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Review

Nutritional regulation of leptin in humans

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Overview

Positional cloning of the defective gene in genetically obese *ob*/*ob* mice identified leptin as the long-sought hormone that communicates information about adipocyte metabolism to the brain [1]. Supplying leptin to genetically deficient *ob*/*ob* mice increases metabolic rate, body temperature and general activity and decreases food intake, body weight and adiposity [1–4]. Additionally, the importance of leptin to reproduction has been highlighted by the lack of sexual maturation in people who are genetically deficient in leptin [5] or the leptin receptor [6]. Although many of leptin's effects are centrally mediated, there is growing evidence that peripheral leptin receptors mediate leptin's proliferative effects on haemopoietic cells [7] and its anti-lipogenic effects on muscle and pancreatic islets [8, 9].

Because leptin has a critical role in regulating energy metabolism, adipose stores and body weight, questions have been raised concerning the control of leptin synthesis and secretion and the function of leptin in controlling satiety and food intake. Basal or fasting leptin values are higher in women than men, increase during the course of pregnancy and, in both healthy adults and those with Type II (non-insulin-dependent) diabetes mellitus, are strongly correlated with per cent body fat or BMI [10, 11] and adipose tissue mass [12–15]. Despite these strong correlations, people with similar degrees of adiposity have circulating leptin concentrations that vary considerably. Thus, factors other than adipocyte size and fat content must influence leptin production. Further, the regulation of leptin's diurnal pattern of secretion is still to be explained.

In this review we summarize current data, primarily from human studies, that address the nutritional controls on leptin synthesis and secretion and point out fruitful areas for future study. Excellent recent reviews should be consulted for information on the leptin receptor, other aspects of leptin's effects, and the relation of leptin to other hormones that regulate food intake and energy expenditure [16–22].

Leptin regulation by fasting, refeeding and overfeeding

Fasting and refeeding. During short-term energy restriction, plasma leptin concentrations decrease much more than the amount of weight or adipose tissue lost. For example, lean and obese adults who fasted for 52 to 96 h lost less than 4 % of body weight but the leptin concentrations decreased 54 to 72 % [23–25]. In careful long-term studies of lean and obese people who were either losing weight or maintaining a defined weight loss, leptin concentrations were lower during the period of loss than the period of weight maintenance at the same body composition [26]. After a 4 day fast followed by an additional 6 days of fasting during which an intravenous infusion of 5 % glucose was given, leptin increased 80 % within the first 24 h, even though glucose provided only 338 kcal/day [25]. Although it is possible that the previous 4 day fast confounded these results [27], taken together, these studies suggest that the serum leptin concentration reflects ongoing triacylglycerol synthesis or glucose uptake by fat cells, rather than actual adipocyte stores, because a large fat mass still exists after short-term fasting. Further, when subjects are

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refed after a 3 day fast, leptin values return to baseline within 12 h [24]. Thus, the amount of energy intake and fat store replenishment do not seem to be as critical as the presence of insulin-enhanced glucose metabolism.

Discordant values between weight loss and serum leptin are also observed during energy restriction. When lean adults were restricted for 7 day to 68% of their daily energy requirements, weight loss averaged 4% but leptin decreased 36% [28]. The decrease in leptin during fasting correlated independently with the 22% decrease in glucose and the rise in beta-hydroxybutyrate, factors that reflect decreased glucose availability and increased lipolysis.

Discrepancies are also apparent in the relation between decreased serum leptin during fasting and changes in *ob* gene expression. For example, abdominal subcutaneous adipose tissue leptin mRNA did not change after a 6 day fast even though serum leptin had decreased 40% [29]. A hypocaloric diet for 5 days did not change the leptin mRNA content in abdominal subcutaneous fat despite a loss of 3.7% of body weight; serum leptin values were not reported [30]. These studies suggest that serum leptin is regulated either by changes in leptin clearance or by post-transcriptional control.

The mechanism by which fasting lowers leptin concentrations is not known. Although serum beta-hydroxybutyrate increases fivefold over baseline during 60 h of fasting and is inversely correlated with serum leptin concentrations, there was no association between serum leptin and glucose, insulin or fatty acid concentrations [31]. Further, plasma free fatty acid concentrations themselves do not appear to regulate leptin concentration [32, 33].

Overfeeding. When normal adults are overfed for 12 h at 120 kcal/kg (55% fat, 15% protein, 35% carbohydrate), glucose increases minimally, the fasting insulin concentration doubles and the increase in nocturnal leptin persists until the following morning instead of declining [34]. Overfeeding normal men enough to achieve a 10% weight gain and an increase in body fat from 15.8 to 19.4%, was accompanied by a threefold increase in fasting leptin concentrations [34]. Although there was a strong linear correlation between leptin and body fat, there was little correlation with insulin in this study.

