

Leptin regulation of lipid homeostasis: dietary and metabolic implications

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Leptin is a 16 kDa protein synthesized and secreted primarily from adipocytes. Leptin acts centrally to regulate appetite and energy expenditure and has peripheral effects to coordinate whole-body energy metabolism. Leptin acts as an energy balance and nutrient sensor; its expression and function are regulated by endocrine and dietary factors. Leptin regulates lipid metabolism, specifically lipid storage in adipocytes as well as in skeletal muscle, liver and the pancreas. Effects of leptin on tissue lipid metabolism include regulation of lipogenesis and fatty acid oxidation. Leptin resistance is a hallmark of rodent and human obesity and appears to be due to defects in leptin-receptor signalling (proximal and perhaps distal) as well as impaired leptin transport into the brain. Dietary composition, especially dietary fatty acid intake and profile, impacts on the expression of genes encoding leptin and leptin receptors as well as downstream leptin effectors. Dietary fat consumption, especially consumption of saturated fatty acids, can induce leptin resistance. Leptin has been implicated in nutritional programming during fetal and neonatal growth with long-term effects on susceptibility to obesity, diabetes and CHD in adults.

Leptin: Lipid homeostasis: Energy metabolism: Obesity: Nutritional programming

Introduction

Global rates of adult and childhood obesity have reached epidemic proportions (for a review, see Kopelman, 2000). Given the associated risk for diabetes and cardiovascular disease in obese patients, considerable effort has been spent in understanding candidate genes and pathways involved in the regulation and dysregulation of whole-body energy homeostasis and adiposity. The discovery of leptin, the product of the *ob* gene, in 1994 (Zhang *et al.* 1994) changed the field of obesity and metabolic disease research as it provided the first adipocyte-derived endocrine signal linking body fat stores to central feeding and metabolic control centres. A large and growing body of literature details the expression of leptin in lean and obese animals and man and is delineating the integrative role that this energy and nutrient sensor plays in the regulation of whole-body energy homeostasis (for reviews, see Houseknecht & Portocarrero, 1998; Ahima & Flier, 2000). In fact, leptin has been shown to play a pivotal role not only in the regulation of body-weight maintenance but is also implicated in immune function, reproduction, bone growth and develop-

ment, haematopoiesis, etc. (for reviews, see Fantuzzi & Faggioni, 2000; Barb *et al.* 2001; Faggioni *et al.* 2001; Moschos *et al.* 2002; Thomas & Burguera, 2002). It is beyond the scope of the present review to cover the myriad roles of leptin in regulation of these diverse physiological functions. The present review highlights the role of leptin as energy and nutrient sensor, its role in the regulation of lipid homeostasis as well as the impact of nutrition in the regulation of leptin biology.

Leptin was originally cast as an obesity hormone at the time of its discovery due to the profound phenotype observed in mice that lack functional leptin (*ob/ob* mice; Zhang *et al.* 1994). Accordingly, leptin biology has been at the centre of intense research effort by the obesity research community. Clearly, mutations in the leptin and leptin-receptor genes are associated with morbid obesity and related pathologies in multiple rodent models (for reviews, see Houseknecht & Portocarrero, 1998; Ahima & Flier, 2000). However, in the case of man, with the exception of a few individuals (for a review, see Barsh *et al.* 2000), mutations in the leptin or leptin-receptor genes are not responsible for obesity. In contrast, mutations of pivotal genes in

Abbreviations: AGRP, agouti-related protein; ERK, extracellular signal regulated kinase; MCR, melanocortin receptor; NEFA, non-esterified fatty acid; NPY, neuropeptide Y; POMC, pro-opiomelanocortin; PPAR, peroxisome proliferator activated receptor; SHP-2, SH2-domain containing protein tyrosine phosphatase; SOCS, suppressors of cytokine signalling; SREBP, sterol regulatory element-binding protein; STAT, signal transducers and activators of transcription; ZDF rat, Zucker diabetic fatty rat.

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pathways downstream of leptin such as the melanocortin receptor (MCR)-4 gene have been implicated in human obese populations (Barsh *et al.* 2000; Farooqi *et al.* 2000).

Leptin receptor and signalling

Leptin is synthesized as an 18 kDa protein with a signal sequence that is cleaved to yield the mature 16 kDa hormone. Leptin circulates bound to proteins in serum and is a four-helix bundle cytokine (for reviews, see Houseknecht & Portocarrero, 1998; Ahima & Flier, 2000). The leptin receptor (Tartaglia *et al.* 1995) is a member of the class 1 cytokine receptor family (Tartaglia, 1997; Houseknecht & Portocarrero, 1998; Sweeney, 2002). Leptin-receptor isoforms (Ob-Ra, Ob-Rb, Ob-Rc, Ob-Rd, Ob-Re, Ob-Rf) arise from alternative splicing and represent variants that differ at the carboxy terminus, all resulting in truncated intracellular domains except for the 'long-form' leptin receptor, Ob-Rb. The Ob-Rb isoform is highly expressed in feeding centres in the hypothalamus and is believed to mediate the effects of leptin on feeding behaviour and metabolic rate.

The various short forms of the leptin receptor are widely expressed in many tissues, yet the physiological role(s) of these receptors remain to be fully elucidated. A soluble leptin receptor (Ob-Re) that lacks both the trans-membrane and intracellular domains is one of the plasma proteins that bind leptin in circulation (Tartaglia *et al.* 1995; Houseknecht *et al.* 1996; Sinha *et al.* 1996; Friedman & Halaas, 1998). Ob-Re binds leptin at a 1:1 ratio and has been hypothesized to modulate leptin activity. The expression of Ob-Re changes in various physiological and pathophysiological states in animals and man including weight loss and in eating disorders such as anorexia nervosa and bulimia nervosa (Laimer *et al.* 2002; Monteleone *et al.* 2002; Ogier *et al.* 2002) but the physiological implications of the soluble receptor are currently unknown.

Fig. 1 illustrates our current understanding of the basic signalling pathways emanating from leptin receptors.

Leptin binding to its receptors results in receptor dimerization, an apparent prerequisite for signalling (White *et al.* 1997). Receptor homodimerization of Ob-Rb (long-form receptor) signals activation of the janus-activated kinases-STAT pathway, consistent with other members of this class of cytokine receptors (for a comprehensive review, see Sweeney, 2002).

