Eur J Nutr 39 : 127–144 (2000)

REVIEW

A. Palou
F. Serra
M. L. Bonet
C. Picó

Obesity: molecular bases of a multifactorial problem

Received: 15 September 1999
Accepted: 10 June 2000

A. Palou (✉) · F. Serra · M. L. Bonet
C. Picó
Departament de Biologia Fonamental i Ciències de la Salut (Bioquímica i Biologia Molecular)
Universitat de les Illes Balears
Campus UIB, Edifici Guillem Colom
Ctra. de Valldemossa km. 7.5
07071 Palma de Mallorca – Spain
e-mail: dbfapo0@ps.uib.es

Summary Obesity could well become the most common health problem of the 21st century. There are more opportunities to consume large quantities of food: big portions of tasty, varied food, at reasonable prices, are available everywhere. Moreover, our bodies are better adapted to combat weight loss than to combat weight gain, since for thousands of years our species evolved in circumstances where nutrients were in short supply.

The response of each individual to diet and other environmental factors varies considerably, depending on the characteristics of his/her body weight control mechanisms. The differentiating element in the future, especially as regards the dietary and pharmacological control of obesity, will be knowledge of an individual's possible response depending on his/her genetic background.

Obesity can occur as a result of genetic or acquired changes in three main types of biochemical processes, which are the main focus of this review: a) *feeding control*, which determines the sensations of satiety and hunger through processes that depend on an interplay between internal signals (notably leptin) and environmental factors; b) *energy efficiency*, in particular the activation of thermogenesis mediated by uncoupling proteins (UCPs) that makes it possible to dissipate part of the energy contained in food as heat instead of accumulating it as fat, and c) *adipogenesis*, the process by which cells specialised in fat storage (adipocytes) are formed, which is controlled by an interplay of transcription factors, including members of the C/EBP, PPARγ and ADD families.

The knowledge of a growing number of genes and molecules implicated in these three types of processes and of their metabolic relationships is leading toward a molecular understanding of the body weight regulatory system, and is paving the way for new methods of obesity control, especially pharmacological but also nutritional and possibly involving genetic intervention.

Key words Obesity – feeding control – adipogenesis – thermogenesis – obesity genes

The genetic-molecular bases of obesity

Obesity occurs when energy intake exceeds energy expenditure as a result of genetic or acquired changes in feeding control, energy efficiency or adipogenesis. The genes associated with these three types of biochemical processes and the metabolic location and links between their protein products are the main focus of this review:

- *Feeding control* [1, 2]: i. e. the biochemical processes that determine the sensations of satiety and hunger, in respect of both quantity and quality, including prefer-

ence for certain types of food, appetite, frequency of food intake etc. These processes depend in turn on internal and environmental factors, including social habits. The state and activity of energy reserves, mainly in adipose tissue, are communicated to the CNS by leptin and other signals.

- *Control of energy efficiency* [3]: i. e. the biochemical processes that control the degree to which energy from food is used. Of particular interest is the control of energy efficiency through changes in thermogenesis mediated by uncoupling proteins (UCPs) [4]. Activation of thermogenesis makes it possible to dissipate part of the energy contained in food as heat instead of accumulating it as fat.
- *Adipogenesis* [5, 6]: i. e. the process by which cells specialised in fat storage (adipocytes) are formed. The adipose tissue is at the centre of a key regulatory system exerting a pivotal influence on hormone-regulated fuel partitioning in peripheral tissues, and it relates to many metabolic complications of obesity.

Feeding control

There are various aspects to be considered in the regulation of feeding behaviour [1, 2]. There is the time aspect of appetite control mechanisms: short-term control, by physical signals and release of digestive peptides in response to food, and chronic or medium-long term control, effected by signals (such as leptin) that indicate the levels of the energy reserves in the body. There are also qualitative aspects of appetite control, i. e. mechanisms underlying the selection of certain specific nutrients or groups of nutrients. Finally, it must be remembered that these processes are coordinated by integrating mechanisms.

Signals indicating the availability of energy resources

There may be various types of biomolecules linking all the information regarding the external energy situation (food available) with the internal energy situation (energy-nutritional reserves). Various possibilities were first suggested for several metabolites and hormones (glucose, ketone bodies, amino acids, insulin, glucocorticoids etc.), and more recently for oleoyl-estrone and related compounds [7, 8]. Nevertheless, although other possible signals should be kept in mind, only for leptin – and linked with it, insulin – is a sufficient body of knowledge available to enable us to interpret physiologically the processes involved in the control of energy balance and to explain them, at least partly, in genetic-molecular terms.

Leptin as indicator of internal energy reserves and regulator of energy balance

The ob gene, expressed by adipocytes, encodes the hormone leptin, which is released by the adipose tissue to the circulatory system and which, in the brain, acts on the leptin receptors and provides information about the level of fat reserves. Leptin determines changes in feeding behaviour, with suppression of appetite, and an increase in metabolic activity and energy expenditure (thermogenesis). It also affects different aspects of hormonal action which regulate the break up of nutrients and their metabolism in different tissues [9].

When the ob gene was cloned in December 1994 [10] and its protein product – later named leptin, from the Greek leptos, meaning thin – was described, the complex molecular mechanisms integrating the regulation of food intake, energy expenditure and fat reserves began to be understood. A year later, the db (or lepR) gene, which encodes the leptin receptors (expressed in the hypothalamus, in other parts of the CNS and also in peripheral tissues), was also identified [11] and characterised [12, 13]. Subsequently, several orexigenic and anorexigenic neuropeptides were described in the CNS, whose function is interlinked and connected with that of leptin [1, 2, 14–16; Fig. 1].

The data deriving from the leptin mechanism fit in with the lipostatic theory [17], which stated that the size of the body fat depots was regulated by a product of these depots which, via the circulatory system, acted on the CNS to con-

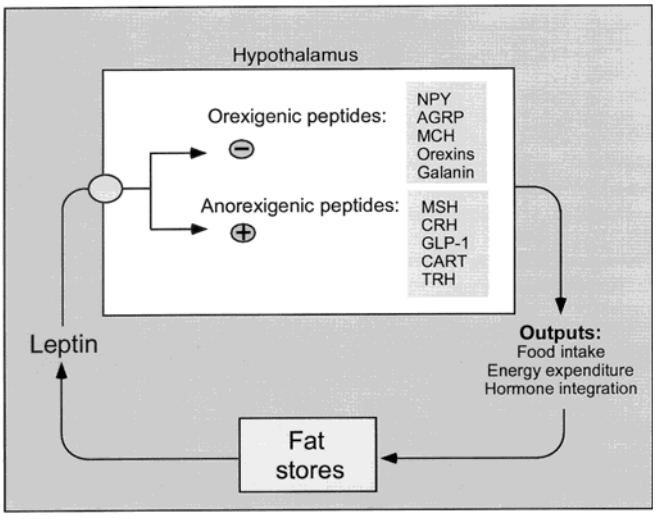

Fig. 1 Orexigenic and anorexigenic neuropeptides involved in the control of food intake and energy expenditure. *NPY* neuropeptide Y; *AGRP* agouti related protein; *MCH* melanine-concentrating hormone; *MSH* melanocyte-stimulating hormone; *CRH* corticotropin-releasing hormone; *GLP-1* glucagon-like peptide-1; *CART* cocaine- and amphetamine-regulated transcript; *TRH* thyrotropin-releasing hormone.

trol food intake. The hypothesis of Coleman [18] that ob/ob genetically obese mice lacked this hormone while db/db genetically obese mice were insensitive to it, with both types of the mutant having identical obese phenotypes, has been now confirmed with the discovery that the ob mutation is located in the gene encoding leptin, and the db mutation is located in the gene encoding the leptin receptor [12, 13, 19].

Effectiveness of administered leptin in normalising the metabolic parameters and reducing the body weight of ob/ob genetically obese mice was confirmed in three studies published simultaneously [20–22], which used either biologically active forms of recombinant leptin expressed in bacteria, or leptin purified from blood plasma. These studies also showed that there was no slimming effect of leptin on db/db mice [22, 23], confirming Coleman's idea [18] that in these mice the receptor for the lipostatic signal had mutated. Mice obese because of hypothalamic damage and mutants on the db locus showed increased ob-mRNA expression in adipose tissues [24], such as could be expected from secondary overexpression to compensate lack of sensitivity to leptin. It was finally concluded that leptin is a hormone generated in adipose tissue, present in the blood of the normal mouse and in human blood and able (at doses of 0.1 mg to 1 g/kg of body weight) to correct obesity in the ob/ob mouse, thus normalising its main metabolic parameters (glycemia, insulinemia) through effects on the CNS.

Based on anatomical and functional data, it appears that leptin exerts its effects on energy balance mainly by acting in the brain. Thus, intravenous leptin injection activates neurons in the arcuate, ventromedial and dorsomedial hypothalamic nuclei and in brainstem neuronal circuits implicated in the regulation of feeding behaviour and energy balance [25, 26]. Intracerebroventricular leptin injection inhibits food intake and decreases adiposity more potently than peripheral leptin administration [22]. However, there are several variants of the leptin receptor, resulting from differential db mRNA processing, distributed in many tissues outside the CNS [12, 13, 19], including adipose tissue, which could mediate direct effects of leptin in these tissues. For example, leptin stimulates lipolysis and the expression of fatty acid oxidation enzymes in isolated adipocytes, an effect that depends on the expression of functionally active leptin receptors in the cell membrane of these cells [27].

Besides its effects on food intake, leptin increases energy expenditure by activating thermogenesis; in particular, it increases norepinephrine turnover in thermogenic tissues [28] and favours the expression of uncoupling proteins [29–34]. Moreover, leptin also stimulates the rate of lipolysis and the expression of enzymes of fatty acid oxidation in adipose [27, 35] and pancreatic cells [29], causing a reduction of the triglyceride content of these cells which is not accompanied by a parallel release of fatty acids [27]. Taken together, these results suggest that leptin favours the internal consumption of fatty acids as thermogenic fuels. Some of these effects of leptin appear to be direct (extraneural) effects, since they are seen in isolated cells or cells in culture [27, 29, 35] and depend on the expression of functionally active leptin receptors in the cell membrane [27]. The effect of leptin to stimulate thermogenesis is the basis of perhaps one of the most potentially interesting aspects of the exogenous administration of leptin, since it allows the maintenance of a high metabolic rate and a gradual and extensive elimination of fat reserves even under conditions of low energy intake (dieting) [36].

Initial data showed that leptin production reflects the size of fat depots, but more recently leptin production in non-adipose tissues such as murine [37] and human stomach [38], placenta [39, 40], skeletal muscle [41], and mammary epithelium [42] was reported. Brain was also suggested to be a source of leptin, based on arteriovenous differences in human studies [43], although actual expression of leptin has not been detected in human brain.

