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Role of adipose tissue in body-weight regulation: mechanisms regulating leptin production and energy balance

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Adipose tissue performs complex metabolic and endocrine functions. Among the endocrine products produced by adipose tissue are tumour necrosis factor α, interleukin 6, acylation-stimulating protein and leptin. The present review will focus primarily on mechanisms regulating leptin production and leptin action, and the implications of this regulation in the control of energy balance. Leptin acts in the central nervous system where it interacts with a number of hypothalamic neuropeptide systems to regulate feeding behaviour and energy expenditure. The presence of extreme obesity in animals and human subjects with mutations of the leptin gene or the leptin receptor demonstrates that normal leptin production and action are critical for maintaining energy balance. Insulin is the major regulator of leptin production by adipose tissue. Insulin infusions increase circulating leptin concentrations in human subjects. Plasma leptin levels are markedly decreased in insulin-deficient diabetic rodents, and the low leptin levels contribute to diabetic hyperphagia. Based on *in vitro* studies, the effect of insulin to stimulate leptin production appears to involve increased glucose metabolism. Blockade of glucose transport or glycolysis inhibits leptin expression and secretion in isolated adipocytes. Evidence suggests that anaerobic metabolism of glucose to lactate does not stimulate leptin production. Alterations in insulin-mediated glucose metabolism in adipose tissue are likely to mediate the effects of energy restriction to decrease, and refeeding to increase, circulating leptin levels. Changes in glucose metabolism may also explain the observation that high-fat meals lower 24 h circulating leptin levels relative to high-carbohydrate meals in human subjects, suggesting a mechanism that may contribute to the effects that high-fat diets have in promoting increased energy intake, weight gain and obesity. The decreased circulating leptin observed during energy restriction is related to increased sensations of hunger in human subjects. Thus, decreases in leptin during energy-restricted weight-loss regimens may contribute to the strong propensity for weight regain. A better understanding of the precise mechanisms regulating leptin production and leptin action may lead to new approaches for managing obesity.

Leptin: Adipose tissue: Obesity: Food intake: Energy expenditure: Glucose metabolism

Endocrine and metabolic functions of adipose tissue

Adipose tissue, once considered to be a relatively passive site of lipid storage is now known to carry out a number of complex metabolic and endocrine functions. For example, fatty acids released from adipose tissue contribute to the regulation of hepatic glucose production (Rebrin *et al*. 1995; Sindelar *et al*. 1997) and to changes in uncoupling protein 3 expression in skeletal muscle (Weigle *et al*. 1998*b*). The endocrine products produced by adipose tissue include cytokines, such as tumour necrosis factor α and interleukin 6, acylation-stimulating protein, and aromatized steroid hormones (for review, see Mohamed-Ali *et al*. 1998), as well as plasminogen activator inhibitor-1 (Wiman & Hamsten, 1990) and adiponectin (Funahashi *et al*. 1999), which are thought to have a role in the pathogenesis of atherosclerosis. These adipocyte-derived factors can have a number of significant metabolic effects. For example, tumour necrosis factor α has been implicated in the insulin resistance associated with obesity and type 2 diabetes (Hotamisligil, 1999), and has been shown to influence adipocyte glucose and lipid metabolism as well as to directly inhibit leptin expression and secretion in isolated adipocytes (Medina *et al*. 1999). Acylation-stimulating protein which is

Abbreviations: CNS, central nervous system.

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released from adipose tissue in the postprandial state appears to primarily exert paracrine and autocrine effects on adipocytes, in which it increases glucose transport and stimulates triacylglycerol synthesis (Cianflone *et al*. 1999). Among the known endocrine products produced by adipocytes, the strongest evidence exists for leptin to have a critical role in regulating energy balance via its actions on food intake and energy expenditure. Thus, the primary focus of the present review will be to discuss the mechanisms regulating leptin production and leptin action, and the implications for leptin production and action in the control of energy balance and body weight.

Evidence that body weight (adiposity) is regulated

Several lines of evidence have led to the idea that body weight and energy stored as body fat content are tightly regulated. First, in most adult human subjects and animals body adiposity remains relatively constant over prolonged periods of time, despite large short-term fluctuations in food intake. Although marked increases or decreases in body weight can be induced in human subjects or animals by forced overfeeding or energy restriction, body weight returns very close to preintervention levels when *ad libitum* feeding is resumed. Kennedy (1953) proposed that body weight is regulated over long periods of time by a factor produced by adipocytes, and that production of this factor is proportional to the triacylglycerol content of adipose tissue. In a series of elegant parabiosis experiments conducted by Coleman and colleagues (Coleman & Hummel, 1969; Coleman, 1973), it was discovered that a genetically-obese rodent model, the *ob/ob* mouse, failed to produce a humoral factor that inhibits food intake, whereas another obese mouse model, the *db/db* mouse, produces this factor but failed to respond to it. In non-obese rats forced overfeeding of one member of a parabiotic pair led to decreased voluntary food intake by its pair-mate (Harris & Martin, 1984), again suggesting a role for a humoral factor in the regulation of feeding. However, until quite recently, identification of the humoral signal of body adiposity and energy status remained elusive.

