ADIPOSE TISSUE AS AN ENDOCRINE / PARACRINE ORGAN

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Until recently, adipose tissue has been considered to act only as a passive energy storage site, it is now known that adipocytes secrete a number of metabolically active molecules which act in an autocrine, paracrine or endocrine fashion. These include proteins such as adipsin, agouti protein, apolipoprotein E, cholesterol ester transfer protein (CETP), lipoprotein lipase, retinol binding protein, non-esterified fatty acids (NEFA), angiotensinogen, transforming growth factor-β (TGFβ), type 1 plasminogen activator inhibitor (PAI-1), tumour necrosis factor-a (TNF-α), interleukin-6 and leptin. Non-peptide compounds such as eicosanoids and monobutyrin are also produced.

LEPTIN

Kennedy[1] in 1953 was the first to propose the existence of a circulating factor, generated in proportion to body fat stores and influencing appetite and energy expenditure. Coleman[2] provided the evidence for the existence of this circulating factor in 1973 and Zhang *et al*[3] in 1994 cloned the ob gene and demonstrated that it encodes a circulating 16-kDa protein (ob protein or leptin).

The ob gene is expressed in white adipose tissue,[4] the stomach, placenta,[5] and the mammary gland. Two different mutations in the mouse leptin gene have been reported, one of which abolishes leptin gene transcription and thus prevents leptin synthesis, the other results in the production of truncated, inactive protein. These mutations, when present in the homozygous form, lead to the ob/ob phenotype. The leptin receptor gene has been cloned and is a member of the cytokine receptor family[6] and it exists in two forms: short (Ra) and long (Rb). The long form of the receptor (Rb) has been identified in several brain regions including arcuate and the paraventricular nuclei.[7] The four short isoforms (Ra) have been found in choroid plexus and the brain's capillary endothelium. Short isoforms are also present in a range of other tissues including liver, lung, kidney, adrenals, ovaries, haematopoietic stem cells, skeletal muscle and pancreas.[8] Extrahypothalamic leptin receptors are also found in cerebellum, thalamus, parabrachial nucleus and nucleus of the solitary tract.

The full array of leptin's actions through activation of these receptors is not clearly understood, however, the short receptor isoforms present in the kidney may mediate leptin clearance.[9] Recently a mutation in the leptin receptor gene has been described. Patients homozygous for this mutation present with early onset obesity, absence of pubertal development and dysfunction of growth and thyroid axes hormone activity.[10]

Leptin is a cytokine that binds to the transmembrane receptors and transmit their information inside the cell, after dimerisation. Leptin uses the short form of the receptor for transport across the blood-brain barrier. Then leptin binds to the long-form of the leptin receptor in the hypothalamus and decreases the production of neuropeptide Y (NPY). The long-form of leptin receptor transmits its information via the Janus Kinases (JAK) which subsequently phosphorylate transcription factors of the STAT family.

REGULATION OF LEPTIN AND INTERACTION WITH OTHER HORMONES

The obesity of the ob/ob mouse is attributable to mutations in the ob gene, which result in the production of a non-functional protein: the signal to the feeding control centres is absent or defective. These animals perceive incorrectly that the fat mass is low, resulting in hyperphagia, reduced energy expenditure and weight gain. Daily administration of leptin results in reduced food intake and weight loss. The db/db mouse has a mutation in the leptin receptor gene, which results in production of an abnormal receptor, which cannot respond normally to leptin binding. The db/db mouse shows early onset obesity, excessive food intake and reduced energy expenditure, but unlike the ob/ob mouse, exogenous leptin has no effect on food intake or body weight.

The circulating leptin levels increase exponentially with percent an amount of body fat. Leptin mRNA expression is higher in subcutaneous than in visceral fat depots.[11] Plasma leptin levels respond slowly to fasting, taking 12-24 hours to begin to decrease,[12] but are severely decreased by longer starvation.[13] Similarly, the increase in leptin after feeding is delayed. Massive overfeeding over a 12-hour period increases leptin levels by approximately 50% of initial basal values. Prolonged and strenuous exercise may decrease leptin concentrations,[14] however daily physical activity is not associated in circulating leptin levels in men.[15] Moderate intensity aerobic exercise may independently effect leptin levels in women.[16]

Independent of adiposity, plasma leptin levels are higher in women than in men[17] and show a diurnal rhythm. Females have higher serum leptin concentration before, during and after puberty than males. This sexual dimorphism could be explained by a suppressive action of androgens on leptin concentration in males and a stimulatory effect of oestradiol on leptin concentration in females.[18] Women also have higher concentrations of leptin in the cerebrospinal fluid than men, implying that a differing amount of leptin is delivered to the brain. This raises the possibility that women are relatively resistant to leptin and require increased leptin signalling to regulate body weight.

The peak in serum leptin is observed in the early morning hours and the nadir in the afternoon. In lean subjects, the majority of leptin circulates in the bound form whereas in obese subjects, the majority of leptin is present in the free form.

Several hormones affect leptin production. Postprandial physiological hyperinsulinaemia does not affect serum leptin levels,[20] however more prolonged insulin infusions or supraphysiologic insulin levels produce marked increases of circulating leptin.[21] Glucocorticoids enhance leptin gene

transcription and leptin levels.[22] Since the central effects of leptin have been found to be maximal in the absence of glucocorticoids and are markedly attenuated when glucocorticoids are supplemented, it has been proposed that glucocorticoids may induce a relative leptin resistance.[23] Leptin inhibits cortisol release by reducing basal secretion and blunting the ACTH-induced cortisol release from adrenocortical cells. Expression of CRF gene is increased by leptin administration and the anorectic effects of leptin are decreased by simultaneous administration of the CRF receptor antagonist, alpha-helical CRF.[24] These observations suggest that weight loss induced by leptin involves stimulation of hypothalamic CRF release.