In addition to STAT activation, leptin receptors appear to signal via additional pathways. Leptin receptors can activate insulin receptor substrate phosphorylation, phosphatidylinositol 3-kinase activation, as well as activation of extracellular signal regulated kinase (ERK) (or mitogen-activated protein kinase) pathways (for a review, see Sweeney, 2002). Tyrosine-985 appears to be necessary for maximal leptin-induced activation of the ERK pathway (Banks *et al.* 2000). SHP-2 is a positive effector of ERK activation mediated by both long- and short-form leptin receptors (Bjorbaek *et al.* 2001). SHP-2 binds to tyrosine-985 on Ob-Rb and impacts ERK activation by two distinct pathways (Bjorbaek *et al.* 2001).

Signalling via leptin receptors is subject to negative regulation. One method of negative feedback is via SOCS (Krebs & Hilton, 2000; Nicola & Greenhalgh, 2000),

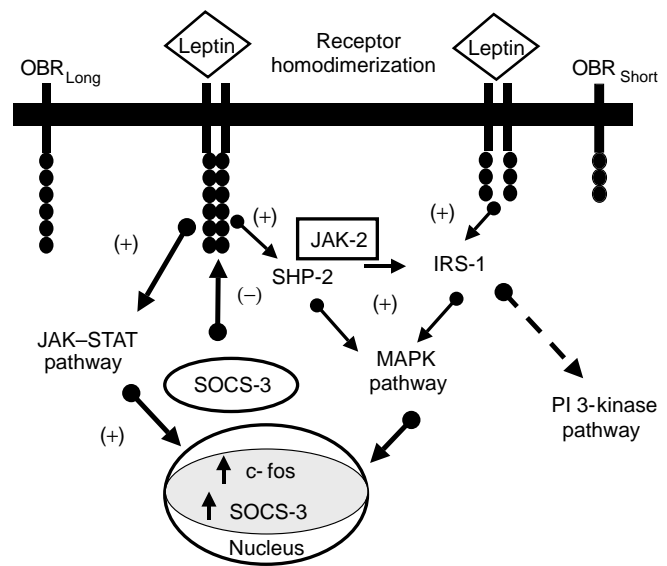


Fig. 1. Signalling pathways emanating from leptin receptors. OBR, leptin receptor; JAK, janus-activated kinases; SHP-2, SH2-domain containing protein tyrosine phosphatase; IRS, insulin receptor substrate; STAT, signal transducers and activators of transcription; SOCS-3, suppressors of cytokine signalling-3; MAPK, mitogen-activated protein kinase; PI, phosphatidylinositol; (+), stimulate; (-), inhibit.

specifically SOCS-3 (Bjorbaek *et al.* 1999, 2000). The Ob-R tyrosine-985 residue plays a pivotal role not only in leptin-receptor activation, but is also pivotal in the feedback inhibition of the leptin receptor by SOCS-3 as it specifically binds to Ob-R tyrosine-985 (Bjorbaek *et al.* 2000). Attenuation of leptin signalling via leptin-induced SOCS-3 expression in hypothalamic neurons is at least one mechanism underlying leptin resistance, as discussed on p. 87.

Leptin-receptor signalling in the hypothalamus

Leptin receptors (Ob-Rb) are expressed in neurons of the arcuate nucleus of the hypothalamus, neurons pivotal to the regulation of appetite, energy expenditure and ultimately body weight (for detailed reviews, see Ahima *et al.* 2000; Schwartz, 2001). Both appetite-stimulating (orexigenic) and appetite-inhibiting (anorexigenic) neurons express Ob-Rb. Activation of leptin signalling in the orexigenic neurons culminates in down regulation of the expression of the neurotransmitters neuropeptide Y (NPY) and agouti-related protein (AGRP). Activation of the NPY pathway profoundly stimulates appetite and lowers energy expenditure resulting in obesity. Leptin-induced down regulation of NPY expression is at least one mechanism by which leptin regulates appetite and energy expenditure (Ahima *et al.* 2000).

AGRP is the naturally occurring antagonist of MCR; antagonism of MCR-4 by AGRP results in hyperphagia and attenuation of leptin-induced anorexia (Fan *et al.* 1997). Conversely, agonism of MCR by the endogenous agonist, α -melanocyte stimulating hormone, culminates in the inhibition of feeding and stimulation of thermogenesis.

Activation of leptin signalling pathways results in an increase in expression of pro-opiomelanocortin (POMC), the precursor of α -MSH, as well as the anorexigenic peptide cocaine- and amphetamine-related transcript (Schwartz *et al.* 1997; Elias *et al.* 1999; Elmquist *et al.* 1999). As leptin regulates the expression of both the endogenous agonist and antagonist of MCR-4, a hypothalamic receptor pivotal in regulation of body weight in rodents (Huszar *et al.* 1997) and a significant site of mutations found in obese human populations (4–5 % of morbid obesity in the population; Farooqi *et al.* 2000), it appears that this is a major downstream effector pathway for leptin regulation of appetite and energy homeostasis.

Leptin: energy-balance sensor

The ability of exogenous leptin to regulate appetite and energy expenditure in rodents with subsequent loss of adipose tissue but not lean body mass has led to the notion that leptin is an anti-obesity hormone (for reviews, see Friedman & Halaas, 1998; Houseknecht & Portocarrero, 1998). The observation that obesity can occur despite high circulating leptin concentrations in rodents and man has led to the notion of leptin resistance (discussed in detail on p. 87). It has been proposed that obesity in the face of leptin resistance reflects the evolutionary advantage of being able to store excess energy as lipid during times of nutritional plenty (Flier, 1998). This has led to the notion that leptin's primary physiological role is in sensing negative energy balance and coordinating physiological signals leading to whole-body adaptation to fasting (Flier, 1998; Ahima & Flier, 2000).

Studies from several groups (Ahima *et al.* 1996; Legrady *et al.* 1997; Yu *et al.* 1997; Finn *et al.* 1998; Lord *et al.* 1998; Nagatani *et al.* 1998) have shown that, indeed, prevention of the fasting-induced fall in leptin concentrations by the administration of exogenous leptin treatment during fasting is able to at least partially overcome the metabolic, endocrine, reproductive and immune system adaptations to starvation.

Leptin as a nutrient sensor

Intense study is underway to determine the mechanism(s) by which leptin 'senses' energy balance. Linked to energy sensing is the notion that leptin serves as a nutrient sensor, specifically sensing nutrient flux in adipocytes and other cells. One way this could be accomplished is via changes in circulating insulin concentrations. Insulin levels in blood change rapidly in response to feeding and insulin has been shown to regulate leptin gene expression in many species (for reviews, see Houseknecht & Portocarrero, 1998; Ahima & Flier, 2000).

