CLINICAL STUDY

Total parenteral nutrition after surgery rapidly increases serum leptin levels

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Abstract

Objective: In humans, leptin is regulated by long-term changes in energy intake. However, short-term regulation of serum leptin by nutrients has been difficult to show. The aim of this study was to investigate whether short periods of fasting and stress sensitise the leptin response to nutrients.

Subjects and experimental protocol: Fourteen patients of normal weight undergoing elective open cholecystectomy were randomised into two groups. One group received saline infusion during surgery and for 24 h postoperatively. The other group also received saline during the surgical procedure, but total parenteral nutrition (TPN) was started immediately after surgery. Blood samples were drawn before as well as 2, 4, 8, 16, and 24 h after the start of surgery to determine the serum levels of leptin and other hormones.

Results: Postoperative TPN induced a significant rise in serum leptin within 6 h, reaching a more than fourfold increase within 14 h ($P < 0.001$). Serum glucose and insulin levels increased within 2 h. Growth hormone and IGF-1 serum levels also increased significantly in the group receiving TPN. Serum cortisol levels increased postoperatively in both groups, which may explain why no significant reduction in serum leptin was observed in the group receiving saline. Free tri-iodothyronine (T3) decreased in both groups, while catecholamines were similar in the groups.

Conclusion: During fasting and surgical stress, nutrients rapidly increased the serum leptin levels in humans in a manner similar to that previously reported in rodents. This may be mediated by increases in serum glucose, insulin and cortisol.

European Journal of Endocrinology 144 123–128

Introduction

Leptin is an adipocyte-derived hormone that contributes to body weight regulation by modulating feeding behaviour and energy expenditure (1–3). Defects in the genes encoding leptin or the leptin receptor lead to a spectrum of manifestations, indicating a critical role for leptin in regulation of body weight and the hypothalamic-hypophyseal axis (4, 5). The leptin receptor is widely distributed in the body (6), indicating that the functions of the hormone reach beyond a simple lipostatic model. Recently, leptin has been reported to affect haematopoiesis, sexual maturation and reproduction (7, 8).

Although body fat content is the major determinant of circulating leptin levels, other factors must contribute because serum leptin levels vary greatly for a given body-fat content (9). In rodents, insulin is a major short-term regulator of leptin (10), but whether insulin directly modulates leptin in humans is controversial. *In vitro* studies have shown that insulin

stimulates adipocyte leptin production (11, 12). In contrast, studies in healthy controls and diabetic patients have shown that serum leptin is upregulated only after prolonged exposure to hyperinsulinaemia suggesting an indirect trophic effect on the adipocytes (13–15). However, rapid effects of insulin, i.e. within 4 h, have also been reported during clamp studies, suggesting a direct effect on leptin production or metabolism (16–18).

Additional endocrine factors also regulate leptin. Most studies have shown that glucocorticoids upregulate leptin levels (19–22). However, unchanged serum leptin levels after high doses of prednisolone given to healthy volunteers, have also been reported (23), and it has been questioned whether the effects of glucocorticoids on leptin in humans are restricted to acute pharmacological dosing (24). The effects of growth hormone (GH) on leptin are unclear and both lower and higher leptin levels secondary to changes in body composition or insulin and IGF-1 levels have been reported (25–27). Some studies have reported results

suggesting a direct effect of GH on leptin (28–30). Adrenergic agonists reduce leptin (31–34), while triiodothyronine (T3) increases leptin RNA expression and secretion in murine 3T3-L1 adipocytes (35). Patients with hypothyroidism have low serum leptin levels that are normalised by substitution treatment (36, 37).

Circulating leptin has a diurnal profile with a nocturnal peak (38). In rodents, feeding upregulates while fasting downregulates leptin within 3 h, and the upregulation of leptin by nutrients is similar whether given enterally or parenterally (39). In man, serum leptin levels are affected by fasting and overfeeding, but the acute effects observed in rodents have not been shown (40–42). Starvation might sensitise the response of leptin secretion to subsequent energy intake, since serum leptin increased within 24 h after refeeding or glucose infusion, when given after a prolonged period of fasting (43, 44).

To determine whether short periods of fasting and stress sensitise the leptin response to nutrients, we compared the effects of TPN and saline given after surgery on the profile of circulating leptin levels in relation to other endocrine axes.