Discovery of leptin and effects on food intake

In an attempt to identify an adipose tissue factor regulating food intake, Wilson *et al*. (1990) utilized a subtraction cDNA cloning strategy to identify cDNA segments coding for RNA that is overexpressed in adipose tissue from chronically-overfed pig-tailed macaques (*Maccaca nemestrina*). A partial cDNA for a sequence with enhanced expression was isolated; however, the putative protein was not ultimately identified using this technique. In December 1994 a landmark paper from Jeffrey Friedman's laboratory (Zhang *et al*. 1994) was published in *Nature*, reporting the cloning of the gene which, when defective, leads to the obesity phenotype observed in the *ob/ob* mouse. The *ob* gene is expressed in adipose tissue and codes for a 16 kDa protein product that was given the name leptin, from the Greek word 'leptos' for thin. In June 1995 it was reported that administration of recombinant leptin to *ob/ob* mice reduced food intake and body weight in *ob/ob* and wild-type mice, but not in *db/db* mice (Campfield *et al*. 1995; Halaas *et al*. 1995; Pelleymounter *et al*. 1995), which were later shown to have a defect in the leptin receptor (Chen *et al*. 1996*a*). The leptin receptor is expressed in several regions of the central nervous system (CNS), including the hypothalamus (Tartaglia *et al*. 1995), as well as in a number of peripheral tissues, and has been shown to signal via a JAK-STAT second messenger transduction pathway common to other cytokine receptors (Tartaglia, 1997). The efficacy of leptin to inhibit food intake when administered into the CNS of rodents (Campfield *et al*. 1995) and non-human primates (Tang-Christensen *et al*. 1999*a*) at doses which are ineffective when given peripherally, demonstrates that the brain is an important site of leptin's actions to regulate energy balance. The hypothalamus is considered to be the primary central location where leptin acts to inhibit feeding (Jacob *et al*. 1997; Satoh *et al*. 1997; Tang-Christensen *et al*. 1999*b*). However, leptin receptors are present in brain areas outside the hypothalamus (Schwartz *et al*. 1996*b*), and direct administration of leptin into at least one other brain area, the prepiriform cortex, inhibits food intake in rats (Blevins *et al*. 1999). A large body of work is emerging that is defining the central mechanisms by which leptin exerts its actions on food intake and energy expenditure. These effects of leptin are thought to be largely mediated by hypothalamic neuropeptide systems regulating energy balance (Woods *et al*. 1998; Schwartz *et al*. 1999). Thus, leptin, along with insulin which also has direct actions in the CNS to regulate food intake and energy expenditure (Schwartz *et al*. 1994; Woods *et al*. 1996), functions as a negative feedback signal to the CNS to regulate energy balance (Fig. 1). It should be noted that leptin and insulin act as medium- to long-term regulators of energy balance, and not as short-term satiety signals. In fact, short-term satiety signals such as cholecystokinin regulate the amount of food consumed in a single meal, but are not by themselves sufficient to alter long-term energy intake and body weight (West *et al*. 1984). Rather, it appears more likely that the short-term and long-term signals interact in an integrated manner to regulate energy intake and expenditure such that energy balance is achieved. For example, leptin has been shown to increase the sensitivity to the satiety-producing effects of exogenous cholecystokinin (Matson *et al*. 1997; Emond *et al*. 1999). The possibility that there may be other, as yet unidentified, factors produced by adipose tissue (Weigle *et al*. 1998*a*) that are involved in the regulation of energy balance is also worthy of consideration.

Leptin and energy expenditure

In addition to its well-characterized effect to inhibit food intake, there is also evidence that leptin can regulate energy balance by influencing energy expenditure. Early studies reported that leptin administration in *ob/ob* mice increased body temperature and physical activity (Pelleymounter *et al*. 1995). Studies performed with groups of animals that were pair-fed to the leptin-treated animals showed that decreases in body weight induced by chronic leptin administration were larger than could be explained by the reduction of food intake alone (Levin *et al*. 1996). It appears that the role of

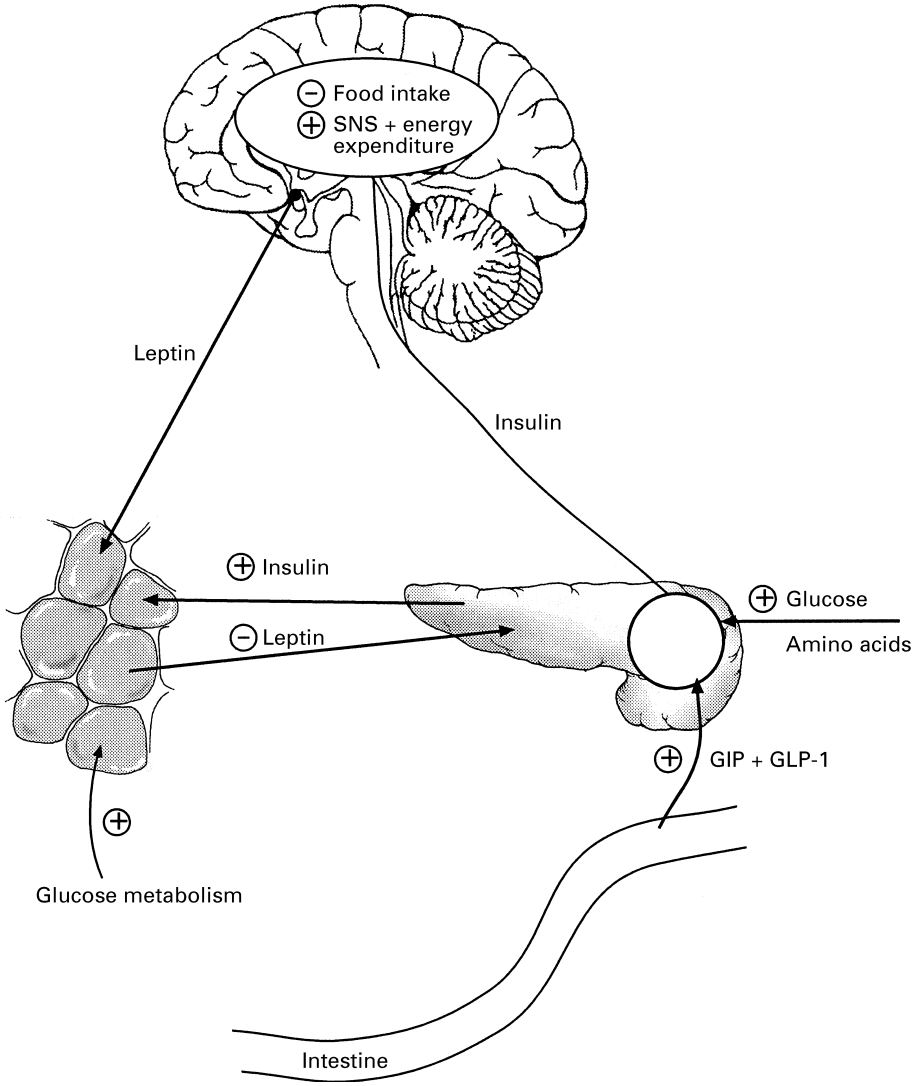

Fig. 1. Long-term signals regulating energy balance. Insulin and leptin are the two hormones that act as long-term regulators of food intake and energy balance. Both insulin and leptin act in the central nervous system to inhibit food intake and to increase energy expenditure. Activation of the sympathetic nervous system (SNS) is likely to contribute to the increase in energy expenditure. Insulin is secreted from β-cells in the endocrine pancreas in response to circulating nutrients (glucose and amino acids) and to the incretin hormones, glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) which are released during meal ingestion and absorption. Insulin stimulates leptin production from adipose tissue, most probably by increasing glucose uptake and metabolism. There is also evidence that leptin can act to inhibit insulin secretion. (Reproduced with permission from Havel *et al.* 2000.)

