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Perspective

Leptin, a Pleiotropic Hormone: Physiology, Pharmacology, and Strategies for Discovery of Leptin Modulators

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Introduction

White adipose tissue (WAT) is mainly composed of adipocytes, cells which store energy in the form of triglycerides in times of nutritional affluence and release free fatty acids during nutritional deprivation.[1,2] WAT mass is determined by the balance between energy intake and expenditure and is controlled by genetic, neuroendocrine, and environmental factors.[1,2] Under normal conditions the balance between energy intake and expenditure is carefully regulated so that adipose tissue mass remains constant and remains close to a well-defined "set-point". Alterations in body weight, such as seen after weight reduction, are spontaneously corrected, and weight tends to return to its original set-point. This contributes, for example, to the low long-term succes rate of weight reduction programs. Under certain conditions, however, perturbances of this steady state can lead to a chronically increased or decreased quantity of WAT, such as seen in obesity or in lipodystrophic syndromes, respectively.[1,2] Obesity affects between 25% and 30% of the population in industrialized countries, and its frequency is still rising at an alarming rate.[3,4] It is associated with increased risk of death from any cause[5] and is furthermore an independent risk factor for insulin resistance, non-insulin-dependent diabetes mellitus, and coronary artery disease. Conversely, decreased amounts of adipose tissue, such as seen in malnutrition, cachexia, or anorexia nervosa, are also associated with important morbidity and mortality.

This tight coupling between energy intake and expenditure implies the necessity of a regulatory system that keeps the body constantly informed on its energy stores. This formed the basis of a long quest to identify such signaling molecules. In theory, such a regulatory system needs to be capable of both sensing energy intake, storage, and usage and relaying this sensory input to an effector system, capable of integrating all this information and of generating an adequate compensatory response. Solid evidence for such a signaling system in rodents was provided more then 20 years ago by parabiosis (cross-circulation) experiments between *ob/ob* and *db/db* mice.[6] In this condition only *ob/ob* mice reduced their food intake and lost weight. This led Coleman to suggest that the phenotype in the *ob/ob* mouse was caused by the absence of a circulating signaling molecule. The *ob/ob* mice can, however, respond to this molecule when it is provided, whereas *db/db* mice produce this signaling factor but cannot respond to it. The *obese* gene, which is mutated in the *ob/ob* mice and which codes for this signaling factor later called leptin (Greek for 'thin'), was isolated in 1994 by Jeffrey Friedman's group after a long positional cloning effort.[7] Very few proteins received so much scientific and media coverage as leptin. Between its initial discovery at the end of 1994 and August 1998, more than 1200 papers have appeared on the subject, and its discovery completely changed our perception of body weight homeostasis. This discovery furthermore revitalized the obesity field, where new molecules playing an important role in energy homeostasis are now being reported almost monthly.

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In the present Perspective, we will summarize the recent developments in the leptin field with particular attention to clinical aspects and drug discovery strategies. In view of the wealth of the existing literature, many important contributions to this field cannot be cited because of space limitations.

Leptin Biology

Leptin, a Cytokine Acting on a Cytokine-Type Receptor. Mice homozygous for the *obese* mutation (*ob/ob* mice) have several characteristics in common with starved animals: hyperphagia, decreased energy expenditure, reproductive deficiency, and delayed growth. Leptin was first cloned in an effort to identify the molecular defect underlying the phenotype in the *ob/ob* mouse[7] and was suggested to be the signaling factor from Coleman's experiments.[6] The human leptin protein, a polypeptide of 167 amino acids, is encoded by a gene on chromosome 7q31,[8−10] which is composed of three exons covering more than 15 kb.[10−12] The sequence coding for the leptin protein is contained in exons 2 and 3, whereas exon 1 codes for the 5′-untranslated region.[10−12] Leptin transcription is controlled by a promoter located 5′ of the transcription initiation site in exon 1.[11,12] The description of the gene structure in the mouse also facilitated the definition of the genetic abnormalities in the two types of *ob/ob* mice. The first type has a nonsense mutation, resulting in the production of a nonfunctional gene product (C57Bl/6J *ob/ob*).[7] The second strain carries a genomic mutation resulting in the complete absence of ob mRNA (SM/Ckc-$^{+Dac}$-ob^{2J}/ob^{2J}).[7]

Considerable progress has been made in the determination of the tertiary structure of the leptin protein. The crystal structure at 2.4-Å resolution of a mutant human leptin protein (leptin-E-100), which has comparable biological activity to that of wild-type leptin but crystallizes more readily, has been reported.[13] The structure reveals a four-helix bundle similar to that of the long-chain helical cytokine family, which also includes IL-6, IL-11, IL-12, LIF, G-CSF, CNTF, and oncostatin M.[13] The leptin protein contains two cysteine residues, Cys^{96} and Cys^{146}, which form a disulfide bond and hence are in close spatial proximity.[13] This disulfide bond is crucial for structural integrity and stability of the protein.[14]

Leptin was originally thought to be exclusively produced in adipose tissue, with high levels in white adipose tissue and low levels expressed in brown adipose tissue.[7] Recently it was, however, shown that two other tissues also produce significant amounts of leptin. In pregnant women substantial amounts of leptin are produced in the placenta,[15−17] resulting in an increase in circulating leptin levels. Gastric epithelium is another site of leptin production, although the levels of leptin mRNA and protein seem somewhat lower than those in white adipose tissue.[18] After synthesis leptin is secreted in a pulsatile fashion from adipose tissue into the bloodstream.[19,20] The release of leptin by the gastric epithelium is triggered by food intake and CCK-4.[18] In the plasma leptin circulates, either free or complexed to a binding protein,[21,22] which might be a truncated form of its receptor.[23] The proportion of bound/free leptin varies with body mass. In humans, higher proportions of total leptin circulate in the bound form in lean, relative to obese, subjects and free leptin increases with body mass index (BMI).[21,22] This property of leptin adds to the variability in the determination of leptin concentration and also affects the physiological impact of the protein. During pregnancy the amount of bound leptin also increases in the maternal circulation.[23]

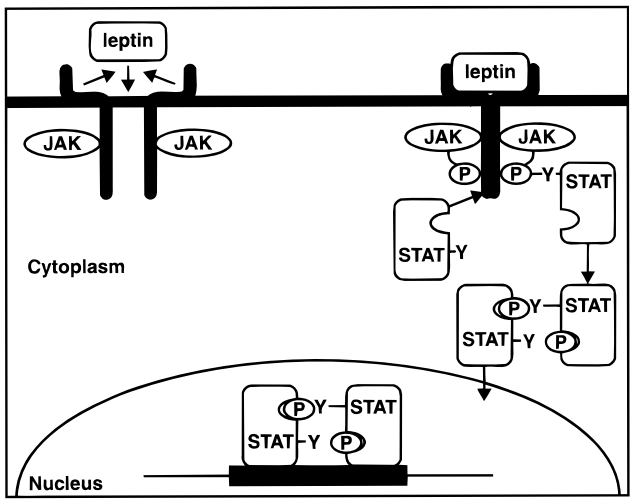


Figure 1. Signaling by the leptin receptor. Leptin binding induces dimerization of the receptor and activation of the associated JAK-2 which phosphorylates tyrosine (Y) residues on the receptor, creating phosphotyrosine docking sites for STAT proteins. After phosphorylation, these STAT proteins dissociate from the receptor and form dimers. STAT dimers are transported into the nucleus and control expression of responsive genes via binding to response elements (black box).

The multiple metabolic effects of leptin are achieved by its interaction with specific leptin receptors, located both in the central nervous system as well as in peripheral tissues[24−26] (Figure 1). The leptin receptor belongs to the class I cytokine receptor family, which uses gp130 as a signal-transduction component in its receptor complex. Ligand activation of this group of receptors, which also contains among others the IL-6, LIF, GH, CNTF, and oncostatin M receptors, results ultimately in activation of signal transducers and activators of transcription (STATs) (reviewed in refs 27 and 28). Although induction of receptor oligomerization by leptin still has to be demonstrated, the structural similarity of the ligands of this class of cytokine receptors suggests that leptin is no exception concerning its interaction with its receptor. Once activated the leptin receptor transmits its signal via *janus* kinase 2 (JAK-2)[29] to STAT 3, 5, and 6.[30−33] This subset of STATs has received the name of "fat-STATS".[34] One additional interesting aspect of the activation of the leptin receptor is the fact that it induces a fast activation of ATP-sensitive potassium channels both in the central nervous system[35] and in peripheral tissues.[36,37] It is at present unknown how the activity of leptin on cellular channels ties back to its reported activity on JAK-2 and STAT action.

Multiple splice variants of the leptin receptor are produced from the same gene, during a complex tissue-specific splicing process.[25,26,38] The different leptin receptors include a long form, which is present in the central nervous system,[24−26] on endothelial cells,[33] and on CD4+ T cells[39] and which is presumed to mediate

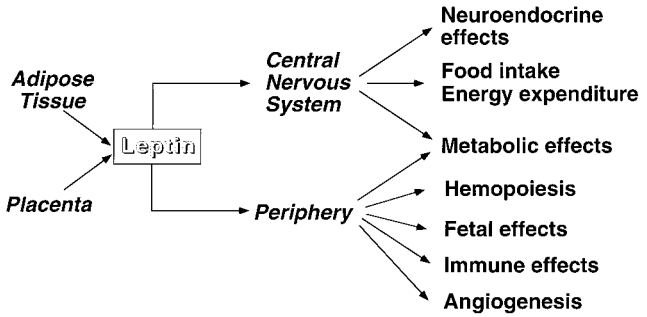


Figure 2. Schematic model of the various signaling pathways in which leptin is implicated. Neuroendocrine effects include effects on the reproductive system as well as effects on the thyroid gland and adrenal gland. The metabolic effects include effects on glucose homeostasis which could be mediated via either the central nervous system or the peripheral nervous system.

most or all of leptin's effect. In addition, several shorter forms of the leptin receptor have been described, which although substantially weaker than the long form, are still able to transmit signal transduction via the JAK/STAT pathway.[40] The leptin receptor has been shown to be mutated in *db/db* mice[25,26,38] as well as in the Zucker fatty (*fa/fa*) rat[38,41−43] and the spontaneously hypertensive obese Koletsky rat,[44] and its malfunctioning underlies the extreme leptin resistance in these animals.

Transport through the Blood−Brain Barrier and Central Effects of Leptin. To reach its central site of action (Figure 2), leptin crosses the blood−brain barrier via a saturable transport system which might involve the short form of the leptin receptor.[45−48] Leptin concentration in the cerebrospinal fluid (CSF) is strongly correlated in a nonlinear manner with plasma leptin levels and BMI.[47,48] In obesity, there is a less important increase in the CSF leptin levels relative to the increase in serum leptin. When expressed as CSF/serum leptin ratio, higher ratios are found in lean compared to obese subjects, suggesting a reduced efficacy of brain leptin transport and a relative leptin deficiency in the central nervous system in obese individuals.[47,48] Therefore, beyond a certain leptin level (estimated around 25 ng/mL), the CSF leptin levels do not increase accordingly, suggesting that exogenous administration of leptin or stimulation of leptin production might be ineffective to treat obesity.

The central nervous system in general and the hypothalamus in particular have been suggested to be the critical targets for the satiety effects of leptin (Figure 2), as supported by several arguments including: (1) the loss of leptin's satiety effects associated with lesions of the ventromedial hypothalamus;[49,50] (2) the reproduction of the powerful anorexic and metabolic effects of leptin after central administration of minute quantities of leptin which do not influence circulating leptin levels;[51−53] (3) the specific binding of leptin to hypothalamic plasma membranes;[52] (4) the modulation of neuronal activity in the hypothalamus by leptin;[35,54,55] and (5) the expression of the full-length leptin receptor in the hypothalamus.[24,56] The complete set of genes involved in mediating the downstream effects of activation of the central leptin receptor and STAT transcription factors on energy metabolism is currently unknown.

One candidate effector molecule may be the hypothalamic neuropeptide Y (NPY), which is a potent stimulator of food intake and whose synthesis is inhibited by leptin.[52,57,58] From studies in animals with a mutated NPY gene, however, it became evident that NPY cannot be the sole mediator of leptin's actions.[57,59] Another signaling pathway, initially thought to be downstream of the leptin receptor in the brain, includes the melanocortin system composed of melanocyte-stimulating hormone and its receptor, the melanocortin-4 (MC-4) receptor.[60,61] One set of experiments arguing for a potential role of the melanocortin system in leptin signaling relied on the use of a synthetic antagonist of the MC-4 receptor, which blocked leptin's inhibitory effect on food intake.[62] Opposite data were, however, obtained in animals carrying both the *ob/ob* mutation and the *lethal yellow* (A^y/a) mutation, which results in the constitutive ectopic expression of the *agouti* peptide, a potent antagonist of the MC-4 receptor.[63] In these genetic studies both the leptin and melanocortin pathways were shown to have an independent and additive effect on murine obesity,[64] suggesting that obesity in the *lethal yellow* mouse, caused by the *agouti* peptide, is independent of leptin's action on energy homeostasis.[64] Other candidate genes, whose activity could be modulated by leptin and are active in the brain, include the glucagon-like peptide-1 (GLP-1),[65] corticotropin-releasing hormone (CRH or CRF) or the related factor urocortin,[66−68] orexins,[69] and melanin-concentrating hormone (MCH).[70] However, until now, no experimental evidence is available to support a role of these pathways in leptin signaling.