The regulation of leptin expression by changes in nutrient flux appears to involve more than changes in insulin concentrations, however. Work from several laboratories has implicated the hexosamine biosynthetic pathway in leptin's role as cellular nutrient sensor.

Marshall *et al.* (1991) proposed that cellular hexosamine biosynthesis is an important mechanism by which cellular glucose flux in cells is sensed. Uridine-diphospho-*N*-acetylglucosamine, the endproduct of hexosamine biosynthesis, is

used in *o*-linked glycosylation reactions; important regulatory steps in gene transcription. Specifically, the activity of some transcription factors is modified by *o*-linked glycosylation. Leptin expression in the adipose tissue of man (Considine *et al.* 2000) and rodents (Wang *et al.* 1998, 1999b; McClain *et al.* 2000; Obici *et al.* 2002b) is regulated by the hexosamine biosynthetic pathway, consistent with the observation that leptin expression in adipocytes *in vitro* is more sensitive to glucose concentrations than insulin (Mueller *et al.* 1998). It appears that the mechanisms by which hexosamine biosynthesis regulates leptin production involve the regulation of activity of transcription factors. In experiments using 3T3-L1 adipocytes transfected with a leptin gene promoter-luciferase reporter construct, Zhang *et al.* (2002) found that leptin production in 3T3-L1 adipocytes is stimulated by the metabolism of glucose to hexosamines via transcriptional mechanisms in the proximal promoter of the leptin gene.

In addition to its effects on leptin gene expression, recent work by Obici *et al.* (2002b) reveals that the hexosamine pathway is also involved in regulating the expression of a cluster of mitochondrial genes involved in oxidative phosphorylation in skeletal muscle and linked to whole-body energy expenditure. Short-term overfeeding experiments in rodents replicated this transcriptional pattern suggesting that this adaptation to nutrient abundance occurs under physiological conditions. These data reinforce the notion that the hexosamine biosynthetic pathway plays a major role in cellular nutrient sensing, and in the regulation of whole-body energy balance due to its modulation of leptin expression and insulin action in adipose tissue and skeletal muscle (Wang *et al.* 1998, 1999b; McClain *et al.* 2000).

Nutritional regulation of leptin biology

As leptin has been implicated as a major sensor of energy and nutrient balance it is not surprising that the leptin axis is regulated by nutritional status. The vast majority of published manuscripts examining the impact of nutrition on leptin expression and activity have focused on energy intake, namely comparison of *ad libitum* feeding of highly palatable, and often high-fat, diets *v.* low-fat diets. Additionally, a large body of literature has compared effects of *ad libitum* feeding with food deprivation (acute or chronic fasting) on leptin expression. Data in species spanning from rodents to livestock to man have shown that chronic over-consumption of energy leads to increased leptin gene expression coincident with increasing adiposity (for reviews, see Houseknecht & Portocarrero, 1998; Ahima & Flier, 2000; Barb *et al.* 2001). Likewise, many laboratories have shown that fasting results in significant down regulation of leptin expression in many species. Indeed, food deprivation has a more profound and immediate impact on leptin expression compared with chronic overfeeding. Although relatively few in number, studies examining the role of specific nutrients on leptin gene expression and activity are beginning to appear in the published literature. Those studies will be the focus of the present review.

Effect of dietary fatty acids on the regulation of appetite and leptin biology

A large body of literature documents that consumption of diets high in fat affects feeding behaviour and the expression of many genes involved in energy homeostasis. As nutrient intake and composition regulate circulating insulin and leptin concentrations, it has been generally accepted that these hormones modulate the effects of nutrients on feeding behaviour and energy homeostasis. A recent report, however, indicates that fatty acids, *per se*, can act at the site of hypothalamic feeding centres to regulate feeding and insulin action (Obici *et al.* 2002a).

Obici *et al.* (2002a) reported that a single bolus injection of the long-chain fatty acid, oleic acid, into the third cerebral ventricle resulted in a reduction of food intake by 24 and 48 h post-dose in Sprague–Dawley rats. This change in feeding behaviour was independent of plasma leptin concentrations and was associated with a reduction in hypothalamic NPY mRNA abundance. These data indicate that this fatty acid serves as a direct signal of nutrient abundance within hypothalamic feeding and metabolic centres. In addition to feeding, intracerebroventricular oleic acid treatment also markedly and rapidly inhibited hepatic glucose production with associated lowering of plasma glucose and insulin. Furthermore, these effects required activation of central nervous system K_{ATP} channels (Obici *et al.* 2002a). These data provide additional evidence that fatty acids can serve as a direct signal of nutrient abundance to the central nervous system, and these effects share common central mechanistic pathways with hormones such as leptin and insulin that regulate whole-body energy homeostasis.

Many groups have examined the effect of high fat feeding on leptin expression; consumption of high-fat diets leads to increased leptin gene expression in adipose tissue (for reviews, see Caro *et al.* 1996; Houseknecht & Portocarrero, 1998). Many studies are confounded by differences in energy intake among treatment groups, and the role of specific fatty acids on leptin biology is not widely documented.

Raclot *et al.* (1997) provided the first evidence that fatty acid profile, specifically consumption of *n*-3 polyunsaturated fatty acids, resulted in the down regulation of rat adipocyte genes involved in lipid metabolism, adipogenesis and feeding, including leptin. In contrast, Cha & Jones (1998) reported that rats fed diets high in *n*-3 polyunsaturated acids (fish oil or safflower-seed oil) developed hyperleptinaemia despite reduced perirenal fat mass and adipocyte size compared with animals fed beef tallow-supplemented diets. These authors did not quantify leptin expression in adipose tissue, however, so it is not clear if their results are in conflict with those reported by Raclot *et al.* (1997). Recently, Peyron-Caso *et al.* (2002) examined the effect of consumption of fish oil on leptin expression in rats made insulin resistant by consumption of high-sucrose diets. They found that consumption of fish oil increased plasma leptin concentrations compared with controls, and prevented the sucrose-induced increase in adiposity and plasma triacylglycerol and non-esterified fatty acid (NEFA) concentrations. These authors (Peyron-Casa *et al.* 2002)

concluded that plasma leptin concentrations could be inappropriately high relative to body fat mass in rats fed diets supplemented with *n*-3 polyunsaturated fatty acids. Finally, Takahashi & Ide (2000) reported that leptin expression is differentially regulated by dietary fat (safflower-seed oil) in various adipose tissue depots in rats; it is not clear which adipose depots provide the greatest contribution of leptin found in circulation in these nutritional experiments.

