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

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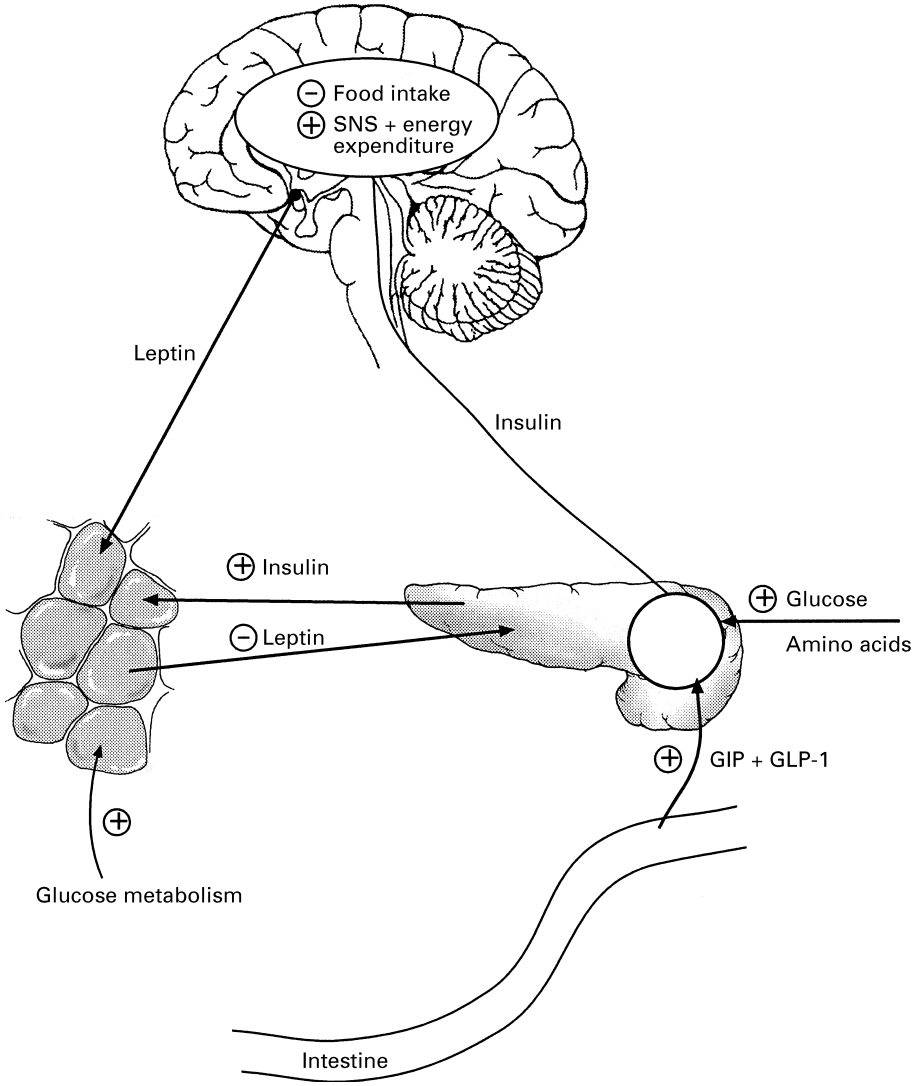

Fig. 1. Long-term signals regulating energy balance. Insulin and leptin are the two hormones that act as long-term regulators of food intake and energy balance. Both insulin and leptin act in the central nervous system to inhibit food intake and to increase energy expenditure. Activation of the sympathetic 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

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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

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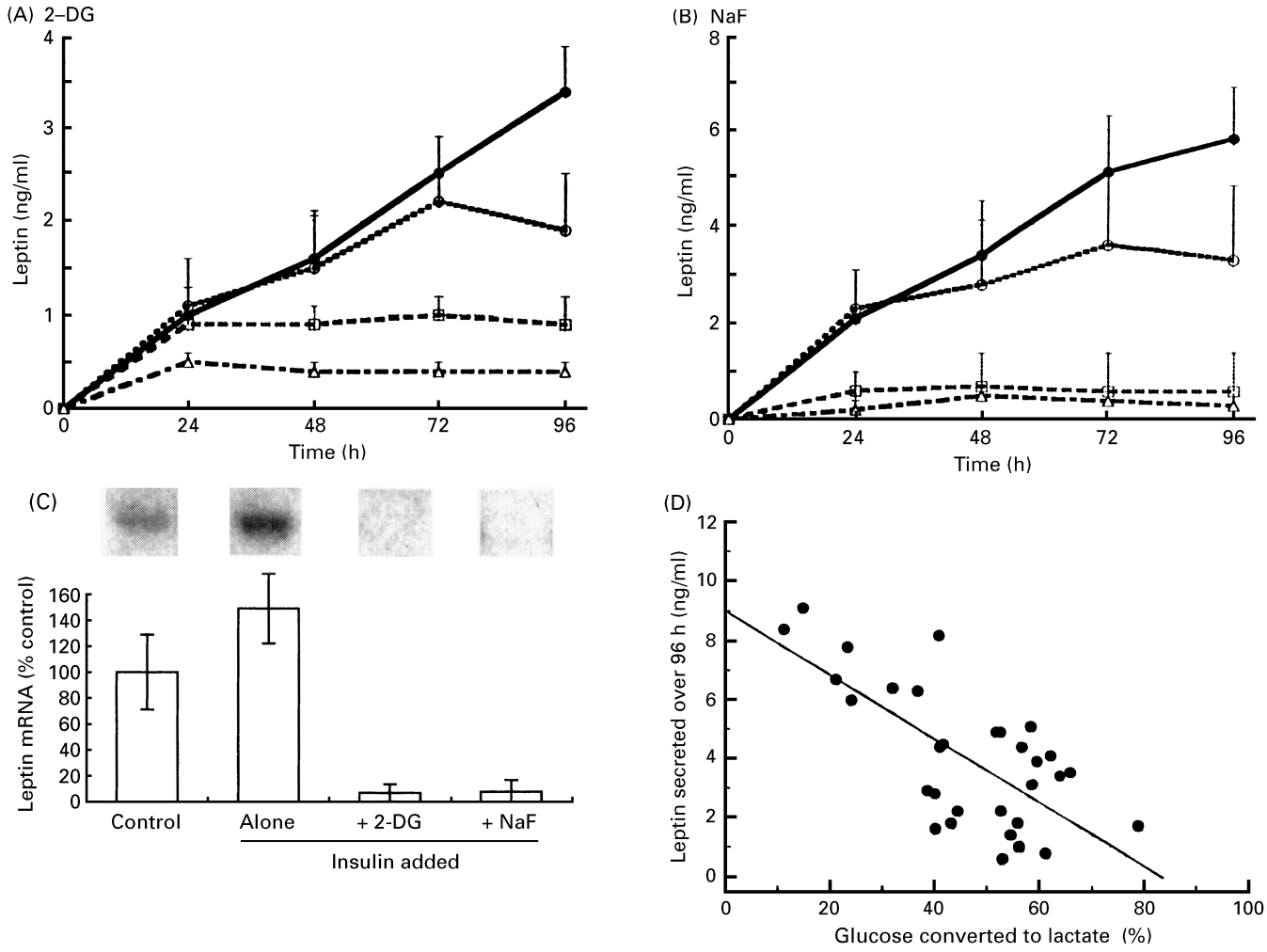

