

# Adiposity signals, genetic and body weight regulation in humans

R Canello\*, A Tounian\*, Ch Poitou, K Clément

## SUMMARY

Numerous signals convey information about body fat status from the periphery to the brain areas that control energy homeostasis so that, throughout life, body weight remains nearly stable. These signals mainly originate, either from the adipose tissue, like leptin and to a lesser extent interleukin 6, or from the pancreas, like insulin and amylin. These factors circulate in proportion to body fat mass and they are referred to as "adiposity signals". It is well established, at least for leptin and insulin, that they enter the brain from the plasma where they induce/repress a network of important neuropeptide regulators of energy intake and expenditure. Beside these endocrine signals, a growing amount of literature show data relative to adipocyte-derived molecules, most of them belonging to the cytokine family, like IL6, TNF $\alpha$ , IL8, IL10 whose secretion also correlates with body fat mass and that may locally regulate fat mass expansion. Others, like Adiponectin, are negatively correlated with body fat mass. These "adiposity molecules" have already been involved in insulin resistance associated with obesity and inflammatory process. They may participate to a complex inter organ dialogue. In this review, we will synthesize data relative to the role played by insulin, leptin and amylin, either alone or through a cross talk, in "energy level sensing" at the brain level. Furthermore, we will develop how "adiposity molecules" through their paracrin and/or autocrin action may contribute to maintain fat mass expansion, therefore representing new adiposity molecules *per se*. Lastly, since any distortion in the metabolic circuitry of energy homeostasis is susceptible to lead to a pathological status like obesity, the impact of known genetic polymorphisms in genes encoding the adiposity signals will be discussed.

**Key-words:** Adiposity signal · Metabolic signals · Obesity · White adipose tissue · Adipocytes · Inflammation · Insulin · Leptin.

Canello R, Tounian A, Poitou Ch, Clément K. Adiposity signals, genetic and body weight regulation in humans  
*Diabetes Metab* 2004,30,215-27

## RÉSUMÉ

### Signaux d'adiposité, génétique et régulation du poids corporel chez l'homme

De nombreux signaux transmettent au cerveau des informations sur le statut des réserves d'énergie contribuant ainsi au contrôle de la balance énergétique et au maintien d'un poids relativement stable. Ces signaux sont produits par le tissu adipeux comme la leptine et l'interleukine 6 ou par le pancréas, comme l'insuline et l'amyline. Ces facteurs circulent en proportion de la masse grasse et sont appelés classiquement des signaux d'adiposité. Il est bien établi, au moins pour la leptine et l'insuline, que ces molécules sont transférées de la circulation vers le cerveau où elles induisent/répriment des circuits neuronaux clés qui contrôlent la prise alimentaire et la dépense énergétique. En dehors de ces molécules endocrines, un nombre croissant de molécules produites par le tissu adipeux sont décrites dans la littérature ; beaucoup d'entre elles appartiennent à la famille des cytokines comme IL6, TNF $\alpha$ , IL8, IL10 or d'autres comme l'adiponectine. La plupart est exprimé ou sécrété en proportion de la masse grasse et pourraient contribuer à contrôler localement son expansion. L'adiponectine, elle, est synthétisée en proportion inverse de la masse grasse. Ces molécules d'adiposité sont supposées impliquées dans l'insulino résistance associée à l'obésité et à des processus d'inflammation. Elles participent probablement au dialogue complexe entre les organes. Nous résumons les informations sur le rôle joué par l'insuline, la leptine et l'amyline, soit seules ou *via* un dialogue complexe impliquant plusieurs d'entre elles, permettant ainsi au cerveau de percevoir les variations des réserves d'énergie. Nous citerons également le rôle d'autres molécules d'adiposité ayant un rôle paracrine et/ou autocrine mais pouvant également contribuer à l'équilibre énergétique. Elles représentent d'une certaine façon des « molécules signales d'adiposité ». Enfin, comme toute modification même modérée des circuits contrôlant les réserves d'énergie est susceptible de conduire à des situations pathologiques comme l'obésité, le rôle de polymorphismes situés dans les gènes codant pour ces molécules d'adiposité est discuté.

**Mots-clés :** Signal d'adiposité · Signaux métaboliques · Obésité · Tissu adipeux blanc · Adipocytes · Inflammation · Insuline · Leptine.

EA3502, et INSERM "Avenir", Laboratoire de nutrition, Service de nutrition Hôtel Dieu, 1, place du parvis Notre-Dame, 75181 Paris Cedex 04, France.

\* authors equally contributed to this work.

Address correspondence and reprint requests to:

K Clément. Service de nutrition Hôtel Dieu, EA3502, Laboratoire de nutrition, 1, place du parvis Notre Dame, 75181 Paris Cedex 04, France.  
karine.clement2@wanadoo.fr

Received: February 27th, 2004

## “Adiposity signals” in the context of body weight regulation

Throughout life, body weight in humans varies within a very narrow range despite large day-to-day fluctuations in food intake [1]. Energy expenditure, classically, adjusts to energy intake and thanks to this mechanism energy balance remains stable over long periods of time [2, 3]. It has been suggested that individuals possess a predefined energy store status, referred to as body weight set point, determined by the combination of environmental and genetic factors. Any attempt to move away from the body weight set point remains vain [4]. The existence of this regulatory system implies a permanent and complex dialogue between the brain and peripheral tissues, including adipose tissues, but also other organs, such as the pancreas, the liver and muscles, that reflect the status of energy stores. The brain, and especially the hypothalamic nuclei, integrates this information from the periphery and in return, coordinates the adaptive response to energy imbalance. The hypothesis of the existence of an endocrine feedback loop, implying factors that circulate in proportion to body-fat content and act in the brain to reduce food intake was confirmed by the discovery of leptin ten years ago [5]. The fact that adiposity signals exist could thus no longer be doubted. To be considered as an adiposity signal, a molecule has to be secreted into the plasma in proportion to the body fat stores, be transported into the brain from the bloodstream, trigger expression of signal-transducing molecules in well-characterized hypothalamic and brainstem centres that regulate energy homeostasis and exert long-acting catabolite effects by decreasing food intake and increasing energy expenditure [6, 7]. Although these molecules have different modes of action in peripheral tissues, three of them meet the “classic” criteria of adiposity signals; insulin and amylin produced by the pancreatic  $\beta$ -cells and the hormone leptin, secreted by the adipose cells. Reproductive hormones have also been considered as adiposity signals but we will mainly focus on adipose tissue and pancreas produced signals. Genetic screening of the adiposity signal genes led to the discovery of genetic variations in their coding or regulatory regions that were sometimes associated with the modulation of adiposity signal circulating levels. This opened the question of the genetic contribution of adiposity signals to the control of body weight. Growing knowledge of adipose tissue biology and the possibility of studying gene expression on a large scale in human adipose tissue has offered — or will offer — new targets to be considered as adipocyte-derived signals. These factors can bear properties of adiposity signals and thus intervene in the long-term control of body weight. However for many of these new adiposity signals, proof of their role in a feedback loop of body weight regulation and especially their role at the brain level, is still to be provided. In this review, we will report data regarding well-known adiposity signals, mainly insulin, leptin and amylin and discuss the fact that there are

also grounds for considering new molecules as possible adiposity signals, i.e. peptide hormones and cytokines that the adipocyte secretes and the fluctuation in the secretion of which reflects the variations in adipose state. The knowledge contributed by the discovery of genetic variation in these adiposity signals is also discussed.