Spurlock *et al.* (2000) examined the effect of specific dietary fat sources (beef tallow, safflower-seed oil and fish oil) on leptin expression in porcine adipose tissue. It was found that leptin gene expression in porcine subcutaneous adipose tissue is unresponsive to consumption of diets supplemented with saturated or polyunsaturated fats. It is important to note that for pigs, unlike many rodent strains, consumption of high-fat diets leads to reduction of food intake due to the increased energy density of the diet compared with diets not supplemented with dietary fat. This may explain the different responses to dietary fat supplementation between species.

Specific isomers of fatty acids may also have differential effects on leptin gene expression. Studies where animals are treated with conjugated linoleic acid, a group of positional isomers of linoleic acid, have revealed that coincident with anti-obesity (Park *et al.* 1997, 1999; West *et al.* 1998; Tsuboyama-Kasaoka *et al.* 2000; Rahman *et al.* 2001; Ryder *et al.* 2001) and anti-diabetic effects in rodents (Houseknecht *et al.* 1998; Ryder *et al.* 2001) is the observation that plasma and tissue leptin content are, for the most part, down regulated compared with controls (Medina *et al.* 2000; Tsuboyama-Kasaoka *et al.* 2000; Yamasaki *et al.* 2000; Ryder *et al.* 2001). The mechanism(s) underlying the conjugated linoleic acid effects remain to be elucidated but could be linked to peroxisome proliferator activated receptor (PPAR) activation and/or changes in food intake and metabolic rate. Additionally, Kang & Pariza (2001) have shown that leptin secretion from 3T3-L1 adipocytes treated with the *trans*-10, *cis*-12 isomer of conjugated linoleic acid was reduced compared with control, implicating effects on both gene expression and perhaps protein secretion.

In addition to regulating the expression of leptin, consumption of diets high in fat also regulates the expression of leptin receptors and neuropeptides downstream of leptin signalling including POMC and NPY. Expression of leptin-receptor mRNA in the arcuate nucleus and choroid plexus was increased in obese mice fed high-fat diets (8 weeks) compared with those fed low-fat diets (Lin *et al.* 2000). The increase in leptin-receptor expression was coincident with a significant down regulation in the abundance of NPY mRNA. However, following 19 weeks on the high-fat diet, leptin-receptor expression was down regulated. Consistent with the notion that leptin-receptor expression is regulated by high fat feeding, Boado *et al.* (1998) reported that the short isoform of the leptin receptor, Ob-Ra, was up regulated in microvessels of the blood–brain barrier in rats fed high-fat diets. Finally, as dietary fats are able to alter the phospholipid composition of brain membranes (Gibson *et al.* 1984), which in turn can alter receptor function, it is possible that consumption of diets high in saturated or unsaturated fatty acids could have effects on leptin-receptor function.

In addition to leptin receptors, high fat feeding regulates expression of neurotransmitters downstream of leptin such as NPY, AGRP and POMC (Giraudo *et al.* 1994; Guan *et al.* 1998; Lin *et al.* 2000; Wang *et al.* 2002). Furthermore, specific fatty acids differentially affect neurotransmitter expression in mice, as the consumption (for 1 or 7 weeks) of diets high in saturated fat down regulated NPY and AGRP expression compared with diets high in polyunsaturated fats or a low-fat diet (Wang *et al.* 2002).

Consumption of fatty acids not only regulates expression of neurotransmitters involved in feeding, but also has an impact on hypothalamic neuronal activity. Wang *et al.* (1999a) examined the effect of dietary fat intake on the function of hypothalamic neurons, using c-fos-like immunoreactivity as a biomarker. They reported that consumption of diets high in saturated fat resulted in an increase in lateral, and decrease in ventromedial, hypothalamic neuronal activity, predictive of, and associated with, hyperphagia, lower metabolic rate and body-weight gain. These authors also found that in contrast, consumption of diets high in polyunsaturated fatty acids did not alter hypothalamic neuronal function, when compared with animals fed a low-fat diet. Taken together, these data support the notion that dietary fat composition and quantity can profoundly regulate leptin biology as well as hypothalamic signalling pathways downstream of the leptin receptor.

Leptin resistance: receptor signalling attenuation and leptin transport attenuation

The notion of 'leptin resistance' originated with the observations of many investigators that in most obese mammals, including man, circulating leptin concentrations are high, indicating that the organism is resistant to the effects of leptin to inhibit feeding and increase metabolic rate. Leptin resistance is associated with diet-induced obesity (Campfield *et al.* 1995; Van Heek *et al.* 1997; Widdowson *et al.* 1997; El-Haschimi *et al.* 2000; Lin *et al.* 2001b), acute overfeeding (Wang *et al.* 2001a) and ageing (Ahren *et al.* 1997; Li *et al.* 1997; Qian *et al.* 1998a; Sanchez-Rodriguez *et al.* 2000; Scarpace & Tumer, 2001; Wang *et al.* 2001b; Gabriely *et al.* 2002). Also, various dietary and physiological and pathophysiological states as well as exogenous leptin administration can differentially induce central or peripheral leptin resistance (Van Heek *et al.* 1997; Lin *et al.* 2001b; Scarpace *et al.* 2002a). The mechanism(s) responsible for leptin resistance are under intense investigation; current concepts are illustrated in Fig. 2.

At least one mechanism underlying the phenotype of leptin resistance is the apparent saturation of the blood–brain barrier transport system in obese individuals. Leptin crosses the blood–brain barrier via a saturable system (Banks *et al.* 1996; Bartolome & Couce, 2001). The precise mechanisms are not fully elucidated, but the expression of short-form leptin receptors (Bjorbaek *et al.* 1998; Hileman *et al.* 2002) in brain microvessels comprising the blood–brain barrier is consistent with the notion that leptin-receptor isoforms are involved in this transport system (for a review, see Burguera & Couce, 2001).