Fig. 2. Effects of inhibiting glucose transport and metabolism with 2-deoxy-D-glucose (2-DG; insulin alone (●▒●; *n* 13), insulin + 2-DG (mg/l; 2 (○▒○; *n* 8), 10 (□––□; *n* 12), 50 (△––△; *n* 10); A) or glycolysis with NaF (insulin alone (●▒●; *n* 6), insulin + NaF (mM; 0·5 (○▒○; *n* 3), 1·0 (□––□; *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

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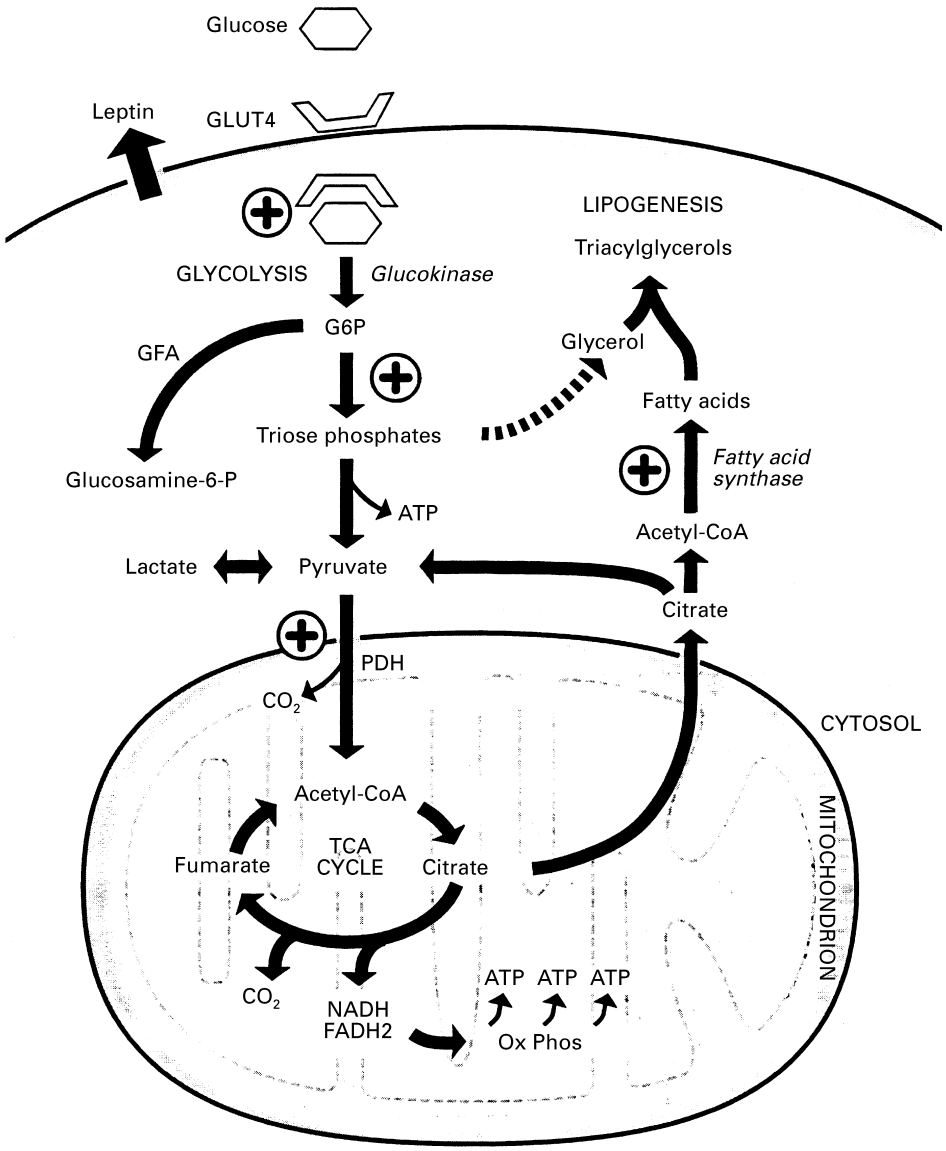

Fig. 3. Insulin-mediated glucose metabolism in adipocytes: insulin stimulates glucose uptake by increasing glucose transporter (GLUT4) translocation to the adipocyte membrane. Insulin also stimulates glycolysis, and via its actions on pyruvate dehydrogenase (PDH) kinase to activate PDH, increases glucose 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.