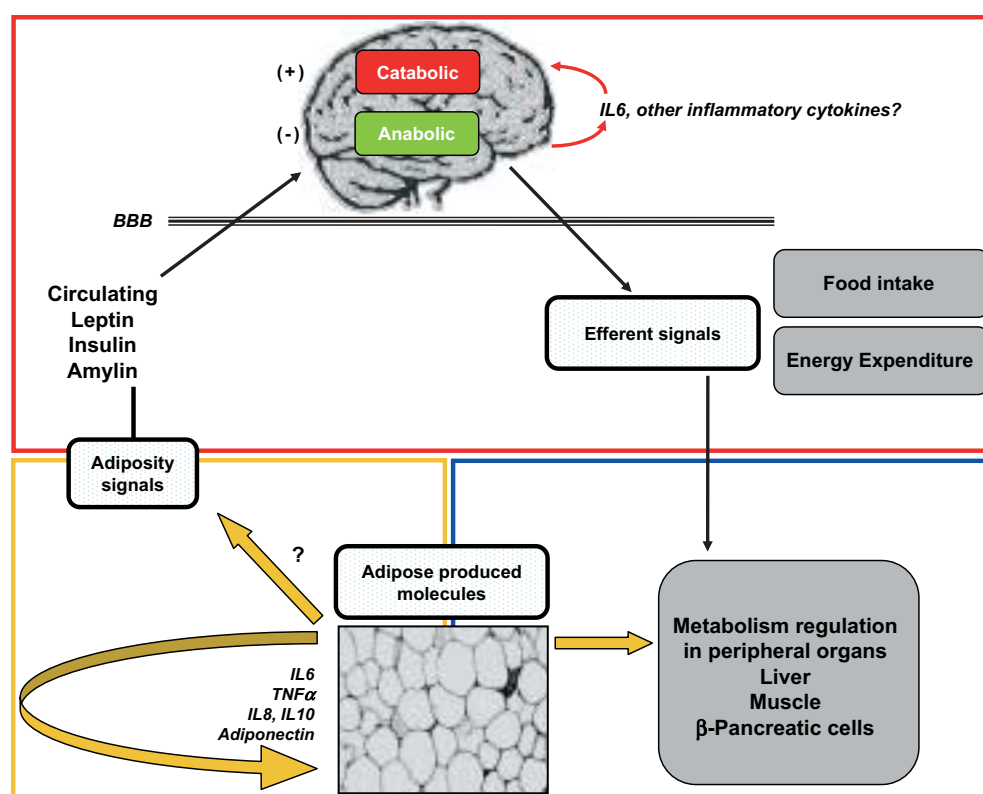
## Recognized adiposity signals

### Leptin

While historically insulin was the first adiposity signal discovered, the “gold standard” in the field remains leptin, discovered ten years ago [5]. This adipocyte-derived hormone fully satisfies the criteria for an adiposity signal. In healthy animals [8], as in humans [9], circulating concentrations of leptin are highly correlated with body fat mass, it crosses the blood-brain barrier and interacts with neurons known to decrease food intake and stimulate thermogenesis [10]. The physiologically active isoforms of the leptin receptor (LEPR) are expressed in these neurons receiving the leptin signal from the periphery. Since its discovery, an increasing amount of knowledge has been gained in particular regarding the mode of action of leptin in the brain. Several brain pathways targeted by leptin have been described. Leptin globally activates anorexigenic neurons (such as proopiomelanocortin [POMC] derived neurons) through a neural network in the hypothalamic nuclei while it inhibits orexigenic neurons (such as NPY/AGRP neurons).

However, the simple model of negative feedback existing between body fat and the brain carried by leptin, that would avoid obesity development, appears more complicated than at first suggested. Substantial data suggest that the basal levels of leptin in the fed state represent a signal of energy sufficiency [10] (*Fig 1*). A relatively short-term period of food restriction, independent of reduction of fat stores, produces a clear-cut decrease in leptin levels. Reduced leptin levels trigger a neural response characteristic of starvation with food seeking behaviour, efficient metabolism and an array of neuroendocrine responses that help survival in periods of food scarcity [10]. In the brain, the response to weight loss induced decreased leptin leads to the activation of anabolic pathways and the inhibition of catabolic pathways. Conversely, circulating leptin concentrations rapidly increase after reintroduction of energy supplies [11] or overfeeding [12], and the process cited is immediately aborted. However, the neuronal responses to weight loss are more efficient than the response to weight gain since at basal state (stable weight) catabolic pathways are already activated and anabolic pathways suppressed.

In parallel to its central action on food intake and energy expenditure, leptin may also be endowed with direct peripheral effects that limit fat accumulation in non-adipose tissue (muscle, liver) [13]. Thus leptin appears as the survival hormone, which aims at maintaining energy balance. In the

**Figure 1**

Body weight regulation: complex inter-organ dialogue through adiposity signals and adipose-derived signals. The adipose tissue provides multiple signals (e.g. leptin, adiponectin, inflammatory markers, various cytokines, etc.) to the brain (endocrine role, red square), to the adipose tissue depots (autocrine role) and to other peripheral organs such as the muscles, the liver, and the beta cells (paracrine role, blue square). Some signals are recognized as adiposity signals, some are not. BBB stands for the blood-brain barrier.

light of these observations leptin first appeared as a great hope for obesity treatment. Unfortunately, except for the rare forms of monogenic obesity due to genetic leptin deficiency, the majority of cases of human obesity are associated with high levels of leptin generally related to the degree of adiposity in each case. The idea that patients are resistant to the action of leptin [14] was then brought to the fore, even though the genesis of leptin resistance still remains to be discovered. Human trials performed with leptin treatment have shown that most subjects are unlikely to respond to pharmacological treatment with the hormone [15]. Several assumptions have been made to explain the concept of leptin resistance and the inefficiency of leptin treatment. Decreased leptin transport into the CNS has been suggested since obese people have lower leptin levels in the cerebrospinal fluid than in the plasma [16]. Since the leptin transport mechanism is saturated at low plasma leptin concentrations, it might limit the effectiveness of peripherally administered hormone. In addition, the molecular events downstream of the receptor in key hypothalamic neurons have been under focus [17]. The binding of leptin to its full-length receptor triggers the activation of the Janus kinase family (Jak2), which leads to the translocation of STAT3 to the nucleus where it regulates gene transcription. Concomitantly, the activation of the leptin receptor induces the expression of a “suppressor of cytokine signalling” (SOCS-3). While putative alterations in the JAK-STAT signalling either in animal or human obesity are still to be highlighted, the SOCS-3

could appear as a potential mediator of leptin resistance in obesity. The expression of SOCS-3 mRNA in the arcuate and dorsomedial hypothalamic nuclei is for example increased in Ay/a mice, a model of leptin-resistant murine obesity [18]. For leptin to have therapeutic potential, it either needs to be modified or the transport system by which leptin enters the brain needs to be upregulated to allow leptin to enter the brain more easily. It may also be necessary to overcome “central leptin insensitivity” by developing agents that act downstream of leptin activity. In this regard, the CNTF ciliary neurotrophic factor (rhvCNTF) that acts through leptin-like pathways in the hypothalamus, bypassing leptin resistance, is being used in different human trials [19].

## Insulin

Insulin was the first putative adiposity signal discovered but it took time to consider it as such since the action of the hormone in the peripheral systems is the opposite of that in the central systems. While insulin shows unambiguously anabolic effects in peripheral tissues, the hormone contributes to food intake control in the brain. Experiments performed in animals proved very conclusive in the field. In the late 1970s, Woods and Porte first proposed that insulin could be a long-term regulator of food intake and energy stores [20]. Continuous intracerebroventricular infusions of insulin in free-feeding baboons clearly reduced food intake and

body weight over a period of 20 days. Since then, many other clinical observations and fundamental experiments have confirmed the Woods and Porte hypothesis. Over a 24-hour period, fasting plasma insulin levels, insulin responses to meal ingestion and overall insulin secretion are correlated with the magnitude of body fat stores [21]. Type 1 diabetic patients with a deficit of insulin secretion show pronounced hyperphagia known as “diabetic hyperphagia” and in streptozotocin-induced diabetic rats infusion of a low dose of insulin (6 days) reduced food intake by 58% and body weight by 69% [22]. Very recently, in an attempt to develop new compounds to prevent the development of obesity, Air *et al.* showed that central or oral administration of an insulin mimetic, which enters the brain more readily than insulin, resulted in a dose-dependent reduction of food intake and body weight and limited body weight gain in a mouse model of high-fat diet-induced obesity [23]. It is now clearly acknowledged that insulin from serum is transported into the cerebrospinal fluid by a regulated and saturated mechanism and exerts its effects through binding to specific receptors. Insulin receptors have been identified in many brain areas involved in the regulation of feeding behaviour and energy expenditure, particularly the arcuate nucleus of the hypothalamus. The hormone in combination with leptin activates the release of alpha-MSH and inhibits neuropeptide Y (NPY) and agouti-related peptide (AgRP) from hypothalamic neurons [24]. Experiments resulting in the absence of any central insulin receptor in mice, after either a neuron-specific knock-out [25] or perihypothalamic injections of insulin receptor antisense oligonucleotides [26], have confirmed to an enormous extent the role played by insulin in the regulation of energy balance since, in both cases, animals exhibited hyperphagia and increased body fat expansion. In the light of these data, and since, in addition to inhibiting food intake, the hormone also increases energy expenditure to restore a body weight set point, insulin appears to be an important adiposity signal. The recent studies that focus on the hypothesis that insulin and leptin signals interact at cellular level are most interesting. In the central nervous system, both insulin and leptin inhibit arcuate nucleus NPY gene expression while they increase expression of proopiomelanocortin. Neuron-specific leptin or insulin receptor knock-out mice both have increased food intake and heavier weight than controls [25]. Several cell culture and in vivo studies have suggested that leptin, like insulin, is capable of activating insulin-receptor-substrate (IRS) protein-phosphatidylinositol 3 kinase (PI3K) signalling, albeit to a lesser extent than insulin [27]. Moreover leptin, like insulin, hyperpolarizes the “glucose-responsive” arcuate nucleus neurons through a mechanism that is again PI3K dependent. The obvious cross-talk between insulin and leptin and specifically the role of the PI3K as a signalling convergence constitutes, at present, a wide field for investigation and a potent target for anti-obesity drugs.

