered hyperglycemic relative to the other 13 from this group. These two subgroups could be regarded as models of NIDDM and

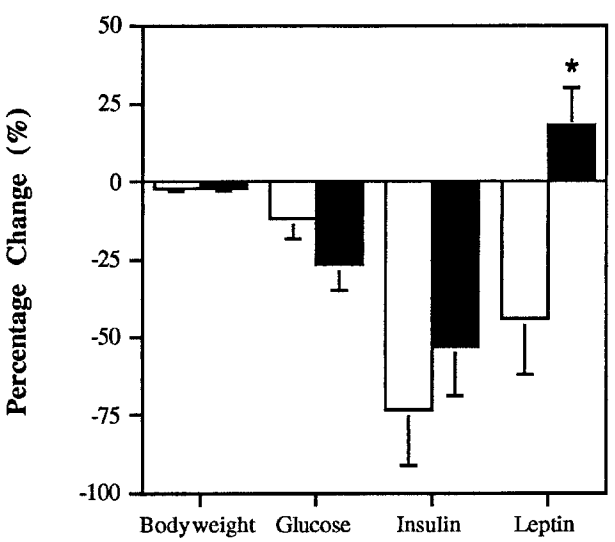

Fig 1. Percentage changes in body weight and blood glucose, plasma insulin, and leptin concentrations in (□) insulin-sensitive (n = 25) and (●) insulin-resistant (n = 24) *P obesus* after a 24-hour fast. *$P = .003$ *v* insulin-sensitive group.

impaired glucose tolerance (IGT), respectively. No differences between IGT and NIDDM animals were apparent in terms of fed or fasted leptin levels or the change in leptin levels after a 24-hour fast (Fig 2 and Table 2). The hyperglycemic, insulin-resistant animals had an increase in plasma leptin after a 24-hour fast of 11.7 ± 7.3 ng/mL, and the corresponding change in normoglycemic, insulin-resistant animals was 10.9 ± 11.6 ng/mL.

DISCUSSION

In this study, we have shown that obese, insulin-resistant *P obesus* have significantly elevated fasting and fed plasma leptin concentrations compared with their lean, insulin-sensitive littermates (Table 1). Using the fasting plasma insulin concentration as a marker of insulin sensitivity, we found that obese, insulin-resistant *P obesus* failed to show a decrease, and indeed tended to show an increase, in the plasma leptin concentration after a 24-hour fast (Fig 1). This is opposite to the results found in lean, insulin-sensitive *P obesus* and other lean animals and humans,[13-20] which have shown decreases of 40% to 80% in circulating plasma leptin after fasting for 1 to 3 days.

It is currently proposed that the principal physiological function of leptin is the regulation of energy balance, especially the control of food intake. Because circulating leptin levels appear to reflect the amount of body fat and do not change postprandially, leptin is thought to act as a "lipostat" in the long-term control of body weight homeostasis. It has also been suggested that leptin may have a second function as an acute sensor of energy balance,[19] whereby the circulating leptin concentration is regulated in an acute manner in response to departures from normal energy balance, including fasting. In this capacity, leptin appears to act independently of body fat stores, suggesting a dual role for this hormone in the control of energy balance.[19]

However, the effects of obesity and insulin resistance on the response of leptin to a short-term fast are still unclear. One small study (n = 3) in obese subjects found a significant decrease in plasma leptin after a 24-hour fast.[20] However, no data regarding plasma insulin concentrations were provided in the study. Two further studies, also with small numbers (n = 3 and n = 5) of obese subjects, found significant decreases in plasma leptin after a 52- or 60-hour fast.[18,19] In both of these studies, obese subjects did not appear to be significantly hyperinsulinemic relative to lean control subjects. Given the small number of subjects in these studies and the apparent lack of significant hyperinsulinemia and (probably) insulin resistance, we believe these data do not exclude the possibility that a subgroup of obese humans with relatively severe insulin resistance may also have a dysregulation in the response of leptin to acute starvation, as seen in *P obesus* in this study.

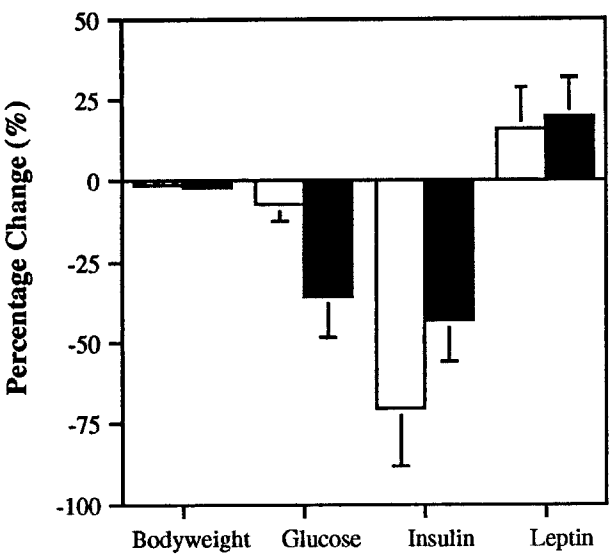

Fig 2. Percentage changes in body weight and blood glucose, plasma insulin, and leptin concentrations in (□) IGT (n = 13) and (●) NIDDM (n = 11) *P obesus* after a 24-hour fast.