Leptin has diverse effects in addition to long-term regulation of body weight [9, 44]. In particular, leptin plays significant physiological roles in various aspects of reproduction in humans, and appears to be necessary for the maturation of the reproductive axis [45]. Leptin also exerts acute effects on glucose and lipid metabolism [27, 35]. Local leptin expression in the stomach could regulate satiety [37, see below], and leptin signalling in the intestine could be involved in the regulation of nutrient absorption and intestinal motility [46]. Leptin has also been implicated in the regulation of the cardiovascular and renal function and in the functioning of the immune system [47]. It may also play an important role in development, as suggested by the formation of leptin in placenta, widespread expression of leptin and its receptors in fetal tissues and stimulation of hematopoiesis and angiogenesis by leptin [39, 40, 48, 49].

In summary, the initial view of leptin as an anti-obesity hormone has changed to a more complex view: leptin is produced in a variety of tissues, it is targeted to a variety of tissues, and it is involved in the regulation of a variety of functions, including energy balance, metabolism, neuroendocrine and immune function, and development.

Satiety signals from the digestive system as indicators of external energy resources

The living organisms can accommodate to a wide variety of foods and eating habits – with meals varying in size, number and composition, taken at different times – and nonetheless maintain the right energy balance. This points to the existence of acute, finely tuned food intake control mechanisms, which are activated once food intake has begun. These control mechanisms are based on satiety signals triggered by food, which contribute to ending the meal [1].

Cholecystokinin (CCK) is the best known and the most representative of the peptides secreted by the digestive tract during meals. Over 25 years ago it was shown that, when

administered to rats before meals, CCK causes a dose-dependent reduction of food intake [50]. Since then, the role of this intestinal peptide has been extensively studied, but there are others with similar effects, such as gastrin and the peptides of the bombesin family (bombesin, gastrin-releasing peptide, neuromedin B) and the glucagon family. Satiety peptides combine with other signals, e. g. physical signals such as gastric distension, to synergistically reduce meal size. With regard to their therapeutic use in obesity treatment, pharmacological studies have shown that satiety peptides are generally well tolerated in humans [1, 51], although repeated administration does not seem to alter body weight, since their action is offset by more frequent meals [52].

Satiety peptides carry their signal to the CNS via peripheral nerves such as the afferent vagal fibres, or via the circulatory system, which allow these peptides to reach specific receptors in the brain.

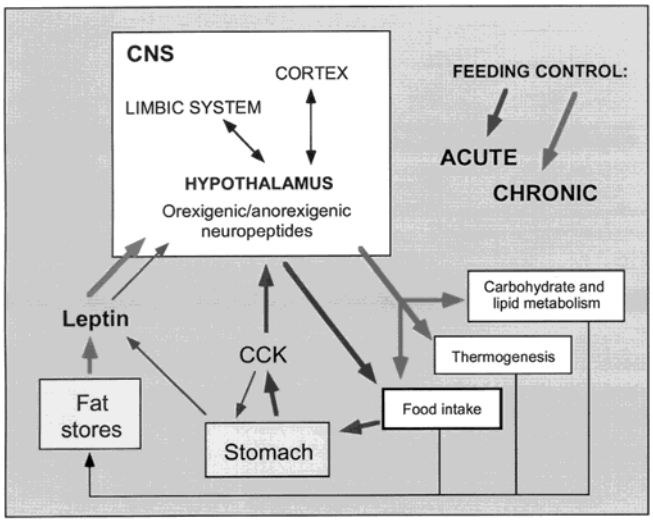

Fig. 2 General system of acute and chronic feeding control. Chronic feeding control depends on leptin released from fat stores, while acute control is based on gastric satiety peptides such as cholecystokinin (CCK). Leptin released from stomach in response to food intake may represent a link between acute and chronic control systems.

Leptin as a potential link between internal and external energy resources

Besides indicating the size of the body fat stores, leptin may help indicate that food is available. Thus, recently it was reported that the stomach of rats can produce and store leptin and release it into the blood in response to food intake, leading (in a few minutes) to an increase in plasma leptin levels [37]. Two satiety signals, CCK and gastrin, were found to stimulate the secretion of the leptin stored in the rat stomach [37]. More recently, we reported the presence of significant leptin levels in the stomach glands of the human [38]. Ultrastructural immunocitochemistry showed leptin immunoreactivity both in the pepsinogen granules of chief cells and in the granules of a specific endocrine cell type, suggesting the use of leptin in both endocrine and exocrine ways. Interestingly, in one of the patients studied (who did not follow the medical request before endoscopia and was not under food deprivation conditions) gastric glands appeared depleted of leptin. All together these findings suggest a role for gastric leptin and that leptin may mediate, at least in part, the satiety response produced by gastrointestinal peptides. Thus, leptin may somehow be linking acute and chronic regulation of feeding behaviour, connecting information from both external (food intake) and internal (fat stores) energy resources and the CNS (Fig. 2).

Resistance to the action of leptin in obese humans

If leptin plays a central role in regulating energy balance and body weight, its function is expected altered in the obese. By homology with the mouse, deficiencies in leptin production or leptin reception might cause energy imbalances and obesity in humans. However, leptin production is not deficient in most obese humans, neither do frequent mutations of the leptin gene or the leptin receptor gene occur in humans (see below), so the alterations must be either in the transport of leptin to the CNS or in post-receptor events. These alterations will lead to a failure in leptin action that could be responsible for the hyperleptinemia, which is a general feature of obesity [53–58].

The first data reported from extremely obese humans indicated high levels of ob-mRNA expression in their fat depots, and that both the levels of leptin expression in adipocytes and the circulating leptin levels correlated positively with the degree of obesity [53–58]. Since the expected response to increased leptin levels is a reduction of energy intake and an increase of energy expenditure, it follows that obese people are insensitive to their endogenous leptin.

In the majority of obese humans, the leptin (ob) gene is not mutated; actually, only two of the several thousand families studied were shown to have mutations that stop leptin production and are responsible for their familiar obesity [59, 60]. Changes in the leptin receptor (db) gene do not appear either to be a common factor in human obesity, except in the isolated case of a Kabyle family [61] in which this gene has mutated at a splicing site, so that the receptor lacks transmembrane and intracellular domains essential for signal transduction and is therefore not functional.

The transport of leptin into the CNS is a regulatory element to consider. Leptin is transported into the CNS by a saturable system located in the endothelial cells of the brain [62], which is encoded by one of the mRNA splicing variants (the smallest one) of the leptin receptor (db) gene

[63, 64]. In obese humans, high serum leptin levels are not matched by proportionally high levels of leptin in the cerebrospinal fluid, suggesting a causal relationship between a deficit in the transport system bringing leptin to the CNS and obesity [65], although this has not yet been fully established at the molecular level.

Regulation of leptin synthesis

Leptin expression is influenced by the status of fat stores, as evidenced by increased adipose *ob* mRNA and serum leptin levels in obese humans and other mammals [9] and by the existence of a correlation, at the adipocyte level, between fat content and leptin expression [55]. However, leptin production is not equal in the different adipose tissue depots, since each has a distinct ontogenic pattern of leptin expression [66].

Plasma leptin levels are higher in women than in men [67], are subjected to daily rhythms in both sexes [68], and they drop with fasting and increase with food intake [36].

Insulin plays a key although indirect role in the leptin system. The administration of insulin stimulates leptin expression [69], but it takes several hours for this increase to be reflected in the circulating leptin levels [70]. Leptin and insulin have marked similarities as signals since the concentration of both of them is proportional to the degree of obesity, both reach the brain via saturable systems located in the endothelial cells, and both act on hypothalamic receptors, triggering similar responses [1]. Moreover, secretion of both insulin and leptin depends on the level of energy reserves of the organism and immediate changes in the energy balance [1], including food intake [37]. However, several observations suggest that leptin has a more important role than insulin in the central control of energy homeostasis [2]. For example, leptin deficiency causes severe obesity, with hyperphagia that persists despite high insulin levels. In contrast, obesity is not induced by insulin deficiency. In addition, in rats with pharmacologically induced insulin-deficient diabetes (that have low levels of both insulin and leptin), the administration of leptin at basal plasma concentrations prevents the development of diabetic hyperphagia, indicating that the latter is due to deficiency of leptin, rather than of insulin [71].

Leptin production in adipocytes is stimulated by certain cytokines in anorexia associated with infection [72] and by corticosteroids [73], whereas it appears to be suppressed by activation of the sympathetic nervous system (SNS), with intervention of the ß3-adrenergic receptors [74]. Secretion of stomach leptin, on the other hand, is stimulated by two satiety signals related to food intake, CCK and gastrin [37]; the latter was also found to activate leptin expression and secretion in rat adipose tissues, through activation of an adipocyte gastrin/CCK-B receptor [75].

UDP-N-acetylglucosamine (UDP-GlcNAc), the end product of the hexosamine biosynthetic pathway, may be the metabolic intermediary responsible for stimulating the expression, synthesis and secretion of leptin in adipocytes and muscle cells [41]. The effect could be mediated by the n-acetylglycosylation of proteins that positively regulate leptin gene expression. UDP-GlcNAc accumulates in conditions in which flow through the hexosamine biosynthetic pathway increases, as for example after blockage of glycolysis due to an increase of intracellular fatty acid levels. Thus, UDP-GlcNAc could link the specific energy status of each cell with its leptin production [41].

Organisation of feeding control in the central nervous system

Neuronal circuits organised in the arcuate nucleus (ARC) of the hypothalamus have highly specialised roles in energy homeostasis [2]. The ARC is a collection of neuronal cell bodies occupying approximately one-half of the length of the hypothalamus, to the floor of the third ventricle. Two orexigenic neuropeptides, NPY (neuropeptide Y) and AGRP (Agouti-related peptide) are co-localised in ARC neurons [76, 77]. The anorexigenic peptides POMC (pro-opiomelanocortin) and CART (cocaine-amphetamine related transcript) are co-localised in a distinct, but adjacent, subset of ARC neurons [78]. A majority of both NPY/AGRP and POMC/CART neurons coexpress leptin receptors [79, 80] and both types of neurons are regulated by leptin, but in an opposing manner. Conditions characterised by reduced insulin or leptin levels activate NPY/AGRP neurons [76, 77, 81–83] and inhibit POMC/CART neurons [78, 84–86]. Taken together, these findings indicate that the ARC is a major site for transduction of adiposity signals into a neuronal response [2]. Other hypothalamic areas which are richly supplied by axons from ARC neurons such as the paraventricular nucleus (PVN), zona incerta, perifornical area and lateral hypothalamus (LH) may also participate in the energy homeostasis circuit [87, 88]. The link between the LH and the higher centres of the brain which regulate hunger and satiety is an important aspect of the regulatory system, and two types of neuropeptides associated with neurons apparently exclusive to the LH have been characterised: the MCH (melanin-concentrating hormone) [89] and the orexins [90, 91].

The neuropeptide Y pathway

Leptin, acting through its hypothalamic receptor, determines a suppression of NPY expression and release, leading to a decrease in food intake and an increase in metabolic activity (Fig. 3). This was the first hypothalamic pathway for leptin action that was suggested after the leptin gene was cloned [82].