Peripheral Actions of Leptin. In addition to its actions in the central nervous system, leptin also has direct effects on peripheral tissues (Figure 2). This observation is supported by the expression of several of the leptin receptor isoforms in peripheral tissues.[24−26,33,39] Opponents of this hypothesis, however, point out that the majority of the leptin receptors present in peripheral tissues are truncated and have less signaling capacity.[40] The observation that addition of leptin generates profound biological responses in cultured hepatocytes,[71] adipocytes,[72−75] muscle cells,[76,77] hemopoietic cells,[29,39,78,79] granulosa cells,[80] adrenocortical cells,[81] endothelial cells,[33] and pancreatic islet cells,[36,37,74] however, supports a direct peripheral action of leptin. Further support for a direct effect of leptin on peripheral tissues came from its action on isolated soleus and extensor digitorum muscles.[82,83]

The peripheral actions of leptin have been mainly evaluated in a metabolic perspective. The effects of leptin on lipid metabolism in cultured cells is especially intriguing in this context. Leptin directly decreases intracellular lipid concentration in several tissues through a reduction of fatty acid and triglyceride synthesis and a concomitant increase in lipid oxidation.[74,83,84] It is postulated that this effect on lipid metabolism is mediated by an inhibitory effect of leptin on acetyl-CoA carboxylase (ACC) activity, the rate-limiting enzyme in fatty acid synthesis, whose expression decreases after leptin administration.[72,84] Inhibition of ACC leads to a reduction in malonyl-CoA levels, a known inhibitor of carnitylacyltransferase I (CPT-1) and mitochondrial β-oxidation.[84] In addition to this indirect

stimulatory effect on CPT-1 activity, leptin also increases the CPT-1 mRNA levels.[84] Altogether the inhibition of ACC and stimulation of CPT-1 will block fatty acid synthesis and favor mitochondrial fatty acid uptake and β-oxidation resulting in lower intracellular fatty acid and triglyceride levels.[74,83,84] Furthermore, also, enzymes involved in peroxisomal β-oxidation, such as acyl-CoA oxidase, are induced by leptin.[84] Hence both mitochondrial and peroxisomal β-oxidation will favor the lowering of intracellular lipid content.[84] Unger and coworkers therefore rightfully suggest that leptin, through reversal of lipid accumulation in several tissues, could have beneficial effects on insulin resistance via the Randle cycle[85] and on pancreatic β-cell function, via a reduction of "lipotoxicity and lipoapoptosis" and interleukin (IL)-1β-mediated cytotoxicity,[86,87] ultimately improving glucose homeostasis.[74,84] Furthermore mitochondrial oxidation could be uncoupled from ATP synthesis, in view of the increase in uncoupling protein (UCP-1, -2, and -3) expression induced by leptin, resulting in increased thermogenesis which will contribute to energy dissipation.[84,88−91] The recent observation that leptin has important angiogenic activity, an effect mediated through the long form of the leptin receptor which is expressed on endothelial cells, is also interesting in this context.[33] Leptin, produced in the adipocytes, is not only secreted into the blood stream but could also act locally upon endothelial cells in a paracrine fashion to induce angiogenesis, which may assist in heat dissipation at sites of active thermogenesis, such as the adipose tissue.[33]

Besides these metabolic effects, leptin has several other peripheral effects. One of the potential peripheral effects of leptin, outside the metabolic arena, is its effects on the hematopoietic and immune systems. In fact, leptin has been reported to have a specific effect on CD4+ T lymphocytes (Th cells), which express high levels of the long form of the leptin receptor.[39] Leptin differentially modulates naive and memory CD4+ T cells (Th).[39] Furthermore, leptin seems to increase Th1 cytokine production (proinflamatory cytokines, such as IL-2 and interferon γ (IFNγ)) and to decrease Th2 cytokine production (regulatory cytokines, such as IL-4). Administration of leptin furthermore reverses the immunosuppressive effects of starvation.[39] In addition, in view of its cytokine-like structure, it was no surprise that leptin also has a role in proliferation, differentiation, and functional activation of macrophages.[29,78,79] Whether these proliferative effects in the hemopoietic system are related to the capacity of leptin to induce MAP kinase-dependent cell proliferation of certain unrelated cell lines, such as C3H10T1/2 cells, awaits further study.[75] Leptin also seems to affect steroid hormone production, since it can directly inhibit cortisol release from the adrenocortical cells gland[81] and inhibit insulin-induced progesterone and estradiol production by granulosa cells.[80]

Leptin, from an "Adipostat" to a "Gauge of Energy Reserves". Classically, leptin is thought of as an adipocyte-derived signaling molecule, which limits food intake and increases energy expenditure[7] (see also Figure 2). Evidence for the "adipostat" hypothesis was provided by leptin's capacity to reduce body weight and to improve metabolic control in rodents with either genetic obesity[52,92−96] or diet-induced obesity.[92] Interestingly, leptin seemed even capable of decreasing adipose tissue mass in normal weight animals.[97,98] Based on all the above evidence, it has been suggested that leptin has an adipostatic function and can reduce fat mass and body weight in the obese.

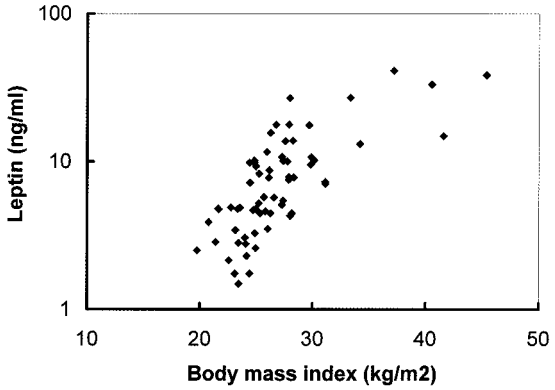
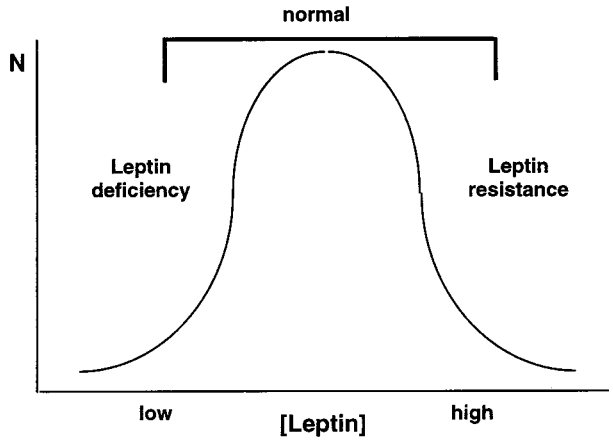


Figure 3. Correlation between leptin levels and BMI. Leptin levels were measured in 65 subjects and plotted against BMI. Correlation between BMI and leptin levels is evident.

Two key observations, however, questioned whether prevention of obesity or weight gain is leptin's prime or only function and suggested that leptin might rather serve as a gauge of body energy reserves. The first observation was the close correlation between adipose tissue leptin mRNA and plasma leptin with the size of the adipose tissue depot,[99−103] which suggested that most forms of obesity, unlike the obesity in *ob/ob* mice, are not caused by an absolute deficiency in leptin levels itself but are instead associated with increased levels of leptin (see Figure 3). These findings gave rise to the "leptin resistance" hypothesis (reviewed in refs 51 and 104), which states that obesity might be the result of inadequate levels of leptin signaling for a given leptin concentration (Figure 4). A second piece of evidence, in favor of leptin's role as a gauge of energy reserves, came from the discovery that leptin triggered an important neuroendocrine and immune response, adapting the function of several organ systems to changes in energy reserves. In fact, from a number of studies of the immune and endocrine systems, it became clear that low levels of leptin, such as seen during starvation or after weight loss, are perceived by the body as harmful and lead to a number of compensatory changes mainly mediated through inhibition of the hypothalamic−pituitary axis[100,105−111] and suppression of the finely tuned cognate immune response, which requires energy-expensive large-scale clonal expansion.[39] Raising leptin levels could bypass these effects and restore both a normal hypothalamic−pituitary axis[105,106,109] and immune response.[39]

Hence, both these arguments suggested that leptin levels might merely reflect adipose tissue energy stores, and leptin became a "signal of plenty" or an "antistarvation hormone".[104] High leptin levels could inform the body that energy reserves are sufficient. Low leptin levels, in contrast, warn the body about limited energy supplies and protect certain vital functions, such as the central nervous system, whereas they suppress certain functions such as reproduction and immune response, which are considered less immediately essential.

A



B

Leptin deficiency	*Normal*	*Leptin resistance*
Inadequate WAT stores Neuroendocrine deficiencies	Normal humans	Obesity
ob/ob mice	Normal animals	db/db mice fa/fa rats

Figure 4. Leptin resistance in mice and humans. A. Distribution of leptin levels in humans: A relative leptin resistance is observed in human obese subjects with high circulating steady-state leptin levels. Low steady-state leptin levels are associated with inadequate adipose tissue mass and can cause neuroendocrine disturbances, such as abnormalities of the reproductive system. B. Table giving examples of leptin deficiency and leptin resistance in humans and animals: Inadequate adipose tissue stores in humans are seen in situations such as cachexia and anorexia nervosa. The animal models represent extreme situations: *ob/ob* mice on one side and *db/db* mice or Zucker fatty rats on the other side. These last animals are extremely leptin-resistant.

Again, a Regulatory Role for Leptin? Most studies addressing leptin's role in obesity indicated an association between established obesity and high levels of leptin mRNA or protein at a certain given point in time and reflect a static evaluation. Conclusions based on such associations, however, tend to mask any regulatory or dynamic effects which leptin might have in the early development of changes in body weight. Therefore, we feel that the characterization of the metabolic phenotype before and during the onset of obesity will be of utmost importance. Several recent studies start to address this issue and demonstrate a dissociation between body fat mass and leptin levels, suggesting a potentially important regulatory role for leptin in the control of body weight homeostasis.

An initial group of data relates to human studies. In a first study leptin levels were measured in Pima Indians, in whom weight evolution was prospectively monitored.[112] In persons with comparable initial body weight, high leptin levels at the onset of the study identified the subjects which remained slim, whereas low leptin levels were predictive for the development of obesity at a later age.[112] This observation seems, however, dependent upon the genetic background of the study population, since a similar study in Mexicans failed to demonstrate such a relationship.[113] The observation in the Pima Indians, however, suggests that diminished production of leptin may, under certain conditions, play a role in the pathogenesis of obesity and that leptin levels before the development of obesity not only reflect adipose tissue size as was suggested in initial "steady-state" studies. Similar conclusions were obtained from a second human study showing that, in children with chronic renal insufficiency, elevated leptin levels do not correlate with body weight. The high leptin levels in these children, which could be due to disturbed leptin clearance via the kidney,[114−116] might underlie the anorexia and resulting body weight loss associated with chronic renal insufficiency. Finally a recent genetic study suggested that a polymorphic allele in the PPARγ gene (for a review, see ref 117) was associated with increased serum leptin levels.[118] Interestingly, the increase in plasma leptin levels in the bearers of the rare PPARγ allele was not associated with an increase in BMI, as would have been expected from the elevated leptin levels in these subjects. This suggests that the relative higher leptin levels in the bearers of the rare allele seemed to protect these subjects against the further development of obesity.[118]

These human data, underscoring a regulatory role for leptin in the control of body weight, are supported by a number of studies in animal models also showing discoordinate changes of leptin levels and body fat. A good example of this is found in rodents treated with thiazolidinedione insulin sensitizers. Thiazolidinediones, such as troglitazone or rosiglitazone (BRL 49,563), bind to and activate the nuclear hormone receptor PPARγ (reviewed in ref 117). In rodents, these compounds are known to induce weight gain mainly by activating PPARγ, a key trigger of adipogenesis (reviewed in refs 1 and 2). Interestingly, these PPARγ activators reduce leptin gene transcription resulting in a decrease in leptin mRNA and plasma levels.[119−121] In contrast to the well-documented effects of the thiazolidinediones on rodent leptin expression, only two small studies on this in humans are published. Whereas one of these studies found no effect of thiazolidinediones on weight gain or circulating leptin levels,[122] another study demonstrated that thiazolidinediones reduce leptin levels and increase BMI.[123] Both data sets are, however,

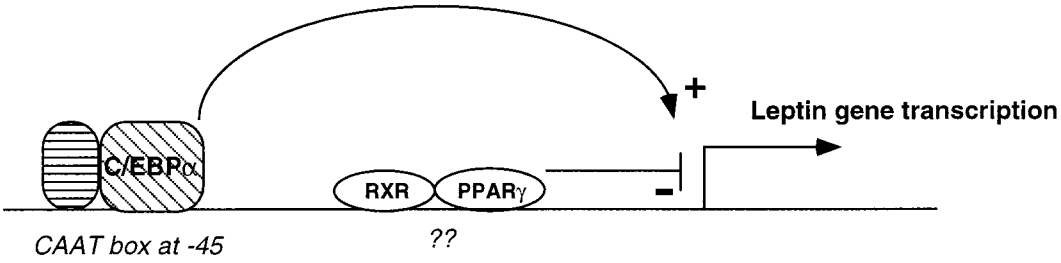


Figure 5. Hypothetical scheme illustrating the opposing effects of two adipogenic transcription factors, C/EBPα and PPARγ, on leptin transcription. C/EBPα induces whereas PPARγ inhibits leptin gene transcription. Although it has been shown that C/EBPα can bind to the leptin promoter, no evidence for binding of PPARγ is present.

rather small, and a definite answer on the question whether PPARγ activation affects leptin production in humans will have to await a more comprehensive study. It is, however, important to stress that the decrease in rodent leptin levels after thiazolidinedione treatment occurs despite the fact that these compounds induce weight gain and increase adipose tissue mass and food intake. Other examples in which body mass and leptin levels are regulated in opposite fashion are provided by treatment of animals with LPS,[124] cytokines,[124] or high doses of glucocorticoids,[125] which all induce leptin levels despite a reduction in body weight.