leptin in regulating energy expenditure may be more to prevent the fall in energy expenditure associated with energy restriction rather than to induce an increase above basal rates of energy expenditure (Scarpace *et al*. 1997; Doring *et al*. 1998). Further results from studies in rodents suggest that the effects of leptin on energy expenditure are mediated by activation of sympathetic nerves innervating thermogenically-active brown adipose tissue (Scarpace & Matheny, 1998), which results in the dissipation of energy as heat. Thus, the sympathetic nervous system appears to be involved in the effects of leptin to regulate energy expenditure. Accordingly, leptin administration increases the firing rate of sympathetic nerves innervating several tissues in rodents (Haynes *et al*. 1997) and increases circulating noradrenaline concentrations in non-human primates (Tang-Christensen *et al*. 1999*a*). Furthermore, the metabolic effects of leptin in increasing circulating glucose and lactate levels in monkeys can be blocked by the administration of adrenergic receptor antagonists (Havel & Pelleymounter, 1997). In addition, leptin attenuates decreases in glucose, insulin and glucagon during fasting in mice, and this effect is prevented by sympathectomy with 6-hydroxydopamine (Ahren & Havel, 1999*a*). Together these results provide evidence that sympathetic mechanisms are involved in mediating some of the metabolic effects of leptin.

Relationship of circulating leptin to adiposity and gender differences

Numerous studies have reported that circulating leptin concentrations are highly correlated with indices of

adiposity, such as BMI, percentage body fat and total fat mass in human subjects (Maffei *et al*. 1995; Considine *et al*. 1996*b*; Havel *et al*. 1996*c*), and in animals (Maffei *et al*. 1995; Ahren *et al*. 1997). The presence of high plasma leptin concentrations in most obese subjects has been interpreted to suggest that human obesity is most often associated with resistance to the actions of leptin (Caro *et al*. 1996*b*). Circulating leptin levels are higher in women than in men, even after correcting for the greater extent of adiposity in women (Havel *et al*. 1996*b*; Rosenbaum *et al*. 1996). In addition, in a study utilizing frequent blood sampling and pulse analysis, it was reported that the amplitude of leptin pulses is larger in women than in men (Saad *et al*. 1998*b*). Absolute and adiposity-corrected leptin levels are similar in pre- and post-menopausal women and in post-menopausal women who are either receiving or not receiving hormone-replacement therapy (Havel *et al*. 1996*b*), indicating that it is unlikely that the gender difference is due to an effect of female reproductive hormones. It is possible that the gender difference is a result of an inhibitory effect of androgens and/or differences in body fat distribution between men and women. The gender difference is reversed in rats, with male rats having higher leptin concentrations than female rats (Landt *et al*. 1998). This difference is likely to be due to the greater amount of body fat in male rats (Havel *et al*. 1996*a*).

Regulation of leptin production

As previously discussed, circulating leptin concentrations are closely related to adipose tissue mass in both human subjects (Maffei *et al*. 1995; Havel *et al*. 1996*c*) and animals (Maffei *et al*. 1995; Ahren *et al*. 1997). However, adipose tissue mass is not the only determinant of circulating leptin concentrations. Recent energy intake also has a major influence on plasma leptin levels. Plasma leptin decreases acutely during fasting (Boden *et al*. 1996; Weigle *et al*. 1997) or energy restriction (Dubuc *et al*. 1998; Keim *et al*. 1998; Wisse *et al*. 1999), whereas refeeding (Kolaczynski *et al*. 1996*a*; Weigle *et al*. 1997) and overfeeding (Kolaczynski *et al*. 1996*c*) acutely increase leptin. These changes are disproportionate to the relatively small changes in body fat induced by these short-term interventions (Dubuc *et al*. 1998; Wisse *et al*. 1999). Like leptin, insulin secretion is also decreased by fasting and energy restriction, and is increased during refeeding. The increased insulin secretion is mediated by stimulatory effects on the β-cell of ingested glucose and amino acids, and insulinotropic gastrointestinal hormones (Fig. 1). Since insulin responses to energy intake precede changes in circulating leptin, insulin is a good candidate hormone to act as a regulator of changes in leptin secretion resulting from alterations in energy intake.

A number of early experiments showed that insulin increases *ob* gene expression and leptin secretion *in vitro* (Hardie *et al*. 1996*a*; Leroy *et al*. 1996; Rentsch & Chiesi, 1996; Wabitsch *et al*. 1996) and *in vivo* (Cusin *et al*. 1995; Saladin *et al*. 1995; Hardie *et al*. 1996*b*). Infusions of insulin in human subjects at rates producing supraphysiological (Malmstrom *et al*. 1996; Utriainen *et al*. 1996) or physiological (Saad *et al*. 1998*a*) increases in circulating insulin concentrations result in an increase in circulating leptin concentrations. This increase in leptin is detectable approximately 4 h after the start of the insulin infusions, suggesting that these effects may be mediated at the level of transcription and translation. This time-course is likely to explain why changes in circulating leptin were not seen during more short-term insulin infusions (Dagogo-Jack *et al*. 1996; Kolaczynski *et al*. 1996*b*). Glucose infusions which increase endogenous insulin secretion have also been shown to increase plasma leptin in human subjects (Sonnenberg *et al*. 1996; Grinspoon *et al*. 1997) and in non-human primates (Havel, 1997). Furthermore, *ob* gene expression and circulating leptin levels are decreased in rodents with insulin-deficient diabetes (Havel *et al*. 1998; Sivitz *et al*. 1998), and the low levels are restored by administration of insulin in proportion to the extent of glucose lowering (Havel *et al*. 1998). Infusion of small amounts of glucose, sufficient to prevent the decline of glycaemia and insulinaemia during fasting in human subjects prevents the decrease in plasma leptin (Boden *et al*. 1996). In addition, decreases in circulating leptin during periods of energy restriction in human subjects are related to the decreases in plasma glucose (Dubuc *et al*. 1998; Keim *et al*. 1998).