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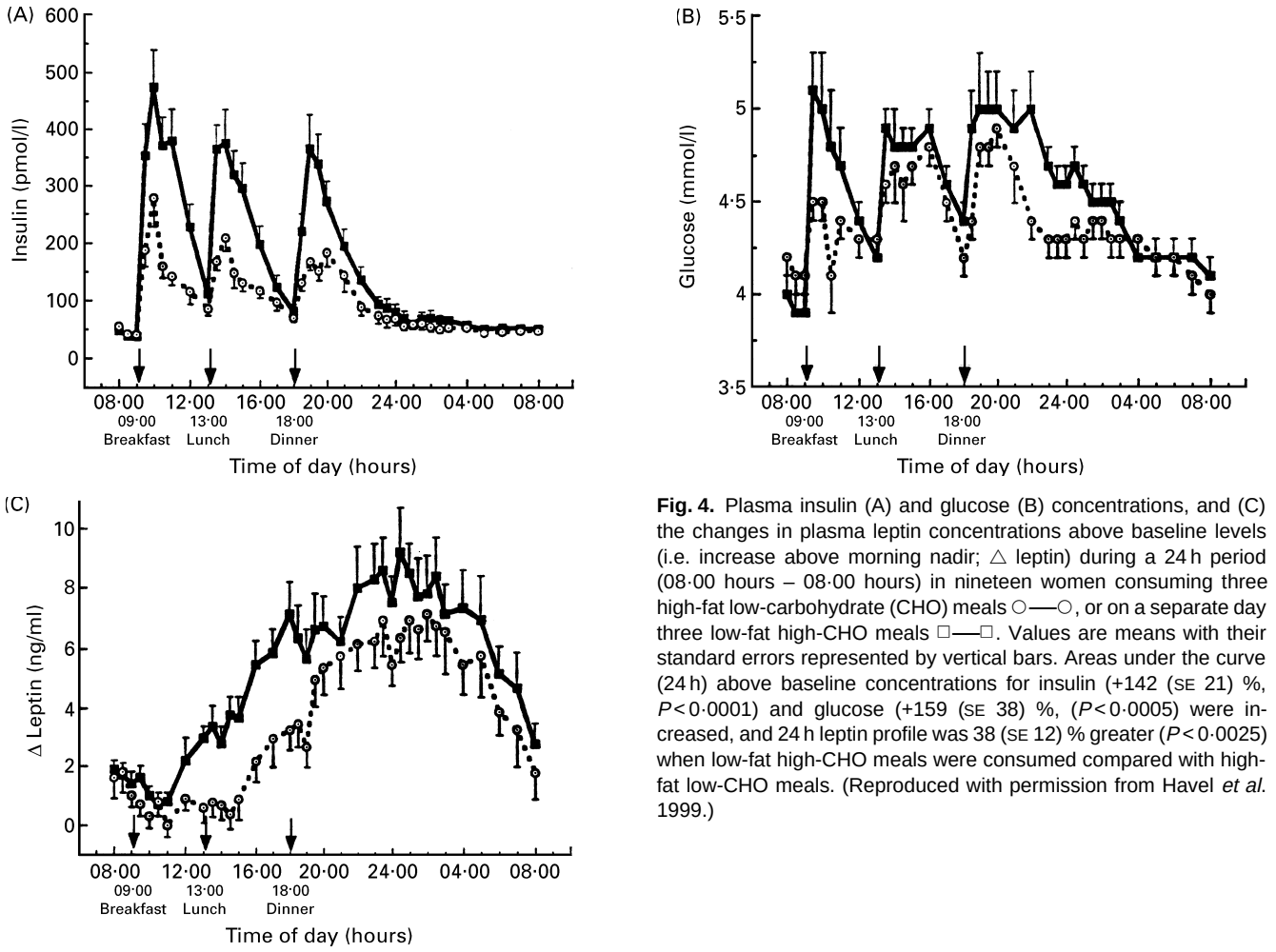

Fig. 4. Plasma insulin (A) and glucose (B) concentrations, and (C) the changes in plasma leptin concentrations above baseline levels (i.e. increase above morning nadir; △ leptin) during a 24 h period (08·00 hours – 08·00 hours) in nineteen women consuming three high-fat low-carbohydrate (CHO) meals ○—○, 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

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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

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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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References

Ahima RS, Prabakaran D, Mantzoros C, Qu D, Lowell B, Maratos-Flier E & Flier JS (1996) Role of leptin in the neuroendocrine response to fasting. *Nature* **382**, 250–252.

Ahren B (1999) Plasma leptin and insulin in C57BI/6J mice on a high-fat diet: relation to subsequent changes in body weight. *Acta Physiologica Scandinavica* **165**, 233–240.

Ahren B & Havel PJ (1999*a*) Leptin increases circulating glucose, insulin and glucagon via sympathetic neural activation in fasted mice. *International Journal of Obesity and Related Metabolic Disorders* **23**, 660–665.

Ahren B & Havel PJ (1999*b*) Leptin inhibits insulin secretion induced by cellular cAMP in INS-1 cells. *American Journal of Physiology* **277**, R959–R966.

Ahren B, Mansson S, Gingerich RL & Havel PJ (1997) Regulation of plasma leptin in mice: Influence of age, high-fat diet and fasting. *American Journal of Physiology* **273**, R113–R120.

Barash IA, Cheung CC, Weigle DS, Ren H, Kabigting EB, Kuijper JL, Clifton DK & Steiner RA (1996) Leptin is a metabolic signal to the reproductive system. *Endocrinology* **137**, 3144–3147.

Barzilai N, Wang J, Massilon D, Vuguin P, Hawkins M & Rossetti L (1997) Leptin selectively decreases visceral adiposity and enhances insulin action. *Journal of Clinical Investigation* **100**, 3105–3110.

Blevins JE, Havel PJ & Gietzen DW (1999) Injections of leptin into the anterior piriform cortex inhibit food intake in rats. *Nutritional Neuroscience* **2**, 357–367.

Boden G, Chen X, Mozzoli M & Ryan I (1996) Effect of fasting on serum leptin in normal human subjects. *Journal of Clinical Endocrinology and Metabolism* **81**, 3419–3423.

Bornstein SR, Uhlmann K, Haidan A, Ehrhart-Bornstein M & Scherbaum WA (1997) Evidence for a novel peripheral action of leptin as a metabolic signal to the adrenal gland: leptin inhibits cortisol release directly. *Diabetes* **46**, 1235–1238.