These human as well as animal studies suggest that one cannot reduce leptin to a simple "gauge of energy stores" and suggest that leptin also has a regulatory role. To establish such regulatory effects of leptin unequivocally in humans, however, careful intervention as well as longitudinal studies need to be performed in different populations. The importance of evaluating the dynamics of the leptin system needs to be stressed since only consecutive leptin measurements will demonstrate whether the changes in leptin levels precede the changes in body weight. Conclusions about leptin's role in body weight control cannot rely only on single steady-state plasma leptin measurements. Previous experience with measurement of insulin or thyroxine levels during development of NIDDM or thyroid disease, for example, suggests that the measurement of a leptin response to a challenge and the performance of multiple leptin measurements over a period of time will undoubtly be more informative than a single measurement.

Control of Leptin Gene Expression. In view of the regulatory role of leptin in the control of metabolism, it would be helpful to understand the genetic and environmental factors contributing to the variability in basal leptin mRNA and plasma levels. Since basal leptin levels are shown to be closely related to triglyceride stores and adipose tissue mass, it was important to define the molecular mechanism underlying adipose tissue-specific gene expression. Leptin gene expression is regulated in an opposite fashion by PPARγ and C/EBPα, two transcription factors controlling adipocyte differentiation (Figure 5). Leptin gene expression is induced by C/EBPα, an effect mediated by a C/EBP-binding site in the proximal leptin gene promoter.[11,126,127] In contrast antidiabetic thiazolidinediones, ligands and activators of PPARβ, reduce leptin expression,[119−121] via a direct effect on the leptin promoter[119,127] (Figure 5). Therefore, it seems that PPARγ not only favors adipocyte differentiation on a local adipocyte level but in addition would also trigger a systemic response, mediated by adipocyte-derived signaling molecules. Part of this response consists of a decrease in leptin production, associated with an increase in food intake, which will provide substrate to be stored in these adipocytes. Leptin hence also appears to be functioning in this adipocyte-sustaining positive feedback loop. In addition to this functional evidence supporting a role of PPARγ in controlling leptin levels, a large genetic study demonstrated that a variant in the PPARγ gene was important to determine circulating leptin levels.[118]

Furthermore, in addition to adipocyte transcription factors, leptin gene expression is controlled by a nutrient-sensing pathway. In fact, it was recently suggested that leptin expression is induced by the end product of the hexosamine biosynthetic pathway, UDP-*N*-acetylglucosamine (UDP-GlcNAc).[128] This regulation was observed both in adipose tissue and in skeletal muscle, where no leptin is found under basal conditions.[128] The hexosamine biosynthetic pathway is a cellular sensor of energy availability and is responsible for mediating the effects of glucose and lipids on the expression of several genes. Further studies are, however, needed to determine the relative contribution of this pathway to the overall regulation of leptin gene expression and to identify the transcription factors mediating this increase.

Besides the evidence supporting a role for C/EBPα and PPARγ in controlling leptin production, a recent genetic study demonstrated that a locus on chromosome 2p21 (D2S1788) is a major determining factor for circulating leptin levels.[129] Although the exact gene responsible for this regulation has not yet been identified, a possible candidate gene in this region is the pro-opiomelanocortin (POMC) gene.[129] From a single precursor protein, POMC, prohormone-type cleavage can generate several hormones. The melanocyte-stimulating hormone, the primary agonist of the MC-4 receptor, which has a tonic inhibitory role in feeding and energy storage, is one of the products of the POMC gene.[61] POMC is also the precursor for adrenocorticotropic hormone (ACTH), which regulates the production of glucocorticoid hormones in the adrenal gland. High levels of glucocorticoids have been demonstrated to regulate leptin gene expression[125,130] and decrease food in take in vivo.[125] In addition, also in cultured adipocytes of rat or human origin, glucocorticoids induce leptin expression, arguing for a direct receptor-mediated effect of glucocorticoids on leptin gene expression.[130−133] Recent studies in our laboratory, utilizing luciferase reporter genes driven by the human leptin promoter, confirm a direct transcriptional effect of glucocorticoids on the leptin promoter activity. This effect seems not to be mediated by the glucocorticoid receptor but rather

Table 1. Inducers and Suppressors of Leptin Expression

inducer	effect[a]	species
feeding	+	rodent and human
fasting	−	rodent and human
obesity	+	rodent and human
excercise	−	rodent
insulin	+	rodent and human
glucosamine	+	rodent
pertussis toxin	−	rodent
cAMP	−	rodent
?-receptor agonists	−	rodent
thiazolidinediones	−	rodent
cytokines (TNF?)	+	rodent and human
androgens	−	human
glucocorticoids	+	rodent and human
thyroid hormones	nc	human

[a] Code: +, induction; −, suppression; nc, no change.

by an inhibitory effect of glucocorticoids on a negative regulatory protein bound to the human leptin promoter.[134]

In addition to regulatory phenomena linked with adipose tissue, recently a placental enhancer for the human leptin gene was identified in the 5′-regulatory sequence of the gene.[135] This enhancer element binds nuclear proteins present in extracts of placenta and choriocarcinoma cell lines, but not in adipocyte or HeLa cell extracts.[135] Identification of the exact transcription factors interacting with this placental enhancer awaits further study, and it will be interesting to see whether fundamental differences between placental and adipose tissue will be identified.

Besides the tissue-specific regulation associated with adipose tissue and placenta, the expression of the leptin gene appears tightly controlled by multiple environmental and hormonal factors. A summary of regulatory effects on leptin expression can be found in Table 1, whereas more detailed information can be found in a recent review focusing on the regulation of leptin expression,[136] as well as in the section dealing with human biology.

Leptin and Glucose Homeostasis. One particularly remarkable effect of leptin injection is its capacity to improve glucose homeostasis and to reduce circulating insulin levels in vivo. This effect was seen in animals injected with recombinant leptin[52,53,93−95,98,137] and in animals after adenovirus-mediated leptin gene therapy.[26,138] How this beneficial glucose-lowering effect occurs is difficult to address since leptin's effects on glucose metabolism are easily confounded by its weight-reducing effect and hence remain at present a matter of debate. Kamohara et al. suggested that the improved glucose homeostasis results mainly from the central effects of leptin since both intravenous- and intracerebroventricular-administered leptin had similar effects on glucose metabolism.[53] Leptin by both routes of administration caused an increased glucose turnover and glucose uptake, and it decreased hepatic glycogen content,[53] suggesting that central leptin administration increased not only metabolism of lipids and energy but also metabolism of glucose.

The existence of a direct peripheral effect of leptin on cellular lipid[74,83] and/or glucose[76,77] metabolism has provided an alternative hypothesis to explain the improvement in glucose homeostasis. Leptin-mediated reversal of lipid accumulation in several tissues[74,83] could improve insulin-stimulated glucose disposal via a decrease in substrate competition as suggested by Randle.[85] In addition, it could have a direct stimulatory effect on pancreatic β-cell function, via a reduction of "lipotoxicity and lipoapoptosis" and cytotoxic effects,[86,87] and as such also contribute to glucose homeostasis.[74,84] Besides these effects on lipid metabolism, leptin is also able to mimic certain insulin effects on glucose transport and glycogen synthesis.[76] This insulinomimetic effect involves PI-3 kinase and JAK-2- and IRS-2-dependent signaling pathways.[76,77] However, all these peripheral effects of leptin, which suggest that it stimulates directly or indirectly glucose metabolism, are somewhat in contradiction with some other in vitro observations, which suggest that addition of leptin to cultured hepatoma cells,[71] primary adipocyte cells,[73] or isolated soleus muscle[82] interferes with the insulin signaling pathway. It awaits therefore further study to determine whether central, peripheral, or a combination of central and peripheral effects explain the general effects of leptin on glucose homeostasis.

Genetics of Leptin

After the initial discovery of the leptin gene, a large number of studies have screened for mutations in the coding region and evaluated linkage between the region of the leptin gene and some markers of adiposity. Potential linkage of obesity to the leptin gene has been reported in some extremely obese Hispanic[139] and Caucasian[140,141] subjects. This association was, however, not confirmed in a group of Pima Indians, in which obesity and associated pathologies are extremely prevalent.[142] Furthermore, several investigations failed to identify abnormalities in the coding region of the leptin gene.[99,143−145] Recently, however, a mutation in the leptin gene in two severely obese children from the same highly consanguinous pedigree was identified.[146] Their serum leptin levels were very low despite their marked obesity, and in both children a homozygous frame-shift mutation involving codon 133 of the leptin gene was present. The resulting leptin protein lacked the carboxy-terminal cysteine that is required for intrachain disulfide bonding which is necessary for its biological action.[13,14] These patients had, however, some interesting differences relative to the metabolic phenotype observed in the *ob/ob* mouse. Most notably, they had a normal body temperature, unlike the reduced temperature seen in the *ob/ob* mouse. Furthermore they did not display hypercortisolism, which is a typical feature of the *ob/ob* mouse. Although mutations such as this are most likely extremely rare and do not significantly contribute to the majority of obesity syndromes encountered in clinical practice, the severe obesity in these two probands provides direct genetic evidence that leptin plays a key role in energy metabolism in man. Furthermore, the fact that a mutated leptin gene can render both humans and mice obese underscores the important conserved function of leptin through evolution. Further genetic studies should not only aim to identify whether allelic variants at the leptin locus are linked with obesity but also address the genetics of the complex regulatory pathways underlying the control of body weight.

From Basic Research to Human Physiology

Human research on leptin was boosted by the development of reliable leptin immunoassays. These assays allowed researchers to "get more miles" out of banked serum samples of studies, often performed for totally unrelated reasons. In this section we aim to relate these human data on leptin to the clinic. Several more carefully designed studies to evaluate leptin's effects in humans are currently underway and will soon complement the data from these initial studies.