## Amylin

Amylin is a 37 amino acid protein mainly secreted by the pancreas and is co-secreted with insulin in response to food intake [28, 29]. The involvement of amylin in short and long-term effects of the regulation of food intake and body weight is well documented [30]. Evidence for the role of amylin in the regulation of food intake mostly stems from the central or peripheral administration of a low dose of amylin in rodents that potentially reduced food intake in a dose-dependent manner [31]. Amylin crosses the blood-brain barrier and binds to receptors located in different brain areas involved in the regulation of energy homeostasis (nucleus of the solitary tract (NTS), nucleus accumbens and hypothalamus) [32, 33]. Amylin acts only as a humoral signal since neither vagotomy nor destruction of capsaicin-sensitive sensory afferents affect the biological hormone effect [34]. Besides its short-term effect on food intake regulation, amylin probably controls energy balance over the longer term. While an acute bolus of amylin in the 3<sup>rd</sup> ventricle decreases food intake in a dose-dependent manner, the effect seems to persist for 7 days after the administration. Animals treated with amylin lose weight compared with controls [31]. A tonic elevation of central amylin over 10 days or repeated daily injections over 6 days in rodents leads to a marked alteration in food intake with considerable weight loss due to the reduction of body adiposity. Although the exact nature of amylin receptors remains still to be determined, amylin binding sites have been identified [32, 33] and specific antagonists have been developed. When rats were given an acute intracerebroventricular administration of an amylin antagonist, a significant increase in energy intake was observed [31]. The tonic inhibition of any central amylin signal led to a body fat increase of about 30% in treated rats as compared with controls. These experiments have placed amylin among the adiposity signals since the crucial difference between a short-term satiety signal and long-term energy homeostasis regulators resides in the fact that repeated administrations of amylin do not alter body weight because of a compensatory increase in meal frequency.

To date, a growing body of data is converging to prove that amylin possesses many characteristics in common with well-known adiposity signals and also show that it seems to act in cooperation with them. Like insulin and leptin, amylin is rapidly and efficiently transported to brain areas involved in the regulation of energy balance. Amylin secretion and plasma amylin concentrations correlate with the degree of body adiposity [35]. Lastly, amylin, like leptin and insulin, potentially reduces food intake without aversive consequences, and the signal it conveys to the brain grows in importance when it is remembered that sub-threshold doses of amylin and insulin, with no effect on food intake when infused individually, significantly reduce intake when administered together. Even though many points regarding amylin (nature of the receptors, signalling pathways, interactions with other

adiposity signals, etc.) still remain to be elucidated, all these findings pave the way for amylin analogs as new anti-obesity drugs [36], all the more so since body weight gain observed after administration of these compounds was entirely due to fat accumulation.

## Inflammatory cytokines as “adiposity signals”?

Among the cytokines/chemokines that are produced by adipose tissue, are some that may contribute to signalling the amount of fat mass and its variation to the brain. Recent data show that several pro-inflammatory cytokines (such as interleukin-6, interleukin-1, interleukin-8) and chemokines may participate in the control of feeding during physiological conditions, as reviewed recently by Plata-Salaman [37]. Among the cytokines, interleukin-6 (IL6) is probably the one that best displays many characteristics in common with insulin, leptin and amylin as an adiposity signal. During inflammation, IL6 is released from immune cells and elicits pro-inflammatory effects [38-40]. Its secretion is modulated by immune, hormonal and metabolic stimuli in a cell-specific manner. In physiological conditions, the adipose tissue (especially the omental tissue) provides about one third of the circulating levels of IL6 [41]. Besides its role in the immune response, IL6 is characterized by the capacity to regulate energy balance in the short and long-term. IL6 serum levels correlate with adipose tissue mass in animals and humans to the same extent as leptin, and in the brain, it favours a negative energy balance since it decreases food intake and enhances energy expenditure. Rodent IL6 and its receptor are expressed, among other nuclei, in the dorsomedial and ventromedial hypothalamus, two brain areas involved in the regulation of energy balance [42] and there is substantial evidence that this observation can be extended to humans [43]. IL6 is also produced by several parts of the brain [42, 44-46]. Mice lacking the IL6 gene develop mature-onset obesity and obesity-related metabolic disturbances with an increase mainly in subcutaneous adipose tissue [47]. These animals exhibit increased absolute food intake that is corrected by long-term intracerebroventricular administration of IL6 but not by intraperitoneal injection [48]. While the effect of an ICV IL6 treatment on caloric ingestion has not always been consistent (probably because of dose and duration-dependent effects) the animals all exhibited body weight reduction. IL6 injections also decrease body weight in non-human primates [48, 49] and in humans it enhances energy expenditure and lipid oxidation [50-53]. As the slight decrease in food intake could not solely explain weight loss observed in animal models, an associated increase in energy expenditure could be involved. Acute or chronic ICV injection of IL6 suppresses obesity through increase energy expenditure. It was suggested that IL6 could stimulate the

sympathetic nerve system leading to an increase in thermogenesis in brown adipose tissue.

A straight extrapolation of this mechanism to humans, in whom the physiological role of brown adipose tissue is still debated, is questionable. A lot remains to be discovered about the impact of IL6 on energy balance in humans [54]. In obese subjects, serum IL6 levels correlate positively with body mass index [55-57] but cerebrospinal fluid (CSF) IL6 levels correlate negatively with total body fat. Unlike the observation with leptin the CSF levels of which, even if lower than serum ones, remain correlated with body weight [16], CSF IL6 seems to be regulated independently of serum IL6. This observation suggests that CSF IL6 is not serum-derived but could be locally produced. However, the possibility that the blood-brain barrier in obese subjects may become, for reasons still to be discovered, dramatically “IL6-tight” cannot be ruled out. Indeed, it is clearly acknowledged that IL6 crosses the blood-brain barrier by a unidirectional (blood-to-brain) influx system which can be saturated [58]. Taken together, these data provide indications of a role for IL6 in the regulation of energy balance over the long-term. The origin of CNS IL6, the exact brain sites involved and the possible interactions with other adiposity signals are, at present, under investigation.

## Other adipose tissue-derived molecules as adiposity signals?

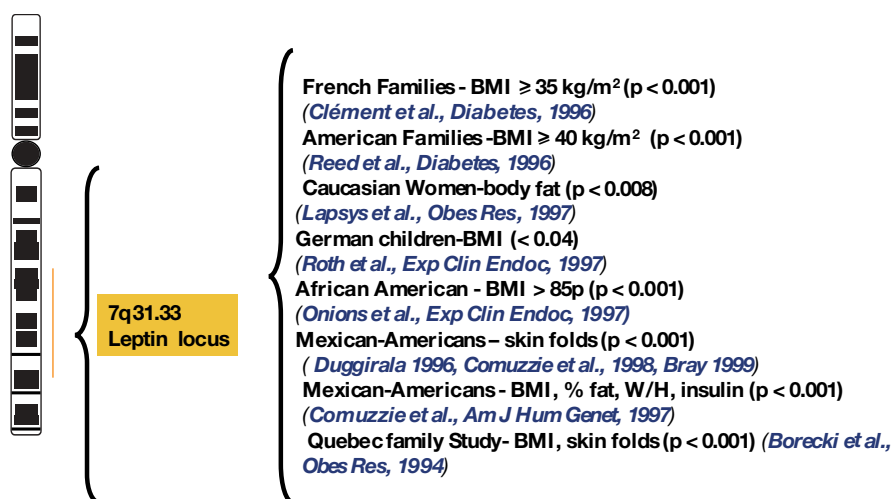
Besides IL6, both in mice and humans, adipose tissue produces and secretes a large number of other cytokines including interleukins, chemokines and related substances with their level of expression and/or synthesis directly correlated with the degree of adiposity. These molecules could be involved in processes mediating adiposity and insulin resistance. We will only cite some of them. Tumour necrosis factor alpha (TNF- $\alpha$ ) is among these. Studies on obese human patients have demonstrated a positive correlation between levels of TNF- $\alpha$ , the extent of obesity, and the level of hyperinsulinaemia observed [59-61]. In vitro cell culture studies have suggested that TNF- $\alpha$  is able to render cells insulin resistant through regulation of the synthesis of the insulin responsive glucose transporter as well as through interference with insulin signalling [62]. Other molecules discovered could also belong to this class of factors [63-65]. Circulating levels of the anti-inflammatory cytokine IL-10, for example, are raised in obese women. Adipocyte production, modulation and secretion of interleukin 8 (IL8), another adipocyte-derived cytokine, has been well described in vitro [66], and in vivo relationships between IL8 levels and obesity have been observed [67]. An increase in plasma IL-8 concentrations after glucose load in obese impaired glucose tolerance subjects in comparison with normoglycaemic weight-matched individuals, also suggests a modulating role both by

insulin sensitivity and glucose tolerance [68]. Interestingly, IL8 was detected in CSF and a role in decreasing food intake and weight was shown several years ago in rodents [69]. Up to now there are, however, few arguments regarding the contribution of TNF $\alpha$  and others as direct messengers between adipose tissue and the brain in physiological conditions. Many authors believe that TNF $\alpha$  has an effect on body weight regulation and that it acts probably through a local action on adipose tissue, although interactions between the brain and the cytokines produced in the periphery are described [58].