Table 2. Effects of a 24-Hour Fast on Body Weight and Blood Glucose, Plasma Insulin, and Leptin Concentrations in IGT (n = 13) and NIDDM (n = 11) *P obesus* (mean ± SEM)

Group	Body Weight (g)	Glucose (mmol/L)	Insulin (μU/mL)	Leptin (ng/mL)
IGT (n = 13)				
Fed	204.2 ± 8.1	4.11 ± 0.27	202.9 ± 37.6	68.5 ± 10.8
Fasted	200.7 ± 8.2	3.82 ± 0.28	60.6 ± 8.7	79.4 ± 20.6
P	.041	.220	.003	.370
NIDDM (n = 11)				
Fed	226.0 ± 6.9	11.38 ± 0.54	426.3 ± 103.3	59.5 ± 5.3
Fasted	221.7 ± 7.8	7.33 ± 1.64	241.1 ± 63.4	71.2 ± 9.7
P	.043	.023	.007	.140

Interestingly, acute dietary restriction (1,045 kJ/d) for 5 days in seven morbidly obese NIDDM patients did not significantly alter *ob* gene expression, with the mean change found to be a 17% increase (range, −46% to +69%).[25] These subjects had NIDDM and presumably were considerably insulin-resistant and the dietary restriction significantly reduced the plasma insulin concentration, analogous to the findings in this study. In addition, in *db/db* mice fasted for 15 days, plasma leptin was unchanged despite a 25% decrease in the body mass index; however, it was reduced after 28 days.[12] Similarly, in obese hyperinsulinemic animal models such as *ob/ob* mice and *fa/fa* rats, fasting for 16 to 72 hours did not significantly change *ob* gene expression.[13,14,16]

The reduction in plasma leptin in the lean Israeli sand rat is almost certainly not related to a decrease in body fat mass or body weight, as both groups had small but identical percentage decreases in body weight after the 24-hour fast (1.8%). Therefore, it appears that the plasma leptin concentration in this case is not simply a reflection of body fat content, as previously suggested.[8]

Previous longitudinal studies in both *P obesus* and humans have indicated that individuals tend to progress from normal glucose tolerance to IGT to NIDDM.[22,24] Within our insulin-resistant group of animals were representatives of both IGT and NIDDM, and it is likely that IGT animals would progress to develop NIDDM over a sufficient period.[22] We were interested in whether IGT animals already had the leptin dysregulation in response to fasting, or whether this developed concomitantly with or as a consequence of hyperglycemia. The results clearly show that the dysregulatory defect in the leptin response to fasting was already present in IGT animals, and it therefore

appears to develop around the same time as hyperinsulinemia and before hyperglycemia in these animals.

The normal physiological response to fasting is characterized by a reduction in blood glucose and insulin, an increase in gluconeogenesis and a decrease in glycogenolysis, and an increase in circulating fatty acids and ketones.[26-28] It is possible that one or more of these factors regulate leptin production in response to fasting; however, it appears from a recent study that hyperketonemia per se does not directly inhibit leptin secretion.[19] We are unable to address this issue with the results of the current study, but it is interesting that the percentage decreases in blood glucose and plasma insulin after fasting were similar between the two groups of animals.

Another potential leptin regulatory factor is insulin,[16,29] which decreases markedly during fasting. Several studies investigating any effects of insulin on leptin production both in vivo and in vitro have failed to demonstrate a significant acute effect.[30,31] However, in the long-term, insulin may enhance leptin production.[30,32] The precise physiological role of insulin in the regulation of leptin production is unclear at this time.

In summary, obese, insulin-resistant *P obesus* are hyperleptinemic relative to their lean, insulin-sensitive littermates, and in response to a 24-hour fast, there is a dysregulation of leptin in the obese animals. The mechanism(s) causing the failure of plasma leptin to decrease in fasted obese *P obesus* is unknown.

ACKNOWLEDGMENT

The authors would like to thank Dr. Margery Nicolson (Amgen, Thousand Oaks, CA) for assistance.

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