Isoproterenol[25] and β_3-adrenergic receptor agonists reduce leptin mRNA expression and circulating levels.[26] Cigarette smoking, which induces a hyperadrenergic state, has been associated with decreased serum leptin levels. Similarly cold exposure induces a sympathetically mediated suppression of the ob gene leading to a rapid decrease in both ob mRNA and serum leptin levels.[27] Long-chain fatty acids and thiazolidinediones inhibit leptin expression. Testosterone, somatostatin and insulin-like growth factor-1 (IGF-1) also decrease leptin levels.

The long leptin receptor isoform activates the JAK (Janus Kinase) signal transducer and alters expression of many hypothalamic neuropeptides.[28, 29] The best studied of these neuropeptides are neuropeptide Y (NPY) in the arcuate nucleus, and thyrotropin and corticotrophin-releasing hormone in the paraventricular nucleus. NPY is downregulated by leptin,[30] which results in reduced appetite, increased sympathetic nervous system outflow, and alteration of peripheral metabolic milieu. Expression of NPY mRNA in the arcuate nucleus is increased in response to fasting and in leptin-deficient ob/ob and leptin-resistant db/db mice. In contrast, in the absence of NPY, ob/ob mice are less obese and are less severely affected by diabetes, sterility and somatotropic defects. Glucocorticoids also stimulate the expression of NPY and plasma levels of glucocorticoids are known to be elevated in circumstances in which NPY levels are augmented.[31]

Other targets of leptin in the hypothalamus include the melanocyte-stimulating hormone (a-MSH) and its competitive antagonist, agouti-related protein (AGRP), cocaine and amphetamine-regulated transcript (CART) peptide.[32]

Orexins, a recently discovered family of neuropeptides from the hypothalamus, stimulate appetite and food consumption. Their genes are expressed bilaterally and symmetrically in the lateral hypothalamus. Orexin immunoreactive terminals originating from the lateral hypothalamus have been found to make direct synaptic contact with neurones of arcuate nucleus that not only express NPY but also contain leptin receptors.[33] In addition, orexin-containing neurones also express leptin receptor immunoreactivity.[34] Leptin has been shown to reduce orexin concentrations in the lateral hypothalamus.[35]

Leptin has also been shown to have an inhibitory action on other hormones involved in the regulation of food intake including galanin and melanin-concentrating hormone (MCH).[36, 37]

A number of cytokines have also been found to regulate leptin gene expression and circulating levels in humans. Tumour necrosis factor α (TNFα) directly induces leptin gene expression,[38] and so does the interleukin-1 (IL-1), either directly or indirectly, by increasing the activity of the hypothalamic-pituitary-adrenal axis.[39] SOCS3, a member of the suppressers of cytokine signalling (SOCS) family, blocks leptin-induced activation of STAT3 in cells expressing the long form of the leptin receptor.[40]

Thus leptin-mediated suppression of feeding is controlled by a complex interaction of orexigenic (NPY and MCH) and anorexigenic signals (alpha-MSH/MC4-receptor, CART and CRF).

ROLE OF LEPTIN IN HUMAN PATHOPHYSIOLOGY

Leptin and Obesity

The levels of leptin are directly related to the quantity of body fat. However, obesity is a complex disorder, involving interaction of endocrine and autonomic nervous system.

It was expected that human obesity might be a leptin-deficient state in the light of dramatic effects of leptin administration to ob/ob mice. However, it was soon realised that that the leptin-deficient persons represent only a minority of obese humans. In contrast, most obese humans have increased leptin levels, indicating that obesity is a leptin-resistant state in most obese people. Unlike marked changes in serum leptin, CSF leptin is only modestly increased in obese subjects and the CSF leptin/serum leptin ratio decreases logarithmically with increasing BMI. Possible receptor and post-receptor defects, interference due to peripheral signals such as glucocorticoids, abnormal serum leptin binding, abnormal leptin catabolism, and leptin transport across the blood-brain barrier are the focus of research to solve this puzzle.

Leptin and Diabetes Mellitus

Insulin resistance and diabetes are consistent features of ob/ob mice and both improve in response to leptin administration.[41] Leptin inhibits insulin secretion from pancreatic islets, reduces insulin-stimulated glucose transport in adipocytes, and increases glucose transport, glycogen synthesis and fatty acid oxidation in skeletal muscle.[42, 43] There is a close correlation between leptin levels and fasting and two-hour post glucose load insulin levels suggesting a possible role for leptin in insulin resistance. This is true in both non-diabetic and diabetic subjects. An independent relationship between leptin and fasting insulin has been described.[44] Serum leptin levels are, however, similar in patients with type 2 diabetes mellitus and controls.[45] In contrast, fasting plasma leptin levels are higher in type 1 diabetes patients than in control subjects.[46] Chronically increased serum insulin levels probably cause this.