Several adipocyte-derived molecules may also participate in the complex dialogue between organs involved in body weight regulation. Adipocyte cells use polypeptide hormones to influence metabolic processes at distant sites, other than the brain. Adiponectin exclusively synthesized in adipose tissue is probably the best example of such a type of molecule. There are, however, weak requirements for considering adiponectin as an adiposity signal, as classically defined above. Adiponectin could then fall into this category of signalling molecules since this adipocytokine contributed to the description of a key role for adipose tissue in fine-tuning hepatic and muscle insulin responsiveness. Contrasting with other molecules, adiponectin plasma levels decrease proportionally with the accumulation of adipose tissue, especially in the visceral depot [70] and with the development of insulin resistance. When administered to mice, it enhances insulin sensitivity and glucose tolerance, and appears to increase free fatty acid oxidation in muscle. The links between visceral adipose tissue adiponectin, insulin resistance, and inflammatory-related disease such as diabetes and arteriosclerosis have been emphasised. Adiponectin is likely to be involved in the regulation of energy homeostasis certainly (if not exclusively) more by its action on triggering peripheral organs than brain circuits [71, 72].

## Genetic studies to identify the contribution of adiposity signal genes

The first gene identification in obese human subjects was linked with screening for genes encoding leptin and leptin receptor. Homozygous carriers of a loss of function mutation in the leptin gene or in the leptin receptor exhibit morbid obesity with onset in the first months of life, hypogonadotropic hypogonadism and central hypothyroidism [73]. Three sisters bearing the leptin receptor mutation also display significant growth retardation due to impaired growth hormone secretion. Affected subjects continuously seek food and eat considerably more than their siblings [73]. A leptin deficient child has been treated successfully by leptin replacement. In this nine-year-old girl, daily subcutaneous injection of recombinant human leptin for a year was well tolerated and led to an important and sustained fat mass loss and an age-appropriate improvement in function of the reproductive axis [74]. Although exceptional, these situations of monogenic obesity have greatly contributed to validating the role of the leptin axis not only in body weight regulation but also in the control of several endocrine functions. Efforts to identify candidate genes for common obesity have concentrated on adiposity signal genes. Association studies have been conducted to identify the association between obesity or obesity-related phenotypes and genetic variants (single nucleotide polymorphisms or SNPs) located in gene-encoding adiposity signal genes. In addition, researchers have looked for regions in the genome including the location of adiposity signal genes [75]. In association studies, the frequency of DNA variations between groups of subjects (i.e. obese vs. non obese) is evaluated on a measurable phenotype (body mass index, fat mass, skin folds, waist/hip ratio, as well as circulating levels of adiposity signal proteins) in subjects carrying or not carrying the given polymorphism being



**Figure 2**

Example of linkage studies between the leptin gene locus and obesity related phenotypes.

D Allison *et al.* [76] suggests that this region is strongly linked with BMI using a meta-analysis.

compared. These association studies have been conducted in children and obese populations in Europe and in North America. Gene-gene and gene-environment interactions have begun to emerge.

As shown in tables I and II, several studies have shown indications of linkage and/or association between adiposity signal (or adipocyte produced molecule) gene variants and obesity related phenotypes. This was particularly the case for leptin gene studies where many linkage studies in independent populations and a meta-analysis suggested that the lep-

tin gene locus is associated with obesity phenotypes [76] (*Fig 2*). An impressive genome wide scan performed in thousands of subjects also found a peak of linkage in this region [77]. However no functional mutation has been discovered to explain these findings. As shown in *Tables I and II*, the results observed have not always been consistent for the markers tested. These studies met with difficulties of interpretation related to statistical power, biased population stratification, false positive results due to multiple testing and the suppression of negative results.

**Table I**

Examples of association studies between recognized adiposity signal gene variants and obesity phenotypes.

Adiposity signal	Polymorphisms	Population	Association with Studied phenotypes	References
Leptin	nucleotide repeat	Japanese	Hypertension (+)	[88]
Leptin	25CAG	Japanese	Obesity (+)	[89]
Leptin	G-2548A	French	Weight response to caloric restriction (+)	[79]
Leptin	A19G	French	Obesity (-)	[78]
Leptin	C(-188)A	Finns	Weight (-)	[90]
EPR	K109R, Q223R,	Dutch	High leptin level (+)	[91]
	K656N	Americans	Weight gain (+)	[92]
	Q223R, K656N	British	Obesity (-)	[93]
	3'-UTR ins/del	French	Morbid obesity (-)	[94]
LEPR	K109R, Q223R,	African-American,	BMI (+)	[95]
	K656N	Caucasian, Danish,	Waist circumference (+)	
		Finns, Canadian and		
		Nigerian		
LEPR	K109R, Q223R,	Caucasian	BMI (+), Fat mass (+)	[96]
	K656N, base repeats			
LEPR	Q223R	Pima Indians	Adiposity (+)	[97]
		Americans	Energy Expenditure (+)	[98]
			Glucose metabolism (+)	
LEPR	Q223R	Caucasian women	BMI, fat mass, serum leptin (+)	[99]
LEPR	Q223R	Greeks	predict BMI and percentage fat mass	[100]
LEPR	K109R, Q223R,	Caucasian women	Insulin glucose metabolism, fat mass, energy	[101, 102]
	K656N		expenditure (+)	
LEPR	T+70C	French	BMI, Fat mass (+)	[103]
	Asp (A) 96 Asp (G)			
	Ser (T) 343 Ser (C)			
LEPR and leptin	Q223R, P1019P	Nauruans	Adiposity (+)	[104; 105]
	Leptin variants		Insulin resistance (+)	
LEPR and leptin	LEP: A19GLEPR	Caucasians	BMI (+)	[106]
	Q223R, P1019P			
IL6	CA repeat	Caucasians	BMI, fat mass (+)	[81]
IL6	C-174G	Finns	Energy expenditure insulin sensitivity (+)	[83; 107]
		French-Canadians	Waist either insulin or glucose (+)	[82]
		Spanish Caucasians	Glucose metabolism, type-2 diabetes (+)	[108; 109]
		Native Americans		

BMI Body Mass Index. (+) refers to positive association while (-) refers to negative association.

More importantly, some association between the genetic variations of adiposity signals and the level of expression of the blood circulation levels of their substrates has been found (*Tab III*). For example although no polymorphisms were detected in the coding region of the leptin gene, a single A-to-G transition and a 2548G-A were found respectively in the untranslated first exon and in the regulatory region. Hager *et al.* showed that patients homozygous for the G allele of the exon 1 variant had significantly lower fasting leptin levels compared with subjects who were either heterozygous (AG) or homozygous for the A allele despite a similar BMI [78]. Men carriers of the 2548 G allele variant had lower leptin concentrations adjusted for fat mass [79]. In 2001, Farooqi *et al.* studied 13 subjects who were heterozygous for the frame shift mutation delta-G133 of the leptin gene [80]. Their serum leptin concentrations were lower than in controls with a similar sex distribution and age. Lower leptin levels in these subjects were characterized by an increased prevalence of obesity. Similar observations

were made between insulin gene variation and insulin circulating levels. Gene polymorphisms in the region of the INS gene were studied in obese children. The authors found that obese patients homozygous for class I VNTR (variable nucleotide tandem repeat polymorphism) alleles secreted more insulin than those with other genotypes [125]. Genetic polymorphisms of the IL-6 gene have also been described [81]. Lower expression of cellular constructs containing the -174C change was found when comparing with the 174G constructs. The IL6 -174G/C polymorphism is associated with some indices of body composition and parameters of glucose and insulin homeostasis [82] but no study has determined relationships between this variant and circulating levels of IL6.

It is possible that a relatively small decrease in this adiposity signal production, associated with the genetic variations, may be sensed by the homeostatic feedback system that controls energy balance and may in turn contribute to some dysregulation in energy balance. This was suggested by Fa-

**Table II**

Examples of association study between molecules produced by the adipocyte and obesity phenotypes.