Relation of Leptin to Body Fat and Body Fat Distribution. One of the earliest observations concerning leptin in human physiology was the correlation between circulating leptin levels and BMI.[147] This correlation is already detectable in the newborn in whom leptin concentration is correlated with weight and length.[148−150] In normal weight humans serum leptin is correlated positively with BMI and more particularly with body fat content.[100−103,147] This explains why in serum of obese subjects leptin is detectable in much higher concentration than in normal weight subjects. The increase in circulating leptin in obese subjects is associated with an increase in leptin mRNA in adipose tissue and points to increased leptin gene expression as the mechanism responsible for elevated leptin levels in obesity.[101−103] The lipid content of fat cells seems to be, more than any other marker of obesity, the main determinant of leptin production.[102,151] These observations, together with the absence of frequently occurring mutations in the leptin gene, led to the conclusion that leptin deficiency is unlikely to play a major role in human obesity.[100,147]

Furthermore leptin production seems to vary depending on the individual adipose tissue depots. Leptin mRNA expression is higher in subcutaneous adipose tissue than in the omental, retroperitoneal, and mesenteric fat depots.[152−154] Interestingly, although visceral adipose tissue produces less leptin than subcutaneous adipose tissue, leptin seems to selectively decrease visceral fat accumulation, suggesting that it might play a role in the pathophysiology of visceral obesity and a number of associated metabolic and hormonal alterations, such as insulin resistance, non-insulin-dependent diabetes, hyperlipidemia, and atherosclerotic vascular disease.[98]

Leptin and Food Intake. It is noteworthy that leptin expression itself is influenced by food intake. In fact, in rodents leptin levels are markedly reduced after fasting and increased by refeeding.[110,155−158] These changes in leptin expression with feeding patterns in rodents are probably accounted for by changes in insulin levels, since leptin gene expression is positively regulated by insulin both in vivo and in vitro.[155,156,159] In contrast to these animal data, early feeding and feeding experiments in humans failed to show any significant changes in postprandial leptin levels[160−162] and adipose tissue leptin mRNA levels.[103,162] Several explanations for the discrepancies between these early human studies and the observations in rodents can be invoked. One particular difference resides in the general short followup of the feeding experiments in humans, where leptin is generally measured within the first 3 h after food intake.[162] Another possible source of disagreement

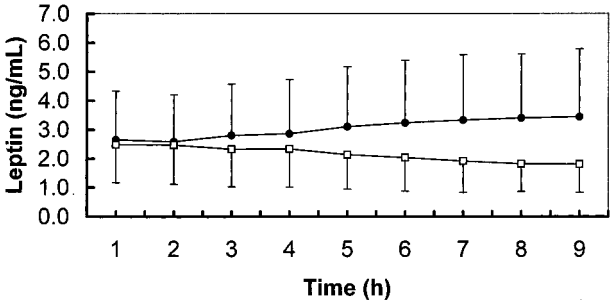


Figure 6. Leptin levels increase after a mixed meal (black circles) and conversely decrease upon fasting. Twenty-six male subjects were either given a mixed meal in the morning or kept fasted. Leptin levels in serum were determined for 8 consecutive hours. ANOVA with repeated measures demonstrated that values of both interventions were significantly different from values at time 0 ($p < 0.005$ for the fasting and $p < 0.0001$ for the postprandial leptin levels).

is the inclusion of obese subjects in the postprandial experiments.[160,162] Most recent studies, however, suggest that feeding has a sustained stimulating effect of leptin production, which in humans shows a rather delayed onset[163−165] (Figure 6). Conversely plasma leptin levels decrease significantly during fasting in both normal subjects and patients with NIDDM.[21,161,166−168] Moreover, free and bound leptin appear to react differently to these physiological stimuli, since after fasting a significant and rapid reduction in free leptin was observed in both lean and obese subjects, whereas bound leptin levels were not affected.[21] This indicates that not only leptin but the entire system regulating the leptin levels responds to extremes of energy balance. Furthermore, the finding that leptin concentration decreased with fasting and increased after feeding in humans, when changes in adipose mass are trivial, suggests that leptin secretion is determined not only by the number and size of adipocytes but perhaps also by nutrient intake, effects potentially mediated by changes in insulin and glucosamine levels.[128]

As a result of the sensitivity of leptin levels to food intake, the diurnal cycle of leptin levels that is observed in humans appears to be highly dependent on the feeding schedule[111,165] (Figure 6). Strong evidence for physiological regulation by meal pattern is provided by a meal-shift protocol, which showed an acute phase shift that directly corresponded to the shift in the timing of meals.[169] These changes were independent of cortisol and sleep-related hormones. Altogether, these results indicated meal pattern as a physiological factor that entrains the diurnal rhythm of leptin levels.

Leptin and Insulin. In rodents insulin was shown to be a potent regulator of leptin gene expression, and changes in insulin levels were shown to underlie the regulation of leptin levels by food intake.[155] Because of insulin's close relationship to appetite and body weight control, its role in controlling human leptin levels has also been studied by several groups. In several studies, insulin and insulin resistance were highly correlated to serum leptin, independently of BMI and body fat distribution.[170−174] Adjustment of this relation for body fat attenuated this relationship, suggesting that at least part of it can be attributed to the collinearity of insulin and BMI.

Short-term studies in humans, using hyperinsulinemic clamp techniques, failed to demonstrate any stimulatory effect of insulin infusion on serum leptin[160,175−177] or on leptin mRNA expression in adipose tissue.[103] Most of these studies were, however, not appropriately designed to fully assess the impact of insulin on circulating leptin, since the duration of insulin infusion was often too short and the amount of insulin administered was too small to provoke any stimulation of leptin production. In more recent studies, the length of infusion and the amount of insulin were increased, resulting in a significant increase in leptin levels.[166,178,179] The changes in leptin levels were independent of changes in glucose or fatty acid concentrations. Furthermore a direct stimulatory effect of insulin on leptin production by cultured human adipocytes was demonstrated.[178,180] It was hence concluded from these studies that long-term insulin exposure was associated in vivo and in vitro with increased leptin secretion.

These studies therefore suggest that chronic alterations in serum insulin levels, such as those that occur in obesity, may be responsible, at least in part, for the high leptin levels and positive correlation between leptin and body fat mass.[179]

Leptin and Excercise. In animals a single bout of exercise seems to be associated with a clear decrease in leptin mRNA levels.[181] Furthermore, exercise training of animals decreased leptin mRNA and serum levels, although the changes in leptin levels were paralleled by changes in body mass.[182] The physiological regulation of leptin expression by exercise may be partially attributed to the increase in activity of the sympathetic nervous system associated with physical activity.[183] Through increased levels in catecholamines and cAMP, this sympathetic activity could reduce leptin expression.[130,184,185] In humans the regulation of leptin levels by exercise is less clear. As of yet, no acute effects of exercise on leptin levels have been demonstrated.[186] Furthermore chronic exercise training did not seem to affect leptin levels independently of the reduction in body mass.[111,187]

Gender Differences in Leptin. The higher serum leptin levels in women relative to men are partially the result of the increased total body fat content in women[100] and partially the result of the differences in body fat distribution, with relatively more subcutaneous versus omental adipose tissue.[152−154] In addition, sex hormones contribute to these gender differences in leptin levels. Androgens have a potent suppressive effect on leptin expression in adipocytes[188] and are negatively correlated to plasma leptin levels.[177] Before puberty no gender differences in leptin levels exist, but in late puberty and adolescence plasma leptin levels decrease in boys and increase in girls.[189,190] This decline in leptin levels in boys is concordant with the rise in testosterone concentration, suggesting that androgens play an important role in the regulation of serum leptin levels.[189] Further evidence for an androgen effect on leptin levels is provided by the observation that androgen treatment in hypogonadal men[191,192] or in female-to-male transsexuals[193] is always accompanied by a decrease in leptin levels. Conversely, increased leptin levels are observed in hypogonadal men with androgen deficiency.[191] This combined evidence suggests that higher testosterone levels are associated with lower circulating leptin levels.

Studies in menopausal women are also suggestive of some effects of female sex steroids. Leptin concentrations are neither significantly different in pre- and postmenopausal women[194] nor affected by hormone replacement,[187,194] suggesting that female sex hormones do not explain the sexual dimorphism in leptin levels. However, when these analyses are adjusted for the lower fat mass in premenopausal women, leptin concentrations were significantly lower in post- versus premenopausal women, suggesting that leptin levels are probably increased by estrogen and/or progesterone.[195] In addition, since postmenopausal women still had significantly higher leptin levels than men, it confirms the concept that androgens have a suppressive effect on leptin in vivo.[195]

Altogether these data suggest that the prevailing sex steroid milieu as well as the differences in body composition, and not the genetic sex, are the main determinants of the sex differences in leptin levels. This difference becomes evident in early puberty in conjunction with the developing dimorphism in the production of sex hormones.

Leptin and Thyroid Hormones. Thyroid hormones increase the basal metabolic rate and have a permissive effect on adaptive thermogenesis. The potential mechanism responsible for thyroid hormone-controlled energy expenditure is not yet fully elucidated. Studies in murine 3T3-L1 cells have shown that T3 increases the expression of leptin mRNA and protein, whereas T4 does not.[196] In primary rat adipocytes isolated from hypothyroid rats, the effect of T3 was shown to be dependent on the feeding status of the animal.[197] In fact, T3 enhanced leptin accumulation in conditions mimicking the fed state but inhibited leptin mRNA in conditions similar to the fasted state.[197] The mechanism of this in vitro stimulation is not yet clearly established. Since there is no evidence for a thyroid hormone receptor binding site on the flanking regions of the leptin gene, it is possible that T3 exerts its effect indirectly as a result of changes of other metabolites or regulatory factors. Until now the results of the clinical data on thyroid hormone and leptin are not very informative. In an uncontrolled clinical study, short-term thyroid hormone administration had no significant impact on circulating leptin concentration despite clinical evidence of treatment efficiency.[198] In another study leptin levels were reported to be higher in hyperthyroid subjects, whereas leptin levels were not different from those of control subjects in hypothyroidism.[199] These rather inconclusive findings suggest that the ability of thyroid hormones to control energy expenditure does not seem to operate through major changes in leptin levels.

Leptin and Steroid Hormones. Obesity is associated with both increased cortisol turnover and insulin resistance. Since glucocorticoids promote obesity mainly via a stimulation of energy intake, it was logical to explore the potential relationship between glucocorticoid hormones and leptin in humans. In patients with Cushing's syndrome, leptin levels are elevated compared to those of control subjects with comparable amounts of body fat.[200−202] However, the acute decrease in cortisol observed in Cushing's patients immediately after cura-

tive surgery was not associated with changes in plasma leptin levels.[202] In the long term, however, adrenal or pituitary tumor resection caused a marked reduction in plasma leptin levels with a concomitant decrease in cortisol and body weight.[201]

These observations in patients with Cushing's syndrome go in line with the demonstration that the induction of mild hypercortisolism by administration of dexamethasone increased leptin mRNA levels,[203] as well as increased circulating leptin levels.[201,203−206] Although this effect was paralleled by an increase in plasma insulin levels, multivariate statistical analysis demonstrated that the increase in leptin was independent of steroid-induced hyperinsulinemia.[206] Since some of these studies included a number of moderately obese subjects, the data suggest that these subjects still have a leptin-secretory reserve.[205,206] In contrast with the studies using dexamethasone, methylprednisolone, another synthetic glucocorticoid, had no acute or prolonged effect on plasma leptin concentration in lean individuals, despite a 3-fold increase in plasma insulin levels,[207] suggesting specific effects of different synthetic glucocorticoids.

Leptin and Reproduction. Chehab et al. were the first to demonstrate that leptin had an impact on the reproductive system, when they demonstrated that the sterility defect in *ob/ob* female mice could be corrected with leptin administration.[105] In followup studies it became clear that leptin triggered the onset of puberty and sexual maturity in both female and male mice, supporting the hypothesis that fat accumulation enhances the maturation of the reproductive tract.[106−108,111,208−210] These observations were extended by the work of Ahima et al. who showed that leptin controls reproductive functions and also many other neuroendocrine functions, such as thyroid and adrenal steroid production.[109] From these studies it became clear that low levels of leptin are perceived by the body as harmful and preclude reproduction through a number of adaptive changes mediated by the hypothalamic−pituitary axis. Raising leptin levels could bypass these effects and restore a normal hypothalamic-pituitary axis.[109]

An interesting twist to leptin's role in reproduction was recently provided by the demonstration of the elevation in leptin levels during pregnancy.[211] This elevation in circulating leptin levels was attributed to high-level leptin expression by the placental trophoblasts and amnion cells from the uteri of pregnant women.[15−17] Although the increase in body mass seen during pregnancy could contribute to the rise in serum leptin levels during pregnancy, placental and uterine production accounts most likely for the majority of the rise in plasma leptin in pregnancy in humans, although different results were obtained in rodents.[212] Interestingly the hyperleptinemia observed during pregnancy is associated with an increased expression of the leptin receptor[17,23] and the appearance of a circulating form of the leptin receptor, which complexes with the excess leptin.[23] This might help to maintain free leptin levels relatively normal (or even decreased) in the maternal circulation, an effect compatible with the tendency for weight gain during pregnancy. What role placental leptin plays in the fetus is unknown at present, and further investigations are required to establish a role for leptin in neonatal physiology.

In view of the leptin production in the placenta, researchers recently also used plasma leptin levels as a marker for hydatiform mole or choriocarcinoma, a tumor derived from placental tissue.[213] Reduced tumor load, produced by either surgical removal of the tumor or chemotherapy, was associated with a fast reduction in serum leptin levels.[213]

Future Directions

Compared to leptin, very few proteins have benefited from such a swift progress in understanding their physiology. The major promise of the discovery of leptin was that it might offer, in the not too distant future, an efficient therapy for obesity and associated disorders, diseases which occur in epidemic proportions at present and which contribute significantly to mortality.[5] Stimulation of the leptin signaling pathways could in fact be beneficial for diseases associated with excessive adipose tissue mass and, for instance, for inducing weight loss and improving metabolic control. Conversely, inhibition of the leptin signaling pathways could provide ways to interfere with diseases associated with a reduced adipose tissue mass, such as cachexia, anorexia nervosa, lipoatrophy, and various wasting syndromes. Besides its promise as a therapeutic agent in the metabolic arena, recent discoveries suggest that leptin might have applications in other fields, as different as reproduction, angiogenesis, immunology, and hematology. In the following we will give an overview of the current status as well as the potential perspectives for the use of the leptin system as a therapeutic target. Despite the fast progress in this field, it is not yet clear whether and when the potential therapeutic promise of the leptin system will be fulfilled. Furthermore the complexity of leptin's biology offers multiple potential strategies for using the leptin system therapeutically.