In summary, the available data on leptin and *ob* gene mRNA in humans suggest that serum leptin is regulated by energy availability. This regulation is not immediate, however, and the adaptive response of leptin to energy availability is not mediated entirely through changes in serum insulin, fatty acids or beta-hydroxybutyrate. During energy restriction or fasting, leptin does not reflect actual triacylglycerol stores or adipocyte numbers. Instead, the amount of circulating leptin appears to reflect the presence of energy that is sufficient to promote triacylglycerol synthesis rather than lipolysis.

High fat diets and other nutrients

High fat diets. Although the positive correlation between body fat and leptin may be partly mediated by insulin concentrations in humans, other factors like diet composition could be important. Studies of diets with changed fat and carbohydrate content have yielded mixed results. Rodents have varying susceptibilities to diet composition depending on the strain investigated. In rat strains that gain weight (Osborn-Mendel) or are resistant (S5B/P1) to high fat diets, feeding a diet that contains 56% energy from fat increased serum leptin similarly for both strains within 2 days [35]. Not all rodent strains respond similarly. In A/J mice, which are resistant to weight gain on a high fat diet, high fat feeding for 14 weeks increased leptin concentrations tenfold, whereas in C57BL/6J mice, which are prone to diet fat-induced obesity, leptin increased only twofold [36]. Leptin mRNA increased almost fourfold in 1 week in Wistar suckling rats weaned to a high fat diet compared with rats weaned to a high carbohydrate diet even though there were no differences in adipose tissue mass, adipocyte size or number, or serum insulin [37]. In addition to their effects on leptin, high fat diets also upregulate the short form of the leptin receptor 11-fold at the rat blood brain barrier, perhaps altering uptake of leptin into the brain [38].

Human studies have shown no effect of high fat diets on leptin. Fasting serum leptin did not change during 7 days on a diet that contained 60% of energy as fat, although on an individual basis, the leptin response to the high fat diet appeared to correlate with changes in insulin concentrations [12]. Similarly, diets containing fat at 14, 23, or 31% of energy and which maintained stable weight, had no effect on leptin in lean or overweight women, suggesting that diet fat content at these concentrations does not regulate leptin [13]. In these studies only morning measurements were made and dietary effects on the diurnal leptin pattern were possibly missed. In a study that did compare the 24-h leptin pattern of subjects who ate isocaloric high or low fat meals, the timing of the nocturnal rise in leptin differed substantially (see below), suggesting that diet composition does change serum leptin but that the change would not be apparent between 0700 and 1000 hours [39].

Fibre and other nutrients. Little information is available on the effect of specific nutrients or fibre on serum leptin. Sucrose and amino acids given over 4 h did not change leptin concentrations despite increases in serum glucose and insulin concentrations [40]. Hydrolysed guar gum had no effect on postprandial

glucose, insulin or leptin in obese women eating 800 kcal/day [41]. Zinc restriction does, however, decrease human leptin concentrations [42].

Appetite and satiety

Although initially identified as a satiety hormone, it has been more recently hypothesized that leptin functions to prevent starvation and maintain reproductive capacity. Thus, in non-obese people, an increase in serum leptin may signal that energy stores are replete, whereas a decrease in leptin may signal the need for food intake as well as inadequate reserves to support pregnancy.

In fact, there is no evidence in humans that serum leptin influences satiety. Numerous studies have shown that the high leptin concentration in obese people is associated with excess rather than diminished food intake. Further, postprandial plasma leptin concentrations do not correlate with immediate subjective feelings of hunger and are not statistically significantly different between two meals that induce different effects on subjective feelings of hunger [43]. Another negative study reported that postprandial feelings of hunger do not differ statistically significantly between diets that contain either high-glycaemic or low-glycaemic index carbohydrates, even though the high-glycaemic index carbohydrate diet increased postprandial leptin concentrations [44]. These limited data suggest that leptin does not mediate energy intake in humans either in the short term or related specifically to meals.

Regulation of leptin by insulin

Leptin concentrations appear to be regulated by insulin, although questions remain concerning the insulin dose, the time course and the mechanism of control. In one patient, 6 months after an insulinoma was surgically removed, insulin concentrations decreased from 16.2 to 7.6 μg/l, leptin decreased about 50% and the patient's BMI had only decreased from 31 to 28 [45]. In diabetic pregnancies, placenta leptin mRNA and protein increased threefold to fivefold after the women were treated with insulin [46]. These observations strongly support a role for insulin in long-term regulation of leptin secretion from both adipocytes and placenta.