Leptin and Puberty

Leptin plays a permissive role in the onset of puberty in normal children; leptin levels increase before puberty and reach their peak at the onset of puberty.[47] There is a consistent rise in leptin levels in boys of normal height, weight, and weight-for-height just before a major increase in circulating testosterone concentration or an increase in testicular size.[48] As adolescents approach the end of puberty, leptin levels decline in boys but not in girls. In contrast, subjects with inactivating mutations of leptin receptor have hypogonadotrophic hypogonadism. Adequate levels of leptin in the circulation are essential, but not sufficient, for pubertal progression. Leptin treatment can reverse the delay in sexual maturation caused by food restriction and accelerate the onset of puberty.[49]

Leptin and polycystic ovary syndrome

Serum leptin levels in women with the polycystic ovary syndrome (PCOS) do not differ from those of normal women.[50] In addition, leptin does not appear to play a role in either hyperandrogenaemia or hypersecretion of luteinizing hormone in PCOS patients.[51] Brzechffa *et al*, however, reported that a substantial proportion of women with PCOS have leptin levels that are higher than expected for their body mass index, free testosterone, and insulin sensitivity.[52]

Leptin and the thyroid

Leptin is involved in the regulation of thyroid hormones. In the ob/ob mouse, an early abnormality is a low circulating level of thyroid hormones.[53] Patients with a leptin receptor mutation are hypothyroid, with low levels of free thyroxin, normal levels of TSH and a delayed TSH response to TRH stimulation. The leptin deficient children show slightly elevated TSH levels.[54]

Leptin and other conditions

End-stage renal disease, a catabolic and anorectic state, is associated with marked elevation in plasma leptin levels.[55] Serum leptin levels are similar to those of control subjects in anorexia nervosa and bulimia.[56] However, in anorexia nervosa, the CSF to serum leptin ratio is highest prior to weight gain. This is the mirror image of the disproportionately low CSF uptake of leptin in obese individuals with high serum leptin levels.[57] It is not known whether altered leptin transport across the blood-brain barrier in anorexia nervosa plays a primary role in the pathogenesis of the disease, or is a secondary epiphenomenon accompanying weight loss.

ADIPOSE TISSUE CYTOKINES

TNFα and IL-6 have important effects on lipid and glucose metabolism: both stimulate basal glucose uptake into cultured adipocytes,[58, 59] and inhibit lipoprotein lipase (LPL) activity. TNFα also stimulates lipolysis.[60] IL-6 has been shown to stimulate glucose and fatty acid oxidation as well as release of glucagon and cortisol in humans.[61, 62] Adipose tissue is a significant source of both IL-6 and TNFα. Both these cytokines have been implicated in human obesity as the expression of TNFα is elevated in most rodent models of obesity and the circulating concentrations of IL-6 increase with obesity.[63, 64] TNFα impairs insulin signalling, but the mechanism of this effect is unclear.[65, 66] Several studies have observed a relationship between hyperinsulinaemia and elevated circulating levels of cytokines, the mechanisms responsible for these relationships are poorly understood.[67] TNFα has been shown to increase basal glucose uptake by adipose tissue.[59] High concentrations of both TNFα and IL-6 increase basal intracellular calcium, which can alter phosphorylation of GLUT 4, thus blocking insulin-stimulated glucose uptake.[68] Increase in TNFα may cause insulin resistance by inhibiting insulin receptor substrate (IRS)-1- and IRS-2-mediated phosphatidylinositol-3-kinase activation.[69] Hydrogen peroxide at low concentrations may be involved in the development of insulin resistance in response to TNFα.[70]

Different studies have shown contrasting results regarding catecholamine regulation of adipose tissue cytokines. Both stimulatory and inhibitory effects of catecholamines on these cytokines have been demonstrated.[71, 72] Both TNFα and IL-6 inhibit LPL activity as well as increase lipolysis.[73] This may down-regulate triglyceride deposition and increase fuel mobilisation from the adipose tissue. This is supported by the observations that repeated injections of both TNFα and IL-6 cause weight loss in mice and this is inhibited by pre-treatment with either anti-TNFα or anti-IL-6 monoclonal antibodies.[74]

TNFα induces the release of both leptin and IL-6 from adipose tissue.[75] In another study, however, short-term exposure of isolated adipocytes to TNFα did not affect leptin concentration, but a prolonged exposure produced a concentration-dependent inhibition of leptin secretion and gene expression.[76] The acute effects of TNFα to increase circulating leptin levels *in vivo* may be indirect, as TNFα appears to induce dissociation between adipocyte glucose metabolism and leptin production.

IL-6 has a direct central role, as IL-6 receptors are present in the hypothalamus.[77] Cytokines, particularly IL-1 and IL-6, act as endogenous pyrogens in the brain and stimulate thermogenesis via synthesis of prostaglandins and CRF.[78] Peptides such as lipocortin-1, arginine vasopressin, and alpha MSH potently inhibit central effects of cytokines.

Both TNFα and IL-6 stimulate the hypothalamic-pituitary-adrenal axis (HPA). IL-6 stimulates release of CRH, which is mediated by an eicosanoid cyclo-oxygenase pathway.[79] IL-6 and TNFα also stimulate ACTH release in rats.[80, 81] These actions are mediated by CRH as co-administration of an anti-CRH antibody with the TNFα or IL-6 blocks the effect of these cytokines on ACTH secretion.[81] IL-6 has been shown to stimulate CRH release from rat medial basal hypothalamus and ACTH secretion by human foetal pituitary cultures.[82, 83]

The effects of IL-6 on hypothalamic-pituitary-thyroid axis are not clear. IL-6 inhibits TSH release, whereas it stimulates TRH release.[84, 85] TNFα has a direct inhibitory effect on thyroid hormone secretion and deiodinase activity in thyroid gland.[86, 87]

NON-ESTERIFIED FATTY ACIDS (NEFA)

Elevated concentrations of NEFA in the circulation are associated with impaired insulin sensitivity.[88] NEFA impairs insulin-mediated glucose uptake and glycogen synthase activity in skeletal muscle. They also enhance hepatic gluconeogenesis and hepatic glucose output. NEFA also stimulates β-cell insulin secretion.[89] Local NEFA in adipose tissue stimulates the activity of LPL.[90]

CHOLESTERYL ESTER TRANSFER PROTEIN (CETP)