Bouloumie A, Drexler HC, Lafontan M & Busse R (1998) Leptin, the product of Ob gene, promotes angiogenesis. *Circulation Research* **83**, 1059–1066.

Bray GA & Popkin BM (1998) Dietary fat intake does affect obesity! *American Journal of Clinical Nutrition* **68**, 1157–1173.

Campfield LA, Smith FJ, Guisez Y, Devos R & Burn P (1995) Recombinant mouse OB protein: evidence for a peripheral signal linking adiposity and central neural networks. *Science* **269**, 546–549.

Cao GY, Considine RV & Lynn RB (1997) Leptin receptors in the adrenal medulla of the rat. *American Journal of Physiology* **273**, E448–E452.

Caro JF, Kolaczynski JW, Nyce MR, Ohannesian JP, Opentanova I, Goldman WH, Lynn RB, Zhang PL, Sinha MK & Considine RV (1996*a*) Decreased cerebrospinal-fluid/serum leptin ratio in obesity: a possible mechanism for leptin resistance. *Lancet* **348**, 159–161.

Caro JR, Sinha MK, Kolaczynski JW, Zhang PL & Considine RV (1996*b*) Leptin: the tale of an obesity gene. *Diabetes* **45**, 1455–1461.

Chehab FF, Lim ME & Lu R (1996) Correction of the sterility defect in homozygous obese female mice by treatment with the human recombinant leptin. *Nature Genetics* **12**, 318–320.

Chehab FF, Mounzih K, Lu R & Lim ME (1997) Early onset of reproductive function in normal female mice treated with leptin. *Science* **275**, 88–90.

Chen H, Charlat O, Tartaglia LA, Woolf EA, Weng X, Ellis SJ, Lakey ND, Culpepper J, Moore KJ, Breitbart RE, Duyk GM, Tepper RI & Morgenstern JP (1996*a*) Evidence that the diabetes gene encodes the leptin receptor: identification of a mutation in the leptin receptor gene in db/db mice. *Cell* **84**, 491–495.

Chen G, Koyama K, Yuan X, Lee Y, Zhou YT, O'Doherty R, Newgard CB & Unger RH (1996*b*) Disappearance of body fat in normal rats induced by adenovirus-mediated leptin gene therapy. *Proceedings of the National Academy of Sciences* **93**, 14795–14799.

Chessler SD, Fujimoto WY, Shofer JB, Boyko EJ & Weigle DS (1998) Increased plasma leptin levels are associated with fat accumulation in Japanese Americans. *Diabetes* **47**, 239–243.

Cheung CC, Thornton JE, Kuijper JL, Weigle DS, Clifton DK & Steiner RA (1997) Leptin is a metabolic gate for the onset of puberty in the female rat. *Endocrinology* **138**, 855–858.

Cianflone K, Maslowska M & Sniderman AD (1999) Acylation stimulating protein (ASP), an adipocyte autocrine: new directions. *Seminars in Cell and Development Biology* **10**, 31–41.

Clement K, Vaisse C, Lahlou N, Cabrol S, Pelloux V, Cassuto D, Gourmelen M, Dina C, Chambaz J, Lacorte JM, Basdevant A, Bougneres P, Lebouc Y, Froguel P & Guy-Grand B (1998) A mutation in the human leptin receptor gene causes obesity and pituitary dysfunction. *Nature* **392**, 398–401.

Coleman DL (1973) Effects of parabiosis of obese with diabetes and normal mice. *Diabetologia* **9**, 294–298.

Coleman DL & Hummel KP (1969) Effects of parabiosis of normal with genetically diabetic mice. *American Journal of Physiology* **217**, 1298–1304.

Considine RV, Considine EL, Williams CJ, Hyde TM & Caro JF (1996*a*) The hypothalamic leptin receptor in humans: identification of incidental sequence polymorphisms and absence of the db/db mouse and fa/fa rat mutations. *Diabetes* **45**, 992–994.

Considine RV, Sinha MK, Heiman ML, Kríauciunas A, Stephens TW, Nyce MR, Ohannesian JP, Marco CC, McKee LJ, Bauer TL & Caro JF (1996*b*) Serum immunoreactive-leptin concentrations in normal-weight and obese humans. *New England Journal of Medicine* **334**, 292–295.

Cunningham MJ, Clifton DK & Steiner RA (1999) Leptin's actions on the reproductive axis: perspectives and mechanisms. *Biology of Reproduction* **60**, 216–222.

Cusin I, Sainsbury A, Doyle P, Rohner-Jeanrenaud F & Jeanrenaud B (1995) The ob gene and insulin: a relationship leading to clues to the understanding of obesity. *Diabetes* **44**, 1467–1470.

Dagogo-Jack S, Fanelli C, Paramore D, Brothers J & Landt M (1996) Plasma leptin and insulin relationships in obese and nonobese humans. *Diabetes* **45**, 695–698.

Doring H, Schwarzer K, Nuesslein-Hildesheim B & Schmidt I (1998) Leptin selectively increases energy expenditure of food-restricted lean mice. *International Journal of Obesity and Related Metabolic Disorders* **22**, 83–88.

Dubuc GR, Phinney SD, Stern JS & Havel PJ (1998) Changes of serum leptin and endocrine and metabolic parameters after 7 days of energy restriction in men and women. *Metabolism* **47**, 429–434.

Echwald SM, Sorensen TD, Sorensen TI, Tybjaerg-Hansen A, Andersen T, Chung WK, Leibel RL & Pedersen O (1997) Amino acid variants in the human leptin receptor: lack of association to juvenile onset obesity. *Biochemical and Biophysical Research Communications* **233**, 248–252.

Emilsson V, Liu YL, Cawthorne MA, Morton NM & Davenport M (1997) Expression of the functional leptin receptor mRNA in pancreatic islets and direct inhibitory action of leptin on insulin secretion. *Diabetes* **46**, 313–316.

Emond M, Schwartz GJ, Ladenheim EE & Moran TH (1999) Central leptin modulates behavioral and neural responsivity to CCK. *American Journal of Physiology* **276**, R1545–R1549.