NPY is a well-known potent stimulator of food intake

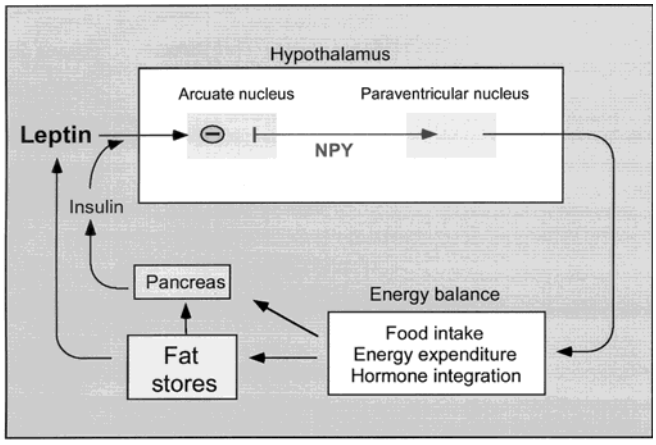

Fig. 3 The NPY pathway. Feedback regulation of body fat stores integrating the action of leptin, secreted by the adipocytes, and insulin, secreted by the endocrine pancreas, both acting in the brain controlling energy balance via a mechanism that likely involves inhibition of NPY.

when injected directly into the brain [92]. The action of NPY is channelled via the parasympathetic nervous system, producing hyperinsulinemia and an increase in glucocorticoid production, leading to an accumulation of fat in the adipose tissue, and reduced thermogenesis and muscular uptake of glucose. In conditions associated with weight loss or with a negative balance, such as caloric restriction, lactation and intense exercise, the NPY pathway becomes activated, causing increased appetite. This is a response mediated, at least in part, by a reduction of the negative feedback from leptin, with possible intervention of changes in insulin sensitivity [1, 93].

Central injection of NPY virtually evokes all features of leptin deficiency, including hyperphagia, hyperinsulinemia with insulin resistance, and decreased thermogenesis [1], and repeated NPY central injection produces obesity within a matter of days [92]. In ob/ob mice (which lack functional leptin and have high levels of NPY), the knock-out of the NPY gene attenuates the degree of obesity [94], demonstrating the importance of the pathway; however, these mice display nonetheless a severe obesity and they respond normally to the satiety effects of leptin, indicating that, in addition to NPY, other downstream components must be involved in the leptin pathway.

NPY acts via Y1, Y2 and Y5 G-protein coupled receptors expressed in hypothalamic neurons. The Y5, and also the Y1, receptors mediate the stimulatory effects of NPY on food intake [95]. The Y2 receptor, on the other hand, appears to mediate an inhibitory effect of NPY at low concentrations that could be important for basal control of body weight, in view that Y2 null mutant mice developed both increased food intake and body weight [96]. The action of NPY on its receptors can be affected by other neurotransmitters, such as GLP–1 (glucagon-like peptide–1), which inhibits food intake and diminishes the orexigenic effect of NPY, probably by antagonising NPY receptors Y5 and Y1 [97]. NPY receptors are a main focus of research in the development of new drugs for controlling appetite.

The glucocorticoids produced in the adrenal cortex participate in energy regulation potentiating the NPY orexigenic pathway, probably as endogenous antagonists of leptin and insulin [1]. Thus, adrenalectomy attenuates the effect of fasting to increase both appetite and NPY expression, and potentiates the anorexigenic and slimming effects of insulin and leptin, and these effects are offset by administration of glucocorticoids [1].

The agouti gene and the MC4 melanocortin receptor pathway

Several dominant mutations in the agouti (Ay) gene cause obesity, yellow colouring and a non-insulin dependent form of diabetes mellitus in mice. All these mutations give rise to generalised expression of the agouti protein in tissues in which it is not normally expressed (it is normally found only in the hair follicles of the skin) [98]. The agouti protein antagonises various melanocortin receptors, and it normally functions as an antagonist of the cutaneous MC1 melanocortin receptor (MC1-R), thus inhibiting pigment synthesis (hence the yellow coat colour of the agouti mutants) [99]. The agouti protein is also a specific antagonist of the MC4 melanocortin receptor (MC4-R), which is expressed primarily in the hypothalamus and other areas of the brain, and this antagonistic action could cause obesity, since melanocortinergic neurons have been shown to exert a tonic inhibition on food intake [100]. Thus, chronic blockage of this inhibitory (anorexigenic) signal – owing to ectopic agouti production within the brain – seems to be the most logical explanation of the agouti obesity syndrome (Fig. 4).

At the MC4-R a regulatory system would therefore be established, with two types of opposing binders: agonists (melanocortins, in particular the melanocyte-stimulating hormone, αMSH), with anorexigenic action, and antagonists (proteins similar to that encoded by the agouti gene but normally expressed in the brain, like the Agouti Related Peptide, AGRP [101]), with orexigenic action. This hypothesis is supported by sound experimental evidence, such as the fact that genetic knock-out of the MC4-R causes obesity similar to that of the Ay mouse but without affecting pigmentation [102], and the observation that food intake is reduced after central administration of a MC4-R agonist (αMSH) and increased after administration of a synthetic antagonist of this receptor [100]. In addition, mutations in the MC4-R gene have been shown to cause obesity in humans [103].

αMSH is formed from pro-opiomelanocortin (POMC) in arcuate nucleus (ARC) neurons that express the leptin receptor and project their axons (containing αMSH) to

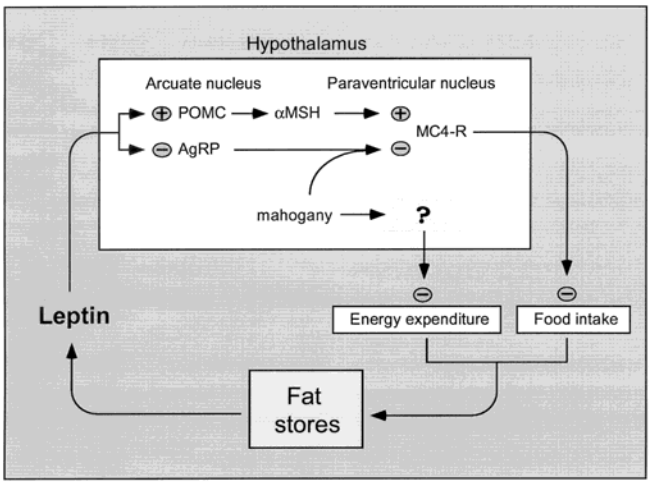

Fig. 4 The MC4-R pathway. A model of leptin action in the hypothalamus throughout the MC4-R melanocortin receptor pathway. Leptin positively regulates POMC (the precursor of αMSH), and negatively regulates AGRP. Neuronal release of αMSH activates MC4-R and thereby reduces food intake. Conversely, reduced MC4-R signalling induced by AGRP causes hyperphagia and obesity. *POMC* pro-opiomelanocortin.

MC4 post-synaptic neurons in other areas of the hypothalamus. Production of αMSH is potentiated by leptin, which stimulates the expression of POMC in the ARC neurons [84, 2]. AGRP is also characteristically expressed in the ARC and also appears to be regulated by leptin, but in an opposing manner, since its expression is stimulated by leptin deficiency, as in ob/ob mice [104, 2]. It is significant that overexpression of AGRP causes obesity similar to that of MC4 receptor knock-out mice [101].

The latter results establish a prominent role of the MC4-R pathway in leptin action. However, not all leptin action seems to be mediated through this pathway, since knock-out or blockage of the MC4-R does not produce hypercortisolemia, or have any effects on the reproductive system such as those produced by leptin deficiency.

Another gene involved in energy homeostasis that appears to be related to melanocortin signalling is the mahogany gene. This gene is mainly expressed in neurons of the ventromedial hypothalamus, encodes a single-transmembrane-domain receptor-like protein, and its mutation can suppress diet-induced obesity [105] as well as the obesity and yellow-coat colour of the agouti mutant mice [106]. The mahogany protein normally functions to potentiate signalling from antagonists (like agouti protein and AGRP) on MC4-R [105]. Thus, the loss-of-function of the mahogany protein favours signalling from anorexigenic agonists (α-MSH) and can compensate for antagonist overexpression.

MCH and the orexins of the lateral hypothalamus (LH) participate in integrated appetite control

Two types of orexigenic neuropeptides located primarily in the LH have been described: the melanin-concentrating hormone (MCH) [89] and the A and B orexins [90, 91].

MCH neurons project from the LH to the nucleus of the solitary tract and the parabrachial nucleus, but they also have monosynaptic projections to the medial prefrontal cortex, which suggests that MCH may be involved in complex integrative behaviours. Renewed interest in MCH came from the observation that its mRNA is overexpressed in the hypothalamus of obese ob/ob mice compared with controls [89], and that MCH-knockout mice have reduced food intake and are excessively lean [107]. A G-protein-coupled receptor previously known as SLC-1 has been identified as the natural receptor of MCH [108]. This receptor is expressed in several brain regions, in particular those involved in olfactory learning and reinforcement mechanisms, further suggesting the involvement of MCH in the neuronal regulation of food consumption.

Two additional neuropeptides involved in the regulation of food intake were discovered simultaneously by two groups, the so-called A and B orexins [90], also known as hypocretins 1 and 2 [91]. Orexins are derived (by proteolysis) from a common precursor, and function through G-protein coupled receptors. Their expression seems to be limited to the neurons of the LH and nearby region, although there is also some expression in the testicles. Central administration of orexins stimulates food intake, and orexin production increases with fasting [90].

Control of energy efficiency by adaptive thermogenesis

Although some energy is released as heat (thermogenesis) in all bioenergetic transformations, here we will refer only to adaptive or facultative thermogenesis mechanisms, which specifically function to produce heat in a physiologically regulatable manner. It is easy to see why the regulation of thermogenesis and the degree of efficiency in the utilisation of nutrients has become one of the main focuses of obesity research.

Almost everyone who is trying or has tried to lose weight envies those who seem able to eat whatever they like and stay slim. Higher or lower metabolic efficiency, with a substantial inherited component according to Bouchard's studies [109], may be the key. We have been familiar with the thermogenin or UCP of the brown adipose tissue (today UCP1) [110, 111] in small mammals for twenty years, and with UCP2 [112, 113] and UCP3 [114–116] in different tissues of rodents and humans since autumn 1997 [4]. This family of genes governs energy use during the mitochondrial oxidation of nutrients.