Since leptin is a cytokine-like signaling factor, recombinant leptin protein could be injected and used as a therapeutic agent, as is the case for other cytokines such as GM-CSF or erythropoitin. In this context, leptin has undergone phase I clinical trials in the United States, after an Investigational New Drug Application was submitted to the Federal Drug Administration. None of the results of these trials are published, and the brief summary below is based on presentations at meetings and press releases of the companies involved. This first clinical trial was designed to establish the tolerability of subcutaneous (sc) injections of leptin. The most commonly reported adverse events were mild-to-moderate injection site reactions, whereas no systemic toxicities were reported at any dose. The development of antibodies at higher doses of leptin in some subjects was not considered relevant since they were not neutralizing. Interestingly, preliminary results of this study using sc leptin injection at a dose range with an acceptable safety profile were already showing a moderate effect on body weight reduction, suggesting its potential usefulness for the treatment of obesity. We will, however, need to wait until the results of the ongoing phase II efficacy trials for leptin, in subjects with both obesity and type 2 diabetes, will be available before its efficacy can be judged.

One potential drawback of leptin as a therapeutic could be its route of administration and pharmacokinetic properties. Developments exploring alternative routes of administration, such as inhalation, which now have been successfully used for insulin could, however, remedy this inconvenience. In addition, several researchers also focused on the production of peptide analogues or peptidomimetics of leptin, which might have better pharmacological or pharmacokinetic characteristics. Previous experience with modified protein drugs, such as insulin lispro, suggests that relatively minor modifications in structure can result in major differences in absorption rate from the site of injection and duration of action, relative to the original molecule. Several peptides, corresponding to restricted domains within the primary structure of leptin, are biologically active and have the ability to alter feeding behavior and energy balance in animal models.[214] These peptides localized, leptin's biological activity between amino acid residues 106 and 140.[214] Interestingly, a mutant leptin protein, with an Arg-to-Gln substitution at position 128 in this active domain, lacks biological activity.[215] Injection of normal mice with this leptin mutant seems to have a dominant negative effect on the activity of endogenous leptin and results in a progressive increase in body weight.[215] This demonstrates that this mutant leptin can potentially have therapeutic use for wasting disorders, such as anorexia and cachexia.

Another approach for interfering with the leptin signaling pathways would be to design small molecule leptin receptor modulators with either agonistic or antagonistic activity. Recently, the feasability of such an approach has been demonstrated for a similar cytokine signaling pathway controlled by the granulocyte colony-stimulating factor (G-CSF).[216] A small nonpeptidyl molecule, SB 247464, was identified which was capable of mimicking G-CSF actions in inducing tyrosine phosphorylation of multiple signaling proteins, in stimulating primary bone marrow cells to form granulocytic colonies in vitro, and in increasing peripheral blood neutrophil counts in mice.[216] Since the extracellular domain of the G-CSF receptor was required for the activity of this compound, it was concluded that it oligomerized receptor chains, proving that a small molecule can activate a receptor that normally binds a relatively large protein ligand. Similar approaches for the discovery of small molecule leptin receptor modulators are worthwhile in view of the better pharmacological properties of such small molecules.

Changing the expression or activity of either endogenous leptin or its receptor, by designing drugs specifically affecting transcription, synthesis, or activity of the various components of the leptin signaling pathway, would be an alternative approach. Both the leptin gene and the leptin receptor gene have been cloned. Therefore tools are available for screening compounds which can modulate the transcription rate of leptin or its receptor. Most progress has been made toward changing the transcription and hence expression of the leptin protein. In fact, a number of studies have shown that the expression levels of leptin can be modified by nutritional, hormonal, and/or pharmacological interventions. Although several compounds, such as insulin, glucocorticoids, and thiazolidinediones, have been shown to modulate leptin gene expression both in vivo and in vitro, these compounds are not specific enough to be used as drugs at present, and further pharmacological developments are necessary before such compounds can be evaluated for clinical use. In addition to changing leptin gene expression itself, an alternative viable approach might consist of modifying leptin secretion or release from cells. The efficacy of agents affecting leptin release was recently highlighted by the robust effects of CCK-8, the biologically active carboxy-terminal end of cholecystokinin, in releasing leptin from gastric stores into the circulation.[18]

The expression or activity of the leptin receptor, or other effector pathways in the leptin signaling cascade, can also be targeted by drugs, but so far much less progress has been made toward achieving this goal. An approach, focused on receptor expression, could facilitate central and/or peripheral leptin receptor levels and also improve the transport of leptin through the blood−brain barrier toward its central site of action. A final strategy for therapeutic intervention could consist of modifying the various effector pathways modified by leptin. A thorough understanding of these pathways would, however, be necessary to achieve this. Considering the speed of discoveries in this field, several downstream pathways will undoubtedly be identified soon. Modifying leptin effector pathways might allow the design of compounds which will only modify a subset of the pleiotropic responses normally elicited by leptin and hence achieve more specificity with less undesired side effects.

Conclusions

Leptin was originally described as an adipocyte-derived signaling factor which, after interaction with specific cytokine-like receptors, induces a pleiotropic response including control of body weight and energy expenditure. Although leptin research is moving at a rapid pace, there are still many more questions than definite answers. The near future will be very exciting since we will know the outcome of the clinical studies using either leptin or leptinomimetics to determine leptin's effect on body weight and metabolic control in humans. In addition to this, it will be important to determine leptin's exact role in neuroendocrine, reproductive, hemopoietic, and metabolic control pathways and to identify which other organ systems are affected in addition to the central nervous system. The definition of the full array of downstream effector molecules transducing the leptin signal will be very important in this respect. Finally, it will be important to determine the regulatory pathways controlling leptin levels, since they might provide ways to modulate leptin expression. Therefore, we expect a wealth of new information to become available in the years to come, not the least of which will consist of the effect of leptin administration in humans.

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Jean-Charles Fruchart graduated in pharmacy at the University of Lille, France, where he also obtained a specialization in clinical chemistry, a Ph.D. in biochemistry, and a doctorate in human biology. Since 1974, he has been a professor in pharmacy at the University of Lille, where he also directs INSERM Unit 325 at the Institut Pasteur. He is a member of the Academy of Medicine, Paris, France, is "Chevalier de la Légion d'Honneur", and serves as the actual president of the Association for Research on Cholesterol, France.

Johan Auwerx received his M.D. and Ph.D. in molecular endocrinology at the Katholieke Universiteit in Leuven, Belgium. He is a specialist in endocrinology, metabolism, and nutrition. He spent 3 years as a research scientist at the Departments of Medical Genetics and Metabolism, University of Washington, Seattle, WA. After his training in 1994, he became a Research Director for CNRS at the Institut Pasteur in Lille, France. He is associate professor in medicine at the University of Leuven, Belgium. He has received over a dozen of international scientific awards, among which is the Minkowski Award from the European Association for the Study of Diabetes (EASD). His current research interests include metabolic diseases in general and the study of nutritional control of gene expression by nuclear receptors in particular.

References

(1) Spiegelman, B. M.; Flier, J. S. Adipogenesis and obesity: rounding out the big picture. *Cell* **1996**, *87*, 377−389.
(2) Fajas, L.; Fruchart, J. C.; Auwerx, J. Transcriptional control of adipogenesis. *Curr. Opin. Cell Biol.* **1998**, *10*, 165−173.
(3) Rosenbaum, M.; Leibel, R. L.; Hirsch, J. Obesity. *N. Engl. J. Med.* **1997**, *337*, 396−407.
(4) Bjorntorp, P. Obesity. *Lancet* **1997**, *350*, 423−426.
(5) Stevens, J.; et al. The effect of age on the association between body mass index and mortality. *N. Engl. J. Med.* **1998**, *338*, 1−7.
(6) Coleman, D. L. Obese and diabetes: two mutant genes causing diabetes-obesity syndromes in mice. *Diabetologia* **1978**, *14*, 141−148.
(7) Zhang, Y.; et al. Positional cloning of the mouse obese gene and its human homologue. *Nature* **1994**, *372*, 425−432.
(8) Geffroy, S.; et al. Localization of the human ob gene to chromosome 7q32 by fluorescence in situ hybridization. *Genomics* **1995**, *28*, 603−604.
(9) Green, E. D.; et al. The human obese gene: RNA expression pattern and mapping on the physical, cytogenetic, and genetic maps of chromosome 7. *Genome Res.* **1995**, *5*, 5−12.
(10) Isse, N.; et al. Structural organization and chromosomal assignment of the human *obese* gene. *J. Biol. Chem.* **1995**, *270*, 27728−27733.
(11) Miller, S. G.; et al. The adipocyte specific transcription factor, C/EBPα modulates human ob gene expression. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 5507−5511.
(12) Gong, D. W.; Bi, S.; Pratley, R. E.; Weintraub, B. D. Genomic structure and promoter analysis of the human *obese* gene. *J. Biol. Chem.* **1996**, *271*, 3971−3974.
(13) Zhang, F.; et al. Crystal structure of the *obese* protein leptin-E100. *Nature* **1997**, *387*, 206−209.
(14) Rock, F. L.; Altman, S. W.; van Heek, M.; Kastelein, R. A.; Bazan, J. F. The leptin haemopoietic cytokine fold is stabilized by an intrachain disulfide bond. *Horm. Metab. Res.* **1996**, *28*, 649−652.
(15) Masuzaki, H.; et al. Nonadipose tissue production of leptin: leptin as a novel placenta-derived hormone in humans. *Nature Med.* **1997**, *3*, 1029−1033.
(16) Senaris, R.; et al. Synthesis of leptin in human placenta. *Endocrinology* **1997**, *138*, 4501−4504.
(17) Hoggard, N.; et al. Leptin and leptin receptor mRNA and protein expression in the murine fetus and placenta. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 11073−11078.
(18) Bado, A.; et al. The stomach is a source of leptin. *Nature* **1998**, *394*, 790−793.
(19) Sinha, M. K.; et al. Ultradian oscillations of leptin secretion in humans. *Biochem. Biophys. Res. Commun.* **1996**, *228*, 733−738.
(20) Licinio, J.; et al. Human leptin levels are pulsatile and inversely related to pituitary-adrenal function. *Nature Med.* **1997**, *3*, 575−579.
(21) Sinha, M. K.; et al. Evidence of free and bound leptin in human circulation. *J. Clin. Invest.* **1996**, *98*, 1277−1282.
(22) Houseknecht, K. L.; et al. Evidence for leptin binding to proteins in serum of rodents and humans: modulation with obesity. *Diabetes* **1996**, *45*, 1638−1643.
(23) Gavrilova, O.; Barr, V.; Marcus-Samuels, B.; Reitman, M. Hyperleptinemia of pregnancy associated with the appearance of a circulating form of the leptin receptor. *J. Biol. Chem.* **1997**, *272*, 30546−30551.
(24) Tartaglia, L. A.; et al. Identification and expression cloning of a leptin receptor, OB-R. *Cell* **1995**, *83*, 1263−1271.
(25) Lee, G. H.; et al. Abnormal splicing of the leptin receptor in diabetic mice. *Nature* **1996**, *379*, 632−635.
(26) Chen, H.; et al. Evidence that the diabetes gene encodes the leptin receptor: identification of a mutation in the leptin receptor gene in *db/db* mice. *Cell* **1996**, *84*, 491−495.
(27) Schindler, C.; Darnell, J. E., Jr. Transcriptional responses to polypeptide ligands: the JAK−STAT pathway. *Annu. Rev. Biochem.* **1995**, *64*, 621−651.
(28) Ihle, J. STATs: signal transducers and activators of transcription. *Cell* **1996**, *84*, 331−334.
(29) Ghilardi, N.; Skoda, R. C. The leptin receptor activates janus kinase 2 and signals for proliferation in a factor-dependent cell line. *Mol. Endocrinol.* **1997**, *11*, 393−399.
(30) Ghilardi, N.; et al. Defective STAT signaling by the leptin receptor in diabetic mice. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 6231−6235.
(31) Baumann, H.; et al. The full length leptin receptor has signaling capabilities of interleukin 6-type receptors. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 8374−8378.
(32) Vaisse, C.; et al. Leptin activation of Stat3 in the hypothalamus of wild-type and *ob/ob* mice but not *db/db* mice. *Nature Genet.* **1996**, *14*, 95−97.
(33) Sierra-Honigmann, M. R.; et al. Biological action of leptin as an angiogenic factor. *Science* **1998**, *281*, 1683−1686.
(34) Darnell, J. E. Reflections on STAT3, STAT5, and STAT6 as fat STATs. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 6221−6224.
(35) Spanswick, D.; Smith, M. A.; Groppi, V. E.; Logan, S. D.; Ashford, M. L. Leptin inhibits hypothalamic neurons by activation of ATP-sensitive potassium channels. *Nature* **1997**, *390*, 521−525.
(36) Kieffer, T. J.; Heller, R. S.; Leech, C. A.; Holz, G. G.; Habener, J. F. Leptin suppression of insulin secretion by activation of ATP-sensitive K+ channels in pancreatic beta-cells. *Diabetes* **1997**, *46*, 1087−1093.
(37) Harvey, J.; McKenna, F.; Herson, P. S.; Spanswick, D.; Ashford, M. L. Leptin activates ATP-sensitive potassium channels in the rat insulin-secreting cell line, CRI-G1. *J. Physiol.* **1997**, *504*, 527−535.
(38) Chua, S. C.; et al. Phenotypes of mouse *diabetes* and rat *fatty* due to mutations in the OB (leptin) receptor. *Science* **1996**, *271*, 994−996.
(39) Lord, G. M.; et al. Leptin modulates the T-cell immune response and reverses starvation-induced immunosuppression. *Nature* **1998**, *394*, 897−901.
(40) Bjorbaek, C.; Uotani, S.; de Silva, B.; Flier, J. S. Divergent signaling capacities of the long and short isoforms of the leptin receptor. *J. Biol. Chem.* **1997**, *272*, 32686−32695.
(41) Philips, M. S.; et al. Leptin receptor missense mutations in the fatty Zucker rat. *Nature Genet.* **1996**, *13*, 18−19.
(42) Iida, M.; et al. Phenotype-linked amino acid alteration in leptin receptor cDNA from Zucker fatty (fa/fa) rat. *Biochem. Biophys. Res. Commun.* **1996**, *222*, 19−26.
(43) Takaya, K.; et al. Molecular cloning of rat leptin receptor isoform complementary DNAs-identification of a missense mutation in Zucker fatty (fa/fa) rats. *Biochem. Biophys. Res. Commun.* **1996**, *225*, 75−83.
(44) Takaya, K.; et al. Nonsense mutation of the leptin receptor in the obese spontaneously hypertensive Koletsky rat. *Nature Genet.* **1996**, *14*, 130−131.
(45) Banks, W. A.; Kastin, A. J.; Huang, W.; Jaspan, J. B.; Maness, L. M. Leptin enters the brain by a saturable system independent of insulin. *Peptides* **1996**, *17*, 305−311.
(46) Golden, P. L.; Maccagnan, T. J.; Pardridge, W. M. Human blood-brain barrier leptin receptor; binding and endocytosis in isolated human brain microvessels. *J. Clin. Invest.* **1997**, *99*, 14−18.
(47) Schwartz, M.; Peskind, E.; Raskind, M.; Boyko, E. J.; Porte, D. Cerebrospinal fluid leptin levels: relationship to plasma levels and to adiposity in humans. *Nature Med.* **1996**, *2*, 589−593.
(48) Caro, J. F.; et al. Decreased cerebrospinal fluid/serum leptin ratio in obesity: a possible mechanism for leptin resistance. *Lancet* **1996**, *348*, 159−161.