The notion that impaired leptin transport into the brain underlies leptin resistance is supported by the findings that

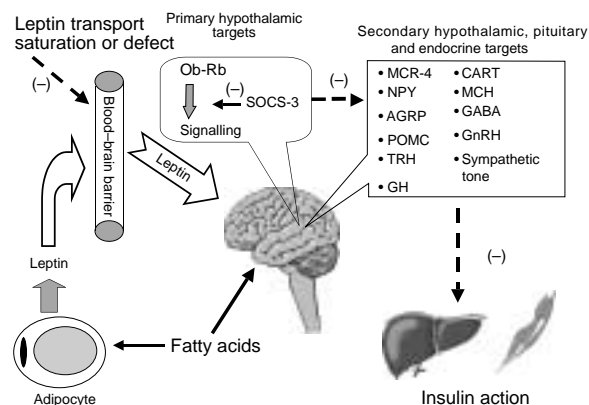


Fig. 2. Current concepts of leptin resistance Ob-Rb, long-form leptin receptor; SOCS-3, suppressors of cytokine signalling-3; MCR-4, melanocortin receptor-4; NPY, neuropeptide Y; AGRP, agouti-related protein; POMC, pro-opiomelanocortin; TRH, thyrotropin-releasing hormone; GH, growth hormone; CART, cocaine and amphetamine-related transcript; MCH, melanin concentrating hormone; GABA, γ amino butyric acid; GnRH, gonadotropin-releasing hormone; (-), lesions involved in leptin resistance mechanism(s).

centrally administered leptin was more efficacious than peripherally administered leptin in stimulating hypophagia and hypothalamic signalling events (Van Heek *et al.* 1997; El-Haschimi *et al.* 2000). Hileman *et al.* (2002) recently reported that leptin uptake into the brain is reduced in mice lacking leptin receptors or rendered obese due to dietary manipulation. Additionally, they reported that New Zealand obese mice have reduced leptin uptake into the brain yet respond to exogenous leptin administered via an intracerebroventricular cannula, again consistent with the hypothesis that leptin resistance is due, at least in part, to a defect in leptin transport into the brain.

A second mechanism underlying leptin resistance appears to be attenuation of leptin-receptor signal transduction. The precise mechanisms underlying the attenuation in leptin signalling observed with leptin resistance (El-Haschimi *et al.* 2000; Scarpace & Tumer, 2001) are not known; however, SOCS-3 expression and activity may play a role. SOCS-3 expression is induced by leptin-stimulated STAT-3 activation and is up regulated in the hypothalamus of obese animals (Bjorbaek *et al.* 1998, 1999; Banks *et al.* 2000) and in cell models of leptin resistance (Sweeney, 2002). In addition to the hypothalamus, SOCS-3 is over-expressed in white adipose tissue of obese rodents and inhibits insulin signalling in adipocytes (Emanuelli *et al.* 2001).

Recent evidence (Scarpace *et al.* 2002a,b) indicates dissociation in the effects of leptin resistance on appetite and thermogenesis. Chronic leptin treatment (via adenovirus) induced leptin resistance in terms of appetite regulation, while the thermogenic effect of leptin was sufficient to maintain the weight loss achieved during the period of hypophagia. These authors also reported that STAT-3 phosphorylation was maintained over the course of the study (in the face of elevated SOCS-3), indicating that mechanisms downstream of STAT activation are responsible for the differential leptin resistance.

The concept of leptin resistance is growing in complexity with the steady emergence of data (predominantly from the rodent literature) indicating that the onset of leptin resistance can be quite rapid (days; well before the development of obesity), and the rate and duration of onset and degree of hyperleptinaemia associated with leptin resistance may differ depending upon the physiological response being studied. Some authors (Arch *et al.* 1998) have argued that leptin resistance may not be a relevant physiological phenomenon in obese human individuals given the fact that diminished response to leptin (at least in terms of feeding) can occur within the normal range of plasma leptin concentrations. As leptin has many physiological roles in addition to appetite regulation, the notion of leptin resistance is being expanded by many scientists to encompass the complexity of leptin action in multiple physiological and pathophysiological systems. Recent work by Mark *et al.* (2002) advances the concept of selective leptin resistance in man in the context of physiological effects of leptin on cardiovascular biology. They suggest that obese individuals can be resistant to the appetite-regulating effects of leptin yet remain sensitive to the increased sympathetic activity and arterial pressure induced by hyperleptinaemia. Clearly, additional research is needed to elucidate the mechanisms underlying leptin resistance and to determine the full cohort of physiological implications of hyperleptinaemia associated with obesity and the metabolic syndrome in man.

Leptin resistance: role of nutrition

The majority of work examining leptin resistance *in vivo* has examined the effect of diet-induced obesity on leptin action. In fact, the first published evidence of leptin resistance described the phenomenon in mice fed a high-fat diet (diet-induced obesity; Campfield *et al.* 1995). Specifically, mice made obese by diet required a higher dose of exogenous leptin than ob/ob mice to elicit similar weight loss (30 *v.* 6 µg leptin, respectively). Mooradian *et al.* (2000) found that consumption of monosaccharide-enriched diets caused hyperleptinaemia with no change in food intake; the hallmark of leptin resistance. With the exception of the work of Mooradian *et al.* (2000), the majority of the work examining the effect of nutrition on leptin resistance has examined the effect of dietary fat consumption on leptin resistance.

The ability of high fat feeding to induce leptin resistance (lack or attenuated feeding response to intracerebroventricular or peripheral leptin administration) has been observed in rats (Widdowson *et al.* 1997; Lin *et al.* 1998, 2001*b*) as well as mice, and the effect of fat feeding is reversible upon dietary change to a low-fat diet, in the absence of reduced body weight (Widdowson *et al.* 1997). Furthermore the dietary fat-induced leptin resistance occurs quickly (within 5 d), as does the reversal of leptin resistance following the change from a high-fat to a low-fat, high-carbohydrate diet (within 1–5 d; Lin *et al.* 2001*b*).

The effect of high fat feeding to induce leptin resistance is complex in that in some rodent models the effect is to induce peripheral but not central leptin resistance (Van Heek *et al.* 1997; Lin *et al.* 1998; El-Haschimi *et al.* 2000). The reasons for differences among rodent strains in terms

of presentation of central *v.* peripheral leptin resistance are not clear; however, the data suggest that multiple mechanisms including attenuated signalling and transport into the brain underlie leptin resistance in diet-induced obesity.

Nutritional programming of obesity: leptin as candidate gene

The concept that nutritional exposure during critical developmental windows pre- and postnatally can influence or 'programme' long-term growth, development and disease susceptibility is known as 'nutritional programming' (for a brief review, see Lucas, 1994). Nutritional programming has been well described in animals and evidence is beginning to mount that it may play a role in human development as well. Given the recent increase in global rates of obesity and the coincident negative impact upon public health, the search for candidate genes that may be involved in the regulation of adiposity and the predisposition to obesity, insulin resistance, hypertension and other sequelae associated with the metabolic syndrome has accelerated. Data suggest that leptin may be a candidate gene involved in regulation of fetal programming for obesity and diabetes.