European Journal of Nutrition, Vol. 39, Number 4 (2000)

The adaptive thermogenesis mechanism

The adaptive thermogenesis mechanism is well understood only in a particular type of adipose tissue, the brown adipose tissue (BAT), which, unlike the white, contains many nerve endings of the SNS, and it is very vascularised [3, 111, 117]. Also, brown adipocytes contain several vacuoles of fat instead of just one, are rich in mitochondria and contain a unique protein, the thermogenin or uncoupling protein (UCP1) [3, 111, 117]. The production of heat in BAT is stimulated by cold (CIT or cold-induced thermogenesis) and diet (DIT or diet-induced thermogenesis) [118, 119] and driving the mechanism is the UCP1, which is located at the inner mitochondrial membrane of brown adipocyte mitochondria, and whose function is to short-circuit the proton gradient generated by the oxidation of nutrients (mainly fatty acids) in the respiratory chain [111]. Finally, the energy obtained from nutrients is dissipated, in an adjustable way, as heat via the UCP1, instead of the proton gradient being channelled through the ATP-synthase and used in ATP synthesis (Fig. 5).

Up to now it has been possible to characterise this type of mechanism only in the BAT of mammals. However, results obtained by the group of Nagase [120] and in our laboratory [121, 122] have shown that the basic gene in this process, the one encoding UCP1, and thus probably adaptive thermogenesis, can appear in other tissues (white adipose tissue and muscle) with appropriate stimulus. Even more important, it has been shown that proteins similar to thermogenin, such as UCP2 [112, 113] and UCP3 [114–116], are expressed in a variety of non-BAT tissues (white adipose tissue, muscle and others). Several studies indicate that these UCPs also have proton transport activity [112, 113, 116, 123, 124]. This has opened up new possibilities and encouraged interest in adaptive thermogenesis, especially because these new UCPs are widely expressed in human tissues. However, the *in vivo* function of these UCP homologues, with respect to thermogenesis and regulation of mitochondrial energy metabolism, is presently uncertain [125], and it is an active area of investigation (see appendix). A so-called UCP4 has also been described [126, 127]; it is present in the brain but its homology with UCP1 is poor and its function unknown.

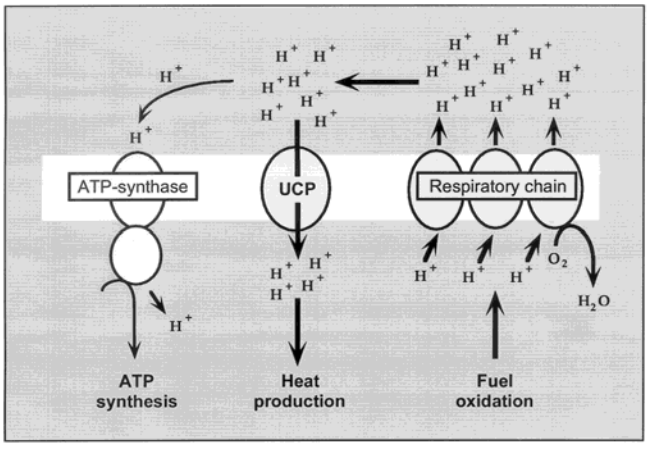

Fig. 5 The three conceptual elements involved in the function of the uncoupling proteins: fuel oxidation, energy dissipation and energy conversion. (Modified from [111]).

Regulation of adaptive thermogenesis

Adaptive thermogenesis is deficient in almost all the animal models of obesity studied [117, 128]. In addition, when an animal model with low BAT content was developed (using the UCP1 promoter to direct expression of the A chain of the diphtheria toxin) transgenic mice with less BAT, marked obesity and increased susceptibility to develop diet-induced obesity were obtained [129].

The regulation of adaptive thermogenesis by exogenous factors depends primarily on stimulation of SNS, which densely innervates BAT [130]. In general, BAT thermogenesis can be regulated through changes in the intrinsic activity of UCP1 (seconds), the quantity of UCP1 (hours), the number of mitochondria and adipocytes (days) or by generalised hyperplasia of the BAT (days/weeks) [130, 131]. Norepinephrine (NE) released by the SNS has a major role on brown adipocytes, stimulating UCP1 activity and synthesis, and also cell division [111, 131]. NE increases UCP1 and UCP1-mRNA levels mainly by affecting transcription of the UCP1 gene [132, 133]; this effect is mediated mainly through β3 adrenergic receptor (β3-AR) and an increase of the intracellular cAMP levels [132, 134, 135] and, to a lesser degree, through the al adrenergic receptor [132]. A putative role for a recently described atypical β-AR, named β4-AR, has also been suggested [136]. In addition NE has an effect in potentiating the stability of UCP1-mRNA [137], and increasing UCP1 protein half-life [138]. Besides NE, triiodothyronine (T3) also stimulates UCP1 transcription, depending on the function of type II tyroxine 5′-desiodase in the brown adipocytes, the activity of which is increased by NE [139].

Actual activation of thermogenesis basically depends on the presence of fatty acids, which directly act on UCP1 stimulating its proton transport activity and also serve as the main thermogenic fuel. The availability of fatty acids depends in turn on cAMP levels and adrenergic regulation [111].

In laboratory animals, long-term overfeeding with the so-called cafeteria diet – a variety of energy-rich, palatable foods, offered in excess quantities [composition and other details in 140, 141] – is accompanied by hyperplasia of BAT and increased UCP1 levels [142–144], and also of UCP2 mRNA expression [144], a response that confers a relative protection against the development of obesity (hypothesis first put forward by Rothwell and Stock [118]). In fact, an impairment of this DIT response was seen in fe-

male rats, which gained more weight than male rats after cafeteria diet feeding [144]. Although cafeteria diet-induced overweight is usually lost when the animals are put back on a normal diet, we have observed that under certain conditions (prolonged feeding of a cafeteria diet during the period of development) a persistent dietary obesity can develop, with changes in the thermogenic mechanism and other biochemical parameters [141, 142, 145, 146].

Studies carried out in our laboratory show that different mitochondrial subpopulations, which likely represent different stages of mitochondriogenesis, display different thermogenic capacity [147–150] and point to the importance of the regulation of mitochondrial biogenesis in obesity. Changes in the control of mitochondrial turnover may be an important factor in the modulation of energy balance, although to gain a better understanding of these aspects more has to be learned about the genesis and recycling of mitochondria in BAT and other tissues.

In summary, adaptive thermogenesis represents a chapter of energy expenditure of critical importance in the overall energy balance, and considerable interest has been raised on uncoupling proteins as the target of antiobesity drugs or treatments aimed at enhancing thermogenesis. Several questions are still waiting for answers, in particular, what are the physiological roles of UCP2 and UCP3, and also whether other mechanisms independent of BAT and of the uncoupling protein systems are dominant components in human thermogenesis. The knowledge of such mechanisms and their integration in the complex apparatus controlling body weight should help in designing new strategies against obesity and its complications.

Adipogenesis

When considering the possible causes of obesity (and increased fat deposition in localised zones) the factors that regulate the number of adipocytes and their maturity and differentiation should be taken into account. Moderate obesity results mainly from an increase in the size of the adipocytes due to increased triglyceride content (hypertrophic obesity), while more extreme obesity, or obesity which occurs at an earlier age, also implies an increase in the number of adipose cells (hyperplastic obesity) [151, 152]. The capacity to make new adipocytes continues throughout life, and can be activated by the size, frequency and composition of meals, and by other environmental factors.

The adipocyte cell line derives from a multipotent embryonic precursor, which can differentiate into various types of mesodermic cells [151]. In recent years, a great deal has been learned about the bases of differentiation and gene expression in adipocytes; in particular transcription factors which promote adipogenesis have been identified. These factors belong to three families: C/EBP, PPAR and ADD (or SREBP) [reviewed in 5, 6, 151].

The C/EBPs are a family of basic leucine zipper transcription factors; two members of this family, C/EBPβ and C/EBPδ, are induced early in the adipogenesis program, in response to hormonal stimulation, and they cooperate to induce the expression of another transcription factor PPARγ, which is a key activator of the entire adipogenesis program [5, 6], capable of promoting not only the conversion of fibroblasts into adipocytes [153], but also the transdifferentiation of committed myoblasts into adipocytes [154]. PPARs (peroxisome proliferator-activated receptors α, β and γ) are a subfamily of the nuclear hormone receptor superfamily that form heterodimers with retinoid X receptors (RXRs); the heterodimeric complexes recognise and bind to particular sequences in target genes and activate transcription upon ligand binding [155]. One of the two isoforms of PPARγ, PPARγ2, is characteristically expressed in fat cells and, after ligand activation, functions as a direct regulator of many fat-specific genes such as the adipocyte fatty acid-binding protein (aP2) and phosphoenolpyruvate carboxykinase genes [152]. Given the central role of PPARγ in adipocyte development (and also in glucose homeostasis), PPARγ is seen as a target for new drugs to control obesity-related metabolic disorders.

PPARγ can be activated by a variety of synthetic ligands including clofibrate, ETYA, Wy 14643 and antidiabetic drugs of the tiazolidinedione group [5], and by natural metabolites such as one prostaglandin of the J series (15-deoxy-Δ 12, 14- prostaglandin J2) [156, 157] and certain eicosanoids and polyunsaturated fatty acids [158]. It appears that the endogenous ligand must be a fatty acid derivative, and that a member of the ADD/SREBP (adipocyte determination differentiation, also known as sterol regulatory element binding protein) family of transcription factors, ADD1/SREBP1, which specifically regulates aspects of cholesterol and fatty acid metabolism, plays a key role in the generation of the endogenous ligand of PPARγ [159].

A recently described co-activator of PPARγ, named PGC-1 [160], could be important in the developmental bifurcation between white and brown fat cells [3, 5]. PGC-1 is highly expressed in brown but not white fat, and also expressed in other tissues such as heart, kidney, brain and skeletal muscle. This factor was initially connected to adaptive thermogenesis because of its marked and rapid induction in BAT and in muscle upon cold exposure of mice. In addition to PPARγ, PGC-1 also binds to a variety of other nuclear receptors including the retinoic acid and thyroid hormone receptors, both of which positively regulate expression of UCP1 [111]. Ectopic expression of PGC-1 in cultured cells activates and co-ordinates multiple aspects of the adaptive thermogenesis programme. Mitochondrial biogenesis is induced, and also many genes of the electron transport system and the expression of UCPs, in a cell-selective manner [161]. UCP1 but not UCP2 or UCP3 is induced when PGC-1 is introduced into white fat cells, whereas UCP2 but not UCP1 or UCP3 is induced when PGC-1 is expressed in muscle cells. In both fat and muscle

cells, these changes in gene expression are reflected in increased respiration, both coupled and uncoupled [161].

A third member of the C/EBP family, C/EBPα, is also involved in adipogenesis. Ectopic expression of C/EPBα at high concentrations can stimulate adipogenesis in many types of fibroblastic cell lines [162]. However, C/EBPα is unlikely to be a primary signal in adipogenesis, since it is induced late during this process, following PPARγ induction. C/EBPα does play a role, however, in maintaining terminal differentiation, probably through its interaction with the tumour suppressor retinoblastoma protein (pRB), according to a hypothesis put forward in 1994 by our group [163]. Thus, we have shown that the expression of pRB increases during differentiation of adipose cells and have demonstrated a physical and functional interaction between pRB and C/EBPα that is linked to the expression of adipocyte marker genes, such as UCP1 in brown adipocytes [163–165].