Subjects and methods

Fourteen, otherwise healthy, patients undergoing elective open cholecystectomy participated in the study. All had normal blood counts, fasting glucose levels, and liver as well as renal function tests. The Ethics Committee of the Karolinska Institute approved the study protocol, and informed consent was obtained from all participants. The patients were randomised into two groups (Table 1). Both groups received saline infusion during surgery (3 ml/kg body weight (BW) per h). Then, one group was given TPN for 24 h while the other group remained on saline (35 ml/kg BW per 24 h). The TPN consisted of 135 kJ/kg BW per 24 h. The non-protein calories were provided as equal amounts of glucose (Glucose 20%, Kabi Pharmacia) and fat (Intralipid 20%, Kabi Pharmacia). The dose of nitrogen was 0.2 g N/kg BW per 24 h (Vamin-glukos, Kabi Pharmacia). Saline and TPN were administered continuously by an infusion pump (281; IVAC, San Diego, CA, USA). No vitamins, trace elements, electrolytes, or other nutrients apart from the TPN formula

were given. No interventions were made regarding diet before arrival at the hospital. After an overnight fast, the operation started at 0830 h in 11 of the patients, at 1300 h in two patients, and at 1500 h in one patient (median 0830 h). The duration of surgery was 1.0–5.0 h (median 1.5 h) and did not differ significantly between the groups. TPN was initiated immediately postoperatively, i.e. approximately 1.5 h after starting surgery. The duration of fasting, i.e. from midnight to the start of TPN, ranged between 10 and 16 h (median 11 h).

Anaesthesia and surgery

Anaesthesia was induced with 250 mg thiopental (Pentothal, Natrium; Abbott, North Chicago, IL, USA), 0.2 mg fentanyl (Leptanal; Janssen Pharmaceutica, Meckenheim-Merl, Germany), 5 mg diazepam (Stesolid, Novum; Dumex, Copenhagen, Denmark), and 0.1 mg/kg BW pancuronium (Pavulon; Organon, Teknika, Boxtel, Holland). Anaesthesia was then maintained with 70% nitrous oxide in oxygen, together with intermittent doses of fentanyl and pancuronium to provide stable anaesthesia and muscle relaxation. No patient required a blood transfusion or antibiotics. Neuromuscular blockade was reversed by an i.v. injection of 0.5 mg glycopyrron and 2.5 mg neostigmin (Rubinul–neostigmin; Wyeth, Philadelphia, PA, USA). Intravenous injections of pethidine (Petidin; Kabi Pharmacia) were given to the patients for relief of postoperative pain (45).

Hormone measurements

Blood samples were drawn before as well as 2, 4, 8, 16 and 24 h after initiation of surgery for determination of serum leptin, insulin, glucose, GH, cortisol, adrenaline, noradrenaline, dopamine, and free T3. Serum IGF-1 levels were determined before and 24 h after surgery. Serum leptin levels were determined by radioimmunoassay (Linco Research Inc, St Charles, MO, USA). All samples were within the linear detection range, i.e. 0.5–100 ng/ml, and analysed in duplicate in the same assay run. The intra-assay coefficient of variance was 6.8% at the low leptin concentration (2.4 ng/ml) and 3.5% at the high leptin concentration (14.6 ng/ml). Serum glucose was determined with an enzymatic hexokinase method (Gluco-quant, Roche Diagnostics, Mannheim, Germany). Serum insulin and GH levels were determined by radioimmunoassay, Pharmacia Insulin RIA and Pharmacia HGH RIA, respectively (Pharmacia & Upjohn, Uppsala, Sweden). Serum IGF-I levels were measured as described (46). Serum cortisol and free T3 levels were determined by time-resolved fluoroimmunoassay (Wallac Oy, Turku, Finland). Serum levels of catecholamines were determined by HPLC with electrochemical detection (47).

Table 1 Patients' characteristics.

	Saline	TPN
Men/Women	3/4	5/2
Age (years)	42 ± 4	43 ± 5
Weight (kg)	70 ± 4	78 ± 5
Height (cm)	168 ± 3	176 ± 2
Body mass index (kg/m ²)	24.8 ± 0.7	24.9 ± 1.1

Values are means ± s.e.