A historical cohort study of men born during the Dutch famine of 1944–5 revealed elevated rates of obesity in young adult men born of mothers who were exposed to undernutrition during the first half of gestation whereas men whose mothers were exposed to famine during the last trimester and during the early postnatal period had lower rates of adult obesity (Ravelli *et al.* 1976). Others have reported that low birth weight in human infants is associated with adult insulin resistance, hypertension and CHD (Lucas, 1994; Barker, 1995). Additionally, infants born of mothers with non-insulin-dependent or insulin-dependent diabetes mellitus or glucose intolerance have a higher risk of developing childhood obesity (Silverman *et al.* 1991).

The mechanism(s) underlying the effects of fetal programming on subsequent obesity remain to be fully elucidated. It has been proposed that undernutrition during a critical period of hypothalamic development plays a critical role in this process (Reynolds & Phillips, 1998). Additionally, leptin is now implicated in playing a role in fetal programming (Ekert *et al.* 2000). Infants from diabetic mothers have elevated cord blood leptin concentrations, and these concentrations are positively correlated with infant adiposity (Cetin *et al.* 2000). Vickers *et al.* (2000) developed a rodent model of fetal undernutrition to predict effects of prenatal nutritional programming associated with intra-uterine growth retardation in man. These authors reported that offspring from underfed mothers were smaller at birth and had higher food intake early in the postnatal period that was maintained throughout life. Additionally, consumption of a high-energy fat-supplemented diet postnatally exacerbated the hyperphagia in these animals. In addition to adult hyperphagia, offspring from underfed mothers presented hyperinsulinaemia, hyperleptinaemia and elevated systolic blood pressure. These data are consistent with the notion that fetal programming plays a key role in predisposing offspring to obesity and metabolic disease as adults. In a subsequent report, these authors provide evidence that the negative effects of fetal undernutrition on the

programming of adult metabolic disease could be at least partially overcome by exogenous insulin-like growth factor-1 treatment (Vickers *et al.* 2001).

Finally, Singhal *et al.* (2002) examined the effect of nutritional programming during the early postnatal period and found that a high plane of nutrition during this period is positively correlated with the development of adult obesity. Specifically, serum leptin concentrations were quantified in adolescent children who had been born preterm and assigned at birth to receive either banked breast milk, nutrient-enriched preterm formula or normal formula. The leptin:body-fat mass value was greater in children receiving higher-energy preterm formula compared with either standard formula or breast milk despite no significant differences in adiposity. These authors suggest that the leptin:fat mass ratio is an early indicator of leptin resistance and subsequent obesity.

Leptin regulation of lipid metabolism

In this section, the regulation of lipogenesis and lipolysis in pre-adipocytes and adipocytes by leptin will be discussed. The discovery of the *obese* gene (Zhang *et al.* 1994) was followed quickly by the determination that exogenous leptin reduced adiposity in the leptin-deficient *obese* mouse (Pellemounter *et al.* 1995), decreased feed intake (Campfield *et al.* 1995), and stimulated energy expenditure (Halaas *et al.* 1995). When Bai *et al.* (1996) reported that incubating pre-adipocyte cultures with leptin reduced acetyl-CoA carboxylase expression and activity, and decreased lipogenesis, it became apparent that leptin might act directly upon the adipocyte. These findings, coupled with the presence of leptin receptors in adipose tissue and skeletal muscle of several species (Tartaglia *et al.* 1995; Löllmann *et al.* 1997; Lin *et al.* 2000), prompted investigations of the role of leptin in lipid metabolism in the adipocyte. Results of several investigations have established unequivocal linkages of leptin to lipid metabolism in the adipocyte.

In rodent models, leptin acts directly to suppress the inhibition of β -adrenoceptor-mediated lipolysis by insulin, and simultaneously reduces insulin-induced glucose transport and lipogenesis (Müller *et al.* 1997), and similar effects have been reported in porcine adipocytes derived from stromal-vascular cultures (Ramsay, 2001). However, leptin also acts directly to stimulate lipolysis in adipocytes in the absence of insulin (Frühbeck *et al.* 1997, 1998). Mechanistically, several things must be considered. Hormone-sensitive lipase is activated by protein kinase A as a result of the production of cAMP by adenylate cyclase, and is the major mediator of lipolysis within the adipocyte (Ramsay, 1996). Although the effect of leptin on the expression of hormone-sensitive lipase in adipose tissue has been addressed in two rodent studies, one in which expression was increased (Sarmiento *et al.* 1997), and one in which it was not (Scarpace *et al.* 1998), the regulation of the adenylate cyclase-cAMP system has not been studied. Leptin expression itself is down regulated by cAMP (Sliker *et al.* 1996), and in the study of Wang *et al.* (1999c), leptin stimulated lipolysis and also decreased leptin expression. If noradrenaline, a potent inducer of cAMP

production, had reduced leptin expression, one might conclude that leptin acts solely through the production of cAMP to activate lipolysis. However, this is not the case. Recent work published by Frühbeck *et al.* (2001) indicates that leptin antagonizes the tonic inhibition of lipolysis by adenosine. Leptin is a member of the class-I family of helical cytokines (Madej *et al.* 1995), as is growth hormone. It is thus interesting that growth hormone, with similarities to leptin in receptor signalling (Tartaglia *et al.* 1995), alters the functionality of the inhibitory G protein (i.e. diminished ADP-ribosylation by pertussis toxin) in adipose tissue (Houseknecht & Bauman, 1997). In this study, the diminished functionality of inhibitory G protein was associated with an enhancement of lipolysis by growth hormone. Although similar results were not obtained in ovine adipocytes, growth hormone nonetheless antagonized the anti-lipolytic action of an adenosine analogue, an effect probably stemming from a stifled interaction between the inhibitory G protein α subunit and adenylate cyclase (Doris *et al.* 1998).

It is also important to consider recent publications in which leptin has been reported to stimulate phosphodiesterase activity (Zhao *et al.* 1998), the mechanism that underlies the suppression of cAMP-mediated insulin secretion by leptin in pancreatic islets (Ahren & Havel, 1999). It is perplexing that leptin mimics insulin signalling and antagonizes the stimulation of cAMP production by glucagon in isolated hepatocytes (Zhao *et al.* 2000). However, the absence of the long form of the leptin receptor in these hepatocytes indicates that this response is mediated by the short receptor isoform. This is consistent with the findings of Müller *et al.* (1997), in which leptin failed to influence lipolysis in the *db/db* mouse, an obese model that does not express the long form of the leptin receptor, but does express the short form. It is probable that the stimulation of lipolysis and attenuation of lipogenesis in the adipocyte is mediated via the long form of the leptin receptor.