In summary, the conversion of pre-adipocytes into mature adipose cells follows a series of linked steps:

- Stimulation of C/EBPβ and C/EBPδ expression by hormonal stimulation.
- Activation of PPARγ expression mediated by C/EBPβ and C/EBPδ, and production of the PPARγ endogenous ligand, stimulated by ADD1/SREBP1.
- Increase in insulin sensitivity and progress in differentiation, stimulated by activated PPARγ.
- Induction of C/EBPα and pRB expression, required for terminal differentiation and for maintenance of the differentiated phenotype.

A further look at the obesity genes

Obesity represents the archetype of phenotypic complexity [166]: body weight can be affected by very diverse factors that affect the size and composition of any tissue, organ or individual system in our organism.

Several simple mutations of individual genes that cause obesity have been identified in laboratory animals (see monogenic obesity in Table 1). The situation in humans is much more complex. The most common forms of human obesity depend on the interaction of many genes (Table 1 and Fig. 1), environmental factors, behavioural habits and lifestyle.

Only a small set of concrete mutations causing human obesity are known, each one representing a tiny percentage of obesity cases, but could be the object of a very specific therapeutic treatment. However, more than 200 genes and other markers associated or linked with human obesity phenotypes have been identified [167, 168]. A future goal will be to look at the roles they play, together with other genes not yet discovered.

Study of candidate genes

One of the strategies of current research is to identify and characterise candidate genes. These are genes identified on the basis of the obesity that their alteration causes in animals, or genes suspected to be linked with some physiological process on which obesity depends.

Simple mutations that cause obesity have only been found in a few human families. These include two families with defects in the ob (leptin) gene [59, 60], and one family with a mutation in the db (leptin receptor) gene [61]. A mutation in the prohormone convertase 1 gene was found associated with obesity in a single female [169]; this abnormality is related to that caused in the rat by the fat (carboxypeptidase E) mutation, which leads to defective processing of several neuropeptides and prohormones [169]. Mutations in the MC4-R gene [103] and the POMC gene [170] also cause obesity in humans, reflecting the importance of the melanocortin system in the control of human body weight. The individuals with loss-of-function POMC mutations are not only obese but they also display altered pigmentation and adrenal insufficiency because of the absence of αMSH and ACTH, melanocortin agonists for MC1-R and MC2-R, respectively [168, 170]. Taking into account the low incidence of these mutations in humans, their study does not directly address genetic causes in the population as a whole, but it provides important insight into the underlying physiological pathways.

In addition, some interesting associations have been described between obesity and/or obesity-related traits and the presence of polymorphic markers in or around certain genes. In Mexican-Americans, for example, the sum of skinfolds was statistically associated with a fragment close to the leptin gene on chromosome 7 [171]; also, body mass index (BMI) was associated with markers of the leptin chromosomal region [172]. A recent study has shown the linkage of serum leptin levels with chromosome 2 at band 21 in a population of African-Americans [173]. Interestingly, this region of chromosome 2 includes the POMC gene, the loss-of-function of which results in monogenic obesity in mice and humans [170].

Certain mutations of the ß-adrenergic receptors also appear to be related to obesity. Thus, the Trp64Arg ß3-AR mutation that occurs frequently in the Pima Indian population [174] – a group with high incidence of obesity – and in other human populations [174–176] is significantly associated with a certain predisposition to obesity and its complications. Specifically, this mutation is associated with a lower metabolic rate and various characteristics of the insulin resistance syndrome: increased BMI, abdominal adiposity, hyperinsulinemia, increased blood pressure and earlier appearance of diabetes. However, these results do not hold for all the populations studied [e. g. 177] and it is accepted that this ß3-AR mutation cannot be a major determining factor in the predisposition to obesity. In addition, a clear association has been established between poly-

Table 1 The main gene products and other mediators involved in obesity

1. Monogenic obesity	
Ob, Lep	Leptin or OB protein
Db, LepR	Leptin receptor
Ay, ASIP	Agouti protein and related proteins
Tub	Tubby hypothalamic protein
Fat, CPE	Carboxypeptidase E
PC1	Prohormone convertase 1
POMC	Pro-opiomelanocortin
MC4-R	Melanocortin-4 receptor
2. Adipocytes and peripheral thermogenesis	
ß3AR	ß3-adrenergic receptor
ß2AR	ß2-adrenergic receptor
UCP1	Uncoupling protein 1
UCP2	Uncoupling protein 2
UCP3	Uncoupling protein 3
PKA	Protein kinase A
LPL	Lipoprotein lipase
PPARγ2	Peroxisome proliferation activated receptor γ 2
PRB	Retinoblastoma protein
C/EBPs	CCAAT/enhancer binding proteins
TNFα	Tumor necrosis factor α
3. Feeding control	
3.1. Chronic feeding control	
Orexigenic peptides:	
NPY	Neuropeptide Y
GAL	Galanin
ßEND	ß-Endorphin
DNF	Dinorphine
GH-RH	Growth hormone releasing hormone
OREX	A and B orexins, hypocretins 1, 2
ART, AGRP	Agouti-related peptides
MCH	Melanin-concentrating hormone
Anorexigenic peptides:	
Lep, Ob	Leptin, OB protein
POMC	Pro-opiomelanocortin
αMSH	α Melanocyte-stimulating hormone
CART	Cocaine-amphetamine related transcript
IGF-I and II	Insulin-like growth factors
MOT	Motilin
BOM	Bombesin
OXT	Oxytocin
NTS	Neurotensin
TRH	Tyrotropine releasing hormone
CRH	Corticotropine releasing hormone
SOM	Somatostatin
CCK	Colecistokinin
NPK	Neuropeptide K
GLP-1	Glucagon-like peptide–1
Neurotransmitters in the CNS:	
5HT	Serotonin
NE, NA	Norepinephrine
DPM	Dopamine
Trp	Tryptophan
5HTP	5-hydroxytryptophan
3.2. Short-term feeding control (satiety digestive peptides)	
CCK	Colecistokinin
GAST	Gastrin
GLP	Glucagon
NMD	Neuromedin
BOM	Bombesin
LEP	Leptin, OB protein
3.3. Main receptors linked with feeding control	
MCH-R, MC4-R, Y5, Y1, CRH-R, GAL-R, OREX-R, GH-R, CCK-A-R, GLP-1-R, LepR, Mahogany.	

morphic variants of the ß2-AR gene and a predisposition to obesity, the level of energy expenditure and the lipolytic rate [178–181].

A linkage between obesity and polymorphic markers in the genes encoding uncoupling proteins has also been described. A greater tendency to gain weight and to have a lower metabolic rate associated with the presence of one of the two main alleles of the UCP1 gene in humans was first reported in 1994 [182]. This genetic variant of UCP1 was also associated with weight loss resistance [183]. More recently, it has been shown that UCP1 polymorphisms of the UCP1 gene, and also of the LPL and ß3-AR genes, are more closely linked with the medical complications of obesity than with obesity per se, with variants of individual genes or combinations of these being associated with the incidence of risk factors [184]. Also, in French Canadians, an area around the UCP2 gene on the D11S911 chromosome was found to be linked with the basal metabolic rate, BMI and the percentage of body fat [185]. In any case, variants of thermogenic genes do not appear to be the main cause of widespread obesity, although they may be partly responsible for it and for related medical complications.

Prospecting or unspecific genomic scanning

This means investigating the genotypical association between obesity and a series of polymorphisms, located at regular intervals on the genome, e. g. using some 300 markers, with no prior indication that they could be linked with obesity [166]. The results allow the identification of chromosomal regions or, in some cases, candidate positional genes. Inter alia, an association has been described between obesity and two interesting regions of chromosomes 2 and 8, which are very significant: a region of chromosome 2 that includes the gene encoding POMC [186] – which is the precursor of various hormones some directly related with feeding behaviour, such as αMSH – and a re-

gion of chromosome 8 which contains the gene for the ß3-AR [187].

Future trends and therapeutic implications

The extraordinary progress made in the molecular understanding of the mechanisms regulating body weight is paving the way for new methods of obesity treatment, especially pharmacological but also nutritional and possibly involving genetic intervention.

Some drug-based strategies to combat obesity are foreseen. Table 2 lists the basic pharmacological agents that are now or will shortly be available. It also lists possible genes and basic compounds that are the subject of extensive research and pharmacological development. However, given the complexity of the metabolic network controlling energy balance, the idea of having just one drug to control weight in the majority of obese people looks unrealistic. At any case, some strategies can be outlined [188]: 1) inhibition of food intake, by blocking orexigenic signals or enhancing anorexigenic signals; 2) stimulation of energy expenditure, by enhancing the levels and activity of UCPs; 3) activation of fat mobilisation, while maintaining the body protein; 4) blocking nutrient absorption, particularly fat, as does the recently developed Orlistat [189–191], whose action inhibits pancreatic and digestive lipases, reducing the intestinal digestion of fats and, consequently, their absorption and use. In any case, these pharmacological approaches should be complemented with exercising, nutritional advice and behavioural advice for full success.

Table 2 Potential types of anti-obesity drugs (and their specific targets)

1. **Inhibitors of food intake:**
   Up-regulators of neurotransmitter levels (serotonin, norepinephrine, dopamine) in the CNS.
   Agonists of receptors such as LepR, MC4-R, CRH-R, CCK-A-R, GLP-1-R, Bombesin R.
   Antagonists of receptors such as Y5, Y1, MCH-R, Gal-R, Orexin-R.
   Products developed: Phenfluramine (withdrawn), Dexfenfluramine (withdrawn), Phentermine, Fen/Phen (withdrawn), Sibutramine.
2. **Stimulators of energy expenditure (thermogenesis)**
   Activators of the expression or activity of UCPs and PKA.
   ß3-AR agonists.
   Products developed: none
3. **Activators of fat mobilisation**
   Agonists of LepR, ß3-AR, GH-R.
   Activators of PKA.
   Products developed: Leptin
4. **Inhibitors of fat absorption**
   Products developed: Orlistat

The nutritional approach to obesity should take into account not only the energy and/or plastic properties of foods, but also their selective effects on the expression of specific genes. Phenotypic expression – both the degree of obesity and various pathological manifestations – must depend to a great extent on the regulation of the obesity genes by exogenous factors, mainly diet. In our laboratory, we have described the positive effect of retinoic acid, an active form of vitamin A, on the thermogenic capacity of rodents [192, 193], and a similar effect of ß-carotene and several other carotenoids [194], and certain fatty acids [195]. Knowledge of nutrients with greater thermogenic properties could be useful in designing diets to help control body weight.