Together, these results suggest that the effects of insulin which increase leptin production could be mediated through increased glucose utilization by adipocytes. Results from *in vitro* experiments have provided evidence for this hypothesis. Inhibition of glucose transport with 2-deoxy-D-glucose (Fig. 2A) or phloretin, or glycolysis with NaF (Fig. 2B) or iodoacetate, reduces leptin secretion in proportion to the reduction in glucose utilization in isolated rat adipocytes (Mueller *et al*. 1998). Both 2-deoxy-D-glucose and NaF inhibited insulin-mediated *ob* gene expression in isolated adipocytes (Fig. 2C). The reductions in *ob* gene expression and leptin secretion are observed even in the presence of high concentrations of insulin, suggesting that glucose utilization, rather than a direct effect of insulin *per se*, is an important determinant of insulin-mediated production. Thus, shifts in adipose tissue glucose metabolism resulting from changes of insulin secretion and plasma glucose levels are likely to be involved in the effects of fasting and refeeding on circulating leptin concentrations *in vivo* (Havel, 1998, 1999). Glucose transport alone does not appear to be the regulatory step by which insulin-mediated glucose metabolism stimulates leptin production. The uptake of glucose does not increase leptin secretion if the glucose is metabolized anaerobically and released as lactate (Mueller *et al*. 1998). Accordingly, leptin secretion is inversely related to the proportion of glucose metabolized to lactate (Fig. 2D). Thus, it appears that glucose must be metabolized beyond pyruvate, to a metabolic fate other than lactate, in order to increase leptin production. One study (Wang *et al*. 1998) has suggested that the flux of glucose into the hexosamine biosynthetic pathway (Fig. 3) is involved in stimulating leptin production; however, other metabolic fates of glucose in adipocytes such as *de novo* lipogenesis and/or glucose oxidation may also be involved (Fig. 3).

Input from the sympathetic nervous system is considered to have an inhibitory influence on leptin production (Hardie *et al*. 1996*a*; Trayhurn *et al*. 1998). Although