Leptin regulation of fatty acid oxidation

Here, the fatty acid oxidation, adipose ablation and apoptosis in adipose tissue as caused by leptin will be considered. Early work with models in which hyperleptinaemia was induced (Chen *et al.* 1996; Shimabukuro *et al.* 1997) indicated that specific adipose depots were virtually obliterated. Furthermore, the recovery of body fat in hyperleptinaemic rats was much slower than in diet-matched controls (Higa *et al.* 2000). The implications of these results were that there was a sustained impact of hyperleptinaemia on the ability of adipocytes to accumulate and store lipid. A portion of this response is possibly related to an induction of apoptosis and loss of adipocytes caused by elevated leptin concentrations within the brain (Qian *et al.* 1998b), if the higher circulating concentrations also achieve higher concentrations within the central nervous system. However, the majority of the response is probably due to an induction of fatty acid release, and concomitant up regulation of genes that regulate fatty acid oxidation (i.e. acyl-CoA oxidase and carnitine palmitoyl transferase) that is driven by increased PPAR α expression (Wang *et al.* 1999c). In fact, an up regu-

lation of PPAR α has recently been shown to be a prerequisite for depletion of body fat by leptin. Using wild-type and PPAR $\alpha^{-/-}$ mice, Lee *et al.* (2002) determined that the depletion of body fat in the null mice during hyperleptinaemia is markedly lower than in wild-type mice. This effect was associated in part with the failure of hyperleptinaemia to achieve an up regulation of carnitine palmitoyl transferase in adipose tissue due to the absence of PPAR α .

The physiological changes associated with hyperleptinaemia shift the metabolic goal of adipocytes from lipid storage to lipid disposal (Fig. 3). Leptin binding to the long form of the receptor attenuates lipogenic activity through the suppression of acetyl-CoA carboxylase and fatty acid synthase activity. Lipolysis is stimulated by direct or indirect activation of hormone-sensitive lipase through antagonisms of the inhibitory effects of adenosine and insulin. The expression of PPAR α is increased, and up regulates the expression of oxidative genes, acyl-CoA oxidase, carnitine palmitoyl transferase-1, and uncoupling proteins. Consequently, the cellular oxidation of NEFA is increased and the majority of NEFA are consumed internally. Also of considerable significance is that leptin ultimately results in a de-differentiation of adipocytes (Zhou *et al.* 1999), as indicated by the loss of adipocyte marker (aP2) and concomitant appearance of the pre-adipocyte marker, Pref-1. Collectively, these findings indicate that leptin may be used to reduce adipose mass, but it is important to note that these extreme results are achieved only with pharmacological circulating concentrations of leptin (Higa *et al.* 2000) and are not indefinite.

Although the most pronounced effects of leptin on body fat are achieved with pharmacological blood levels, significant shifts in metabolism have been noted under less extreme conditions. Specifically, Chen & Heiman (2000, 2001) have shown in rodent models that daily peripheral

injections of leptin caused a sustained stimulation of lipid utilization that was reflected in reduced plasma NEFA and triacylglycerol concentrations, and in lower respiratory quotients relative to controls. Furthermore, Reidy & Weber (2002) determined in rabbits that a single intravenous leptin bolus stimulated lipolysis (increased serum NEFA concentrations) and enhanced triacylglycerol-fatty acid cycling as a means of energy expenditure.

Leptin regulation of lipid accumulation: beyond the adipocyte

Multiple metabolic complications arise when the lipid content of non-adipocytes rises above tightly controlled normal levels (for a detailed review, see Unger, 2002). Thus, it is of considerable interest that the regulation of lipid metabolism by leptin extends beyond the adipocyte. Leptin deficiency or leptin resistance results in an over-accumulation of triacylglycerol in non-adipocytes, such as pancreatic islet cells, whereas leptin administration or improvements in sensitivity normalize triacylglycerol homeostasis. Early in the development of diet-induced obesity, circulating leptin concentrations rise. Previous work has shown that with an intact leptin-leptin receptor coupling, non-adipocytes maintain normal concentrations of triacylglycerol reserves, even when fed as much as 60 % dietary fat (Zhou *et al.* 1998); however, in the absence of an intact leptin-leptin receptor axis, excessive accumulation of lipid occurs in islets on as little as 6 % dietary fat. These findings and observations are the foundation on which Unger *et al.* (1999) proposed that confining intracellular lipid stores to appropriate and non-detrimental concentrations is a major function of leptin in non-adipocytes.

The Zucker diabetic fatty (ZDF) rat has been of considerable interest as a model to study this biological role for leptin. These rats markedly over-accumulate lipid in pancreatic islets, in part because they have tremendous capability for esterification of fatty acids (Lee *et al.* 1997), and for *de novo* fatty acid synthesis (Zhou *et al.* 1998). Chronic hyperleptinaemia in normal controls down regulates lipogenic enzymes and suppresses lipid accumulation from labelled glucose, but has no effect in ZDF rats. Furthermore, islets from normal rats respond similarly to recombinant leptin, *in vitro*, whereas islets from the receptor-deficient ZDF rats are largely unresponsive (Zhou *et al.* 1998). Transgenic over-expression of leptin receptors (confined to the liver) of the ZDF rat sensitizes the liver to the anti-steatotic effects of leptin, and further substantiates that this is a direct effect of leptin, rather than a centrally mediated action (Lee *et al.* 2001).

The mechanism for this critical role of leptin is such that there is a coordinated shift in metabolism from storage to oxidation of fatty acids. Generally, this transition from storage to oxidation involves an enhancement of systems that transport and oxidize fatty acids, and a repression of lipogenic systems similar to what is seen in adipocytes (Fig. 3). However, the principal mechanism differs by tissue, and seems to reflect whether pre-existing oxidative machinery within a given cell type is capable of handling the increased oxidation of lipid brought about by leptin (Lee *et al.* 2001).