Adiposity signal	Polymorphisms	Population	Association with studied phenotypes	References
TNF $\alpha$	-857C/A -863C/A		adipose tissue TNF secretion (+)	[86]
TNF $\alpha$	G-308A	Caucasian	BMI (+)	[110] [111]
TNF $\alpha$	G-308A	Swedish, Finnish subjects, Polish Caucasians, Australian	Fat accumulation (+) Metabolic parameters (+)	[83, 85] [107, 112, 113]
TNF $\alpha$	G-308A G-238A	Danish	Insulin resistance (+) Birth weight (+)	[114]
TNF $\alpha$	A + 252G G-308A	Obese Korean subjects	decreased waist/hipBMI (-)	[115]
TNF $\alpha$	Nco I		Insulin resistance (-)	[116, 117]
TNF $\alpha$	G-308A	Japanese, Chinese Caucasians	Obesity, metabolic parameters (-)	[11-120]
TGF $\beta$	T29C	Swedish men	Obesity (+) Insulin resistance (+)	[121]
Adiponectin	T94G	Chinese (Taiwan)	BMI (+)	[122]
Adiponectin	T45G IVS2 + G62T	Obese Swedish Subjects	Cholesterol, waist circumference (+). Blood glucose, BMI, diastolic blood pressure, sagittal diameter (+)	[123]
Adiponectin	haplotype Snips -11391 and -11377, 5' sequence rare non-synonymous mutations of exon 3 45T $\rightarrow$ G, 276G $\rightarrow$ T	Caucasians	type 2 diabetes (+) BMI, insulin resistance (+)	[124] [125]

BMI Body Mass Index. (+) refers to positive association while (-) refers to negative association.

**Table III**

Examples of the study of association between genetic variants of molecules produced by the adipocyte (either adiposity signal or adipocyte produced molecules) and circulating levels.

Molecules	Gene variant and type of study	Population	Genetic association or linkage with circulating levels	References
Leptin	G-2548A Association	French	Serum leptin levels (+)	[79; 126]
Leptin	A19G Association	French	Serum leptin levels (+)	[78]
Leptin	Lep -2, 549 Association	Caucasians	Relationship between serum leptin and body fatness modified	[127; 128]
Leptin	Linkage Estimation of heritability	Monozygotic and dizygotic twins	Serum leptin levels (estimated heritability 55%)	[129]
Leptin	CA repeat D2S1788 Genome wide scan	Mexicans Americans	Serum leptin levels (Iod score 4.95)	[130]
LEPR	K109R, Q223R, K656N Association	Dutch	Serum leptin levels (+)	[91]
IL6	G-597A, G-572C, G -174C, -373A(n)T(n) Association	Spanish women	Interleukine 6 levels (+)	[131]
Adiponectin	-1139 & -11377 haplotypes one non-synonymous mutation in exon 3	Caucasians	Serum adiponectin levels (+)	[124]
Adiponectin	Missense mutation (R112C) in exon 3 G/T polymorphism in exon 2 Association	Japanese	Serum adiponectin levels (+) Serum adiponectin R112C (-)	[132]
Adiponectin	Missense mutation (I164T) Association	Japanese	Serum adiponectin levels (+)	[133]
Adiponectin	PolyCA Linkage analysis	Pima population	Serum adiponectin levels (QTL on chromosomes 2, 3, 9, 10)	[134]
Adiponectin	PolyCA Linkage analysis	Northern European ancestry	Serum adiponectin levels (LOD score: 4.06 on chromosome 5 and 3.2 on chromosome 14)	[135]

QTL = quantitative trait linkage.

(+) refers to positive association while (-) refers to negative association.

rooqi *et al.* when analysing the phenotypes of the frame shift mutation delta-G133 carrier with relatively decreased leptin. It was suggested that in these subjects fat mass would be increased in an attempt [80] to restore leptin levels to some "set" point.

Is SNP in other signal molecules associated with modification of their transcription, production or circulating levels and does this perhaps influence body weight regulation? There is evidence for association between the -308A polymorphism located in the promoter of the TNF- $\alpha$  gene and obesity, with high rates of glucose oxidation in normal weight subjects and with lipid storage in overweight subjects [83-85]. Another polymorphism described in the promoter region of the TNF- $\alpha$  gene (a C  $\rightarrow$  A substitution at position

-863) is associated with lower transcriptional activity and with down-regulation of the basal rate of transcription of the TNF $\alpha$  gene *in vitro*. In men (carriers of the rare A allele having a significantly lower TNF- $\alpha$  level), the -863C/A polymorphism seems to be associated with serum TNF $\alpha$  concentrations [86, 87]. Nevertheless, these polymorphisms have not been sufficiently investigated in human obesity to date. Adiponectin gene variations have also been studied in different populations. A combination of SNPs (haplotype including 2 5-prime SNPs) was associated with adiponectin levels and with type II diabetes. Interestingly, the presence of at least 1 non-synonymous mutation in exon 3 showed evidence of association with adiponectin levels. The results have been quite consistent in different populations (*Tab III*).

It is possible that combinations of polymorphisms, encoding adiposity signal genes and signalling molecules that may contribute to the complex dialogue between organs, may intervene in the accuracy and synergy of signal transmission. Slight modification of this molecule expression and production by the peripheral organ may bias the way the brain senses the amount of fat mass and its variation. Similarly at the peripheral level, slight modification of circulating molecules may interfere with inter-organ dialogue. Systematic analysis of these gene variants and genotype combinations (haplotype) on a large scale may be necessary to explore this hypothesis especially in the context of the dynamic variation of body weight. Up to now most genetic studies include the search for genotype/phenotype associations without taking into account the influence of the environment (diet) in the relationship. Among the limitations, the availability of large sample numbers should be mentioned, as well as the bio-informatics tools that are still in their infancy for accessing the question of multiple interactions with no "a priori hypothesis". This picture will probably change rapidly in the future. Large databases and DNA and biological sample banks will be available with updated environmental information and precise phenotypes especially thanks to European working groups. The capacity for studying multiple genes at once at the level of DNA or RNA is rapidly growing. Finally, tremendous (rapid) progress in bio-informatics will allow information of different natures to be integrated (biological sources) (i.e. environment, phenotype, genotype, expression) improving our capacity to deal with complexity.