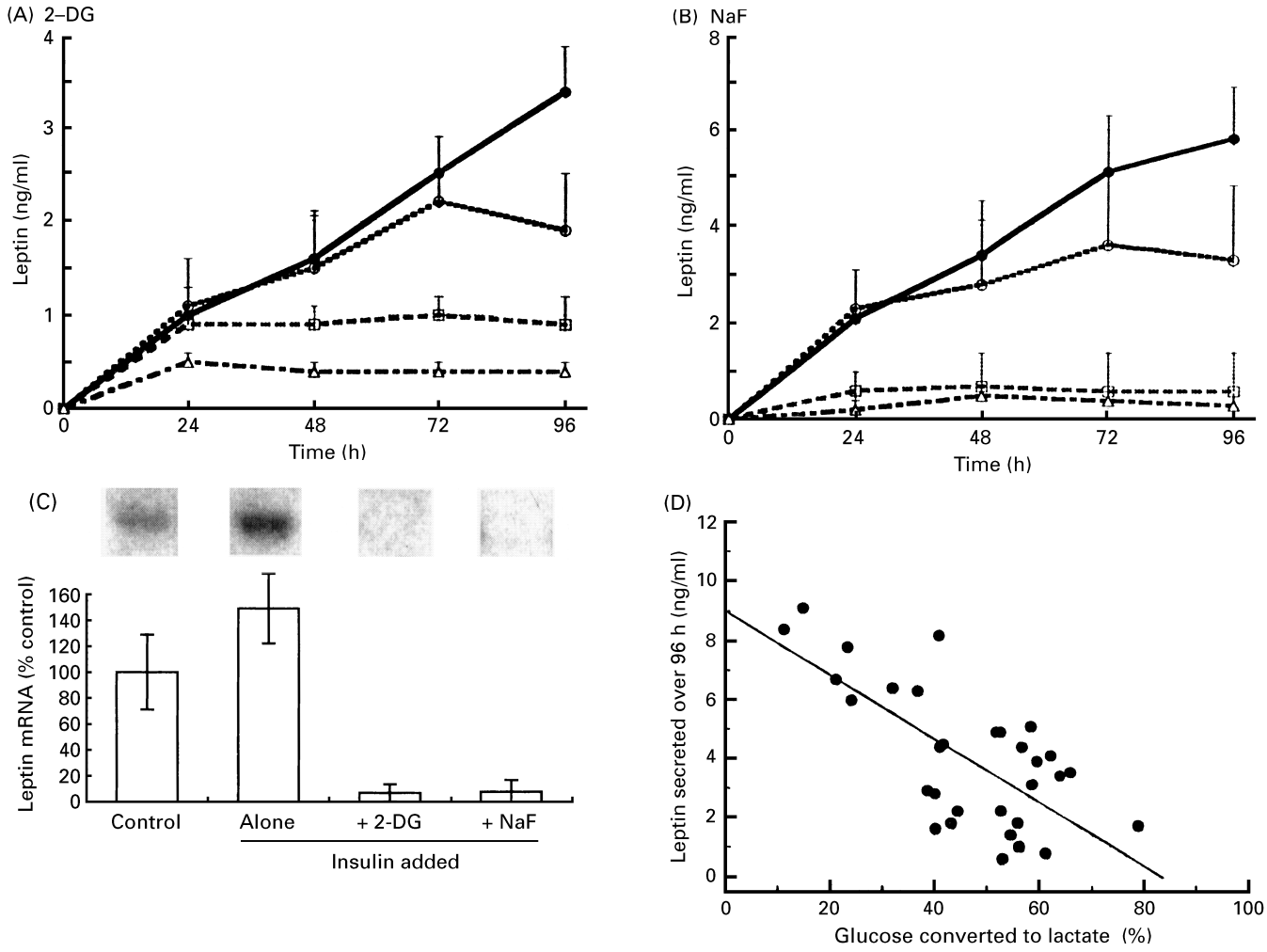

Fig. 2. Effects of inhibiting glucose transport and metabolism with 2-deoxy-D-glucose (2-DG; insulin alone (●▒●; *n* 13), insulin + 2-DG (mg/l; 2 (○▒○; *n* 8), 10 (□––□; *n* 12), 50 (△––△; *n* 10); A) or glycolysis with NaF (insulin alone (●▒●; *n* 6), insulin + NaF (mM; 0·5 (○▒○; *n* 3), 1·0 (□––□; *n* 6), 5·0 (△––△; *n* 6); B) on leptin concentrations from 0 to 96 h in media from isolated rat adipocytes incubated in primary culture with 1·6 nM-insulin. (C) Effects of 1·6 nM-insulin and 1·6 nM-insulin + 100 mg 2-DG/l or 1·0 mM-NaF on leptin (*ob*) mRNA after 48 h of incubation, as assessed by Northern blots. For A-C values are means with their standard errors represented by vertical bars. (D) Relationship between the proportion of glucose converted to lactate and leptin secretion over 96 h during incubation of adipocytes with 0·16 nM-insulin (*n* 32; *r* –0·73; $P < 0{\cdot}001$). (Reproduced with permission from Mueller *et al.* 1998.)

glucocorticoids have been reported to stimulate leptin production in some studies (Hardie *et al.* 1996*a*; Rentsch & Chiesi, 1996), it would seem unlikely that endogenous glucocorticoids would have a physiological role in increasing leptin production, since in conditions in which glucocorticoid levels are increased, e.g. fasting and uncontrolled diabetes, leptin production and circulating leptin concentrations are decreased (Ahren *et al.* 1997; Weigle *et al.* 1997; Dubuc *et al.* 1998; Havel *et al.* 1998).

Diurnal pattern of circulating leptin and effects of macronutrients

Circulating leptin concentrations are not constant over the course of 1 d, and in human subjects exhibit a diurnal pattern with a nocturnal peak that typically occurs after midnight (Sinha *et al.* 1996). This nocturnal peak is not due to a true circadian rhythm, since it does not occur when subjects are fasted (Boden *et al.* 1996) and is related to meal-induced insulin secretion (Laughlin & Yen, 1997; Saad *et al.* 1998*b*) The diurnal pattern can be entrained to meal timing such that shifting the time at which meals are consumed forward by 5–6 h delays the timing of the nocturnal peak by a similar amount of time (Schoeller *et al.* 1997). Some studies which examined fasting morning leptin concentrations did not find an effect on circulating leptin levels of altering the macronutrient content of the diet with respect to the fat : carbohydrate ratio (Havel *et al.* 1996*b*; Weigle *et al.* 1997). However, in a more recent study it was reported that human subjects consuming low-fat high-carbohydrate meals, which induce large variations in insulin (Fig. 4A) and glucose (Fig. 4B), produced postprandial increases in leptin (Fig. 4C) occurring 4–6 h after meals and a 24 h leptin profile that was 40 % greater than when the same subjects