(49) Funahashi, T.; et al. Enhanced expression of rat obese (ob) gene in adipose tissues of ventromedial hypothalamus (VMH)-lesioned rats. *Biochem. Biophys. Res. Commun.* **1995**, *211*, 469−475.
(50) Satoh, N.; et al. Pathophysiological significance of the obese gene product, leptin, in ventromedial hypothalamus (VMH)-lesioned rats: evidence for loss of its satiety effect in VMH-lesioned rats. *Endocrinology* **1997**, *138*, 947−954.
(51) Campfield, L. A.; Smith, F. J.; Burn, P. The ob protein (leptin) pathway-a link between adipose tissue mass and central neural networks. *Horm. Metab. Res.* **1996**, *28*, 619−632.
(52) Stephens, T. W.; et al. The role of neuropeptide Y in the antiobesity action of the *obese* gene product. *Nature* **1995**, *377*, 530−532.
(53) Kamohara, S.; Burcelin, R.; Halaas, J. L.; Friedman, J. M.; Charron, M. J. Acute stimulation of glucose metabolism in mice by leptin treatment. *Nature* **1997**, *389*, 374−377.
(54) Glaum, S. R.; et al. Leptin, the obese gene product, rapidly modulates synaptic transmission in the hypothalamus. *Mol. Pharmacol.* **1996**, *50*, 230−235.
(55) Elmquist, J. K.; Ahima, R. S.; Maratos-Flier, E.; Flier, J. S.; Saper, C. B. Leptin activates neurons in the ventrobasal hypothalamus and brainstem. *Endocrinology* **1997**, *138*, 839−842.
(56) Schwartz, M. W.; Seeley, R. J.; Campfield, L. A.; Burn, P.; Identification of targets of leptin action in rat hypothalamus. *J. Clin. Invest.* **1996**, *98*, 1101−1106.
(57) Erickson, J. C.; Hollopeter, G.; Palmiter, R. D. Attenuation of the obesity syndrome of ob/ob mice by the loss of neuropeptide Y. *Science* **1996**, *274*, 1704−1707.
(58) Wang, Q.; et al. Interactions between leptin and hypothalamic neuropeptide Y neurons in the control of food intake and energy homeostasis in the rat. *Diabetes* **1997**, *46*, 335−341.
(59) Erickson, J. C.; Clegg, K. E.; Palmiter, R. D. Sensitivity to leptin and susceptibility to seizures of mice lacking neuropeptide Y. *Nature* **1996**, *381*, 415−418.
(60) Fan, W.; Boston, B. A.; Kesterton, R. A.; Hruby, V. J.; Cone, R. D. Role of melanocortinergic neurons in feeding and the agouti obesity syndrome. *Nature* **1996**, *385*, 165−168.
(61) Huszar, D.; et al. Targeted disruption of the melanocortin-4 receptor results in obesity in mice. *Cell* **1997**, *88*, 131−141.
(62) Seeley, R. J.; et al. Melanocortin receptors in leptin effects. *Nature* **1997**, *390*, 349.
(63) Bultman, S. J.; Michaud, E. J.; Woychik, R. P. Molecular characterization of the mouse agouti locus. *Cell* **1992**, *71*, 1195−1204.
(64) Boston, B. A.; Blaydon, K. M.; Varnerin, J.; Cone, R. D. Independent and additive effects of central POMC and leptin pathways on murine obesity. *Science* **1997**, *278*, 1641−1644.
(65) Turton, M. D.; et al. A role for glucagon-like peptide-1 in the central regulation of feeding. *Nature* **1996**, *379*, 69−72.
(66) Arase, K.; York, D. A.; Shimizu, H.; Shargill, N.; Bray, G. A. Effect of corticotropin-releasing factor on food intake and brown tissue thermogenesis in rats. *Am. J. Physiol.* **1988**, *255*, E255−E259.
(67) Spina, M.; et al. Appetite-suppressing effects of urocortin a CRF-related neuropeptide. *Science* **1996**, *273*, 1561−1564.
(68) Muglia, L.; Jacobson, L.; Dikkes, P.; Majzoub, J. A. Corticotropin-releasing hormone deficiency reveals major fetal but not adult glucocorticoid need. *Nature* **1995**, *373*, 427−432.
(69) Sakurai, T.; et al. Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior. *Cell* **1998**, *92*, 573−585.
(70) Qu, D.; et al. A role for melanin-concentrating hormone in the central regulation of feeding behaviour. *Nature* **1996**, *380*, 243−247.
(71) Cohen, B.; Novick, D.; Rubinstein, M. Modulation of insulin activities by leptin. *Science* **1996**, *274*, 1185−1188.
(72) Bai, Y.; Zhang, S.; Kim, K. S.; Lee, J. K.; Kim, K. H. Obese gene expression alters the ability of 30A5 preadipocytes to respond to lipolgenic hormones. *J. Biol. Chem.* **1996**, *271*, 13939−13942.
(73) Muller, G.; Ertl, J.; Gerl, M.; Preibisch, G. Leptin impairs metabolic actions of insulin in isolated rat adipocytes. *J. Biol. Chem.* **1997**, *272*, 10585−10593.
(74) Shimabukuro, M.; et al. Direct antidiabetic effect of leptin through triglyceride depletion of tissues. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 4637−4641.
(75) Takahashi, Y.; et al. Leptin induces mitogen-activated protein kinase-dependent proliferation of C3H10T1/2 cells. *J. Biol. Chem.* **1997**, *272*, 12897−12900.
(76) Berti, L.; Kellerer, M.; Capp, E.; Haring, H. U. Leptin stimulates glucose transport and glycogen synthesis in C2C12 myotubes: evidence for a PI-3 kinase mediated effect. *Diabetologia* **1997**, *40*, 606−609.
(77) Kellerer, M.; et al. Leptin activates PI-3 kinase in C2C12 myotubes via janus kinase-2 (JAK-2) and insulin receptor substrate-2 (IRS-2) dependent pathways. *Diabetologia* **1997**, *40*, 1358−1362.
(78) Gainsford, T.; et al. Leptin can induce proliferation, differentiation, and functional activation of hemopoietic cells. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 14564−14568.
(79) Umemoto, Y.; et al. Leptin stimulates the proliferation of murine myelocytic and primitive hematopoietic progenitor cells. *Blood* **1997**, *90*, 3438−3443.
(80) Spicer, L. J.; Fransisco, C. C. The adipose obese gene product, leptin: evidence of a direct inhibitory role in ovarian function. *Endocrinology* **1997**, *138*, 3374−3379.
(81) Bornstein, S. R.; Uhlmann, K.; Haidan, A.; Ehrhart-Bornstein, M.; Scherbaum, W. A. Evidence for a novel peripheral action of leptin as a metabolic signal to the adrenal gland: leptin inhibits cortisol release directly. *Diabetes* **1997**, *46*, 1235−1238.
(82) Liu, Y. L.; Emilson, V.; Cawthorne, M. A. Leptin inhibits glycogen synthesis in isolated soleus muscle of obese (ob/ob) mice. *FEBS Lett.* **1997**, *411*, 351−355.
(83) Muoio, D. M.; Dohn, G. L.; Fiedorek, F. T.; Tapscott, E. B.; Coleman, R. A. Leptin directly alters lipid partitioning in skeletal muscle. *Diabetes* **1997**, *46*, 1360−1363.
(84) Zhou, Y. T.; et al. Induction by leptin of uncoupling protein-2 and enzymes of fatty acid oxidation. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 6383−6390.
(85) Randle, P. J.; Garland, P. B.; Hales, C. N.; Newsholme, E. A. The glucose-fatty acid cycle: its role in insulin sensitivity and metabolic disturbances of diabetes mellitus. *Lancet* **1961**, *I*, 785−789.
(86) Shimabukuro, M.; Koyama, K.; Lee, Y.; Unger, R. H. Leptin- or troglitazone-induced lipopenia protects islets from interleukin 1beta cytotoxicity. *J. Clin. Invest.* **1997**, *100*, 1750−1754.
(87) Shimabukuro, M.; Wang, M. Y.; Zhou, Y. T.; Newgard, C. B.; Unger, R. H. Protection agianst lipoapoptosis of beta cells through leptin-dependent maintainance of Bcl-2 expression. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 9558−9561.
(88) Gong, D. W.; He, Y.; Karas, M.; Reitman, M. Uncoupling protein-3 is a mediator of thermogenesis regulated by thyroid hormone, beta3-adrenergic agonists, and leptin. *J. Biol. Chem.* **1997**, *272*, 24129−24132.
(89) Cusin, I.; et al. Chronic central leptin infusion enhances insulin-stimulated glucose metabolism and favors the expression of uncoupling proteins. *Diabetes* **1998**, *47*, 1014−1019.
(90) Scarpace, P. J.; Matheny, M.; Pollock, B. H.; Tumer, N. Leptin increases uncoupling protein expression and energy expenditure. *Am. J. Physiol.* **1998**, *273*, E226−E230.
(91) Mizuno, A.; Murakami, T.; Otani, S.; Kuwajima, M.; Shima, K. Leptin affects pancreatic endocrine functions through the sympathetic nervous system. *Endocrinology* **1998**, *139*, 3863−3870.
(92) Campfield, L. A.; Smith, F. J.; Guisez, Y.; Devos, R.; Burn, P. Recombinant mouse OB protein; evidence for a peripheral signal linking adiposity and central neural networks. *Science* **1995**, *269*, 546−549.
(93) Halaas, J. L.; et al. Weight-reducing effects of the plasma protein encoded by the *obese* gene. *Science* **1995**, *269*, 543−546.
(94) Pelleymounter, M. A.; et al. Effect of the obese gene product on body weight regulation in *ob/ob* mice. *Science* **1995**, *269*, 540−543.
(95) Weigle, D. S.; et al. Recombinant *ob* protein reduces feeding and body weight in the *ob/ob* mouse. *J. Clin. Invest.* **1995**, *96*, 2065−2070.
(96) Levin, N.; Nelson, C.; Gurney, A.; Vandlen, R.; De Sauvage, F. Decreased food intake does not completely account for adiposity reduction after ob protein infusion. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 1726−1730.
(97) Rentsch, J.; Levens, N.; Chiesi, M. Recombinant ob gene product reduces food intake in fasted mice. *Biochem. Biophys. Res. Commun.* **1995**, *214*, 131−136.
(98) Barzilai, N.; et al. Leptin selectively decreases visceral obesity and enhances insulin action. *J. Clin. Invest.* **1997**, *100*, 3105−3110.
(99) Considine, R. V.; et al. Mutation screening and identification of a sequence variation in the human ob gene coding region. *Biochem. Biophys. Res. Commun.* **1996**, *220*, 735−739.
(100) Maffei, M.; et al. Leptin levels in human and rodent: measurement of plasma leptin and ob RNA in obese and weight-reduced subjects. *Nature Med.* **1995**, *1*, 1155−1161.
(101) Lönnqvist, F.; Arner, P.; Nordfors, L.; Schalling, M. Overexpression of the obese (ob) gene in adipose tissue of human obese subjects. *Nature Med.* **1995**, *1*, 950−953.
(102) Hamilton, B. S.; Paglia, D.; Kwan, A. Y. M.; Deitel, M. Increased *obese* mRNA expression in omental fat cells from massively obese humans. *Nature Med.* **1995**, *1*, 954−956.
(103) Vidal, H.; et al. The expression of ob gene is not acutely regulated by insulin and fasting in human abdominal subcutaneous adipose tissue. *J. Clin. Invest.* **1996**, *98*, 251−255.
(104) Caro, J.; Sinha, M. K.; Kolaczynski, J. W.; Zhang, P. L.; Considine, R. V. Leptin: the tale of an obesity gene. *Diabetes* **1996**, *45*, 1455−1462.