Several studies have been done using euglycaemic hyperinsulinaemic clamps to determine whether insulin infusions rapidly change serum leptin concentrations. Two problems prevent firm conclusions from being drawn. Firstly, the insulin doses (by definition) have been supraphysiological, and secondly, leptin values have usually been compared with an initial value which does not reflect the diurnal changes that occur in serum leptin concentrations. No statistically significant correlations were found between insulin-stimulated glucose uptake and serum leptin concentration, either before or after a 6 day fast in severely obese women [29]. When the 3-h hyperinsulinaemic clamp study (serum insulin at 1.4 nmol/l) was carried out without a preceding fast, serum leptin concentrations did not change; following the 6 day fast, however, serum leptin increased 25% compared with basal values [29]. These data suggest that the effect of insulin on serum leptin concentrations depends on the patient's previous nutritional state. Hyperinsulinaemia (about 900 pmol/l) increased serum leptin concentrations after 8 h in a dose-dependent manner in lean men but hyperglycaemia or high-plasma fatty acid concentrations did not affect leptin [33].

Hyperinsulinaemic clamp studies support the idea that insulin regulates leptin synthesis and secretion in humans. Because the high amounts of insulin used have been unphysiological, disagreements have centred on how much insulin is needed and how long a period is required to produce an effect. In lean, obese and obese adults with Type II (non-insulin-dependent) diabetes mellitus given various insulin infusion rates (serum insulin from 0.5–86 nmol/l) for 5 h, no change in leptin was observed compared with initial concentrations; with a hyperglycaemic clamp, leptin doubled, however, by 50 h [47]. In a 4-h euglycaemic hyperinsulinaemic clamp (serum insulin at ~ 600 pmol/l), compared with basal concentrations after an overnight fast, leptin increased 25% in healthy control subjects but was unchanged in patients with Type I (insulin-dependent) diabetes mellitus [48]. Compared with a 9-h saline infusion during which leptin concentrations fell 30%, insulin infusions (serum insulin at ~ 140 pmol/l) prevented the fall, showing a prompt insulin effect (Fig. 1) [49]. Infusions of higher amounts of insulin caused increases in serum leptin compared with the initial concentration, and with higher insulin doses, leptin rose earlier and reached higher concentrations [49]. In non-obese men and women, a euglycaemic hyperinsulinaemic clamp increased leptin 5, 26, and 62% compared with a control saline infusion during sequential 2-h increasing insulin infusions (serum insulins at 379, 823, and 2843 pmol/l) [50]. With a euglycaemic hyperinsulinaemic clamp (serum insulin at 600 pmol/l), leptin did not change compared with basal for up to 2 h in lean and obese subjects [51]. With a hyperinsulinaemic clamp (serum insulin at 480 pmol/l), leptin rose 37% at 6 h compared with a control saline infusion [52].

Interpretation of these studies has varied. The normal 24-h leptin pattern shows a fall of about 20% between 0800 and 1200 hours and then a plateau (Fig. 1, curve A). Since the insulin infusions were initiated after an overnight fast, leptin concentrations that re-

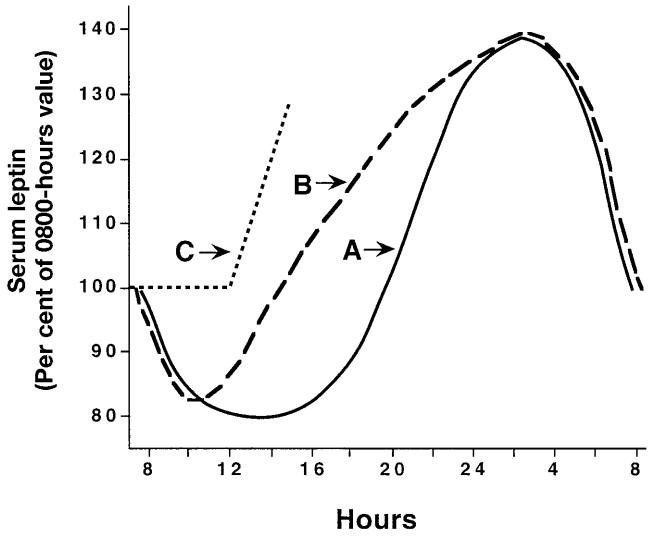

Fig. 1. Plasma leptin during 24 h. Idealized curves are plotted as per cent of the 0800 hours value. Curve A shows the normal curve reported for adults [39, 44, 68]. Curve B shows the curve observed with 3 isocaloric meals of 60% carbohydrate, 20% fat [39] or with a diet that contains 55–65% high glycaemic-index carbohydrate [44]. Curve C shows leptin values during a typical euglycaemic hyperinsulinaemic clamp