## References

1. Bray GA. Weight homeostasis. *Annu Rev Med*, 1991, 42, 205-16.
2. Leibel RL, Rosenbaum M, Hirsch J. Changes in energy expenditure resulting from altered body weight. *N Engl J Med*, 1995, 332, 621-8.
3. Cummings DE, Schwartz MW. Genetics and pathophysiology of human obesity. *Annu Rev Med*, 2003, 54, 453-71.
4. Keesey RE, Hirvonen MD. Body weight set-points: determination and adjustment. *J Nutr*, 1997, 127, 1875S-83S.
5. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. Positional cloning of the mouse obese gene and its human homologue. *Nature*, 1994, 372, 425-32.
6. Schwartz MW, Woods SC, Porte D, Jr., Seeley RJ, Baskin DG. Central nervous system control of food intake. *Nature*, 2000, 404, 661-71.
7. Schwartz MW. Brain pathways controlling food intake and body weight. *Exp Biol Med (Maywood)*, 2001, 226, 978-81.
8. 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.
9. Considine RV, Sinha MK, Heiman ML, *et al.* Serum immunoreactive-leptin concentrations in normal-weight and obese humans. *N Engl J Med*, 1996, 334, 292-5.
10. Schwartz MW, Woods SC, Seeley RJ, Barsh GS, Baskin DG, Leibel RL. Is the energy homeostasis system inherently biased toward weight gain? *Diabetes*, 2003, 52, 232-8.
11. Weigle DS, Duell PB, Connor WE, Steiner RA, Soules MR, Kuijper JL. Effect of fasting, refeeding, and dietary fat restriction on plasma leptin levels. *J Clin Endocrinol Metab*, 1997, 82, 561-5.
12. Kolaczynski JW, Ohannesian JP, Considine RV, Marco CC, Caro JF. Response of leptin to short-term and prolonged overfeeding in humans. *J Clin Endocrinol Metab*, 1996, 81, 4162-5.
13. Fruhbeck G. A heliocentric view of leptin. *Proc Nutr Soc*, 2001, 60, 301-18.
14. Caro JF, Sinha MK, Kolaczynski JW, Zhang PL, Considine RV. Leptin: the tale of an obesity gene. *Diabetes*, 1996, 45, 1455-62.
15. Proietto J, Thorburn AW. The therapeutic potential of leptin. *Expert Opin Investig Drugs*, 2003, 12, 373-8.
16. Caro JF, Kolaczynski JW, Nyce MR, *et al.* Decreased cerebrospinal-fluid/serum leptin ratio in obesity: a possible mechanism for leptin resistance. *Lancet*, 1996, 348, 159-61.
17. El-Haschimi K, Pierroz DD, Hileman SM, Bjorbaek C, Flier JS. Two defects contribute to hypothalamic leptin resistance in mice with diet-induced obesity. *J Clin Invest*, 2000, 105, 1827-32.
18. Bjorbaek C, Elmquist JK, Frantz JD, Shoelson SE, Flier JS. Identification of SOCS-3 as a potential mediator of central leptin resistance. *Mol Cell*, 1998, 1, 619-25.
19. Ettinger MP, Littlejohn TW, Schwartz SL, *et al.* Recombinant variant of ciliary neurotrophic factor for weight loss in obese adults: a randomized, dose-ranging study. *Jama*, 2003, 289, 1826-32.
20. Volk A, Renn W, Overkamp D, *et al.* Insulin action and secretion in healthy, glucose tolerant first degree relatives of patients with type 2 diabetes mellitus. Influence of body weight. *Exp Clin Endocrinol Diabetes*, 1999, 107, 140-7.
21. Porte D, Jr., Woods SC. Regulation of food intake and body weight in insulin. *Diabetologia*, 1981, 20, 274-80.
22. Sipols AJ, Baskin DG, Schwartz MW. Effect of intracerebroventricular insulin infusion on diabetic hyperphagia and hypothalamic neuropeptide gene expression. *Diabetes*, 1995, 44, 147-51.
23. Air EL, Strowski MZ, Benoit SC. Small molecule insulin mimetics reduce food intake and body weight and prevent development of obesity. *Nat Med*, 2002, 8, 179-83.
24. Benoit S, Schwartz M, Baskin D, Woods SC, Seeley RJ. CNS melanocortin system involvement in the regulation of food intake. *Horm Behav*, 2000, 37, 299-305.
25. Bruning JC, Gautam D, Burks DJ. Role of brain insulin receptor in control of body weight and reproduction. *Science*, 2000, 289, 2122-5.
26. Obici S, Feng Z, Karkanias G, Baskin DG, Rossetti L. Decreasing hypothalamic insulin receptors causes hyperphagia and insulin resistance in rats. *Nat Neurosci*, 2002, 5, 566-72.
27. Niswender KD, Schwartz MW. Insulin and leptin revisited: adiposity signals with overlapping physiological and intracellular signaling capabilities. *Front Neuroendocrinol*, 2003, 24, 1-10.
28. Butler PC, Chou J, Carter WB, *et al.* Effects of meal ingestion on plasma amylin concentration in NIDDM and nondiabetic humans. *Diabetes*, 1990, 39, 752-6.
29. Ludvik B, Kautzky-Willer A, Prager R, Thomaseth K, Pacini G. Amylin: history and overview. *Diabet Med*, 1997, 14, S9-13.
30. Geary N. Effects of glucagon, insulin, amylin and CGRP on feeding. *Neuropeptides*, 1999, 33, 400-5.
31. Rushing PA, Hagan MM, Seeley RJ, Lutz TA, Woods SC. Amylin: a novel action in the brain to reduce body weight. *Endocrinology*, 2000, 141, 850-3.
32. Banks WA, Kastin AJ. Differential permeability of the blood-brain barrier to two pancreatic peptides: insulin and amylin. *Peptides*, 1998, 19, 883-9.
33. Banks WA, Kastin AJ, Maness LM, Huang W, Jaspan JB. Permeability of the blood-brain barrier to amylin. *Life Sci*, 1995, 57, 1993-2001.

34. Lutz TA, Althaus J, Rossi R, Scharrer E. Anorectic effect of amylin is not transmitted by capsaicin-sensitive nerve fibers. *Am J Physiol*, 1998, 274, R1777-82.
35. Pieber TR, Roitelman J, Lee Y, Luskey KL, Stein DT. Direct plasma radioimmunoassay for rat amylin-(1-37): concentrations with acquired and genetic obesity. *Am J Physiol*, 1994, 267, E156-64.
36. Reda TK, Geliebter A, Pi-Sunyer FX. Amylin, food intake, and obesity. *Obes Res*, 2002, 10, 1087-91.
37. Plata-Salaman CR. Cytokines and feeding. *Int J Obes Relat Metab Disord*, 2001, 25, 5, S48-52.
38. Pedersen BK, Steensberg A, Schjerling P. Muscle-derived interleukin-6: possible biological effects. *J Physiol*, 2001, 536, 329-37.
39. Wallenius V, Wallenius K, Hisaoka M, *et al.* Retarded liver growth in interleukin-6-deficient and tumor necrosis factor receptor-1-deficient mice. *Endocrinology*, 2001, 142, 2953-60.
40. Cressman DE, Greenbaum LE, DeAngelis RA, Ciliberto G, Furth EE, Poli V, Taub R. Liver failure and defective hepatocyte regeneration in interleukin-6-deficient mice. *Science*, 1996, 274, 1379-83.
41. Fried SK, Bunkin DA, Greenberg AS. Omental and subcutaneous adipose tissues of obese subjects release interleukin-6: depot difference and regulation by glucocorticoid. *J Clin Endocrinol Metab*, 1998, 83, 847-50.
42. Schobitz B, de Kloet ER, Sutanto W, Holsboer F. Cellular localization of interleukin 6 mRNA and interleukin 6 receptor mRNA in rat brain. *Eur J Neurosci*, 1993, 5, 1426-35.
43. Mohamed-Ali V, Pinkney JH, Coppack SW. Adipose tissue as an endocrine and paracrine organ. *Int J Obes Relat Metab Disord*, 1998, 22, 1145-58.
44. Wang W, Lonnroth C, Svanberg E, Lundholm K. Cytokine and cyclooxygenase-2 protein in brain areas of tumor-bearing mice with prostanoid-related anorexia. *Cancer Res*, 2001, 61, 4707-15.
45. Miyahara S, Komori T, Fujiwara R, *et al.* Effects of repeated stress on expression of interleukin-6 (IL-6) and IL-6 receptor mRNAs in rat hypothalamus and midbrain. *Life Sci*, 2000, 66, 93-8.
46. Gao Y, Ng YK, Lin JY, Ling EA. Expression of immunoregulatory cytokines in neurons of the lateral hypothalamic area and amygdaloid nuclear complex of rats immunized against human IgG. *Brain Res*, 2000, 859, 364-8.
47. Wallenius V, Wallenius K, Ahren B, *et al.* Interleukin-6-deficient mice develop mature-onset obesity. *Nat Med*, 2002, 8, 75-9.
48. Wallenius K, Wallenius V, Sunter D, Dickson SL, Jansson JO. Intracerebroventricular interleukin-6 treatment decreases body fat in rats. *Biochem Biophys Res Commun*, 2002, 293, 560-5.
49. Ettinger WH, Jr., Sun WH, Binkley N, Kouba E, Ershler W. Interleukin-6 causes hypocholesterolemia in middle-aged and old rhesus monkeys. *J Gerontol A Biol Sci Med Sci*, 1995, 50, M137-40.
50. Tsigos C, Papanicolaou DA, Kyrou I, Defensor R, Mitsiadis CS, Chrousos GP. Dose-dependent effects of recombinant human interleukin-6 on glucose regulation. *J Clin Endocrinol Metab*, 1997, 82, 4167-70.
51. Tsigos C, Papanicolaou DA, Defensor R, Mitsiadis CS, Kyrou I, Chrousos GP. Dose effects of recombinant human interleukin-6 on pituitary hormone secretion and energy expenditure. *Neuroendocrinology*, 1997, 66, 54-62.
52. Lyngso D, Simonsen L, Bulow J. Interleukin-6 production in human subcutaneous abdominal adipose tissue: the effect of exercise. *J Physiol*, 2002, 543, 373-8.
53. Lyngso D, Simonsen L, Bulow J. Metabolic effects of interleukin-6 in human splanchnic and adipose tissue. *J Physiol*, 2002, 543, 379-86.
54. Mohamed-Ali V, Flower L, Sethi J, *et al.* beta-Adrenergic regulation of IL-6 release from adipose tissue: in vivo and in vitro studies. *J Clin Endocrinol Metab*, 2001, 86, 5864-9.
55. Mohamed-Ali V, Goodrick S, Rawesh A, *et al.* Subcutaneous adipose tissue releases interleukin-6, but not tumor necrosis factor-alpha, in vivo. *J Clin Endocrinol Metab*, 1997, 82, 4196-200.
56. Bastard JP, Jardel C, Bruckert E, *et al.* Elevated levels of interleukin 6 are reduced in serum and subcutaneous adipose tissue of obese women after weight loss. *J Clin Endocrinol Metab*, 2000, 85, 3338-42.
57. Ershler WB, Keller ET. Age-associated increased interleukin-6 gene expression, late-life diseases, and frailty. *Annu Rev Med*, 2000, 51, 245-70.
58. Pan W, Kastin AJ. Interactions of cytokines with the blood-brain barrier: implications for feeding. *Curr Pharm Des*, 2003, 9, 827-31.
59. Hotamisligil GS, Shargill NS, Spiegelman BM. Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. *Science*, 1993, 259, 87-91.
60. Hotamisligil GS, Arner P, Caro JF, Atkinson RL, Spiegelman BM. Increased adipose tissue expression of tumor necrosis factor-alpha in human obesity and insulin resistance. *J Clin Invest*, 1995, 95, 2409-15.
61. Hotamisligil GS, Arner P, Atkinson RL, Spiegelman BM. Differential regulation of the p80 tumor necrosis factor receptor in human obesity and insulin resistance. *Diabetes*, 1997, 46, 451-5.
62. 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.
63. Esposito K, Pontillo A, Ciotola M, *et al.* Weight loss reduces interleukin-18 levels in obese women. *J Clin Endocrinol Metab*, 2002, 87, 3864-6.
64. Esposito K, Pontillo A, Di Palo C, *et al.* Effect of weight loss and lifestyle changes on vascular inflammatory markers in obese women: a randomized trial. *Jama*, 2003, 289, 1799-804.
65. Esposito K, Marfella R, Giugliano D. Plasma interleukin-18 concentrations are elevated in type 2 diabetes. *Diabetes Care*, 2004, 27, 272.
66. Bruun JM, Pedersen SB, Richelsen B. Regulation of interleukin 8 production and gene expression in human adipose tissue in vitro. *J Clin Endocrinol Metab*, 2001, 86, 1267-73.
67. Straczkowski M, Dzieńis-Straczkowska S, Stepień A, Kowalska I, Szelachowska M, Kinałska I. Plasma interleukin-8 concentrations are increased in obese subjects and related to fat mass and tumor necrosis factor-alpha system. *J Clin Endocrinol Metab*, 2002, 87, 4602-6.
68. Straczkowski M, Kowalska I, Nikolajuk A, Dzieńis-Straczkowska S, Szelachowska M, Kinałska I. Plasma interleukin 8 concentrations in obese subjects with impaired glucose tolerance. *Cardiovasc Diabetol*, 2003, 2, 5.
69. Plata-Salaman CR, Borkoski JP. Interleukin-8 modulates feeding by direct action in the central nervous system. *Am J Physiol*, 1993, 265, R877-82.
70. Matsuzawa Y, Funahashi T, Kihara S, Shimomura I. Adiponectin and metabolic syndrome. *Arterioscler Thromb Vasc Biol*, 2004, 24, 29-33.
71. Diez JJ, Iglesias P. The role of the novel adipocyte-derived hormone adiponectin in human disease. *Eur J Endocrinol*, 2003, 148, 293-300.
72. Havel PJ. Update on adipocyte hormones: regulation of energy balance and carbohydrate/lipid metabolism. *Diabetes*, 2004, 53, S143-51.
73. Clement K, Ferre P. Genetics and the pathophysiology of obesity. *Pediatr Res*, 2003, 53, 721-5.
74. Farooqi IS, Jebb SA, Langmack G, *et al.* Effects of recombinant leptin therapy in a child with congenital leptin deficiency. *N Engl J Med*, 1999, 341, 879-84.
75. Clement K, Boutin P, Froguel P. Genetics of obesity. *Am J Pharmacogenomics*, 2002, 2, 177-87.