Transfer of cholesteryl ester between lipoproteins is mediated by a plasma glycoprotein called Cholesteryl ester transfer protein (CETP). CETP mRNA is more abundantly expressed in the immature fat cells of human adipose tissue as compared to lipid rich mature adipocytes, implying that CETP plays an important role in adipocyte cholesterol accumulation from high-density lipoproteins.[91] CETP mRNA increases in subcutaneous adipose tissue in response to cholesterol feeding. High levels of CETP favour decreased plasma high-density lipoprotein cholesterol and increased levels of cholesterol in apolipoprotein B containing lipoproteins. Beta-adrenergic agents increase the secretion of CETP.[92]

PLASMINOGEN ACTIVATOR INHIBITOR 1 (PAI-1)

Plasminogen activator inhibitor 1 (PAI-1) is likely to play a

role in vascular disease, primarily in subjects with android obesity. Elevated Plasma plasminogen activator inhibitor 1 (PAI-1) activity is elevated in obesity. Human adipose tissue, in particular visceral tissue, is an important contributor to the elevated plasma PAI-1 levels observed in central obesity.[93] *In vitro* studies have shown a stimulatory effect of various lipoproteins on PAI-1 release from different cells, including adipocytes.

Insulin and glucocorticoids increase PAI-1 expression in human adipose tissue.[94] Conditions that increase endogenous plasma insulin levels are associated with increase in plasma PAI-1, whereas conditions that reduced endogenous insulin are associated with decreases in plasma PAI-1 levels.[95] There is a strong relationship between PAI-1 and leptin levels, indicating that leptin may increase PAI-1 concentrations in obese subjects.[96] PAI-1 mRNA expression is also stimulated by TNFα, an effect potentiated by insulin.[97, 98] Chronic elevation in TNFα that occurs locally in the adipose tissue in human and rodent obesity may act via an autocrine manner to stimulate PAI-1 biosynthesis by the adipocyte. TGFβ also stimulates PAI-1 biosynthesis in adipocytes.[99]

APOLIPOPROTEIN E

Apolipoprotein E is an important constituent of plasma lipoproteins and a ligand for several lipoprotein receptors. It is produced mainly in the liver but also in several peripheral tissues, such as brain, adrenal glands, kidney, macrophages, and adipocytes.

ANGIOTENSINOGEN

Adipose tissue is an important source of angiotensinogen after liver. Angiotensinogen has been shown to be elevated in adipose tissue of obese (ob/ob and db/db) mice. A potential link between insulin resistance and high blood pressure has been speculated by means of increased angiotensinogen secretion from adipose tissue.

Angiotensinogen mRNA is up regulated by insulin and down regulated by beta-adrenergic stimulation in adipocytes.[100]

GLUCOCORTICOIDS AND SEX STEROIDS

Adipose tissue possesses two enzymes involved in sex steroid metabolism, 17β-hydoxysteroid oxidoreductase and aromatase.[101] 17β-hydoxysteroid oxidoreductase converts androstenedione to testosterone and oestrone to oestradiol. Androgens are also aromatised to estrogens in the adipose tissue by aromatase.

Adipose tissue possesses 11-hydroxysteroid-β dehydrogenase (11-β HSD), which inter-converts cortisol and cortisone. Local 11-β HSD activity may influence local cortisol-induced stimulation of aromatase activity.[102] Increased 11-β HSD expression in visceral adipose tissue has also been suggested to contribute to the development of central obesity.[103]

Adipocytes also express estrogen mRNA and correlate inversely with cytochrome P450 aromatase mRNA levels in adipocytes.[104]

OTHERS

Monobutyrin is a simple lipid secreted by adipocytes that stimulates both angiogenesis and vasodilatation of microvascular beds. Monobutyrin production is increased during lipolysis.

Adipose tissue contains relatively high levels of the specific mRNA for retinol-binding protein (RBP).[105] Adipocytes store retinoids and synthesise and secrete RBP. Retinoic acid, dexamethasone, and triiodothyronine regulate RBP gene expression.[106]

Transforming growth factor β (TGFβ) expression is elevated in the adipose tissue of obese mice.[107] TNFα contributes to this elevated TGFβ expression. It is postulated that TGFβ may play a role in the increased PAI-1 and vascular pathologies associated with obesity.

Fibroblast growth factor-2 (FGF-2) stimulates cell proliferation and capillary growth. Noradrenaline mediates FGF-2 production, in part via the beta-adrenergic receptor, in adipose tissue.[108]

Prostacyclin (PGI-2) plays a key role in the process of preadipose cell differentiation through a paracrine mode of action. Angiotensin-II has been shown to induce the production of PGI-2 in suspensions of isolated adipocytes.[109]

CONCLUSION

Adipocytes synthesise and secrete numerous peptide and non-peptide compounds, suggesting a potential link between excess of adipose tissue mass and various physiopathologic consequences. Increased production from adipose tissue of leptin, cytokines, and NEFA contributes to changes in systemic metabolism of obese subjects causing insulin resistance. Adipocytes are able to secrete proteases, protease inhibitors, hormones, growth factors, and cytokines, and it is likely that some of these proteins contribute to the cardiovascular risk associated with obesity.