The differentiating element in the future, especially as regards the dietary and pharmacological control of obesity, will be the knowledge of an individual's possible response depending on his/her genetic characteristics. It is paradoxical that as a rule excess weight in an obese person is not yet regarded as a specific, individual medical problem, except in cases where it has led to other pathological conditions. This may change considerably, to a more individual approach, on the basis of more direct knowledge of the mechanisms responsible for obesity, the genetic variants involved in each case and the different obesity types and possible complications associated to them.

Acknowledgements At present, the research being carried out by Dr A. Palou's group is being financed mainly by the Spanish Government (DGESIC-PM97-0041) and the European Union (CHRX-CT94-0490 and COST-918). We would like to thank Yolanda Cabello for her technical assistance.

Appendix A direct evidence for a role of UCP3 in thermogenesis has been provided by Clapham et al. [125b]. They showed that male mice overexpressing human UCP3 in skeletal muscle are hyperphagic but they weigh less than their wild type littermates.

References

1. Woods S, Seeley R, Porte DJ, Schwartz M (1998) Signals that regulate food intake and energy homeostasis. Science 280: 1378–1383
2. Schwartz MW, Woods SC, Porte Jr D, Seeley RJ, Baskin DG (2000) Central nervous system control of food intake. Nature 404: 661–71
3. Lowell BB, Spiegelman BM (2000) Towards a molecular understanding of adaptive thermogenesis. Nature 404: 652–660
4. Ricquier D, Bouillaud F (2000) The uncoupling protein homologues: UCP1, UCP2, UCP3, StUCP and AtUCP. Biochem J 345: 161–179
5. Wu Z, Puigserver P, Spiegelman BM (1999) Transcriptional activation of adipogenesis. Curr Opin Cell Biol 11: 689–694
6. Morrison RF, Farmer SR (1999) Insights into the transcriptional control of adipocyte differentiation. J Cell Biochem 32/33: 59–67

7. Sanchis D, Balada F, Picó C, Grasa MM, Virgili J, Farrerons C, Palou A, Fernández-López JA, Remesar X, Alemany M (1997) Rats receiving the slimming agent oleoyl-estrone in liposomes (Merlin-2) decrease food intake but maintain thermogenesis. Arch Physiol Biochem 105: 663–72
8. Adan C, Cabot C, Vila R, Grasa MM, Masanes RM, Esteve M, Estruch J, Fernández-López JA, Remesar X, Alemany M (1999) Oleoyl-estrone treatment affects the ponderostat setting differently in lean and obese Zucker rats. Int J Obes 23: 366–73
9. Ahima RS, Flier JS (2000). Leptin. Annu Rev Physiol 62: 413–437
10. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM (1994) Positional cloning of the mouse obese gene and its human homologue. Nature 372: 425–432
11. Tartaglia LA, Dembski M, Weng X, Deng N, Culpepper J, Devos R, Richards GJ, Campfield LA, Clark FT, Deeds J, Muir C, Sanker S, Moriarty A, Moore KJ, Smutko JS, Mays GG, Woolf EA, Monroe CA, Tepper RI (1995) Identification and expression cloning of a leptin receptor, OB-R. Cell 83: 1263–1271
12. Chen H, Charlat O, Tartaglia LA, Woolf EA, Weng X, Ellis SJ, Lakey ND, Culpepper J, Moore KJ, Breitbart RE, Duyk GM, Tepper RI, Morgenstem JP (1996) Evidence that the diabetes gene encodes the leptin receptor. Identification of a mutation in the leptin receptor gene in db/db mice. Cell 84: 491–495
13. Chua SC Jr, Chung WK, Wu-Peng XSh, Zhang Y, Liu S, Tartaglia L, Leibel RL (1996) Phenotypes of mouse diabetes and rat fatty due to mutations in the OB (Leptin) receptor. Science 271: 994–996
14. Palou A, Picó C (1998) Obesidad y alimentación: nuevos genes de neuropéptidos orexígenos y anorexígenos en el SNC. Nutrición Clínica 18: 21–31
15. Palou A (1998) Los genes de la obesidad. Formación continuada en nutrición y obesidad 1: 280–289
16. Palou A, Picó C (1999) Neuropéptidos orexigenos y anorexígenos. Formación continuada en nutrición y obesidad 2: 54–57
17. Kennedy GC (1953) The role of depot fat in the hypothalamic control of food intake in the rat. Proc R Soc Lond (Biol) 140: 578–592
18. Coleman DM (1978) Obese and diabetes: two mutant genes causing diabetes-obesity syndromes in mice. Diabetologia 14: 141–148
19. Lee GH, Proenca R, Montez JM, Carrol KM, Darvishzadeh JG, Lee JI, Friedman JM (1996) Abnormal splicing of the leptin receptor in diabetic mice. Nature 379: 632–635
20. Halaas J, Gajiwala K, Maffei M, Cohen S, Chait B, Rabinowitz D, Lallone R, Burley S (1995) Weight reducing effects of the plasma protein encoded by the ob gene. Science 269: 543–546
21. Pelleymounter MA, Cullen MJ, Baker MB, Hecht R, Winters D, Boone T, Collins F (1995) Effects of the obese gene product on body weight regulation in ob/ob mice. Science 269: 540–543
22. Campfield L, Smith F, Guisez Y, Devos R, Burn P (1995) Recombinant mouse OB protein: evidence for a peripheral signal linking adiposity and central neural networks. Science 269: 546–548
23. Halaas JL, Boozer C, Blair-West J, Fidahusein N, Denton DA, Friedman JM (1997) Physiological response to long-term peripheral and central leptin infusion in lean and obese mice. Proc Natl Acad Sci USA 94: 8878–8883
24. Maffei M, Fei H, Lee GH, Dani C, Leroy P, Zhang Y, Proencaa R, Negrel R, Ailhaud G, Friedman JM (1995) Increased expression in adipocytes of ob RNA in mice with lesions of the hypothalamus and with mutations at the db locus. Proc Natl Acad Sci USA 92: 6957–6960
25. Elmquist JK, Ahima RS, Maratos-Flier E, Flier JS, Saper CB (1997) Leptin activates neurons in the ventrobasal hypothalamus and brainstem. Endocrinology 138: 839–42
26. Elmquist JK, Maratos-Flier E, Saper C, Flier J (1998) Unravelling the central nervous system pathways underlying responses to leptin. Nature Neurosci 1: 445–450
27. Wang MY, Lee Y, Unger RH (1999) Novel forms of lipolysis induced by leptin. J Biol Chem 274: 17541–17544
28. Collins S, Kuhn C, Petro A, Swich A, Chrunyk B, Surwit R (1996) Role of leptin in fat regulation. Nature 380: 677
29. Zhou YT, Shimabukuro M, Koyama K, Lee Y, Wang MY, Trieu F, Newgard CB, Unger RH (1997) Induction by leptin of uncoupling protein-2 and enzymes of fatty acid oxidation. Proc Natl Acad Sci USA 94: 6386–6390
30. Scarpace PJ, Matheny M, Pollock BH, Tymer N (1997) Leptin increases uncoupling protein expression and energy expenditure. Am J Physiol 273: E226–E230
31. Cusin I, Zakrzewska KE, Boss O, Muzzin P, Giacobino JP, Ricquier D, Jeanrenaud B and Roher-Jeanrenaud F (1998) Chronic central leptin infusion enhances insulin-stimulated glucose metabolism and favors the expression of uncoupling proteins. Diabetes 47: 1014–1019
32. Commins SP, Watson PM, Padgett MA, Dudley A, Argyropoulus G, Gettys TW (1999) Induction of uncoupling protein expression in brown and white adipose tissue by leptin. Endocrinology 140: 292–300
33. Scarpace PJ, Nicolson M, Matheny M (1998) UCP2, UCP3 and leptin gene expression: modulation by food restriction and leptin. J Endocrinol 159: 349–357
34. Rouru J, Cusin I, Zakrzewska KE, Jeanrenaud B, Rohner-Jeanrenaud F (1999) Effects of intravenously infused leptin on insulin sensitivity and on the expression of uncoupling proteins in brown adipose tissue. Endocrinology 140: 3688–3692
35. Siegrist-Kaiser C, Pauli V, Juge-Aubry C, Boss O, Pernin A, Chin WW, Cusin I, Rohner-Jeanrenaud F, Burger AG, Zapf J, Meier CA (1997) Direct effects of leptin on brown and white adipose tissue. J Clin Invest 100: 2858–64
36. Friedman JM (1997) Leptin, leptin receptors and the control of body weight. Eur J Med Res 2: 7–13
37. Bado A, Levasseur S, Attoub S, Kermorgant S, Laigneau JP, Bortoluzzi MN, Moizo L, Lehy T, Guerre-Millo M, Le Marchand-Brustel Y, Lewin MJM (1998) The stomach is a source of leptin. Nature 394: 790–793
38. Cinti S, De Matteis R, Picó C, Ceresi E, Obrador A, Maffeis C, Oliver J, Palou A (2000) Secretory granules of endocrine and chief cells of human stomach mucosa contain leptin. Int J Obes 24: 789–793
39. Masuzaki H, Ogawa Y, Sagawa N, Hosoda K, Matsumoto T, Mise H, Nishimura H, Yoshimasa Y, Tanaka I, Mori T, Nakao K (1997) Non adipose tissue production of leptin: leptin as a novel placenta-derived hormone in humans. Nature Med 3: 1029–1033
40. Ashworth CJ, Hoggard N, Thomas L, Mercer JG, Wallace JM, Lea RG (2000) Placental leptin. Rev Reprod 5: 18–24
41. Wang J, Liu R, Hawkins M, Barzalai N, Rossetti L (1998) A nutrient-sensing pathway regulates leptin gene expression on muscle and fat. Nature 393: 684–688
42. Casabiell X, Pineiro V, Tome MA, Peino R, Dieguez C, Casanueva FF (1997) Presence of leptin in colostrum and/or breast milk from lactating mothers: a potential role in the regulation of neonatal food intake. J Clin Endocrinol Metab 82: 4270–73
43. Esler M, Vaz M, Collier G, Nestel P, Jennings G, Kaye D, Seals D, Lambert G (1998) Leptin in human plasma is derived in part from the brain, and cleared by the kidneys. Lancet 351: 879
44. Himms-Hagen J (1999) Physiological roles of the leptin endocrine system: differences between mice and humans. Crit Rev Clin Lab Sci 36: 575–655
45. Clarke IJ, Henry BA (1999) Leptin and reproduction. Rev Reprod 4: 48–55
46. Morton NM, Emilsson V, Liu YL, Cawthorne MA (1998) Leptin action in intestinal cells. J Biol Chem 273: 26194–201
47. Haynes WG, Sivitz WJ, Morgan DA, Walsh SA, Mark AL (1997) Sympathetic and cardiorenal actions of leptin. Hypertension 30: 619–23