Figure 1 (A) Individual 24 h profiles of serum leptin, insulin, GH and cortisol levels. Surgery was initiated at time 0 and lasted for a median of 1.5 h. TPN was started immediately after surgery. (B) Individual 24 h profiles of serum dopamine, adrenaline, noradrenaline and free T3 levels. Surgery was initiated at time 0 and lasted for a median of 1.5 h. TPN was started immediately after surgery.

Statistics

Individual serum levels of all measured hormones, except IGF-1, are presented (Fig. 1). Serum levels of IGF-1 are presented as means $\pm$ S.E. Analysis of variance for repeated measurements (ANOVA) was used to analyse the intra- and intergroup changes over time in leptin, insulin, free T3, cortisol, and glucose. The area under the curve (AUC) followed by Student's unpaired *t*-test was used to analyse the changes in GH, adrenaline, noradrenaline and dopamine. Student's paired *t*-test was used to evaluate the IGF-1 levels before and 24 h after surgery. The relation between the rise in serum leptin and duration of fasting was assessed using Pearson's correlation coefficient test. Significance was defined as $P \leq 0.05$. Data were analysed with the statistical package for social sciences (SPSS Inc., Chicago, IL, USA).

Results

In the group receiving TPN, serum leptin levels increased uniformly within 6 h and had increased more than fourfold within 14 h ($P < 0.001$, Figs 1A and 2). The duration of fasting prior to TPN correlated with the absolute increase in leptin ($r = 0.9$, $P < 0.01$). Glucose

and insulin levels increased within 2 h ($P < 0.001$, Figs 1A and 2). In addition, serum levels of growth hormone increased significantly ($P < 0.005$, Fig. 1A), and IGF-1 levels measured 24 h after initiation of surgery were significantly higher than the fasting levels, 293.9 ± 22.7 and 207.6 ± 19.7 mg/l, respectively ($P < 0.005$).

No significant fluctuations in serum levels of leptin or insulin were detected in the patients on saline infusion postoperatively (Figs 1A and 2). Glucose levels increased in the immediate postoperative period ($P < 0.001$), but 24 h later the levels were not different from fasting values (Fig. 2). Serum IGF-1 levels measured 24 h after starting surgery were

Table 2 Mean area under the curve (AUC) for growth hormone (GH) (mU min/l), cortisol (nmol min/l), and catecholamines (nmol min/l) in the two groups.

	Saline group	TPN group	<i>P</i> value
GH	113.4 ± 20.3	343.9 ± 45.4	$P < 0.005$
Cortisol	3150.0 ± 415.5	2744.1 ± 264.6	$P = 0.4$
Adrenaline	3.8 ± 1.0	6.0 ± 1.5	$P = 0.3$
Noradrenaline	12.3 ± 2.3	19.0 ± 4.9	$P = 0.2$
Dopamine	1.2 ± 0.3	2.1 ± 0.6	$P = 0.2$

Values are means $\pm$ S.E.

Figure 2 Mean 24 h profiles of serum glucose, insulin and leptin expressed as percentage change from the fasting values. Filled squares – saline postoperatively; open circles – TPN postoperatively. Data are given as mean $\pm$ S.E.. Surgery was initiated at time 0 and lasted for a median of 1.5 h. TPN was started immediately after surgery.

significantly lower than the fasting levels, 171.6 ± 25.0 and 218.9 ± 35.8 mg/l, respectively ($P < 0.05$).

Serum levels of cortisol, adrenaline, noradrenaline and dopamine increased in both groups but did not differ significantly between the groups (Fig. 1A and B, and Table 2). Serum free T3 levels decreased in both groups ($P < 0.001$, Fig. 1B), but although the saline group had lower levels than the TPN group, the difference was not significant.

Discussion

In the present study, TPN after surgery caused a more than fourfold increase in serum leptin, which clearly demonstrates an acute regulation by nutrition. To our knowledge, this is the first report in humans of a marked and uniform rise in leptin within a short period in response to nutrient intake.

Previously, no or only modest changes in serum leptin during fasting, refeeding and clamps have been reported (16–18, 43, 48). The marked increase in the present study is comparable to the leptin response to food intake or insulin stimulation in rodents (10, 49, 50). The fasting and surgical stress during the perioperative period most probably contributed to this

upregulation of serum leptin, but the underlying mechanism is unclear.