REFERENCES

1. Kennedy GC. The role of depot fat in the hypothalamic control of food intake in the rat. *Proc Royal Soc London* 1953; 140B:579-92.
2. Coleman DL. Effects of parabiosis of obese with diabetes and normal mice. *Diabetologia* 1973; 9:294-8.
3. Zhang Y, Proenca R, Maffei M *et al*. Positional cloning of the mouse obese gene and its human homologue. *Nature* 1994; 372:425-32.
4. Cinti S, Frederick RC, Zingaretti MC *et al*. Immuno-histochemical localisation of leptin and uncoupling protein in white and brown adipose tissue. *Endocrinology* 1997; 138:797-804.
5. Masuzaki H, Ogawa Y, Sagawa N *et al*. Nonadipose tissue production of leptin: leptin as a novel placenta-derived hormone in humans. *Nat Med* 1997; 3:1029-33.
6. Baumann H, Morella KK, White DW *et al*. The full-length leptin receptor has signalling capabilities of interleukin 6-type cytokine receptors. *Proc Natl Acad Sci USA* 1996; 93:8374-8.
7. Woods Al, Stock Ml. Leptin activation in hypothalamus. *Nature* 1996; 381:745.
8. Kieffer TJ, Heller RS, Habener JF. Leptin receptors expressed on pancreatic b-cells. *Biochem Biophys Res Commun* 1996; 224:522-7.
9. Cumin F, Baum HP, Levens N. Leptin is cleared from the circulation primarily by the kidney. *Int J Obes Relat Metab Disord* 1996; 20:l120-6.
10. Clement K, Vaiss C, Lahlou N *et al*. A mutation in the human leptin receptor gene causes obesity and pituitary dysfunction. *Nature* 1998; 392:398-401.
11. Hube F, Lietz U, Igel M *et al*. Difference in leptin mRNA levels between omental and subcutaneous abdominal adipose tissue from obese humans. *Horm Res* 1996; 28:690-3.
12. Trayhurn P, Thomas MEA, Duncan JS *et al*. Effects of fasting and refeeding on ob gene expression in white adipose tissue of lean and obese (ob/Ob) mice. *FEBS Lett* 1995; 368:488-90.
13. Maffei M, Halaas J, Ravussin E *et al*. Leptin levels in human and

rodent: Measurement of plasma leptin and ob RNA in obese and weight-reduced subjects. *Nat Med* 1995; 1:1155-61.

[14] Landt M, Lawson GM, Helgeson JM *et al*. Prolonged exercise decreases serum leptin concentrations. *Metabolism* 1997; 46(10):1109-12.

[15] Mantzoros CS, Liolios AD, Tritos NA *et al*. Circulating insulin concentrations, smoking and alcohol intake are important independent predictors of leptin in young healthy men. *Obesity Res* 1998; 6:179-85.

[16] Hickey MS, Houmard JA, Considine RV *et al*. Gender-dependent effects of exercise training on serum leptin levels in humans. *Am J Physiol* 1997; 272:E562-6.

[17] Rosenbaum M, Nicolson M, Hirsch J *et al*. Effects of gender, body composition, and menopause on plasma concentrations of leptin. *J Clin Endocrinol Metab* 1996; 81:3424-7.

[18] Demerath EW, Towne B, Wisemandle W *et al*. Serum leptin concentration, body composition, and gonadal hormones during puberty. *Int J Obes Relat Metab Disord* 1999; 23(7):678-85.

[19] Schwartz MW, Peskind E, Raskind M *et al*. Cerebrospinal fluid leptin levels: relationship to plasma levels and to adiposity in humans. *Nat Med* 1996; 2:589-93.

[20] Clapham JC, Smith SA, Moore GBT *et al*. Plasma leptin concentrations and ob gene expression in subcutaneous adipose tissue are not regulated acutely by physiological hyperinsulinaemia in lean and obese humans. *Int J Obes Relat Metab Disord* 1997; 21:179-83.

[21] Caro JF, Sinha MK, Kolaczynski JW *et al*. Leptin: the tale of an obesity gene. *Diabetes* 1996; 45:1455-62.

[22] De Vos P, Saladin R, Auwerx J *et al*. Induction of ob gene expression by corticosteroids is accompanied by body weight loss and reduced food intake. *J Biol Chem* 1995; 270:15958-61.

[23] Zakrzewska KE, Sainsbury CA, Rohner-Jeanrenaud F *et al*. Glucocorticoids as counterregulatory hormones of leptin. Toward an understanding of leptin resistance. *Diabetes* 1997; 46:717-19.

[24] Uehara Y, Shimizu H, Ohtani K *et al*. Hypothalamic corticotropin-releasing hormone is a mediator of the anorexigenic effect of leptin. *Diabetes* 1998; 47:890-3.

[25] Donahoo WT, Jensen TR, Yost TJ *et al*. Isoproterenol and somatostatin decrease plasma leptin in humans: a novel mechanism regulating leptin secretion. *J Clin Endocrinol Metab* 1997; 82:4139-43.

[26] Mantzoros CS, Qu D, Frederich RC *et al*. Activation of beta(3) adrenergic receptors suppresses leptin expression and mediates a leptin-independent inhibition of food intake in mice. *Diabetes* 1996; 45:909-14.

[27] Trayhurn P, Duncan JS, Rayner DV. Acute cold-induced suppression on ob gene expression in white adipose tissue of mice: mediation by the sympathetic system. *Biochem J* 1995; 311:729-33.

[28] Elmquist JK, Ahima RS, Elias CF *et al*. Leptin activates distinct projections from the dorsomedial and ventromedial hypothalamic nuclei. *Proc Natl Acad Sci USA* 1998; 95:741-6.

[29] Wolf G. Neuropeptides responding to leptin. *Nutr Rev* 1997; 55:85-8.

[30] Yu WH, Kimura M, Walczewska A *et al*. Role of leptin in hypothalamic-pituitary function. *Proc Natl Acad Sci USA* 1997; 94:1023-8.

[31] DalIman MF, Akana SF, Strack AM *et al*. The neural network that regulates energy balance is responsive at a site proximal to CRF neurons. *Ann NY Acad Sci* 1995; 771:730-42.

[32] Flier JS, Maratos-Flier E. Obesity and the hypothalamus: novel peptides for new pathways. *Cell* 1998; 92:437-40.