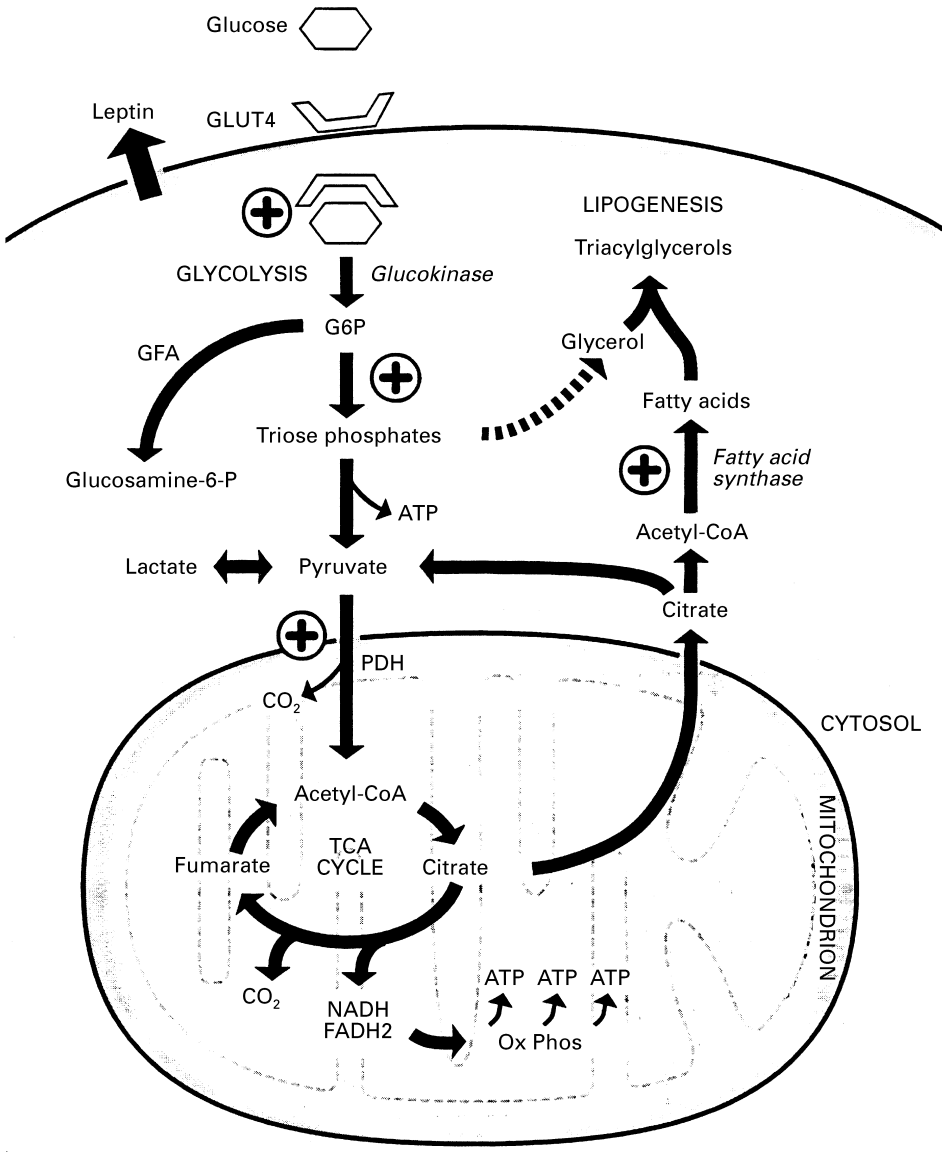

Fig. 3. Insulin-mediated glucose metabolism in adipocytes: insulin stimulates glucose uptake by increasing glucose transporter (GLUT4) translocation to the adipocyte membrane. Insulin also stimulates glycolysis, and via its actions on pyruvate dehydrogenase (PDH) kinase to activate PDH, increases glucose oxidation, and thereby reduces the proportion of glucose-C that is converted to lactate. Insulin stimulates lipogenesis and increases the incorporation of glucose-C from glycerol and fatty acids into triacylglycerol. Blockade of glucose uptake and phosphorylation by glucokinase by 2-deoxy-D-glucose, or inhibition of glycolysis with NaF, inhibit insulin-stimulated leptin expression and secretion (Mueller *et al.* 1998). Anaerobic metabolism of glucose to lactate does not stimulate leptin secretion. The effects of insulin to increase leptin production may involve stimulation of glucose entry into the hexosamine (glucosamine-6-phosphate; glucosamine-6-P) biosynthetic pathway, lipogenesis, or oxidative metabolism in the TCA cycle. G6P, glucose-6-phosphate; GFA, glutamine: fructose-6-phosphate amidotransferase Ox Phos, oxidative phosphorylation; ⊕, stimulation by insulin.

consumed high-fat low-carbohydrate meals (Havel *et al.* 1999). Thus, leptin is acutely regulated by the macronutrient content of meals in proportion to their ability to stimulate insulin secretion. Consequently, energy consumed as fat which does not directly stimulate insulin secretion, and therefore does not indirectly stimulate leptin production, does not signal the CNS via either of these long-term hormonal regulators of energy balance (Fig. 1). Decreased leptin production may contribute to the effects of high-fat diets in promoting increased energy intake, weight gain and obesity in human subjects and animals (Hill *et al.* 1992; Horton *et al.* 1995; Tataranni & Ravussin, 1997; Tremblay *et al.* 1998; Bray & Popkin, 1999). There is evidence that the regulated level of adiposity is influenced by the macronutrient content of the diet. For example, in a weight clamp study, subjects needed to be fed an average of 502 (SE 126) kJ (120 (SE 30) kcal)/d more in order to maintain their body weight when the percentage of energy provided as fat was reduced from approximately 30 % to approximately 15 % (Havel *et al.* 1996*c*). It is possible that augmentation of 24 h leptin production induced by carbohydrate ingestion may have increased energy requirements for weight maintenance by increasing energy expenditure.