main stable compared with the initial value suggest that hyperinsulinaemia does indeed have an immediate effect, even though an actual rise in leptin concentration is not observed until 6 h after the start of the infusion (Fig. 1, curve C). A major problem is the choice of the control comparison group. It is well established that fasting will lower leptin concentrations and a saline infusion merely continues the overnight fast during which the subjects' endogenously produced insulin decreases and counter-regulatory hormones rise. Another option might be to have the comparison group eat normal meals at 0800 and 1200 hours or to select foods that do not increase endogenous serum insulin.

It must also be kept in mind that changes in serum concentrations of leptin depend on both leptin's rate of synthesis and secretion and on its rate of degradation. Adults with chronic renal failure have leptin values that are threefold higher than healthy subjects' who have a similar BMI [53] and in children with chronic renal failure, inappropriately raised leptin concentrations correlate with the declining glomerular filtration rate [54]. Although the kidney is believed to be important in leptin removal and reduced renal clearance was suspected, leptin concentrations in end-stage renal disease do not correlate well with residual renal function [55]. Decreases in leptin values, including the normal morning decline, might result from changes in renal clearance, and stabilization of morning leptin concentrations by insulin could result from effects on the kidney rather than on adipocyte synthesis.

Insulin effects on human adipocytes and adipose tissue. Although hyperinsulinaemia increases serum leptin at 6 h, the responding adipocyte mRNA abundance has not been well studied. After a 3-h euglycaemic hyperinsulinaemic clamp (serum insulin at ~ 850 pmol/l) adipose glucose transporter 4 (GLUT4) mRNA increased threefold but leptin mRNA did not change [30]. Although serum leptin was not measured in this study, 4–6 h of hyperinsulinaemia are usually required to increase serum leptin concentrations.

In studies of isolated human adipocytes, long exposures to insulin are required before leptin mRNA increases. Abdominal adipocytes exposed directly to 100 nmol/l insulin did not increase leptin mRNA and release until 72 h of incubation [47]. In a 48-h incubation of visceral adipose tissue in the presence or absence of insulin (100 nmol/l), leptin mRNA decreased and leptin secretion remained unchanged up to 48 h although GLUT4 and glycerol-P dehydrogenase mRNA increased [56]. Human preadipocytes contain little leptin mRNA or protein but after differentiation, insulin (1000 pmol/l) increased leptin secretion by 24 h; cortisol increased the effect of insulin about threefold [57]. This effect was reversed when the hormones were removed.

Interpretation of these results would be aided by more information about the cellular and molecular biology of leptin. What is the relation between leptin mRNA abundance and the amount of leptin secreted? Does the relatively stable mRNA expression in adipocytes indicate translational control? Is leptin stored in adipocytes and secreted only after a stimulus or is it secreted as soon as it is synthesized?

The mechanism by which insulin exerts its effects on leptin mRNA expression and protein secretion is not known but probably involves enhanced glucose uptake and metabolism [58]. Insulin increases leptin secretion by rat adipocytes when glucose is present but not in the presence of 2-deoxy-D-glucose unless high concentrations of glucose are added [59]. The same study showed that drugs that inhibit glycolysis or glucose transport also inhibit insulin-dependent leptin secretion and decrease the leptin mRNA content of the adipocytes. It has been proposed that the hexosamine pathway and its final product, UDP-N-acetylglucosamine, mediate leptin's response to glucose. Glucosamine, which enters the hexosamine pathway after the rate-limiting step, induces expression of leptin mRNA in 3T3-L1 preadipocytes and even L6 myocytes [60]. Infusions of glucose or a lipid emulsion for 3-h increased leptin mRNA and UDP-N-acetylglucosamine concentrations in fat and skeletal muscle, providing additional links between diet and leptin synthesis [60]. The hexosamine hypothesis is an exciting one but requires further studies in light of the finding that glucosamine also depletes cellular ATP concentrations [61].

Leptin's feedback regulation of insulin. A feedback loop possibly exists between leptin and insulin. In perfused rat pancreas, isolated mouse islets [62], and human islets [63], leptin inhibits glucose-induced insulin secretion. Inhibition in human islets, however, required 20 mmol/l glucose; no effect was observed with concentrations of glucose of 10 mmol/l or lower [63, 64]. As would be expected, leptin blocked insulin secretion by islets from *ob*/*ob* mice but not by *db*/*db* mice which lack the leptin receptor [65]. These effects may be mediated by the ATP-sensitive potassium channel [66, 67].