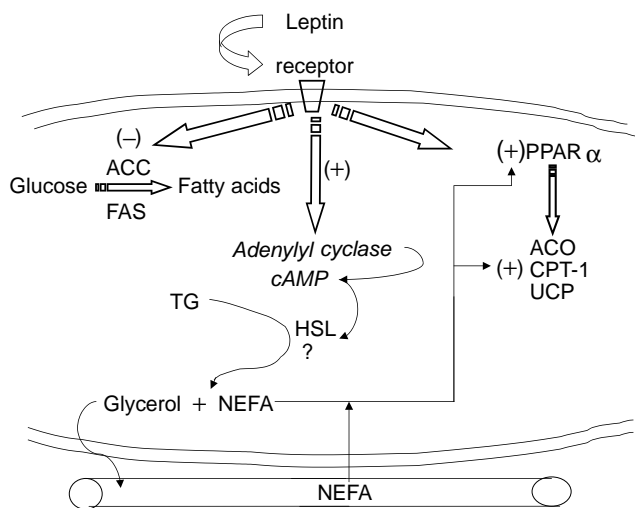


Fig. 3. Model of regulation of lipid metabolism in peripheral tissues by leptin. ACC, acetyl-CoA carboxylase; FAS, fatty acid synthase; PPAR α , peroxisome proliferator activated receptor alpha; TG, triacylglycerols; HSL, hormone-sensitive lipase; ACO, acyl-CoA oxidase; CPT-1, carnitine palmitoyl transferase-1; UCP, uncoupling proteins; NEFA, non-esterified fatty acids; (+), stimulate; (-), inhibit.

Skeletal muscle constitutes the major proportion of whole body mass, and is an important determinant of metabolic rate and energy metabolism (Zurlo *et al.* 1990). There is an intriguing relationship between leptin and lipid metabolism emerging in skeletal muscle that is, overall, quite consistent with the concept that this hormone regulates lipid storage in non-adipocytes and also stimulates skeletal muscle thermogenesis. Early work indicated that leptin acts on skeletal muscle to attenuate the pro-lipogenic actions of insulin (Muioio *et al.* 1997), and to stimulate fatty acid oxidation while diminishing triacylglycerol accumulation (Muioio *et al.* 1997, 1999; Steinberg *et al.* 2002c). Subsequent work has established that with chronic administration of leptin (2 weeks), there is a sustained enhancement of fatty acid oxidation and triacylglycerol hydrolysis in rat muscle that is apparent in both the resting and contracting states (Steinberg *et al.* 2002a). Additional work from this group (Steinberg *et al.* 2002b) now indicates that chronic leptin administration also diminishes fatty acid transport capacity by reducing the abundance of both the fatty acid translocase and fatty acid-binding protein in plasma membrane fractions of red and white muscles. The activity of the 5'-AMP-activated protein kinase seems to be a critical component by which leptin stimulates fatty acid oxidation in skeletal muscle. Recent work (Minokoshi *et al.* 2002) has established quite clearly that leptin activates this kinase in skeletal muscle, which monitors intracellular fuel status, and promotes fatty acid oxidation by inhibiting lipogenic activity at the level of acetyl-CoA carboxylase. Although the long-term regulation of this enzyme by leptin may be mediated through a central mechanism, the acute regulation is a direct effect on the skeletal muscle. It is also of interest that this particular mechanism for leptin may be specific to skeletal muscle. Others (Atkinson *et al.* 2002) determined that whereas leptin also stimulates fatty acid oxidation in cardiac muscle, the effect is achieved independent of activation of the 5'-AMP-activated protein kinase.

The regulation of lipid metabolism in skeletal muscle is conceivably quite important in the development of insulin resistance. Steinberg *et al.* (2002c), using human skeletal muscle preparations, determined that leptin stimulates fatty acid oxidation in lean subjects, but found preparations from obese individuals to be quite resistant to leptin. Likewise, fatty acid uptake and esterification was greater in the obese subjects, despite higher circulating concentrations of leptin. Though it is inappropriate to extend these findings directly to the *in vivo* situation, these data suggest that leptin resistance is a factor in the over-accumulation of triacylglycerol in muscle that is associated with insulin resistance and obesity. It is also significant as regards the regulation of lipid in skeletal muscle that Dulloo *et al.* (2002) have shown that leptin stimulates skeletal muscle thermogenesis in *ex vivo* preparations from lean mice, and that this response is diminished by diet-induced obesity in the fed state.

As with other peripheral tissues, the leptin receptor has been identified in hepatocytes, and the regulation of lipid metabolism in the liver has received considerable attention. The *ob/ob* mouse, which lacks functional leptin protein, is characterized by global defects in hepatic lipid metabolism including the regulation of fatty acid synthesis, fatty acid oxidation, cholesterol and bile salt synthesis and transport.

In an elegant experiment using the *ob/ob* mouse as the model, Liang & Tall (2001) used suppression subtractive hybridization and cDNA microarray technology to identify genes responding to exogenous leptin (*v. pair-fed* controls). Consistent with the concept of leptin as a regulator of lipid metabolism in peripheral tissues, and with the essentiality of PPAR α as a mediator of enhanced lipid oxidation in liver (Lee *et al.* 2002), these researchers identified linkages among PPAR α and genes related to pathways for β - and ω -oxidation of fatty acids. Furthermore, a reduction in lipogenic capacity was indicated by reductions in the mRNA of lipogenic genes, such as fatty acid synthase and sterol regulatory element-binding protein (SREBP)-1, which were probably achieved with a reduction in serum insulin.

Over-accumulation of lipid in the liver of *ob/ob* mice accompanies the leptin deficiency and insulin resistance common to this mouse, and Yahagi *et al.* (2002) have just reported that the fatty liver condition is attenuated in SREBP-1 knockout mice that were generated on an *ob/ob* background. It is also of interest to note that leptin deficiency contributes to the fatty liver condition that results from conditions of lipoatrophy (for a detailed review, see Unger, 2002). Exogenous leptin results in marked improvements in lipid homeostasis in the lipoatrophic A-ZIPF-1 mouse, and in the α P2-n SREBP-1 model (Colombo *et al.* 2002). Thus, the direct or indirect control of SREBP-1 by leptin is critical to the prevention of the fatty liver syndrome that develops with the insulin resistance common to obesity or lipoatrophy. The alterations in gene expression achieved with leptin administration are consistent with an overall suppression of lipogenesis and an enhancement of lipid oxidation, and reflect a metabolic substrate change from carbohydrate to lipid utilization.

Conclusion

Leptin plays a pivotal role in energy balance and nutrient sensing; its expression and function are regulated by energy intake as well as specific dietary fatty acids. Additionally, there is growing evidence that leptin plays an important role in the regulation of lipid metabolism, and specifically the regulation of lipid storage in adipocytes as well as other tissues including skeletal muscle and the pancreas. Finally, leptin is implicated in nutritional programming that may predispose individuals to adult onset obesity and related metabolic disorders such as diabetes. Additional work is needed to enhance our understanding of the role of specific dietary components in the regulation of leptin expression, function and leptin resistance, as well as the interaction between diet and nutrient intake and the genetic background and physiological and pathophysiological state of the organism being studied.

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