Catecholamines and fasting reduce leptin levels (31–34, 43, 44, 51). Thus, it could be argued that the pre-operative fasting levels were already lower than normal, due to the combination of fasting and stress. However, the leptin levels were within the wide normal range for sex and BMI, according to previous studies (52). In addition, the duration of fasting pre-operatively could be expected to result in leptin levels of approximately 80–90% of the basal levels (43), and the stress hormones levels were not markedly elevated. Thus, there is no indication that low starting levels could explain the subsequent fourfold upregulation of serum leptin.

Another explanation may be that the stress and fasting upregulated the reactivity of adipose tissue to nutrient intake or downregulated the clearance rate of leptin. The levels attained being much higher than expected for sex and BMI support the notion of increased reactivity. The preoperative fasting levels corresponded to a BMI that was appropriate to the patients' sex and BMI (median 25.0 kg/m^2) (52), while after TPN, the peak levels corresponded to a significantly higher BMI (median 34.0 kg/m^2). In a previous study, leptin levels fell to approximately 30% of basal levels following a 36 h fast, and refeeding restored them to basal values within 24 h (43). In another study, serum leptin levels increased significantly in response to a 5% glucose infusion following a 4 day fast (44). These findings are in agreement with the hypothesis that prolonged starvation may alter the response of leptin secretion to small manipulations in energy intake. However, in neither of these studies did serum leptin increase beyond basal values.

The effects of insulin on leptin in man are still controversial. Several *in vivo* studies indicate that only prolonged exposure to hyperinsulinaemia upregulates leptin, suggesting an indirect mechanism (13–15). On the other hand, during hyperinsulinaemic clamps, leptin levels increased in a dose-dependent manner, favouring a direct effect of insulin on leptin production or metabolism (18). Modest increases in leptin levels, in the range of 60%, were reported in these clamp studies despite the high serum insulin levels (9000 pmol/l). In our study, TPN increased serum leptin fourfold, while peak serum insulin levels ranged between 300 and 900 pmol/l. The specific components of the TPN, other than glucose, could hardly explain the rise in serum leptin. Short-term changes in free fatty acids have no effect on serum leptin levels (53, 54), and amino acids have only a modest upregulatory effect in rodents (39), while a glucose infusion increases serum leptin significantly (39, 44). In this study, serum glucose levels were high in both groups postoperatively, and in the TPN group the levels were more than double the fasting values. This is most probably caused by transient insulin resistance as a consequence of the

perioperative surge of stress hormones. The combination of high glucose and insulin levels is probably a major mediator of the leptin response seen here.

The saline group showed no significant drop in serum leptin postoperatively. This is in contrast to the pattern previously described during fasting (43). Nevertheless, glucocorticoids are known to upregulate serum leptin (19), and are reported to potentiate the *in vitro* upregulation of leptin by insulin (11). Thus, it is likely that the rise in serum cortisol observed in both groups after surgery counteracted the effect of fasting in the saline group and potentiated the effect of insulin in the TPN group. However, there was a strong correlation between the duration of preoperative fasting and the increase in serum leptin. Thus, it is likely that both factors, i.e. stress-induced upregulation of cortisol and fasting, have contributed to the patterns of serum leptin observed in this study. Gender, age and BMI did not seem to affect the leptin response. Nevertheless, the number of patients in each subgroup was small, and we cannot rule out an effect in a larger cohort.

The present data indicate that in response to the combination of stress and fasting, serum leptin levels could reach high levels following intravenous nutrient intake. Further studies to distinguish between the effects of these two factors are needed. However, it is tempting to speculate that less frequent meals, e.g. 12–16 h apart, might provoke a greater leptin response. The pattern of feeding in rodents is associated with a marked increase in serum leptin shortly after food intake. This hypothesis is in agreement with the finding that in a situation of fasting, energy intake is associated with a rapid upregulation of leptin (44).

In conclusion, postoperative TPN induced a marked rise in serum leptin levels. This may be mediated by insulin and potentiated by the stress-induced rise in cortisol. These findings suggest that in humans, as in rodents, nutrients acutely upregulate leptin.

Acknowledgements

This study was supported by grants from the Wera Ekström Foundation, the Frimurare Barnhuset Foundation, the Jerring Foundation, the Swedish Society for Medical Research, and the Swedish Medical Research Council (9941, 04210, 09101). The authors thank Ulla Engberg in the Department of Surgery, Karolinska Hospital for catecholamine determination and Viveka Gustavsson for invaluable nursing assistance.

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Received 11 July 2000

Accepted 11 October 2000