[33] Horvath Th, Diano S, van den Pol AN. Synaptic interaction between hypocretin (orexin) and neuropeptide Y cells in the rodent and primate hypothalamus: a novel circuit implicated in metabolic and endocrine regulations. *J Neurosci* 1999; 19(3):1072-87.

[34] Hakansson M, de Lecea L, Sutcliffe JO *et al*. Leptin receptor- and STAT3-immunoreactivities in hypocretin/orexin neurones of the lateral hypothalamus. *J Neuroendocrinol* 1999; 11(8):653-63.

[35] Beck B, Richy S. Hypothalamic hypocretin/orexin and neuropeptide Y: divergent interaction with energy depletion and leptin. *Biochem Biophys Res Commun* 1999; 258(1):119-22.

[36] Sahu A. Evidence suggesting that galanin, melanin-concentrating hormone (MCH), neurotensin (NT), proopiomelanocortin (POMC) and neuropeptide Y (NPY) are targets of leptin signalling in the hypothalamus. *Endocrinology* 1998; 139(2):795-8.

[37] Shimada M, Tritos NA, Lowell BB *et al*. Mice lacking melanin-concentrating hormone are hypophagic and lean. *Nature* 1998; 396:670-4.

[38] Zumbach MS, Boehma MWJ, Wahl P *et al*. Tumour necrosis factor increases leptin levels in humans. *J Clin Endocrinol Metab* 1997; 82:4080-2.

[39] Janik JE, Cutri BD, Considine RV *et al*. Interleukin la increases serum leptin concentrations in humans. *Diabetologia* 1997; 40:348-51.

[40] Bjorbaek C, Elmquist JK, Frantz JD *et al*. Identification of SOCS-3 as a potential mediator of leptin resistance. *Molecular Cell* 1998; 1:619-25.

[41] Pelleymounter MA, Cullen Ml, Baker MB *et al*. Effects of the obese gene product on bodyweight regulation in ob/ob mice. *Science* 1996; 269:540-3.

[42] Kieffer TJ, Heller RS, Leech CA *et al*. Suppression of insulin secretion by the activation of ATP-sensitive K channels in pancreatic b-cells. *Diabetes* 1997; 46:1087-93.

[43] Cohen B, Novick D, Rubinstein M. Modulation of insulin activities by leptin. *Science* 1996; 274:1185-8.

[44] Hanley AJO, Harris SB, Gao XJ *et al*. Serum immunoreactive leptin concentrations in a Canadian aboriginal population with high rates of NIDDM. *Diabetes Care* 1997; 20(9):1408-15.

[45] Mcoregor OP, Desaga JF, Ehlenz K *et al*. Radioimmnunological measurement of leptin in plasma of obese and diabetic human subjects. *Endocrinology* 1996; 137:1501-4.

[46] Tuominen JA, Ebeling P, Stenman UH *et al*. Leptin synthesis is resistant to acute effects of insulin in insulin-dependent diabetes mellitus patients. *J Clin Endocrinol Metab* 1997; 82:381-2.

[47] Garcia-Mayor RV, Andrade MA, Rios M *et al*. Serum leptin levels in normal children: relationship to age, gender, body mass index, pituitary-gonadal hormones, and pubertal stage. *J Clin Endocrinol Metab* 1997; 82:2849-55.

[48] Mantzoros CS, Flier JS, Rogol AD. A longitudinal 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; 82:1065-70.

[49] Ahima R, Dushay J, Flier S *et al*. Leptin accelerates the timing of puberty in normal female mice. *J Clin Invest* 1997; 99:391-5.

[50] Rouru J, Anttila L, Koskinen P *et al*. Serum leptin concentrations in women with polycystic ovary syndrome. *J Clin Endocrinol Metab* 1997; 82:1697-700.

[51] Laughlin OA, Morales Al, Yen SSC. Serum leptin levels in women with polycystic ovary syndrome: the role of insulin resistance / hyperinsulinemia. *J Clin Endocrinol Metab* 1997; 82:1692-6.

[52] Brzechffa PR, Jakimiuk J, Agarwal SK *et al*. Serum immunoreactive leptin concentrations women with polycystic ovary syndrome. *J Clin Endocrinol Metab* 1996; 81:4166-9.

[53] York DA, Otto W, Taylor TO. Thyroid status of obese (ob/ob) mice and its relationship to adipose tissue metabolism. *Comp Biochem Physiol* 1078; 590:59-65.

[54] Montagne CT, Farooqi IS, Whitehead JP *et al*. Congenital leptin deficiency is associated with severe early-onset obesity in humans. *Nature* 1997; 387:903-8.

[55] Merabet E, Doggie-Jack S, Coyne DW *et al*. Increased plasma leptin concentrations in end-stage renal disease. *J Clin Endocrinol Metab* 1997; 82:847-50.

[56] Grinspoon S, Gulick T, Askari H *et al*. Serum leptin levels in women with anorexia nervosa. *J Clin Endocrinol Metab* 1996; 81:3861-3.

[57] Mantzoros CS, Flier JS, Lesem MD *et al*. Cerebrospinal fluid in

anorexia nervosa: correlation with nutritional status and potential role in resistance to weight gain. *J Clin Endocrinol Metab* 1997; 82:1845-51.

58 Stouthard JM, Oude-Elferink RP, Sauerwein HP. Interleukin-6 enhances glucose transport in 3T3-L1 adipocytes. *Biochem Biophys Res Commun* 1996; 220:241-5.

59 Wang CN, O'Brien L, Brindley DN. Effects of cell-permeable ceramides and tumor necrosis factor-α on insulin signaling and glucose uptake in 3T3-L1 adipocytes. *Diabetes* 1998; 47:24-31.