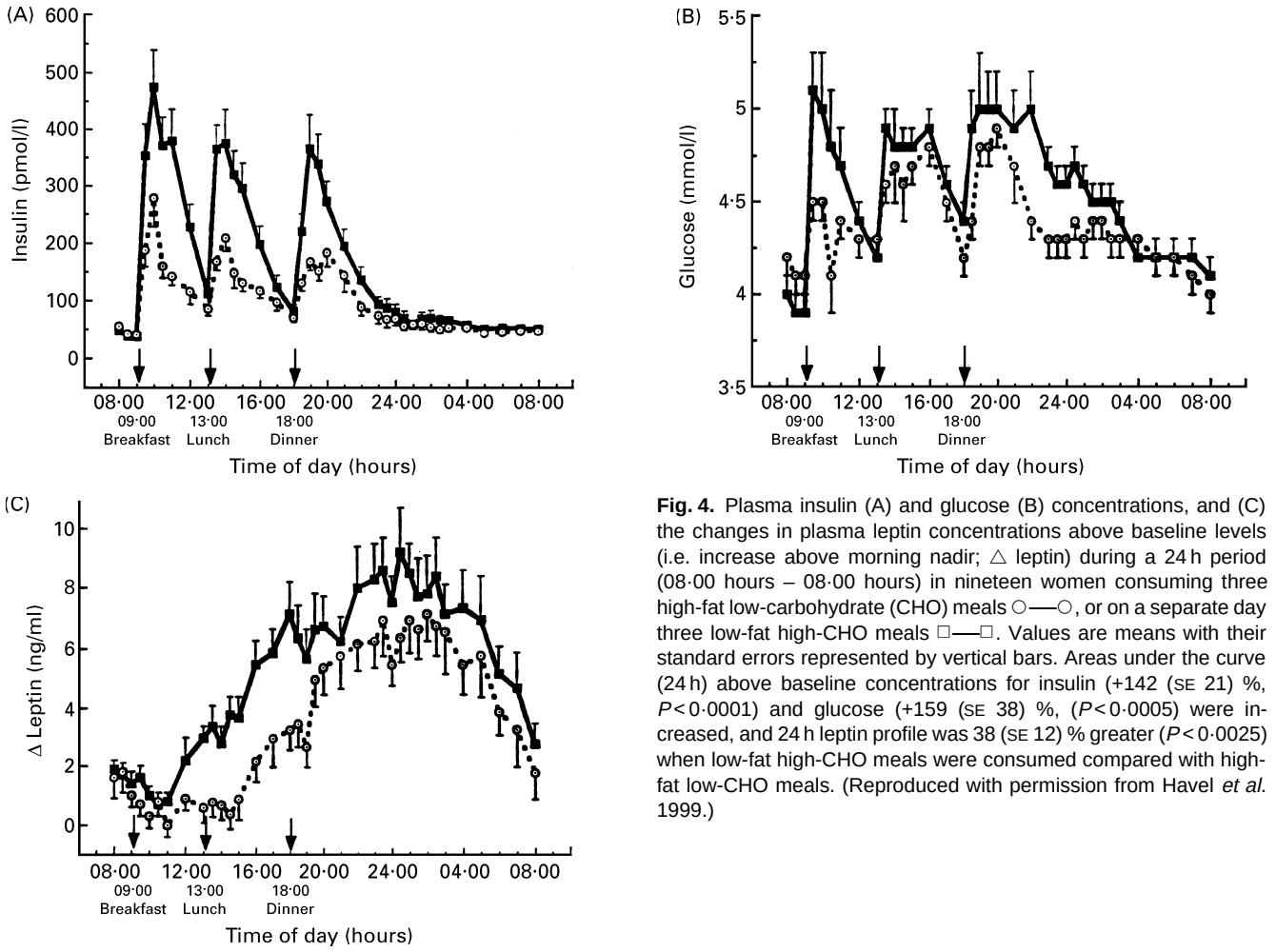

Fig. 4. Plasma insulin (A) and glucose (B) concentrations, and (C) the changes in plasma leptin concentrations above baseline levels (i.e. increase above morning nadir; △ leptin) during a 24 h period (08·00 hours – 08·00 hours) in nineteen women consuming three high-fat low-carbohydrate (CHO) meals ○—○, or on a separate day three low-fat high-CHO meals □—□. Values are means with their standard errors represented by vertical bars. Areas under the curve (24 h) above baseline concentrations for insulin (+142 (SE 21) %, $P < 0{\cdot}0001$) and glucose (+159 (SE 38) %, ($P < 0{\cdot}0005$) were increased, and 24 h leptin profile was 38 (SE 12) % greater ($P < 0{\cdot}0025$) when low-fat high-CHO meals were consumed compared with high-fat low-CHO meals. (Reproduced with permission from Havel *et al.* 1999.)

Consequences of decreased leptin production

The importance of normal leptin production and signalling in regulating energy balance is clearly demonstrated by the hyperphagia, reduced energy expenditure and marked obesity that accompanies genetic leptin deficiency in *ob/ob* mice, or leptin receptor defects in *db/db* mice or fatty Zucker rats. Normal leptin production and action are also critical for the long-term regulation of energy balance in humans subjects, as was convincingly demonstrated by the hyperphagia and obesity in human subjects with mutations in the genes encoding leptin (Montague *et al.* 1997; Strobel *et al.* 1998) or the leptin receptor (Clement *et al.* 1998). Relative deficiency of leptin has been suggested to predict future weight gain in human subjects (Ravussin *et al.* 1996; Matkovic *et al.* 1997) and rodents (Surwit *et al.* 1997; Ahren, 1999); however, such a predictive value has not been observed in all populations examined (Chessler *et al.* 1998; Haffner *et al.* 1998). Ahima *et al.* (1996) reported that leptin administration in mice prevented the decreases in reproductive and thyroid hormones and the activation of the hypothalamic–pituitary–adrenal axis observed in response to fasting. Thus, low leptin levels appear to be involved in the overall neuroendocrine adaptive response to decreased energy availability. Furthermore, leptin administration also prevents the decrease in plasma glucose, insulin and glucagon levels observed in response to fasting in mice (Ahren & Havel, 1999*a*), suggesting that the decrease in leptin is also involved in the metabolic adaptation to restricted energy intake. Less-marked leptin deficiency also has consequences with regard to energy intake. Leptin replacement using osmotic minipumps at a very low rate of delivery, in order to prevent the decrease in leptin after the induction by streptozotocin of diabetes in rats, also prevents the increase in food intake that was observed in untreated animals (Sindelar *et al.* 1999). These results provide evidence that low leptin levels mediate the hyperphagia long known to be a characteristic of insulin-deficient diabetes mellitus.

Decreases in circulating leptin during a prolonged moderate energy deficit are correlated with increased sensation of hunger in women, and this relationship was independent of the changes in body fat content or the extent of reduction in energy intake (Keim *et al.* 1998), further suggesting that leptin also has a role in the regulation of appetite in human subjects. Thus, lowered levels of circulating leptin are likely to function as a signal to the CNS of low energy intake as well as of decreased energy

stores in adipose tissue. The acute reduction in leptin production in response to decreased energy intake before significant decreases in body fat content is likely to have an important adaptive value, in that it would promote compensatory corrections of energy intake and/or expenditure before there are major deviations in body energy stores. This idea is supported by demonstration that leptin replacement prevents the decline in energy expenditure associated with acute fasting in rodents (Scarpace *et al*. 1997; Doring *et al*. 1998).

Leptin in the management of obesity

A salient question is: what is the therapeutic potential of leptin and the leptin system in treating obesity? In one young leptin-deficient individual daily subcutaneous administration of exogenous leptin has reduced food intake and reversed an almost exponential rate of weight gain into a substantial (approximately 15 kg) extent of weight loss after 9 months (Greenberg *et al*. 1999; see also Farooqi *et al*. 1999). Subcutaneous administration of recombinant methionyl human leptin for 24 weeks has been reported to induce a significant ($P < 0{\cdot}02$), but variable, degree of weight loss (–0·7 to –7·1 kg) in normal-weight and obese human subjects in a double-blind placebo-controlled trial (Greenberg *et al*. 1999; Heymsfield *et al*. 1999). The weight loss was primarily due to reductions in body fat mass. The variability in the extent of the weight loss suggests that there are unknown factors which influence the effectiveness of exogenous leptin treatment. Based on the observation that the majority of obese subjects have high circulating leptin levels, it has been hypothesized that obese subjects are resistant to the actions of leptin which normally promote a state of negative energy balance.

One possibility is that the baseline leptin level at the time of treatment may influence sensitivity to exogenous leptin. It seems plausible that the brain would be more sensitive to decreases in circulating leptin than to increases above the levels to which it is normally exposed. If this is the case, leptin may be relatively more effective in subjects in whom endogenous leptin production has first been decreased by dieting. Significant weight loss can be induced in most obese individuals with an energy-restricted diet and exercise. However, the rate of successfully maintaining weight loss is poor at best. Decreases in leptin secondary to reduced adiposity and energy intake during energy-restricted weight-loss regimens may contribute to the strong propensity for weight regain via increased appetite (hunger) (Keim *et al*. 1998) and decreased energy expenditure. Thus, leptin, leptin agonists or leptin secretagogues could potentially help maintain weight loss after successful dieting by decreasing hunger and subsequent food intake, and preventing or reversing the decrease in energy expenditure known to occur during restricted energy intake (Doring *et al*. 1998; Wisse *et al*. 1999).