76. Allison DB, Heo M: Meta-analysis of linkage data under worst-case conditions: a demonstration using the human OB region. *Genetics*, 1998, 148, 859-65.
77. Feitosa MF, Borecki IB, Rich SS, *et al.* Quantitative-trait loci influencing body-mass index reside on chromosomes 7 and 13: the National Heart, Lung, and Blood Institute Family Heart Study. *Am J Hum Genet*, 2002, 70, 72-82.
78. Hager J, Clement K, Francke S, *et al.* A polymorphism in the 5' untranslated region of the human ob gene is associated with low leptin levels. *Int J Obes Relat Metab Disord*, 1998, 22, 200-5.
79. Mammes O, Betoulle D, Aubert R, Herbeth B, Siest G, Fumeron F. Association of the G-2548A polymorphism in the 5' region of the LEP gene with overweight. *Ann Hum Genet*, 2000, 64, 391-4.
80. Farooqi IS, Keogh JM, Kamath S, *et al.* Partial leptin deficiency and human adiposity. *Nature*, 2001, 414, 34-5.
81. Huang QY, Shen H, Deng HY, *et al.* Linkage and association of the CA repeat polymorphism of the IL6 gene, obesity-related phenotypes, and bone mineral density (BMD) in two independent Caucasian populations. *J Hum Genet*, 2003, 48, 430-7.
82. Berthier MT, Paradis AM, Tchernof A, *et al.* The interleukin 6-174G/C polymorphism is associated with indices of obesity in men. *J Hum Genet*, 2003, 48, 14-9.
83. Kubaszek A, Pihlajamaki J, Komarovski V, *et al.* Promoter polymorphisms of the TNF-alpha (G-308A) and IL-6 (C-174G) genes predict the conversion from impaired glucose tolerance to type 2 diabetes: the Finnish Diabetes Prevention Study. *Diabetes*, 2003, 52, 1872-6.
84. Pihlajamaki J, Ylinen M, Karhapaa P, Vauhkonen I, Laakso M. The effect of the -308A allele of the TNF-alpha gene on insulin action is dependent on obesity. *Obes Res*, 2003, 11, 912-7.
85. Hoffstedt J, Eriksson P, Hellstrom L, Rossner S, Ryden M, Arner P. Excessive fat accumulation is associated with the TNF alpha-308 G/A promoter polymorphism in women but not in men. *Diabetologia*, 2000, 43, 117-20.
86. Skoog T, Eriksson P, Hoffstedt J, Ryden M, Hamsten A, Arner P. Tumour necrosis factor-alpha (TNF-alpha) polymorphisms-857C/A and -863C/A are associated with TNF-alpha secretion from human adipose tissue. *Diabetologia*, 2001, 44, 654-5.
87. Skoog T, van't Hooft FM, Kallin B, *et al.* A common functional polymorphism (C→A substitution at position -863) in the promoter region of the tumour necrosis factor-alpha (TNF-alpha) gene associated with reduced circulating levels of TNF-alpha. *Hum Mol Genet*, 1999, 8, 1443-9.
88. Shintani M, Ikegami H, Fujisawa T, *et al.* Leptin gene polymorphism is associated with hypertension independent of obesity. *J Clin Endocrinol Metab*, 2002, 87, 2909-12.
89. Ohshiro Y, Ueda K, Nishi M, *et al.* A polymorphic marker in the leptin gene associated with Japanese morbid obesity. *J Mol Med*, 2000, 78, 516-20.
90. Oksanen L, Kainulainen K, Heiman M, Mustajoki P, Kauppinen-Makelin R, Kontula K. Novel polymorphism of the human ob gene promoter in lean and morbidly obese subjects. *Int J Obes Relat Metab Disord*, 1997, 21, 489-94.
91. van Rossum CT, Hoebel B, van Baak MA, Mars M, Saris WH, Seidell JC. Genetic variation in the leptin receptor gene, leptin, and weight gain in young Dutch adults. *Obes Res*, 2003, 11, 377-86.
92. Silver K, Walston J, Chung WK, *et al.* The Gln223Arg and Lys656Asn polymorphisms in the human leptin receptor do not associate with traits related to obesity. *Diabetes*, 1997, 46, 1898-1900.
93. Gotoda T, Manning BS, Goldstone AP, *et al.* Leptin receptor gene variation and obesity: lack of association in a white British male population. *Hum Mol Genet*, 1997, 6, 869-76.
94. Francke S, Clement K, Dina C, *et al.* Genetic studies of the leptin receptor gene in morbidly obese French Caucasian families. *Hum Genet*, 1997, 100, 491-6.
95. Heo M, Leibel RL, Fontaine KR, *et al.* A meta-analytic investigation of linkage and association of common leptin receptor (LEPR) polymorphisms with body mass index and waist circumference. *Int J Obes Relat Metab Disord*, 2002, 26, 640-6.
96. Chagnon YC, Chung WK, Perusse L, Chagnon M, Leibel RL, Bouchard C. Linkages and associations between the leptin receptor (LEPR) gene and human body composition in the Quebec Family Study. *Int J Obes Relat Metab Disord*, 1999, 23, 278-86.
97. Stefan N, Vozarova B, Del Parigi A, *et al.* The Gln223Arg polymorphism of the leptin receptor in Pima Indians: influence on energy expenditure, physical activity and lipid metabolism. *Int J Obes Relat Metab Disord*, 2002, 26, 1629-32.
98. Ukkola O, Tremblay A, Despres JP, Chagnon YC, Campfield LA, Bouchard C. Leptin receptor Gln223Arg variant is associated with a cluster of metabolic abnormalities in response to long-term overfeeding. *J Intern Med*, 2000, 248, 435-9.
99. Quinton ND, Lee AJ, Ross RJ, Eastell R, Blakemore AI. A single nucleotide polymorphism (SNP) in the leptin receptor is associated with BMI, fat mass and leptin levels in postmenopausal Caucasian women. *Hum Genet*, 2001, 108, 233-6.
100. Yiannakouris N, Yannakoulia M, Melistas L, Chan JL, Klimis-Zacas D, Mantzoros CS. The Q223R polymorphism of the leptin receptor gene is significantly associated with obesity and predicts a small percentage of body weight and body composition variability. *J Clin Endocrinol Metab*, 2001, 86, 4434-9.
101. Wauters M, Mertens I, Chagnon M, *et al.* Polymorphisms in the leptin receptor gene, body composition and fat distribution in overweight and obese women. *Int J Obes Relat Metab Disord*, 2001, 25, 714-20.
102. Wauters M, Mertens I, Rankinen T, Chagnon M, Bouchard C, Van Gaal L. Leptin receptor gene polymorphisms are associated with insulin in obese women with impaired glucose tolerance. *J Clin Endocrinol Metab*, 2001, 86, 3227-32.
103. Mammes O, Aubert R, Betoulle D, *et al.* LEPR gene polymorphisms: associations with overweight, fat mass and response to diet in women. *Eur J Clin Invest*, 2001, 31, 398-404.
104. de Silva AM, Walder KR, Aitman TJ, *et al.* Combination of polymorphisms in OB-R and the OB gene associated with insulin resistance in Nauruan males. *Int J Obes Relat Metab Disord*, 1999, 23, 816-22.
105. de Silva AM, Walder KR, Boyko EJ, *et al.* Genetic variation and obesity in Australian women: a prospective study. *Obes Res*, 2001, 9, 733-40.
106. Mattevi VS, Zembrzuski VM, Hutz MH. Association analysis of genes involved in the leptin-signaling pathway with obesity in Brazil. *Int J Obes Relat Metab Disord*, 2002, 26, 1179-85.
107. Kubaszek A, Pihlajamaki J, Punnonen K, Karhapaa P, Vauhkonen I, Laakso M. The C-174G promoter polymorphism of the IL-6 gene affects energy expenditure and insulin sensitivity. *Diabetes*, 2003, 52, 558-61.
108. Fernandez-Real JM, Broch M, Vendrell J, *et al.* Interleukin-6 gene polymorphism and insulin sensitivity. *Diabetes*, 2000, 49, 517-20.
109. Vozarova B, Fernandez-Real JM, Knowler WC, *et al.* The interleukin-6 (-174) G/C promoter polymorphism is associated with type-2 diabetes mellitus in Native Americans and Caucasians. *Hum Genet*, 2003, 112, 409-13.
110. Brand E, Schorr U, Kunz I, Kertmen E, Ringel J, Distler A, Sharma AM. Tumor necrosis factor-alpha—308 G/A polymorphism in obese Caucasians. *Int J Obes Relat Metab Disord*, 2001, 25, 581-5.
111. Herrmann SM, Ricard S, Nicaud V, *et al.* Polymorphisms of the tumour necrosis factor-alpha gene, coronary heart disease and obesity. *Eur J Clin Invest*, 1998, 28, 59-66.