60 Hauner H, Petruschke T, Russ M *et al*. Effects of tumour necrosis factor alpha (TNFα) on glucose transport and lipid metabolism of newly-differentiated human fat cells in cell culture. *Diabetologia* 1995; 38:764-71.

61 Ritchie DG. Interleukin-6 stimulates hepatic glucose release from prelabeled glycogen pools. *Am J Physiol* 1990; 258:E57-64.

62 Stouthard JM, Romijn JA, Van-der-Poll T *et al*. Endocrinologic and metabolic effects of interleukin-6 in humans. *Am J Physiol* 1995; 268:E813-9.

63 Kern PA, Saghizadeh M, Ong JM *et al*. The expression of tumor necrosis factor in human adipose tissue. Regulation by obesity, weight loss, and relationship to lipoprotein lipase. *J Clin Invest* 1995; 95:2111-9.

64 Hotamisligil GS, Arner P, Caro JF *et al*. Increased adipose tissue expression of tumor necrosis factor-alpha in human obesity and insulin resistance. *J Clin Invest* 1995; 95:2409-15.

65 Hotamisligil GS, Peraldi P, Budavari A *et al*. IRS-1 mediated inhibition of insulin receptor tyrosine kinase activity in TNF-alpha- and obesity-induced insulin resistance. *Science* 1996; 271:665-8.

66 Stephens JM, Lee J, Pilch PF. Tumor necrosis factor-alpha-induced insulin resistance in 3T3-L1 adipocytes is accompanied by a loss of insulin-receptor substrate 1 and GLUT4 expression without a loss of insulin receptor-mediated signal transduction. *J Biol Chem* 1997; 272:971-6.

67 Morohoshi M, Fujisawa K, Uchimura I *et al*. Glucose-dependent interleukin 6 and tumor necrosis factor production by human peripheral blood monocytes in vitro. *Diabetes* 1996; 45:954-9.

68 Sayeed MM. Alterations in calcium signaling and cellular responses in septic injury. *New Horiz* 1996; 4:72-86.

69 del Aguila LF, Claffey KP, Kirwan JP. TNF-alpha impairs insulin signaling and insulinstimulation of glucose uptake in C2C12 muscle cells. *Am J Physiol* 1999; 276(5 Pt 1):E849-55.

70 Hansen LL, Ikeda Y, Olsen GS *et al*. Insulin signaling is inhibited by micromolar concentrations of H(2)O(2). Evidence for a role of H(2)O(2) in tumor necrosis factor alpha-mediated insulin resistance. *J Biol Chem* 1999; 274(35):25078-84.

71 Bruysek L, Houstek J. Beta-adrenergic stimulation of interleukin-1 alpha and interleukin-6 expression in mouse brown adipocytes. *FEBS Lett* 1997; 41:83-6.

72 van-der-Poll T, Jansen J, Endert E *et al*. Noradrenaline inhibits lipopolysaccharide-induced tumor necrosis factor and interleukin-6 production in human whole blood. *Infect Immun* 1994; 62:2046-50.

73 Greenberg AS, Nordon RP, McIntosh J *et al*. Interleukin-6 reduces lipoprotein lipase activity in adipose tissue of mice in vivo and in 3T3-L1 adipocytes: a possible role for interleukin-6 in cancer cachexia. *Cancer Res* 1992; 52:4113-6.

74 Matthys P, Billiau A. Cytokines and cachexia. *Nutrition* 1997; 13:763-70.

75 Grunfeld C, Zhao C, Fuller J *et al*. Endotoxin and cytokines induce expression of leptin, the ob gene product, in hamsters. *J Clin Invest* 1996; 97:2152-7.

76 Medina EA, Stanhope KL, Mizuno TM *et al*. Effect of tumor necrosis factor alpha on leptin secretion and gene expression: relationship to changes of glucose metabolism in isolated rat adipocytes. *Int J Obes Relat Metab Disord* 1999; 23(8):896-903.

77 Jones TH, Kennedy RL. Cytokines and hypothalamic-pituitary function. *Cytokine* 1993; 5:531-8.

78 Rothwell NJ. CNS regulation of thermogenesis. *Crit Rev Neurobiol* 1994; 8:1-10.

79 Navarra P, Tsagarakis S, Faria MS *et al*. Interleukins-1 and -6 stimulate the release of corticotropin-releasing hormone-41 from rat hypothalamus in vitro via the eicosanoid cyclooxygenase pathway. *Endocrinology* 1991; 128:37-44.

80 Lyson K, McCann SM. Induction of adrenocorticotropic hormone release by interleukin-6 in vivo and in vitro. *Ann NY Acad Sci* 1992; 650:182-5.

81 Ebisui O, Fukata J, Murakami N *et al*. Effect of interleukin-1 receptor antagonist and antiserum to TNF-α on LPS-induced plasma ACTH and corticosterone rise in rats. *Am J Physiol* 1994; 266:E986-92.

82 Kageyama K, Watanobe H, Takebe K. In vivo evidence that arginine vasopressin is involved in the adrenocorticotropin response induced by interleukin-6 but not by tumor necrosis factor-alpha in the rat. *Neuroimmunomodulation* 1995; 2:137-40.

83 Shimon I, Yan X, Ray DW, Melmed S. Cytokine-dependent gp130 receptor subunit regulates human fetal pituitary adrenocorticotropin hormone and growth hormone secretion. *J Clin Invest* 1997; 100:357-63.

84 Tsigos C, Papanicolaou DA, Defensor R *et al*. Dose effects of recombinant human interleukin-6 on pituitary hormone secretion and energy expenditure. *Neuroimmunology* 1997; 66:54-62.