48. Gainsford T, Willson TA, Metcalf D, Handman E, McFarlane C, Ng A, Nicola NA, Alexander WS, Hilton DJ (1996) Leptin can induce proliferation, differentiation and functional activation of hemopoietic cells. Proc Natl Acad Sci USA 93: 14564–68
49. Sierra-Honigmann MR, Nath AK, Murakami C, Garcia-Cardena G, Papapetropoulus A, Sessa WC, Madge LA, Schechner JS, Schwabb MB, Polverini PJ, Flores-Riveros JR (1998) Biological action of leptin as an angiogenic factor. Science 281: 1683–85
50. Gibbs J, Young RC, Smith GP (1973) Cholecystokinin decreases food intake in rats. J Comp Physiol Psychol 84: 488–495
51. Muurahainen NE, Kissileft HR, Pi Sunyer FX (1993) Intravenous infusion of bombesin reduces food intake in humans. Am J Physiol 264: R350–R354
52. West DB, Fey D, Woods SC (1984) Cholecystokinin persistently suppresses meal size but not food intake in freefeeding rats. Am J Physiol 246: R776–R787
53. Considine RV, Considine EL, Williams CJ, Nyce MR, Magosin SA, Bauer TL, Rosato EL, Colberg J, Caro JF (1995) Evidence against either a premature stop codon or the absence of obese gene mRNA in human obesity. J Clin Invest 95: 2986–2988
54. Considine RV, Sinha MK, Heiman ML, Kriauciunas A, Stephens TW, Nice MR, Ohannesian JP, Marco CC, McKee LJ, Bauer TL, Caro JF (1996) Serum immunoreactive-leptin concentration in normal-weight and obese humans. N Engl J Med 334: 292–295
55. Hamilton BS, Paglia D, Kwan AYM, Deitel M (1995) Increased obese mRNA expression in omental fat cells from massively obese humans. Nature Med 1: 953–956
56. Maffei M, Halaas J, Ravussin E, Pratley RE, Lee GH, Zhang Y, Fei H, Kim S, Lallone R, Ranganathan S, Kern PA, Friedman JM (1995) Leptin levels in human and rodent: measurement of plasma leptin and ob mRNA in obese and weight-reduced subjects. Nature Med 1: 1155–1161
57. Maffei M, Stoffel M, Barone M, Moon B, Dammerman M, Ravussin E, Bogardus C, Ludwig DS, Flier JS, Talley M, Auerbag S, Friedman JM (1996) Absence of mutations in the human ob gene in obese/diabetic subjects. Diabetes 45: 679–682
58. Lönnquist F, Arner P, Nordfors L, Schalling M (1995) Overexpression of the obese (ob) gene in adipose tissue of human obese subjects. Nature Med 1: 950–953
59. Montague CT, Farooqi IS, Whitehead JP, Soos MA, Rau H, Warenham NJ, Sewter CP, Digby JE, Mohammed SN, Hurst JA, Cheetham CH, Earley AR, Barnett AH, Prins JB, O'Rahilly S (1997) Congenital leptin deficiency is associated with severe early-onset obesity in humans. Nature 387: 903–908
60. Strobel A, Issad T, Camoin L, Ozara M Strosberg AD (1998) A leptin missense mutation associated with hypogonadism and morbid obesity. Nature Genet 18: 213–215
61. Clément K, Vaisse C, Lahlou N, Cabrol S, Pelloux V, Cassuto D, Gourmelen M, Dina C, Chambaz J, Lacorte JM, Basdevant A, Bougnères P, Lebouc Y, Froguel P, Guy-Grand B (1998) A mutation in the human leptin receptor gene causes obesity and pituitary dysfunction. Nature 392: 398–401
62. Banks WA, Kastin AJ, Huang W, Jaspan JP, Maness LM (1996) Leptin enters the brain by a saturable system independent of insulin. Peptides 17: 305–11
63. Bjorbaek C, Elmquist JK, Michl P, Ahima RS, van Bueren A, McCall AL, Flier JS (1998) Expression of leptin receptor isoforms in brain microvessels. Endocrinology 139: 3485–91
64. Golden PL, Maccagnan TJ, Padridge WM (1997) Human blood-brain barrier leptin receptor: binding and endocytosis in isolated human brain microvessels. J Clin Invest 99: 14–18
65. Caro JF, Kolaczynski JW, Nyce MR, Ohannesian JP, Opentanova I, Goldman WH, Lynn RB, Zhang P, Sinha MK, Considine RV (1996) Decreased cerebrospinal-fluid/serum ratio leptin in obesity: a possible mechanism for leptin resistance. Lancet 348: 159–161
66. Li H, Matheny M, Nicolson M, Tümer N, Scarpace PJ (1997) Leptin gene expression increases with age independent of increasing adiposity in rats. Diabetes 46: 2035–2039
67. Rosenbaum M, Nicolson M, Hirsch J, Heymsfield SB, Gallagher D, Chu F, Leibel RL (1996) Effect of gender, body composition, and menopause on plasma concentration of leptin. J Clin Endocr Metab 81: 3424–3427
68. Sinha MK, Ohannesian JP, Helman ML, Kriauciunas A, Stephens TW, Magosin S, Marco C, Caro JF (1996) Nocturnal rise of leptin in lean, obese, and non-insulin-dependent diabetes mellitus subjects. J Clin Invest 97: 1344–1347
69. Saladin R, De Vos P, Guerre-Millo M, Leturque A, Girard J, Staels B, Auwerx J (1995) Transient increase in obese gene expression after food intake or insulin administration. Nature 377: 527–529
70. Bray GA (1995) Obesity, fat intake, and chronic disease. In: Bloom FE, Kipher DJ (eds) Psychopharmacology: The Fourth Generation of Progress. Raven Press, New York, pp 1591–1608
71. Sindelar DK, Havel PJ, Seeley RJ, Wilkinson CW, Woods SC, Schwartz MW (1999) Low plasma leptin levels contribute to diabetic hyperphagia in rats. Diabetes 48: 1275–1280
72. Grunfeld C, Zhao C, Fuller J, Pollock A, Moser A, Friedman J, Feingold KR (1996) Endotoxin and cytokines induce expression of leptin, the ob gene product: a role for leptin in the anorexia of infection. J Clin Invest 97: 2152–2157
73. De Vos P, Saladin R, Auwerx J, Staels B (1995) Induction of ob gene expression by corticosteroids is accompained by body weight loss and reduced food intake. J Biol Chem 270: 15958–15961
74. Trayhurn P, Ducan JS, Rayner DV, Hardie LJ (1996) Rapid inhibition of ob gene expression and circulating leptin levels in lean mice by the ß3-adrenoceptor agonists BRL35135A and ZD2079. Biochem Biophys Res Commun 228: 605–610
75. Attoub S, Levasseur S, Buyse M, Goïot H, Laigneau JP, Moizo L, Hervatin F, Le Marchand-Brustel Y, Lewin JMM, Bado A (1999) Physiological role of cholecystokinin B/gastrin receptor in leptin secretion. Endocrinology 140: 4406–4410
76. Hahn T, Breininger J, Baskin D, Schwartz M (1998) Coexpression of Agrp and NPY in fasting-activated hypothalamic neurons. Nature Neurosci 1: 271–272
77. Broberger C, Johansen J, Johansson, C, Schalling M, Hokfelt T (1998). The neuropeptide Y/agouti gene-related protein (AGRP) brain circuitry in normal, anorectic, and monosodium glutamate-treated mice. Proc Natl Acad Sci USA 95: 15043–15048
78. Elias CF, Lee C, Kelly J, Aschkenasi C, Ahima RS, Couceyro PR, Kuhar MJ, Saper CB, Elmquist JK (1998). Leptin activates hypothalamus CART neurons projecting to the spinal cord. Neuron 21: 1375–1385
79. Baskin D, Breininger J, Schwartz M (1999). Leptin receptor mRNA identifies a subpopulation of neuropeptide Y neurons activated by fasting in rat hypothalamus. Diabetes 48: 828–833
80. Cheung C, Clifton D, Steiner R (1997). Proopiomelanocortin neurons are direct targets for leptin in the hypothalamus. Endocrinology 138: 4489–4492
81. Schwartz MW, Baskin DG, Bukowski TR, Kuijper JL, Foster D, Lasser G, Prunkard DE, Porte D Jr, Woods SC, Seeley RJ, Weigle DS (1996) Specificity of leptin action on elevated blood glucose levels and hypothalamic neuropeptide Y gene expression in ob/ob mice. Diabetes 45: 531–535
82. Stephens TW, Basinski M, Bristow PK, Bue-Valleskey JM, Burgett SG, Craft L, Hale J, Hoffmann J, Hsiung HM, Kriauciunas A, McKellar W, Rosteck PR, Schoner B, Smith D, Tinsley FC, Zhang XY, Heiman M (1995) The role of neuropeptide Y in the antiobesity action of the obese gene product. Nature 377: 530–532