Leptin diurnal rhythm and pulsatility

Leptin's diurnal rhythm was first described in lean, obese, and obese Type II diabetic subjects who had 50–75 % changes in the amplitude of leptin concentrations with nadirs between 1100 and 2000 hours and peaks between 2400 and 0400 hours [68]. This general pattern has been confirmed in adolescent girls [69], young men [33] and lean [70] and obese [71] adults. Some reports describe earlier nadirs at 0900 hours [72, 73], although the overall pattern is similar (Fig. 1, curve A). The diurnal pattern is preserved in growth-hormone deficient hypopituitary adults [74] but reduced or absent in patients with anorexia nervosa [73, 75] and is absent in amenorrheic athletes [72].

In addition to its diurnal pattern, leptin is also secreted in pulses. In lean, obese and obese Type II diabetic subjects pulsatile secretions had means of approximately 3.5 ultradian oscillations/24 h [70, 76]. The relative amplitude had no apparent relation to obesity and more frequent sampling of an obese subset showed 2–5 oscillations/12 h [77].

It is not known whether the diurnal leptin rhythm has a function in weight control or energy balance. Leptin is also highest at night in rodents but unlike the human peak which occurs during the overnight fast, the peak in animals occurs when they are feeding [78]. Of interest in this regard is the finding that in lean suckling rats that were fed identical amounts of milk by an artificial rearing technique, recombinant leptin reduced weight gain by increasing energy expenditure, particularly in the morning when pups usually enter a torpor-like state [79]. The authors suggest that the torpor effect is normal and independent of leptin's effects on food intake. If the diurnal rhythm had a similar function in humans, it might be expected that an abbreviated leptin nadir would increase energy use.

The mechanism that underlies the control of leptin's diurnal rhythm is not known. The inherent rhythm is entrained by day/night reversal but when meals were shifted 6.5 h, leptin's peak and nadir also shifted 6–7 h [80]. In the same study cortisol shifted minimally, indicating both a lack of true circadian control and of regulation by glucocorticoids. In two studies which changed diet composition, the 24-h leptin pattern showed an abbreviated nadir and a premature rise, although the absolute peak values at 0200 hours were not affected (Fig. 1, curve B) [39, 44]. In one of these reports, lean women were fed iso-caloric meals that contained either 60 % carbohydrate and 20 % fat or 60 % fat and 20 % carbohydrate at 0900, 1300 and 1800 hours [39]. Each of the high carbohydrate meals was followed by large increases in serum concentrations of glucose and insulin, and leptin concentrations rose above the 0800 hours baseline by 1300, so that the 24-h area under the curve for leptin was 38 % higher. In the second study, lean men and women were examined in a cross-over design with four different diets, each consumed for 8 days [44]. The principal difference in the diets was the presence of either high-glycaemic or low-glycaemic index carbohydrate. In contrast to the first study, glucose and insulin values measured on the eighth day were virtually identical after meals for all four diets. For the diets that contained high-glycaemic index carbohydrates, however, leptin values began their diurnal increase prematurely at 1300 hours, and the leptin AUC was 17 % higher. These studies suggest three important conclusions: that insulin probably changes the timing of the 24-h leptin pattern, that one or more factors unrelated to insulin is also important in this regulation and that single fasting determinations of leptin are likely to be inadequate for nutritional studies.

Summary and future perspectives

Although leptin was first discussed as an "adipostat" that regulated food intake in accordance with triglyceride stores, it has become clear that leptin's role is much more complex and that a great many unanswered questions persist. Human studies have not shown that serum leptin concentrations can be changed rapidly by meals. Insulin seems to have a modest but immediate effect in attenuating the morning nadir of serum leptin as well as a greater effect manifested after 4–6 h. These changes in serum leptin, like the decreases that occur with fasting, are not accompanied by corresponding changes in adipose leptin mRNA, suggesting regulation by translational control or changes in the rate of leptin degradation, secretion or clearance. There is convincing evidence that insulin increases leptin synthesis and secretion, probably through an insulin-dependent effect on glucose metabolism. This effect of insulin is possibly mediated by the hexosamine pathway. What adipocytes seem to be communicating to the brain is not how much triglyceride they contain but whether they are currently synthesizing or hydrolysing triglyceride.

Confounding many studies is the problem of leptin's diurnal rhythm. Because many studies only measured leptin during its morning nadir or examined the effects of insulin or specific nutrients provided after an overnight fast, important information on regulation may have been lost.

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