Farooqi IS, Jebb SA, Langmack G, Lawrence E, Cheetham CH, Prentice AM, Hughes IA, McCamish MA & O'Rahilly S (1999) Effects of recombinant leptin therapy in a child with congenital leptin deficiency. *New England Journal of Medicine* **341**, 879–884.

Fujioka K, Patane J, Lubina J & Lau D (1999) CSF leptin levels after exogenous administration of recombinant methionyl human leptin. *Journal of the American Medical Association* **282**, 1517–1518.

Funahashi T, Nakamura T, Shimomura I, Maeda K, Kuriyama H, Takahashi M, Arita Y, Kihara S & Matsuzawa Y (1999) Role of adipocytokines on the pathogenesis of atherosclerosis in visceral obesity. *Internal Medicine* **38**, 202–206.

Gainsford T, Willson TA, Metcalf D, Handman E, McFarlane C, Ng A, Nicola NA, Alexander WS & Hilton DJ (1996) Leptin can induce proliferation, differentiation, and functional activation of hemopoietic cells. *Proceedings of the National Academy of Sciences* **93**, 14564–14568.

Greenberg AS, Fujioka K, Heymsfield S, Lau D, Lubina J, Patane J & McCamish M (1999) Clinical effects of leptin in obesity. *Proceedings of the 81st Annual Meeting of the Endocrine Society*, San Diego, CA, pp 53–54, S54–S63 Abstr (http://www.abstracts-on-line.com/abstracts/endo-society/).

Grinspoon SK, Askari H, Landt ML, Nathan DM, Schoenfeld DA, Hayden DL, Laposata M, Hubbard J & Klibanski A (1997) Effects of fasting and glucose infusion on basal and overnight leptin concentrations in normal-weight women. *American Journal of Clinical Nutrition* **6**, 1352–1356.

Haffner SM, Mykkanen LA, Gonzalez CC & Stern MP (1998) Leptin concentrations do not predict weight gain: the Mexico City Diabetes Study. *International Journal of Obesity and Related Metabolic Disorders* **22**, 695–699.

Halaas JL, Boozer C, Blair-West J, Fidahusein N, Denton DA & Friedman JM (1997) Physiological response to long-term peripheral and central leptin infusion in lean and obese mice. *Proceedings of the National Academy of Sciences* **94**, 8878–8883.

Halaas JL, Gajiwala KS, Maffei M, Cohen SL, Chait BT, Rabinowitz D, Lallone RL, Burley SK & Friedman JM (1995) Weight-reducing effects of the plasma protein encoded by the obese gene. *Science* **269**, 543–546.

Hardie LJ, Guilhot N & Trayhurn P (1996*a*) Regulation of leptin production in cultured mature white adipocytes. *Hormone and Metabolic Research* **28**, 685–689.

Hardie LJ, Rayner DV, Holmes S & Trayhurn P (1996*b*) Circulating leptin levels are modulated by fasting, cold exposure and insulin administration in lean but not Zucker (fa/fa) rats as measured by ELISA. *Biochemical and Biophysical Research Communications* **223**, 660–665.

Harris RB & Martin RJ (1984) Specific depletion of body fat in parabiotic partners of tube-fed obese rats. *American Journal of Physiology* **247**, R380–R386.

Havel PJ (1997) Glucose infusion increases circulating leptin in proportion to adipose stores in rhesus monkeys. *Journal of Experimental Endocrinology and Diabetes* **105**, 37–38.

Havel PJ (1998) Leptin production and action: relevance to energy balance in humans. *American Journal of Clinical Nutrition* **67**, 355–356.

Havel PJ (1999) Mechanisms regulating leptin production: implications for the control of energy balance. *American Journal of Clinical Nutrition* **70**, 305–306.

Havel PJ, Busch BL, Curry DL, Johnson PR, Dallman MF & Stern JS (1996*a*) Predominately glucocorticoid agonist actions of RU-486 in young specific-pathogen-free Zucker rats. *American Journal of Physiology* **271**, R710–R717.

Havel PJ, Kasim-Karakas S, Dubuc GR, Mueller WM & Phinney SD (1996*b*) Gender differences in plasma leptin concentrations. *Nature Medicine* **2**, 949–950.

Havel PJ, Kasim-Karakas S, Mueller W, Johnson PR, Gingerich RL & Stern JS (1996*c*) Relationship of plasma leptin to plasma