85 Kennedy JA, Wellby ML, Zotti R. Effect of interleukin-1 beta, tumour necrosis factor-alpha and interleukin-6 on the control of thyrotropin secretion. *Life Sci* 1995; 57:487-501.

86 Ozawa M, Sato K, Han DC *et al*. Effects of tumor necrosis factor-α/cachectin on thyroid hormone metabolism in mice. *Endocrinology* 1988; 123:1461-7.

87 Sato K, Satoh T, Shizume K, Ozawa M *et al*. Inhibition of I^{125} organification and thyroid hormone release by interleukin-1, tumor necrosis factor-1, tumor necrosis factor-α and interferon-γ in human thyrocytes in suspension cultures. *J Clin Endocrinol Metab* 1990; 70:1735-43.

88 Randle PJ, Garland PB, Hales CN *et al*. The glucose fatty acid cycle. Its role in insulin sensitivity and the metabolic disturbances of diabetes mellitus. *Lancet* 1963; 1:785-9.

89 Frayn KN. Role of non-esterified fatty acids in the metabolic changes of obesity. *Int J Obes* 1996; 20(Suppl 4):7-10.

90 Amri EZ, Teboul L, Vannier C *et al*. Fatty acids regulate the expression of lipoprotein lipase gene and activity in preadipose and adipose cells. *Biochem J* 1996; 314:541-6.

91 Radeau T, Robb M, McDonnell M *et al*. Preferential expression of Cholesteryl ester transfer protein mRNA by stromal-vascular cells of human adipose tissue. *Biochimica et Biophysica Acta* 1998; 1392(2-3):245-53.

92 Shen GX, Cai W, Angel A. Increased secretion of Cholesteryl ester transfer protein from hamster adipose tissue: stimulation by beta-adrenergic agents. *Atherosclerosis* 1998; 140(1):113-20.

93 Alessi MC, Peiretti F, Morange P *et al*. Production of plasminogen activator inhibitor 1 by human adipose tissue: possible link between visceral fat accumulation and vascular disease. *Diabetes* 1997; 46(5):860-7.

94 Morange PE, Aubert J, Peiretti F *et al*. Glucocorticoids and insulin promote plasminogen activator inhibitor 1 production by human adipose tissue. *Diabetes* 1999; 48(4):890-5.

95 Juhan-Vague I, Alessi MC. PAI-1, obesity, insulin resistance and risk of cardiovascular events. *Thromb Haemost* 1997; 78:656-60.

96 De Mitrio V, De Pergola G, Vettor R *et al*. Plasma plasminogen activator inhibitor-1 is associated with plasma leptin irrespective of body mass index, body fat mass, and plasma insulin and metabolic parameters in premenopausal women. *Metabolism: clinical and experimental* 1999; 48(8):960-4.

97 Cigolini M, Tonoli M, Frigotto L *et al*. Expression of plasminogen activator inhibitor-1 in human adipose tissue: a role for TNF-alpha? *Atherosclerosis* 1999; 143(1):81-90.

98 Sakamoto T, Woodcock-Mitchell J, Marutsuka K *et al*. TNF-alpha and insulin, alone and synergistically, induce plasminogen activator inhibitor-1 expression in adipocytes. *Am J Physiol* 1999; 276(6 Pt 1):C1391-7.

[99] Sawdey MS, Loskutoff DJ. Regulation of murine type1 plasminogen activator inhibitor gene expression in vivo: tissue specificity and induction by lipopolysaccharide, tumor necrosis factor-α, and transforming growth factor-β *J Clin Invest* 1991; 88:1346-53.

[100] Jones BH, Standridge MK, Taylor JW *et al*. Angiotensinogen gene expression in adipose tissue: analysis of obese models and hormonal and nutritional control. *Am J Physiol* 1997; 273(1 Pt 2):R236-42.

[101] Pedersen SB, Fuglsig S, Sjogren P *et al*. Identification of steroid receptors in human adipose tissue. *Eur J Clin Invest* 1996; 26:1051-6.

[102] Tchernof A, Despres JP, Belanger A *et al*. Reduced testosterone and adrenal C19 steroid levels in obese men. *Metab: Clin Exp* 1995; 44:513-9.

[103] Björntorp P. The regulation of adipose tissue distribution in humans. *Int J Obes* 1996; 20:291-302.

[104] Price TM, O'Brien SN. Determination of estrogen messenger ribonucleic acid (mRNA) and cytochrome P450 aromatase mRNA levels in adipocytes and adipose stromal cells by competitive polymerase chain reaction amplification. *J Clin Endocrinol Metab* 1993; 77(4):1941-5.

[105] Makover A, Soprano DR, Wyatt ML *et al*. Localization of retinol-binding protein messenger RNA in the rat kidney and in the perinephric fat tissue. *J Lipid Res* 1992; 267(3):1805-10.

[106] Okuno M, Caraveo VE, Goodman DS *et al*. Regulation of adipocyte gene expression by retinoic acid and hormones: effects on the gene encoding cellular retinol-binding protein. *J Lipid Res* 1995; 36(1):137-47.

[107] Samad F, Yamamoto K, Loskutoff DJ. Elevated expression of transforming growth factor-beta in adipose tissue from obese mice. *J Mol Med* 1997; 3(1):37-48.

[108] Yamashita H, Sato N, Kizaki T *et al*. Norepinephrine stimulates the expression of fibroblast growth factor-2 in rat brown adipocyte primary culture. *Cell Growth Differ* 1995; 6(11):1457-62.

[109] Darimont C, Vassaux G, Ailhaud G *et al*. Differentiation of preadipose cells: paracrine role of prostacyclin upon stimulation of adipose cells by angiotensin-II. *Endocrinology* 1994; 135(5):2030-6.