(105) Chehab, F. F.; Lim, M. E.; Lu, R. Correction of the sterility defect in homozygous obese female mice by treatment with the human recombinant leptin. *Nature Genet.* **1996**, *12*, 318−320.
(106) Chehab, F. F.; Mounzih, K.; Lu, R.; Lin, M. E. Early onset of reproductive function in normal female mice treated with leptin. *Science* **1997**, *275*, 88−90.
(107) Cheung, C. C.; et al. Leptin is a metabolic gate for the onset of puberty in the female rat. *Endocrinology* **1997**, *138*, 855−888.
(108) Barash, I. A.; et al. Leptin is a signal to the reproductive system. *Endocrinology* **1996**, *137*, 3144−3147.
(109) Ahima, R. S.; et al. Role of leptin in the neuroendocrine response to fasting. *Nature* **1996**, *382*, 250−252.
(110) Frederich, R. C.; et al. Expression of ob mRNA and its encoded protein in rodents. Impact of nutrition and obesity. *J. Clin. Invest.* **1995**, *96*, 1658−1663.
(111) Laughlin, G. A.; Yen, S. S. C. Hypoleptinemia in women athletes: absence of a diurnal rhythm with amenorrhea. *J. Clin. Endocrinol. Metab.* **1997**, *82*, 318−321.
(112) Ravussin, E.; et al. Relatively low plasma leptin concentrations precede weight gain in Pima Indians. *Nature Med.* **1997**, *3*, 238−240.
(113) Haffner, S. M.; Mykkanen, L. A.; Gonzalez, C. C.; Stern, M. P. Leptin concentrations do not predict weight gain: the Mexico City Diabetes Study. *Int. J. Obes. Relat. Metab. Disord.* **1998**, *22*, 695−699.
(114) Cumin, F.; Baum, H. P. Leptin is cleared from the circulation primarily by the kidney. *Int. J. Obes.* **1996**, *20*, 1120−1126.
(115) Merabet, E.; et al. Increased plasma leptin concentration in end-stage renal disease. *J. Clin. Endocrinol. Metab.* **1997**, *82*, 847−850.
(116) Sharma, K.; et al. Plasma leptin is partly cleared by the kidney and is elevated in hemodialysis patients. *Kidney Int.* **1997**, *51*, 1980−1985.
(117) Schoonjans, K.; Martin, G.; Staels, B.; Auwerx, J. Peroxisome proliferator-activated receptors, orphans with ligands and functions. *Curr. Opin. Lipidol.* **1997**, *8*, 159−166.
(118) Meirhaeghe, A.; et al. A genetic polymorphism of the peroxisome proliferator-activated receptor γ gene influences plasma leptin levels in obese humans. *Hum. Mol. Genet.* **1998**, *7*, 435−440.
(119) De Vos, P.; et al. Thiazolidinediones repress *ob* gene expression via activation of PPARγ. *J. Clin. Invest.* **1996**, *98*, 1004−1009.
(120) Zhang, B.; et al. Down-regulation of the expression of the *obese* gene by antidiabetic thiazolidinedione in Zucker diabetic fatty rats and *db*/*db* mice. *J. Biol. Chem.* **1996**, *271*, 9455−9459.
(121) Kallen, C. B.; Lazar, M. A. Antidiabetic thiazolidinediones inhibit leptin (ob) gene expression in 3T3-L1 adipocytes. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 5793−5796.
(122) Nolan, J. J.; Olefsky, J. M.; Nyce, M. R.; Considine, R. V.; Caro, J. F. Effect of troglitazone on leptin production. Studies in vitro and in human subjects. *Diabetes* **1996**, *45*, 1276−1278.
(123) Shimizu, H.; et al. Troglitazone reduces plasma leptin concentration but increases hunger in NIDDM patients. *Diabetes Care* **1998**, *21*, 1470−1474.
(124) Grunfeld, C.; et al. Endotoxin and cytokines induce expression of leptin, the ob gene product, in hamsters. A role for leptin in the anorexia of infection. *J. Clin. Invest.* **1996**, *97*, 2152−2157.
(125) De Vos, P.; Saladin, R.; Auwerx, J.; Staels, B. Induction of *ob* gene expression by corticosteroids is accompanied by body weight loss and reduced food intake. *J. Biol. Chem.* **1995**, *270*, 15958−15961.
(126) He, Y.; Chen, H.; Quon, M. J.; Reitman, M. The mouse *obese* gene. Genomic organization, promoter activity, and activation by CCAAT/enhancer-binding protein alpha. *J. Biol. Chem.* **1995**, *270*, 28887−28891.
(127) Hollenberg, A. N.; et al. Functional antagonism between CCAAT/enhancer binding protein-α and peroxisome proliferator-activated receptor-γ on the leptin promoter. *J. Biol. Chem.* **1997**, *272*, 5283−5290.
(128) Wang, J.; Liu, R.; Hawkins, M.; Barzilai, N.; Rossettti, L. A nutrient-sensing pathway regulates leptin gene expression in muscle and fat. *Nature* **1998**, *393*, 684−688.
(129) Comuzzie, A. G.; et al. A major quantitative trait locus determining serum leptin levels and fat mass is located on human chromosome 2. *Nature Genet.* **1997**, *15*, 273−276.
(130) Slieker, L. J.; et al. Regulation of expression of ob mRNA and protein by glucocorticoids and cAMP. *J. Biol. Chem.* **1996**, *271*, 5301−5304.
(131) Murakami, T.; Iida, M.; Shima, K. Dexamethasone regulates obese expression in isolated rat adipocytes. *Biochem. Biophys. Res. Commun.* **1995**, *214*, 1260−1267.
(132) Hardie, L. J.; Guilhot, N.; Trayhurn, P. Regulation of leptin production in cultured mature white adipocytes. *Horm. Metab. Res.* **1996**, *28*, 685−689.
(133) Considine, R. V.; et al. Dexamethasone stimulates leptin release from human adipocytes: unexpected inhibition by insulin. *J. Cell. Biochem.* **1997**, *65*, 254−258.
(134) De Vos, P.; Lefebvre, A. M.; Shrivo, I.; Fruchart, J. C.; Auwerx, J. Glucocorticoids induce the expression of the leptin gene through a nonclassical mechanism of transcriptional activation. *Eur. J. Biochem.* **1998**, *253*, 619−629.
(135) Bi, S.; Gavrilova, O.; Gong, D. W.; Mason, M. M.; Reitman, M. Identification of a placental enhancer for the human leptin gene. *J. Biol. Chem.* **1997**, *272*, 30583−30588.
(136) Saladin, R.; Staels, B.; Auwerx, J.; Briggs, M. Regulation of ob gene expression in rodents and humans. *Horm. Metab. Res.* **1996**, *28*, 638−641.
(137) Sivitz, W. I.; Walsh, S. A.; Morgan, D. A.; Thomas, M. J.; Haynes, W. G. Effects of leptin on insulin sensitivity in normal rats. *Endocrinology* **1997**, *138*, 3395−3401.
(138) Muzzin, P.; Eisensmith, R. C.; Copeland, K. C.; Woo, S. L. C. Correction of obesity and diabetes in genetically obese mice by leptin gene therapy. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 14804−14808.
(139) Duggirala, R.; et al. Quantitative variation in obesity-related traits and insulin precursors linked to the ob gene region on human chromosome 7. *Am. J. Hum. Genet.* **1996**, *59*, 694−703.
(140) Reed, D. R.; et al. Extreme obesity may be linked to markers flanking the human ob gene. *Diabetes* **1996**, *45*, 691−694.
(141) Clément, K.; et al. Genetic variation in the beta3-adrenergic receptor and an increased capacity to gain weight in patients with morbid obesity. *N. Engl. J. Med.* **1995**, *333*, 352−354.
(142) Norman, R. A.; et al. Absence of linkage of obesity and energy metabolism to markers flanking homologues of rodent obesity genes in Pima Indians. *Diabetes* **1996**, *45*, 1229−1232.
(143) Considine, R. V.; et al. Evidence against either a premature stop codon or the absence of obese gene mRNA in human obesity. *J. Clin. Invest.* **1995**, *95*, 2986−2988.
(144) Maffei, M.; et al. Absence of mutations in the human OB gene in obese/diabetic subjects. *Diabetes* **1996**, *45*, 679−682.
(145) Niki, T.; et al. Human obese gene: molecular screening in Japanese and Asian Indian NIDDM patients associated with diabetes. *Diabetes* **1996**, *45*, 675−678.
(146) Montague, C. T.; et al. Congenital leptin deficiency is associated with severe early-onset obesity in humans. *Nature* **1997**, *387*, 903−908.
(147) Considine, R. V.; et al. Serum immunoreactive-leptin concentrations in normal-weight and obese humans. *N. Engl. J. Med.* **1996**, *334*, 292−295.
(148) Mantzoros, C. S.; Varvarigou, A.; Kaklamani, V. G.; Beratis, N. G.; Flier, J. S. Effect of birth weight and maternal smoking on cord blood leptin concentrations of full term and pre-term newborns. *J. Clin. Endocrinol. Metab.* **1997**, *82*, 2856−2861.
(149) Koistinen, H. A.; et al. Leptin concentration in cord blood correlates with intrauterine growth. *J. Clin. Endocrinol. Metab.* **1997**, *82*, 3328−3330.
(150) Harigaya, A.; Nagashima, K.; Nako, Y.; Morikawa, A. Relationship between concentration of serum leptin and fetal growth. *J. Clin. Endocrinol. Metab.* **1997**, *82*, 3281−3284.
(151) Lonnqvist, F.; et al. Leptin secretion from adipose tissue in woman; relationship to plasma levels and gene expression. *J. Clin. Invest.* **1997**, *99*, 2398−2404.
(152) Hube, F.; et al. Differences in leptin mRNA levels between omental and subcutaneous abdominal adipose tissue from obese humans. *Horm. Metab. Res.* **1996**, *28*, 690−693.
(153) Montague, C. T.; Prins, J. B.; Sanders, L.; Digby, J. E.; O'Rahilly, S. Depot- and sex-specific differences in human leptin mRNA expression; implications for the control of regional fat distribution. *Diabetes* **1997**, *46*, 342−347.
(154) Lefebvre, A. M.; et al. Depot-specific differences in adipose tissue gene expression in lean and obese subjects. *Diabetes* **1998**,, in press.
(155) Saladin, R.; et al. Transient increase in *obese* gene expression after food intake and insulin administration. *Nature* **1995**, *377*, 527−529.
(156) MacDougald, O. A.; Hwang, C. S.; Fan, H.; Lane, M. D. Regulated expression of the obese gene product (leptin) in white adipose tissue and 3T3-L1 adipocytes. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 9034−9037.
(157) Trayhurn, P.; Thomas, M. E. A.; Duncan, J. S.; Rayner, V. D. Effect of fasting and refeeding on ob gene expression in white adipose tissue of lean and (*ob*/*ob*) mice. *FEBS Lett.* **1995**, *368*, 488−490.
(158) Moinat, M.; et al. Modulation of *obese* gene expression in rat brown and white adipose tissues. *FEBS Lett.* **1995**, *373*, 131−134.
(159) Leroy, P.; et al. Expression of *ob* gene in adipose cells: regulation by insulin. *J. Biol. Chem.* **1996**, *271*, 2365−2368.
(160) Dagogo-Jack, S.; Fanelli, C.; Paramore, D.; Brothers, J.; Landt, M. Plasma leptin and insulin relationship in obese and nonobese humans. *Diabetes* **1996**, *45*, 695−698.
(161) Boden, G.; Chen, X.; Mozzoli, M.; Ryan, I. Effect of fasting on serum leptin in normal human subjects. *J. Clin. Endocrinol. Metab.* **1996**, *81*, 3419−3423.