insulin and adiposity in normal weight and overweight women: Effects of dietary fat content and sustained weight loss. *Journal of Clinical Endocrinology and Metabolism* **81**, 4406–4413.
Havel PJ, Larsen PJ & Cameron JL (2000) Control of food intake. In *Neuroendocrinology in Physiology and Medicine*, pp. 335–352 [PM Conn and ME Freeman, editors]. Totowa, NJ: Humana Press.
Havel PJ & Pelleymounter M (1997) Acute adrenergically-mediated increases of circulating glucose and lactate after leptin administration in rhesus monkeys. *Obesity Research* **5**, Suppl. 1, 17S.
Havel PJ, Townsend R, Chaump L & Teff K (1999) High fat meals reduce 24 h circulating leptin concentrations in women. *Diabetes* **48**, 334–341.
Havel PJ, Uriu-Hare JY, Liu T, Stanhope KL, Stern JS, Keen CL & Ahren B (1998) Rapid and marked decreases of circulating leptin in streptozotocin diabetic rats: Reversal by insulin. *American Journal of Physiology* **274**, R1482–R1491.
Haynes WG, Morgan DA, Walsh SA, Mark AL & Sivitz WI (1997) Receptor-mediated regional sympathetic nerve activation by leptin. *Journal of Clinical Investigation* **100**, 270–278.
Heymsfield SB, Greenberg AS, Fujioka K, Dixon RM, Kushner R, Hunt T, Lubina JA, Patane Self B, Hunt P & McCamish (1999) Recombinant leptin for weight loss in lean and obese adults: a randomized, controlled, dose-escalation trial. *Journal of the American Medical Association* **282**, 1568–1575.
Hill JO, Lin D, Yakubu F & Peters JC (1992) Development of dietary obesity in rats: influence of amount and composition of dietary fat. *International Journal of Obesity* **16**, 321–333.
Horton TJ, Drougas H, Brachey A, Reed GW, Peters JC & Hill JO (1995) Fat and carbohydrate overfeeding: different effects on energy storage. *American Journal of Clinical Nutrition* **62**, 19–29.
Hotamisligil GS (1999) The role of TNFalpha and TNF receptors in obesity and insulin resistance. *Journal of Internal Medicine* **245**, 621–625.
Jacob RJ, Dziura J, Medwick MB, Leone P, Caprio S, During M, Shulman GI & Sherwin RS (1997) The effect of leptin is enhanced by microinjection into the ventromedial hypothalamus. *Diabetes* **46**, 150–152.
Keim NL, Stern JS & Havel PJ (1998) Relation between circulating leptin concentrations and appetite during a prolonged, moderate energy deficit in women. *American Journal of Clinical Nutrition* **68**, 794–801.
Kennedy AG (1953) The role of the fat depot in the hypothalamic control of food intake in the rat. *Proceedings of the Royal Society of London* **140B**, 578–592.
Kieffer TJ, Heller RS, Leech CA, Holz GG & Habener JF (1997) Leptin suppression of insulin secretion by the activation of ATP-sensitive K+ channels in pancreatic beta-cells. *Diabetes* **46**, 1087–1093.
Kolaczynski JW, Considine RV, Ohannesian J, Marco C, Opentanova I, Nyce MR, Myint M & Caro JF (1996*a*) Responses of leptin to short-term fasting and refeeding in humans: a link with ketogenesis but not ketones themselves. *Diabetes* **45**, 1511–1515.
Kolaczynski JW, Nyce MR, Considine RV, Boden G, Nolan JJ, Henry R, Mudaliar SR, Olefsky J & Caro JF (1996*b*) Acute and chronic effects of insulin on leptin production in humans: Studies in vivo and in vitro. *Diabetes* **45**, 699–701.
Kolaczynski JW, Ohannesian JP, Considine RV, Marco CC & Caro JF (1996*c*) Response of leptin to short-term and prolonged overfeeding in humans. *Journal of Clinical Endocrinology and Metabolism* **81**, 4162–4165
Kopp W, Blum WF, Ziegler A, Mathiak K, Lubbert H, Herpertz S, Deter HC & Hebebrand J (1998) Serum leptin and body weight in females with anorexia and bulimia nervosa. *Hormone and Metabolic Research* **30**, 5272–5275.
Landt M, Gingerich RL, Havel PJ, Mueller WM, Schoner B, Hale JE & Heiman ML (1998) Radioimmunoassay of rat leptin: sexual dimorphism reversed from humans. *Clinical Chemistry* **44**, 565–570.
Laughlin GA & Yen SSC (1997) Hypoleptinemia in women athletes: absence of diurnal rhythm with amenorrhea. *Journal of Clinical Endocrinology and Metabolism* **82**, 318–321.
Leroy P, Dessolin S, Villageois P, Moon BC, Friedman JM, Ailhaud G & Dani C (1996) Expression of ob gene in adipose cells: regulation by insulin. *Journal of Biological Chemistry* **271**, 2365–2368.
Levin N, Nelson C, Gurney A, Vandlen R & de Sauvage F (1996) Decreased food intake does not completely account for adiposity reduction after ob protein infusion. *Proceedings of the National Academy of Sciences* **93**, 1726–1730.
Loffreda S, Yang SQ, Lin HZ, Karp CL, Brengman ML, Wang DJ, Klein AS, Bulkley GB, Bao C, Noble PW, Lane MD & Diehl AM (1998) Leptin regulates proinflammatory immune responses. *FASEB Journal* **12**, 57–65.
Lord GM, Matarese G, Howard JK, Baker RJ, Bloom SR & Lechler RI (1998) Leptin modulates the T-cell immune response and reverses starvation-induced immunosuppression. *Nature* **394**, 897–901.
Maffei M, Halaas J, Ravussin E, Pratley RE, Lee GH, Zhang Y, Fei H, Lallone R, Ranganathan S, Kern PA & Friedman JM (1995) Leptin levels in human and rodents: measurement of plasma leptin and ob RNA in obese and weight-reduced subjects. *Nature Medicine* **1**, 1155–1161.
Malmstrom R, Taskinen MR, Karonen SL & Yki-Harvinen H (1996) Insulin increases plasma leptin concentrations in normal subjects and in patients with NIDDM. *Diabetologia* **39**, 993–996.
Matkovic V, Ilich JZ, Badenhop NE, Skugor M, Clairmont A, Klisovic D & Landoll JD (1997) Gain in body fat is inversely related to the nocturnal rise in serum leptin level in young females. *Journal of Clinical Endocrinology and Metabolism* **82**, 1368–1372.
Matson CA, Wiater MF, Kuijper JL & Weigle DS (1997) Synergy between leptin and cholecystokinin (CCK) to control daily caloric intake. *Peptides* **18**, 1275–1278.
Matsuoka N, Ogawa Y, Hosoda K, Matsuda J, Masuzaki H, Miyawaki T, Azuma N, Natsui K, Nishimura H, Yoshimasa Y, Nishi S, Thompson DB & Nakao K (1997) Human leptin receptor gene in obese Japanese subjects: evidence against either obesity-causing mutations or association of sequence variants with obesity. *Diabetologia* **40**, 1204–1210.
Medina EA, Stanhope KL, Mizuno TM, Mobbs CV, Gregoire F, Hubbard NE, Erickson KL & Havel PJ (1999) Effects of tumour necrosis factor alpha (TNFα) on leptin secretion and gene expression: relationship to changes of glucose metabolism in isolated rat adipocytes. *International Journal of Obesity and Related Metabolic Disorders* **23**, 896–903.
Mohamed-Ali V, Pinkney JH & Coppack SW (1998) Adipose tissue as an endocrine and paracrine organ. *International Journal of Obesity and Related Metabolic Disorders* **22**, 1145–1158.
Montague CT, Farooqi IS, Whitehead JP, Soos MA, Rau H, Wareham NJ, Sewter CP, Digby JE, Mohammed SN, Hurst JA, Cheetham CH, Earley AR, Barnett AH, Prins JB & O'Rahilly S (1997) Congenital leptin deficiency is associated with severe early-onset obesity in humans. *Nature* **387**, 903–908.
Mueller WM, Gregoire FM, Stanhope KL, Mobbs CV, Mizuno TM, Warden CH, Stern JS & Havel PJ (1998) Evidence that glucose metabolism regulates leptin secretion from cultured adipocytes. *Endocrinology* **139**, 551–558.