While profound defects in the leptin receptor are associated with massive obesity in a few individuals (Clement *et al*. 1998), several studies have failed to associate more subtle leptin receptor polymorphisms with an obesity phenotype in human subjects (Considine *et al*. 1996*a*; Echwald *et al*. 1997; Matsuoka *et al*. 1997). It is possible that post-receptor defects in the leptin signal transduction pathway, or a failure of leptin to fully act on its hypothalamic targets such as neuropeptide Y and melanocortin neurons, or other neuropeptide systems involved in regulating energy balance (Woods *et al*. 1998; Schwartz *et al*. 1999), could result in an apparent resistance to leptin. It has been reported that rodents with diet-induced obesity (Halaas *et al*. 1997; Van Heek *et al*. 1997) and polygenic obesity (Halaas *et al*. 1997) reduce their food intake in response to the administration of leptin into the CNS, but not to peripheral injection of leptin. These results suggest that under some conditions the ability of leptin to reach its targets in the CNS may be impaired. Accordingly, it has been reported that the cerebrospinal fluid: plasma leptin ratio is reduced in obese subjects (Schwartz *et al*. 1996*a*; Caro *et al*. 1996*a*) and that increases in cerebrospinal-fluid leptin levels after leptin administration in human subjects are smaller than would be predicted by the increase in peripheral circulating leptin concentrations (Fujioka *et al*. 1999; Greenberg *et al*. 1999).

An additional possibility is that the leptin signal to the brain can be overcome by the availability of highly-palatable foods. For example, in rats with diet-induced obesity central administration of leptin reduced the consumption of the normal rodent diet, but not consumption of a high-energy high-sucrose diet (Widdowson *et al*. 1997). This observation is likely to be relevant to the aetiology of obesity in human subjects, since palatable high-fat high-energy foods contribute, along with inactivity, to obesity in individuals consuming Western diets (Tataranni & Ravussin, 1997; Tremblay *et al*. 1998; Bray & Popkin, 1998). It is possible from an evolutionary point of view that the ability to override the leptin signal at times when food supplies are readily available would have an adaptive value, in that excess energy could be more readily stored as fat. Nonetheless, the leptin system remains an attractive target for obesity treatment. New strategies which enhance leptin action or leptin transport into the brain may be required to fully realize the clinical potential of this approach for treating obesity.

Other actions of leptin

Leptin has a number of effects other than its central actions causing reduced food intake and increased energy expenditure. There are leptin receptors in many peripheral tissues (for review, see Tartaglia, 1997), including the liver, kidney, adipose tissue, ovary and gastrointestinal tract. Leptin appears to have peripheral actions on fuel metabolism and substrate flux (Barzilai *et al*. 1997; Rossetti *et al*. 1997). These actions may have profound long-term effects, as suggested by studies which showed that 2 weeks of hyperleptinaemia after leptin gene transfection (Chen *et al*. 1996*b*) or during leptin infusion from osmotic minipumps (Barzilai *et al*. 1997) led to a marked loss of body fat in rats, whereas pair-fed animals exhibited much more modest reductions of body fat.

Leptin is also involved in regulating reproductive function (for review, see Cunningham *et al*. 1999), since *ob/ob* mice lacking leptin are infertile, but fertility is restored by leptin treatment (Chehab *et al*. 1996). Obese

human patients with leptin deficiency exhibit hypogonadism (Strobel *et al*. 1998). Furthermore, leptin administration has been shown to accelerate the onset of puberty in rodents (Barash *et al*. 1996; Chehab *et al*. 1997; Cheung *et al*. 1997). It has been proposed that leptin acts as a general signal of low energy status to the neuroendocrine axes; leptin administration reverses the changes in levels of thyrotropin, adrenocorticotrophic hormone, and gonadotropins caused by fasting in mice (Ahima *et al*. 1996). In agreement with this idea, human subjects with leptin receptor defects are not only obese, but have impaired growth hormone and thyrotropin secretion (Clement *et al*. 1998). It is possible that low leptin levels, resulting from very low amounts of body fat and decreased food intake, may contribute to amenorrhoea in women athletes (Laughlin & Yen 1997) or anorexic patients (Kopp *et al*. 1998). Other potential functions of leptin include direct inhibitory effects on insulin secretion (Kieffer *et al*. 1997; Emilsson *et al*. 1997; Ahren & Havel, 1999*b*), actions affecting adrenal function (Bornstein *et al*. 1997; Cao *et al*. 1997), angiogenesis (Bouloumie *et al*. 1998; Sierra-Honigmann *et al*. 1998), haematopoiesis (Gainsford *et al*. 1996), pulmonary function (O'donnell *et al*. 1999) and immune function (Loffreda *et al*. 1998; Lord *et al*. 1998).

Summary and conclusions

Adipose tissue produces a number of endocrine products, including tumour necrosis factor α, acylation-stimulating protein and leptin. Normal production of leptin and leptin action are critical for the long-term regulation of energy balance in animals and human subjects. Circulating leptin concentrations decrease acutely during fasting or energy restriction, and the decreases are proportionally much larger than changes in body adiposity. Leptin production is regulated by insulin responses to meals, and therefore by dietary macronutrient composition. The effects of insulin which stimulate leptin production are likely to involve changes in adipocyte carbohydrate metabolism. Decreases in leptin production contribute to increased hunger and decreased energy expenditure, as well as to hyperphagia in insulin-deficient diabetes. A better understanding of the precise mechanisms regulating leptin production and action is likely to lead new approaches for managing obesity. For example, significant weight loss can usually be achieved in obese patients with energy-restricted diets and exercise; however, the success rate of maintaining weight loss is poor at best. Preventing the decline in circulating leptin during an energy deficit by providing exogenous leptin, a leptin agonist or a leptin secretagogue may attenuate the increased hunger and decreased energy expenditure and help to maintain weight loss.

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