112. Wybranska I, Malczewska-Malec M, Niedbal S, Naskalski JW, Dembinska-Kiec A. The TNF-alpha gene NcoI polymorphism at position -308 of the promoter influences insulin resistance, and increases serum triglycerides after postprandial lipaemia in familiar obesity. *Clin Chem Lab Med*, 2003, 41, 501-10.
113. Dalziel B, Gosby AK, Richman RM, Bryson JM, Caterson ID. Association of the TNF-alpha -308 G/A promoter polymorphism with insulin resistance in obesity. *Obes Res*, 2002, 10, 401-7.
114. Rasmussen SK, Urhammer SA, Jensen JN, Hansen T, Borch-Johnsen K, Pedersen O. The -238 and -308 G→A polymorphisms of the tumor necrosis factor alpha gene promoter are not associated with features of the insulin resistance syndrome or altered birth weight in Danish Caucasians. *J Clin Endocrinol Metab*, 2000, 85, 1731-4.
115. Um JY, Park JH, Kim HM. Gene polymorphisms in tumor necrosis factor locus and waist-hip ratio in obese Koreans. *Clin Chim Acta*, 2003, 338, 117-22.
116. Fernandez-Real JM, Gutierrez C, Ricart W, *et al.* The TNF-beta gene Nco I polymorphism is not associated with hypertriglyceridemia or insulin resistance in lean and obese subjects. *Biochem Biophys Res Commun*, 1997, 236, 829-32.
117. Fernandez-Real JM, Gutierrez C, Ricart W, *et al.* The TNF-alpha gene Nco I polymorphism influences the relationship among insulin resistance, percent body fat, and increased serum leptin levels. *Diabetes*, 1997, 46, 1468-72.
118. Ishii T, Hirose H, Saito I, Nishikai K, Maruyama H, Saruta T. Tumor necrosis factor alpha gene G-308A polymorphism, insulin resistance, and fasting plasma glucose in young, older, and diabetic Japanese men. *Metabolism*, 2000, 49, 1616-8.
119. Lee SC, Pu YB, Thomas GN, *et al.* Tumor necrosis factor alpha gene G-308A polymorphism in the metabolic syndrome. *Metabolism*, 2000, 49, 1021-4.
120. Romeo S, Sentinelli F, Capici F, *et al.* The G-308A variant of the Tumor Necrosis Factor-alpha (TNF-alpha) gene is not associated with obesity, insulin resistance and body fat distribution. *BMC Med Genet*, 2001, 2, 10.
121. Rosmond R, Chagnon M, Bouchard C, Bjorntorp P. Increased abdominal obesity, insulin and glucose levels in nondiabetic subjects with a T29C polymorphism of the transforming growth factor-beta1 gene. *Horm Res*, 2003, 59, 191-4.
122. Yang WS, Tsou PL, Lee WJ, *et al.* Allele-specific differential expression of a common adiponectin gene polymorphism related to obesity. *J Mol Med*, 2003, 81, 428-34.
123. Ukkola O, Ravussin E, Jacobson P, Sjostrom L, Bouchard C. Mutations in the adiponectin gene in lean and obese subjects from the Swedish obese subjects cohort. *Metabolism*, 2003, 52, 881-4.
124. Vasseur F, Helbecque N, Dina C, *et al.* Single-nucleotide polymorphism haplotypes in the both proximal promoter and exon 3 of the APM1 gene modulate adipocyte-secreted adiponectin hormone levels and contribute to the genetic risk for type 2 diabetes in French Caucasians. *Hum Mol Genet*, 2002, 11, 2607-14.
125. Menzaghi C, Ercolino T, Di Paola R, *et al.* A haplotype at the adiponectin locus is associated with obesity and other features of the insulin resistance syndrome. *Diabetes*, 2002, 51, 2306-12.
126. Mammes O, Betoulle D, Aubert R, *et al.* Novel polymorphisms in the 5' region of the LEP gene: association with leptin levels and response to low-calorie diet in human obesity. *Diabetes*, 1998, 47, 487-9.
127. Le Stunff C, Fallin D, Schork NJ, Bougneres P. The insulin gene VNTR is associated with fasting insulin levels and development of juvenile obesity. *Nat Genet*, 2000, 26, 444-6.
128. Le Stunff C, Le Bihan C, Schork NJ, Bougneres P. A common promoter variant of the leptin gene is associated with changes in the relationship between serum leptin and fat mass in obese girls. *Diabetes*, 2000, 49, 2196-200.
129. Narkiewicz K, Szczech R, Winnicki M, *et al.* Heritability of plasma leptin levels: a twin study. *J Hypertens*, 1999, 17, 27-31.
130. Comuzzie AG, Hixson JE, Almasy L, *et al.* A major quantitative trait locus determining serum leptin levels and fat mass is located on human chromosome 2. *Nat Genet*, 1997, 15, 273-6.
131. Villuendas G, San Millan JL, Sancho J, Escobar-Morreale HF. The -597 G→A and -174 G→C polymorphisms in the promoter of the IL-6 gene are associated with hyperandrogenism. *J Clin Endocrinol Metab*, 2002, 87, 1134-41.
132. Takahashi M, Arita Y, Yamagata K, *et al.* Genomic structure and mutations in adipose-specific gene, adiponectin. *Int J Obes Relat Metab Disord*, 2000, 24, 861-8.
133. Kishida K, Nagaretani H, Kondo H, *et al.* Disturbed secretion of mutant adiponectin associated with the metabolic syndrome. *Biochem Biophys Res Commun*, 2003, 306, 286-92.
134. Lindsay RS, Funahashi T, Krakoff J, *et al.* Genome-wide linkage analysis of serum adiponectin in the Pima Indian population. *Diabetes*, 2003, 52, 2419-25.
135. Comuzzie AG, Funahashi T, Sonnenberg G, *et al.* The genetic basis of plasma variation in adiponectin, a global endophenotype for obesity and the metabolic syndrome. *J Clin Endocrinol Metab*, 2001, 86, 4321-5.