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O'donnell CP, Schaub CD, Haines AS, Berkowitz DE, Tankersley CG, Schwartz AR & Smith PL (1999) Leptin prevents respiratory depression in obesity. *American Journal of Respiratory and Critical Care Medicine* **159**, 1477–1484.

Pelleymounter MA, Cullen MJ, Baker MB, Hecht R, Winters D, Boone T & Collins F (1995) Effects of the obese gene product on body weight regulation in ob/ob mice. *Science* **269**, 540–543.

Ravussin E, Pratley RE, Maffei M, Wang H, Friedman JM, Bennett PH & Bogardus C (1996) Relatively low plasma leptin concentrations precede weight gain in Pima Indians. *Nature Medicine* **2**, 238–240.

Rebrin K, Steil GM, Getty L & Bergman RN (1995) Free fatty acid as a link in the regulation of hepatic glucose output by peripheral insulin. *Diabetes* **44**, 1038–1045.

Rentsch J & Chiesi M (1996) Regulation of ob gene mRNA levels in cultured adipocytes. *FEBS Letters* **379**, 55–59.

Rosenbaum M, Nicolson M, Hirsch J, Heymsfield SB, Gallagher D, Chu F & Leibel RL (1996) Effects of gender, body composition, and menopause on plasma concentrations of leptin. *Journal of Clinical Endocrinology and Metabolism* **81**, 3424–3427.

Rossetti L, Massillon D, Barzilai N, Vuguin P, Chen W, Hawkins M, Wu J & Wang J (1997) Short term effects of leptin on hepatic gluconeogenesis and in vivo insulin action. *Journal of Biological Chemistry* **272**, 27758–27763.

Saad MF, Khan A, Sharma A, Michael R, Riad-Gabriel MG, Boyadjian R, Jinagouda SD, Steil GM & Kamdar V (1998*a*) Physiological insulinemia acutely modulates plasma leptin. *Diabetes* **47**, 544–549.

Saad MF, Riad-Gabriel MG, Khan A, Sharma A, Michael R, Jinagouda SD, Boyadjian R & Steil GM (1998*b*) Diurnal and ultradian rhythmicity of plasma leptin: effects of gender and adiposity. *Journal of Clinical Endocrinology and Metabolism* **83**, 2453–2459.

Saladin R, De Vos P, Guerre-Millo M, Leturque A, Girard J, Staels B & Auwerx J (1995) Transient increase in obese gene expression after food intake or insulin administration. *Nature* **377**, 527–529.

Satoh N, Ogawa Y, Katsuura G, Hayase M, Tsuji T, Imagawa K, Yoshimasa Y, Nishi S, Hosoda K & Nakao K (1997) The arcuate nucleus as a primary site of satiety effect of leptin in rats. *Neuroscience Letters* **224**, 149–152.

Scarpace PJ & Matheny M (1998) Leptin induction of UCP1 gene expression is dependent on sympathetic innervation. *American Journal of Physiology* **275**, E259–E264.

Scarpace PJ, Matheny M, Pollock BH & Tumer N (1997) Leptin increases uncoupling protein expression and energy expenditure. *American Journal of Physiology* **273**, E226–E230.

Schoeller DA, Cella LK, Sinha MK & Caro JF (1997) Entrainment of the diurnal rhythm of plasma leptin to meal timing. *Journal of Clinical Investigation* **100**, 1882–1887.

Schwartz MW, Baskin DG, Kaiyala KJ & Woods SC (1999) Model for the regulation of energy balance and adiposity by the central nervous system. *American Journal of Clinical Nutrition* **69**, 584–596.

Schwartz MW, Figlewicz DP, Baskin DG, Woods SC & Porte D (1994) Insulin and the central regulation of energy balance. In *Monographs 2; The Endocrine Pancreas, Insulin Action, and Diabetes. Endocrine Reviews*, pp. 81–113 [A Negro Vilar and LE Underwood, editors]. Bethesda, MD: The Endocrine Society.

Schwartz MW, Peskind E, Raskind M, Boyko EJ & Porte D Jr (1996*a*) Cerebrospinal fluid leptin levels: relationship to plasma levels and to adiposity in humans. *Nature Medicine* **2**, 589–593.

Schwartz MW, Seeley RJ, Campfield LA, Burn P & Baskin DG (1996*b*) Identification of targets of leptin action in rat hypothalamus. *Journal of Clinical Investigation* **98**, 1101–1106.

Sierra-Honigmann MR, Nath AK, Murakami C, Garcia-Cardena G, Papapetropoulos A, Sessa WC, Madge LA, Schechner JS, Schwabb MB, Polverini PJ & Flores-Riveros JR (1998) Biological action of leptin as an angiogenic factor. *Science* **281**, 1683–1686.

Sindelar DK, Chu CA, Rohlie M, Neal DW, Swift LL & Cherrington AD (1997) The role of fatty acids in mediating the effects of peripheral insulin on hepatic glucose production in the conscious dog. *Diabetes* **46**, 187–196.

Sindelar DK, Havel PJ, Seeley RJ, Wilkinson CW, Woods SC & Schwartz MW (1999) Low plasma leptin levels contribute to diabetic hyperphagia in rats. *Diabetes* **48**, 1275–1280.

Sinha MK, Ohannesian JP, Heiman ML, Kriauciunas A, Stephens TW, Magosin S, Marco C & Caro JF (1996) Nocturnal rise of leptin in lean, obese, and non-insulin-dependent diabetes mellitus subjects. *Journal of Clinical Investigation* **97**, 1344–1347.