(162) Clapham, J. C.; et al. Plasma leptin concentrations and ob gene expression in subcutaneous adipose tissue are not regulated acutely by physiological hyperinsulinemia. *Int. J. Obes.* **1997**, *21*, 179−183.
(163) Kolaczynski, J. W.; Ohannesian, J. P.; Considine, R. V.; Marco, C. C.; Caro, J. F. Response of leptin to short-term and prolonged overfeeding in humans. *J. Clin. Endocrinol. Metab.* **1996**, *81*, 4162−4165.
(164) Kolaczynski, J. W.; et al. Response of leptin to short-term fasting and refeeding in humans; a link with ketogenesis but not ketones themselves. *Diabetes* **1996**, *45*, 1511−1515.
(165) Dallongeville, J.; et al. Short-term response of circulating leptin to feeding and fasting in man: influence of ca.dian cycle. *Int. J. Obes. Relat. Metab. Disord.* **1998**, *22*, 728−733.
(166) Malmstrom, R.; Taskinen, M. R.; Karonen, S. L.; Yki-Jarvinen, H. Insulin increases plasma leptin concentrations in normal subjects and patients with NIDDM. *Diabetologia* **1996**, *39*, 993−996.
(167) Weigle, D. S.; et al. Effect of fasting, refeeding, and dietary fat restriction on plasma leptin levels. *J. Clin. Endocrinol. Metab.* **1997**, *82*, 561−565.
(168) Racette, S. B.; Kohrt, W. M.; Landt, M.; Holloszy, J. O. Response of serum leptin concentrations to 7d of energy restriction in centrally obese African Americans with impaired or diabetic glucose tolerance. *Am. J. Clin. Nutr.* **1997**, *66*, 33−37.
(169) Schoeller, D. A.; Cella, L. K.; Sinha, M. K.; Caro, J. Entrainement of the diurnal rhythm of plasma leptin to meal timing. *J. Clin. Invest.* **1997**, *100*, 1882−1887.
(170) Haffner, S. M.; et al. Leptin concentrations and insulin sensitivity in normoglycemic men. *Int. J. Obes.* **1997**, *21*, 393−399.
(171) Segal, K. R.; Landt, M.; Klein, S. Relationship between insulin sensitivity and plasma leptin concentration in lean and obese men. *Diabetes* **1996**, *45*, 988−991.
(172) Widjaja, A.; et al. UKPDS 20: plasma leptin, obesity, and plasma insulin in type 2 diabetic subjects. *J. Clin. Endocrinol. Metab.* **1997**, *82*, 654−657.
(173) Havel, P. J.; et al. Relationship of plasma leptin to plasma insulin and adiposity in normal weight and overweight women: effects of dietary fat content and sustained weight loss. *J. Clin. Endocrinol. Metab.* **1996**, *81*, 4406−4413.
(174) Zimmet, P.; et al. Serum leptin concentration, obesity, and insulin resistance in Western Samoans: cross sectional study. *Br. Med. J.* **1996**, *313*, 965−969.
(175) Pratley, R. E.; Nicolson, M.; Bogardus, C.; Ravussin, E. Effects of acute hyperinsulinemia on plasma leptin concentrations in insulin-sensitive and insulin-resistant Pima Indians. *J. Clin. Endocrinol. Metab.* **1996**, *81*, 4418−4421.
(176) Ryan, A. S.; Elahi, D. The effect of acute hyperglycemia and hyperinsulinemia on plasma leptin levels: its relationships with body fat, visceral adiposity and age in women. *J. Clin. Endocrinol. Metab.* **1996**, *81*, 4433−4438.
(177) Tuominen, J. A.; et al. Leptin synthesis is resistant to acute effects of insulin in insulin-dependent diabetes mellitus patients. *J. Clin. Endocrinol. Metab.* **1997**, *82*, 381−382.
(178) Kolaczynski, J. W.; Nyce, M. R.; Considine, R. V. Acute and chronic effect of insulin on leptin production in humans. Studies in vivo and in vitro. *Diabetes* **1996**, *45*, 699−701.
(179) Boden, G.; Chen, X.; Kolaczynski, J. W.; Polansky, M. Effects of prolonged hyperinsulinemia on serum leptin in normal human subjects. *J. Clin. Invest.* **1997**, *100*, 1107−1113.
(180) Wabitsch, M.; et al. Insulin and cortisol promote leptin production incultured human fat cells. *Diabetes* **1996**, *45*, 1435−1438.
(181) Zheng, D.; Wooter, M. H.; Zhou, Q.; Dohm, G. L. The effect of excercise on ob gene expression. *Biochem. Biophys. Res. Commun.* **1996**, *225*, 747−750.
(182) Zachwieja, J. J.; Hendry, S. L.; Smith, S. R.; Harris, R. B. S. Voluntary wheel running decreases adipose tissue mass and expression of leptin mRNA in Osborne-Mendel rats. *Diabetes* **1997**, *46*, 1159−1166.
(183) Christin, L. M.; O'Connel, M.; Bogardus, C.; Danforth, E.; Ravussin, E. Norepinephrine turnover and energy expenditure in Pima Indian and white men. *Metabolism* **1993**, *42*, 723−729.
(184) Trayhurn, P.; Duncan, J. S.; Rayner, D. V. Acute cold-induced suppression of ob (obese) gene expression in white adipose tissue of mice: mediation by the sympathetic system. *Biochem. J.* **1995**, *311*, 729−733.
(185) Mantzoros, C. S.; et al. Activation of β3 adrenergic receptors suppresses leptin expression and mediates a leptin-independent inhibition of food intake in mice. *Diabetes* **1996**, *45*, 909−914.
(186) Racette, S. B.; Coppack, S. W.; Landt, M.; Klein, S. Leptin production during moderate-intensity aerobic excercise. *J. Clin. Endocrinol. Metab.* **1997**, *82*, 2275−2277.
(187) Kohrt, W. M.; Landt, M.; Birge, S. J. Serum leptin levels are reduced in response to excercise training, but not hormone replacement therapy in older women. *J. Clin. Endocrinol. Metab.* **1996**, *81*, 3980−3985.
(188) Wabitsch, M. M.; et al. Contribution of androgens to the gender difference in leptin production in obese children and adolescents. *J. Clin. Invest.* **1997**, *100*, 808−813.
(189) Garcia-Mayor, R. V.; et al. Serum leptin levels in normal children: relationship to age gender, body massindex, pituitary−gonadal hormones, and pubertal stage. *J. Clin. Endocrinol. Metab.* **1997**, *82*, 2849−2855.
(190) Blum, W. F.; et al. Plasma leptin levels in healthy children and adolescents: dependence on body mass index, body fat mass, gender, pubertal stage, and testoterone. *J. Clin. Endocrinol. Metab.* **1997**, *82*, 2904−2910.
(191) Jockenhovel, F.; et al. Testosterone substitution normalizes elevated serum leptin levels in hypogonadal men. *J. Clin. Endocrinol. Metab.* **1997**, *82*, 2510−2513.
(192) Sih, R.; et al. Testosterone replacement in older hypogonadal men: a 12-month randomized controlled trial. *J. Clin. Endocrinol. Metab.* **1997**, *82*, 1661−1667.
(193) Elbers, J. M.; et al. Reversal of the sex difference in serum leptin levels upon cross-sex hormone administration in transsexuals. *J. Clin. Endocrinol. Metab.* **1997**, *82*, 3267−3270.
(194) Haffner, S. M.; Mykkanen, L.; Ster, M. P. Leptin concentrations in women in the San Antonio Heart study: effect of menopausal status and postmenopausal hormone replacement therapy. *Am. J. Epidemiol.* **1997**, *146*, 581−585.
(195) Rosenbaum, M.; et al. Effects of gender, body composition, and menopause on plasma concentrations of leptin. *J. Clin. Endocrinol. Metab.* **1996**, *81*, 3424−3427.
(196) Yoshida, T.; Monkawa, T.; Hayashi, M.; Saruta, T. Regulation of expression of leptin mRNA and secretion of leptin by thyroid in 3T3-L1 adipocytes. *Biochem. Biophys. Res. Commun.* **1997**, *232*, 822−826.
(197) Fain, J. N.; Bahouth, S. W. Effect of tri-iodothyronine on leptin release and leptin mRNA accumulation in rat adipose tissue. *Biochem. J.* **1998**, *332*, 361−366.
(198) Mantzoros, C. S.; Rosen, H. N.; Greenspan, S. L.; Flier, J. S.; Moses, A. C. Short-term hyperthyroidism has no effect on serum leptin levels in man. *J. Clin. Endocrinol. Metab.* **1997**, *82*, 497−499.
(199) Ozata, M.; Ozisik, G.; Bingol, N.; Corakci, A.; Gundogan, M. A. The effects of thyroid status on plasma leptin levels in women. *J. Endocrinol. Invest.* **1998**, *21*, 337−341.
(200) Leal-Cerro, A.; et al. Serum immunoreactive-leptin levels are increased in patients with Cushing's syndrome. *Horm. Metab. Res.* **1996**, *28*, 711−713.
(201) Masuzaki, H.; et al. Glucocorticoid regulation of leptin synthesis and secretion in humans: elevated plasma leptin levels in Cushing's syndrome. *J. Clin. Endocrinol. Metab.* **1997**, *82*, 2542−2547.
(202) Cizza, G.; Lotsikas, A. J.; Licinio, J.; Gold, P. W.; Chrousos, G. P. Plasma leptin levels do not change in patients with Cushing's disease shorthly after correction of hypercortisolism. *J. Clin. Endocrinol. Metab.* **1997**, *82*, 2747−2750.
(203) Papaspyrou-Rao, S.; Schneider, S. H.; Petersen, R. N.; Fried, S. K. Dexamethasone increases leptin expression in humans in vivo. *J. Clin. Endocrinol. Metab.* **1997**, *82*, 1635−1637.
(204) Miell, J. P.; Englaro, P.; Blum, W. F. Dexamethasone induces an acute and sustained rise in circulating leptin levels in normal human subjects. *Horm. Metab. Res.* **1996**, *28*, 704−707.
(205) Kiess, W.; et al. High leptin concentrations in serum of very obese children are further stimulated by dexamethasone. *Horm. Metab. Res.* **1996**, *28*, 708−710.
(206) Dagogo-Jack, S.; Selke, G.; Melson, A. K.; Newcomer, J. W. Robust leptin secretory responses to dexamethasone in obese subjects. *J. Clin. Endocrinol. Metab.* **1997**, *82*, 3230−3233.
(207) Tataranni, P. A.; Pratley, R.; Maffei, M.; Ravussin, E. Acute and prolonged administration of glucocorticoids (methylprednisolone) does not affect plasma leptin concentration in humans. *Int. J. Obes.* **1997**, *21*, 327−330.
(208) Mantzoros, C. S.; Flier, J. S.; Rogol, A. D. A longitutidinal assessment of hormonal and physical alterations during normal puberty in boys. V. Rising leptin levels may signal the onset of puberty. *J. Clin. Endocrinol. Metab.* **1997**, *82*, 1066−1070.
(209) Ahima, R. S.; Dushay, J.; Flier, S. N.; Prabakaran, D.; Flier, J. S. Leptin accelerates the onset of puberty in normal female mice. *J. Clin. Invest.* **1997**, *99*, 391−395.
(210) Mounzih, K.; Lu, R.; Chehab, F. F. Leptin treatment rescues the sterility of genetically obese ob/ob males. *J. Clin. Endocrinol. Metab.* **1997**, *138*, 1190−1193.
(211) Butte, N. F.; Hopkinson, J. M.; Nicolson, M. A. Leptin in human reproduction: serum leptin levels in pregnant and lactating women. *J. Clin. Endocrinol. Metab.* **1997**, *82*, 585−589.
(212) Kawai, M.; et al. The placenta is not the main source of leptin production in pregnant rat: gestational profile of leptin in plasma and adipose tissues. *Biochem. Biophys. Res. Commun.* **1997**, *240*, 798−802.

(213) Sagawa, N.; Mori, T.; Masuzaki, H.; Ogawa, Y.; Nakao, K. Leptin production by hydatidiform mole. *Lancet* **1997**, *350*, 1518−1519.
(214) Grasso, P.; Leinung, M. C.; Ingher, S. P.; Lee, D. W. In vivo effects of leptin-related synthetic peptides on body weight and food intake in female *ob/ob* mice: localization of leptin activity to domains between amino acid residues 106−140. *J. Clin. Endocrinol. Metab.* **1997**, *138*, 1413−1418.
(215) Verploegen, S. A. B. W.; Plaetinck, G.; Devos, R.; Van der Heyden, J.; Guisez, Y. A human leptin mutant induces weight gain in normal mice. *FEBS Lett.* **1997**, *405*, 237−240.
(216) Tian, S. S.; et al. A small, nonpeptidyl mimic of granulocyte-colony-stimulating factor. *Science* **1998**, *281*, 257−259.

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