Sivitz WI, Walsh S, Morgan D, Donohue P, Haynes W & Leibel RL (1998) Plasma leptin in diabetic and insulin-treated diabetic and normal rats. *Metabolism* **47**, 584–591.

Sonnenberg GE, Krakower GR, Hoffmann RG, Maas DL, Hennes MMI & Kissebah AH (1996) Plasma leptin concentrations: Effects of extended fasting and stepwise increases in glucose infusions. *Obesity Research* **4**, 13S Abstr.

Strobel A, Issad T, Camoin L, Ozata M & Strosberg AD (1998) A leptin missense mutation associated with hypogonadism and morbid obesity. *Nature Genetics* **18**, 213–215.

Surwit RS, Petro AE, Parekhand P & Collins S (1997) Low plasma leptin in response to dietary fat in diabetes- and obesity-prone mice. *Diabetes* **46**, 1516–1520.

Tang-Christensen M, Havel PJ, Jacobs RR, Larsen PJ & Cameron JL (1999*a*) Central administration of leptin inhibits food intake and activates the sympathetic nervous system in rhesus macaques. *Journal of Clinical Endocrinology and Metabolism* **84**, 711–717.

Tang-Christensen M, Holst JJ, Hartmann B & Vrang N (1999*b*) The arcuate nucleus is pivotal in mediating the anorectic effects of centrally administered leptin. *NeuroReport* **10**, 1183–1187.

Tartaglia LA (1997) The leptin receptor. *Journal of Biological Chemistry* **272**, 6093–6096.

Tartaglia LA, Dembski M, Weng X, Deng N, Culpepper J, Devos R, Richards GJ, Campfield LA, Clark FT & Deeds J (1995) Identification and expression cloning of a leptin receptor, OB-R. *Cell* **83**, 1263–1271.

Tataranni PA & Ravussin E (1997) Effect of fat intake on energy balance. *Annals of the New York Academy of Sciences* **819**, 37–43.

Trayhurn P, Duncan JS, Hoggard N & Rayner DV (1998) Regulation of leptin production: a dominant role for the sympathetic nervous system? *Proceedings of the Nutrition Society* **57**, 413–419.

Tremblay A, Plourde G, Despres JP & Bouchard C (1998) Impact of dietary fat content and fat oxidation on energy intake in humans. *American Journal of Clinical Nutrition* **49**, 799–805.

Utriainen R, Malmstrom R, Makimattila S & Yki-Jarvinen H (1996) Supraphysiological hyperinsulinemia increases plasma leptin concentrations after 4 h in normal subjects. *Diabetes* **45**, 1364–1366.

Van Heek M, Compton DS, France CF, Tedesco RP, Fawzi AB, Graziano MP Sybertz EJ, Strader CD & Davis HR Jr (1997) Diet-induced obese mice develop peripheral, but not central, resistance to leptin. *Journal of Clinical Investigation* **99**, 385–390.

Wabitsch M, Jensen PB, Blum WF, Christoffersen CT, Englaro P, Heinze E, Rascher W, Teller W, Tornqvist H & Hauner H (1996) Insulin and cortisol promote leptin production in cultured human fat cells. *Diabetes* **45**, 1435–1438.

Wang J, Liu R, Hawkins M, Barzilai N & Rossetti L (1998) A nutrient-sensing pathway regulates leptin gene expression in muscle and fat. *Nature* **393**, 684–688.

Weigle DS, Duell PB, Connor WE, Steiner RA, Soules MR & Kuijper JL (1997) Effect of fasting, refeeding, and dietary fat restriction on plasma leptin levels. *Journal of Clinical Endocrinology and Metabolism* **82**, 561–565.

Weigle DS, Hutson AM, Kramer JM, Fallon MG, Lehner JM, Lok S & Kuijper JL (1998*a*) Leptin does not fully account for the satiety activity of adipose tissue-conditioned medium. *American Journal of Physiology* **275**, R976–R985.

Weigle DS, Selfridge LE, Schwartz MW, Seeley RJ, Cummings DE, Havel PJ, Kuijper JL & Beltran del Rio H (1998*b*) Elevated free fatty acids induce uncoupling protein 3 expression in muscle: a potential explanation for the effect of fasting. *Diabetes* **147**, 298–302.

West DB, Fey D & Woods SC (1984) Cholecystokinin persistently suppresses meal size but not food intake in free-feeding rats. *American Journal of Physiology* **246**, R776–R787.

Widdowson PS, Upton R, Buckingham R, Arch J & Williams G (1997) Inhibition of food response to intracerebroventricular injection of leptin is attenuated in rats with diet-induced obesity. *Diabetes* **46**, 1782–1785.

Wilson BE, Meyer GE, Cleveland JC Jr & Weigle DS (1990) Identification of candidate genes for a factor regulating body weight in primates. *American Journal of Physiology* **259**, R1148–R1155.

Wiman B & Hamsten A (1990) The fibrinolytic enzyme system and its role in the etiology of thromboembolic disease. *Seminars in Thrombosis and Hemostasis* **16**, 207–216.

Wisse BE, Campfield LA, Marliss EB, Morais JA, Tenenbaum R & Gougeon R (1999) Effects of prolonged moderate and severe energy restriction and refeeding on plasma leptin concentrations in obese women. *American Journal of Clinical Nutrition* **70**, 321–330.

Woods SC, Chavez M, Park CR, Riedy C, Kaiyala K, Richardson RD, Figlewicz DP, Schwartz MW, Porte D & Seeley RJ (1996) The evaluation of insulin as a metabolic signal influencing behavior via the brain. *Neuroscience and Biobehavior Reviews* **20**, 139–144.

Woods SC, Seeley RJ, Porte D Jr & Schwartz MW (1998) Signals that regulate food intake and energy homeostasis. *Science* **280**, 1378–1383.

Zhang Y, Proenca R, Maffei M, Barone M, Leopold L & Friedman JM (1994) Positional cloning of the mouse obese gene and its human homologue. *Nature* **372**, 425–432.

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