83. Sipols AJ, Baskin DG, Schwartz MW (1995) Effect of intracerebroventricular insulin infusion on diabetic hyperphagia and hypothalamic neuropeptide gene expression. Diabetes 44: 147–151
84. Schwartz MW, Seeley RJ, Woods SC, Weigle DS, Campfield LA, Burn P, Baskin DG (1997) Leptin increases hypothalamic proopiomelanocortin (POMC) mRNA expression in the rostral arcuate nucleus. Diabetes 46: 2119–2123
85. Thornton J, Cheung C, Clifton D, Steiner R (1997) Regulation of hypothalamic proopiomelanocortin mRNA by leptin in the ob/ob mice. Endocrinology 138: 5063–5066
86. Kristensen P, Judge ME, Thim L, Ribel U, Christjansen KN, Wulff BS, Clausen JT, Jensen PB, Madsen OD, Vrang N, Larsen PJ, Hastrup S (1998) Hypothalamic CART is a new anorectic peptide regulated by leptin. Nature 393: 72–76
87. Elmquist JK, Ahima RS, Elias CS, Flier JS, Saper CB (1998) Leptin activates distinct projections from the dorsomedial and ventromedial hypothalamic nuclei. Proc Natl Acad Sci USA 95: 741–46
88. Elmquist JK, Elias C, Shaper C (1999) From lesions to leptin: hypothalamus control of food intake and body weight. Neuron 22: 221–232
89. Qu D, Ludwig DS, Gammeltoft S, Piper M, Pelleymounter MA, Cullen MJ, Mathes WF, Przypek J, Kanarek R, Flier EM (1996) A role for melanin-concentrating hormone in the central regulation of feeding behavior. Nature 380: 243–247
90. Sakurai T, Amemiya A, Ishii M, Matsuzaki I, Chemelli RM, Tanaka H, Williams SC, Richardson JA, Kozlowski GP, Wilso S, Arch JRS, Buckingham RE, Haynes AC, Carr SA, Annan RS, McNulty DE, Liu ES, Terret JA, Elshourbagy NA, Bergsma DJ, Yanagisawa M (1998) Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior. Cell 92: 573–585
91. De Lecea L, Kilduff TS, Peyron C, Gao X, Foye PE, Danielson PE, Fukuhara C, Battenberg EL, Gautvik VT, Barlett Fs, Frankel WN, Van den Pol AN, Bloom FE, Gautvik KM, Sutcliffe JG (1998) The hypocretins: hypothalamus-specific peptides with neuroexcitatory activity. Proc Natl Acad Sci USA 95: 322–327
92. Stanley BG, Kyrkouli SE, Kriaciunas A, Leibowitz SF (1986) Neuropeptide Y chronically injected into the hypothalamus: a powerful neurochemical inducer of hyperphagia and obesity. Peptides 7: 1189–1192
93. Flier JS, Maratos-Flier E (1998) Obesity and the hypothalamus: novel peptides for new pathways. Cell 92: 437–440
94. Erickson JC, Hollopeter G, Palmiter RD (1996) Attenuation of the obesity syndrome of ob/ob mice by the loss of neuropeptide Y. Science 274: 1704–1707
95. Gerald C, Walker M, Criscione L, Gustafson EI, Batzl-Hartman C, Smith K, Vaysse P, Durkin M (1996) A receptor subtype involved in neuropeptide-Y-induced food intake. Nature 382: 168–170
96. Naveilhan P, Hassani H, Canals JM, Ekstrand AJ, Larefalk A, Chhajlani V, Arenas E, Gedda K, Svensson L, Thoren P, Ernfors P (1999) Normal feeding behavior, body weight and leptin response require the neuropeptide Y Y2 receptor. Nature Medicine 5: 1188–1193
97. Turton MD, O'Shea D, Gunn I, Beak SA, Edwards CMB, Meeran K, Choi SJ, Taylor GM, Heath MM, Lambert PD, Wilding JPH, Smith DM, Ghatei MA, Herbert J, Bloom SR (1996) A role for glucagon-like peptide-1 in the central regulation of feeding. Nature 379: 69–72
98. Miller MW, Duhl DM, Vrieling H, Cordes SP, Ollmann MM, Winkes BM, Barsh GS (1993) Cloning of the mouse agouti gene predicts a secreted protein ubiquitously expressed in mice carrying the lethal yellow mutation. Genes Dev.7: 454–467
99. Lu D, Willard D, Patel IR, Kadwell S, Overton L, Kost T, Luther M, Chen W, Woychik RP, Wilkison WO, et al (1994) Agouti protein is an antagonist of the melanocyte-stimulating-hormone receptor. Nature 371: 799–802
100. Fan W, Boston BA, Kesterson RA, Hruby VJ, Cone RD (1997) Role of melanocortinergic neurons in feeding and the agouti obesity syndrome. Nature 385: 165–168
101. Ollmann MM, Wilson BD, Yang YK, Kerns JA, Chen Y, Gantz I, Barsh GS (1997) Antagonism of central melanocortin receptors in vitro and in vivo by Agouti-related peptide. Science 278: 135–138
102. Huszar D, Lynch CA, Fairchild-Huntrees V, Dunmore JH, Fang Q, Berkemeier LR, Gu W, Kesterson RA, Boston BA, Cone RE, Smith FJ, Campfield LA, Burn P, Lee F (1997) Targeted disruption of the melanocortin–4 receptor results in obesity in mice. Cell 88: 131–141
103. Hinney A, Schmidt A, Nottebom K, Heibult O, Becker I, Ziegler A, Gerber G, Sina M, Gorg T, Mayer H, Siegfried W, Fichter M, Remschmidt H, Hebebrand J (1999) Several mutations in the melanocortin-4 receptor gene including a nonsense and frameshift mutation associated with dominantly inherited obesity in humans. J Clin Endocrinol Metab 84: 1483–1486
104. Shutter JR, Graham M, Kinsey AC, Scully S, Luthy R, Stark KL (1997). Hypothalamic expression of ART, a novel gene related to agouti, is up-regulated in obese and diabetic mutant mice
105. Nagle DL, McGrail SH, Vitale J, Woolf, EA, Dussault BJ Jr, DiRocco L, Holmgren L, Montagno J, Bork P, Huszar D, Fairchild-Huntress V, Ge P, Keilty J, Ebeling C, Baldini L, Gilchrist J, Burn P, Carlson GA, Moore KJ (1999) The mahogany protein is a receptor involved in suppression of obesity. Nature 398: 148–152
106. Miller KA, Gunn TM, Carrasquillo MM, Lamoreux ML, Galbraith DB, Barsh GS (1997) Genetic studies of the mouse mutations mahogany and mahoganoid. Genetics 146: 1407–1415
107. Shimada M, Tritos N, Lowell B, Flier J, Maratos-Flier E (1998). Mice lacking melanin-concentrating hormone are hypophagic and lean. Nature 39: 670–674
108. Saito Y, Nothacker HP, Wang Z, Lin SH, Leslie F, Civelli O (1999) Molecular characterization of the melanin-concentrating hormone receptor. Nature 400: 265–9
109. Bouchard C, Tremblay A, Despres JP, Nadeau A, Lupien PJ, Theriault G, Dussault J, Moorjani S, Pinault S, Fournier G (1990) The response to long-term overfeeding in identical twins. N Engl J Med 322: 1477–1482
110. Ricquier D, Casteilla L, Bouillard F (1991) Molecular studies of the uncoupling protein. FASEB J 5: 2237–2242
111. Palou A, Picó C, Bonet ML, Oliver P (1998) Molecules in focus. The uncoupling protein thermogenin. Int J Biochem Cell Biol 30: 7–11
112. Fleury C, Neverova M, Collins S, Raimbault S, Champigny O, Levi-Meyrueis C, Bouilllard F, Seldin MF, Surwit RS, Ricquier D, Warden CH (1997) Uncoupling protein-2: a novel gene linked to obesity and hyperinsulinemia. Nature Genet 15: 269–272
113. Gimeno RE, Dembski M, Weng X, Deng N, Shyjan AW, Gimeno CJ, Iris F, Ellis SJ, Woolf EA, Tartaglia LA (1997) Cloning and characterization of an uncoupling protein homolog: a potential molecular mediator of human thermogenesis. Diabetes 46: 900–906
114. Boss O, Samec S, Paoloni-Giacobino A, Rossier C, Dulloo A, Seydoux J, Muzzin P, Giacobino JP (1997) Uncoupling protein-3: a new member of the mitochondrial carrier family with tissue-specific expression. FEBS Lett 408: 39–42
115. Vidal-Puig A, Solanes G, Grujic D, Flier JS, Lowell BB (1997) UCP3: an uncoupling protein homologue expressed preferentially and abundantly in skeletal muscle and brown adipose tissue. Biochem Biophys Res Commun 235: 79–82

116. Gong DW, He Y, Karas M, Reitman M (1997) Uncoupling protein-3 is a mediator of thermogenesis regulated by thyroid hormone, ß3-adrenergic agonists and leptin. J Biol Chem 272: 24129–24132
117. Susulic VS, Lowell BB (1995) Brown adipose tissue and the regulation of body fat stores. Curr Opin Endocrinol Diabetes 3: 44–50
118. Rothwell NJ, Stock MJ (1979) A role for brown adipose tissue in diet induced thermogenesis. Nature 281: 31–35
119. Nicholls DG, Locke RM (1984) Thermogenic mechanisms in brown fat. Physiol Rev 64: 1–84
120. Nagase I, Yoshida T, Kumamoto K, Umekawa T, Sakane N, Nikami H, Kawada T, Saito M (1996) Expression of uncoupling protein in skeletal muscle and white fat of obese mice treated with thermogenic ß3-adrenergic agonist. J Clin Invest 97: 2898–2904
121. Picó C, Bonet ML, Palou A (1998) Stimulation of uncoupling protein synthesis in white adipose tissue of mice treated with the ß3-AR agonist CGP-12177. Cell Mol Life Sci 54: 191–195
122. Oliver P, Picó C, Martínez N, Bonet ML, Palou A (2000) *In vivo* effects of CGP-12177 on the expression of leptin and uncoupling protein genes in mouse brown and white adipose tissues. Int J Obes 24: 423–428
123. Hinz W, Faller B, Gruninger S, Gazzotti P, Chiesi M (1999) Recombinant human uncoupling protein-3 increases thermogenesis in yeast cells. FEBS lett 448: 57–61
124. Zhang CY, Hagen T, Mootha VK, Slieker LJ, Lowell BB (1999) Assessment of uncoupling activity on uncoupling protein 3 using a yeast heterologous expression system. FEBS Lett 449: 129–134
125. Nedergaard J, Matthias A, Golozoubova V, Jacobsson A, Cannon B (1999) UCP1: the original uncoupling protein – and perhaps the only one? J Bioenerg Biomembr 31: 475–491
125b. Clapham JC, Arch JRS, Chapman H, Haynes A, Lister C, Moore GBT, Piercy V, Carter SA, Lehner I, Smith SA, Beeley LJ, Godden RJ, Herrity N, Skehel M, Changani KK, Hodkings PD, Reid DG, Squires SM, Hatcher J, Trail B, Latcham J, Rastan S, Harper AJ, Cadenas S, Buckingham JA, Brand MD, Abuin A (2000) Mice overexpressing human uncoupling protein-3 in skeletal muscle are hyperphagic and lean. Nature 406: 415–418
126. Sanchis D, Fleury C, Chomiki N, Goubern M, Huang Q, Neverova M, Gregoire F, Easlick J, Raimbault S, Levi-Meyrueis C, Miroux B, Collins S, Seldin M, Richard D, Warden C, Bouillaud F, Ricquier D (1998) BMCP1, a novel mitochondrial carrier with high expression in the central nervous system of humans and rodents, and respiration uncoupling activity in recombinant yeast. J Biol Chem 273: 34611–34615
127. Mao W, Yu XX, Zhong A, Li W, Brush J, Sherwood SW, Adams SH, Pan G (1999) UCP4, a novel brain-specific mitochondrial protein that reduces membrane potential in mammalian cells. FEBS Lett 443: 326–330
128. Himms-Hagen J (1989) Brown adipose tissue thermogenesis and obesity. Prog Lipid Res 28: 67–115
129. Lowell BB, Susulic VS, Hamann A, Lawitts JA, Himms-Hagen J, Boyer BB, Kozak LP, Flier JS (1993) Development of obesity in transgenic mice following the genetic ablation of brown adipose tissue. Nature 366: 740–743
130. Himms-Hagen J (1991) Neural control of brown adipose tissue thermogenesis, hypertrophy, and atrophy. Front Neuroendocrinol 12: 38–93
131. Cannon B, Jacobsson A, Rehnmark, Nedergaard J (1996) Signal transduction in brown adipose tissue recruitment: noradrenaline and beyond. Int J Obesity 20: S36–S42
132. Rehnmark S, Néchad M, Herron D, Cannon B, Nedergaard J (1990) α- and ß-adrenergic induction of the expression of uncoupling protein thermogenin in brown adipocytes differentiated in culture. J Biol Chem 265: 16464–16471
133. Ricquier D, Bouillard F, Toumelin P, Mory G, Bazin R, Arch J, Péanicaud L (1986) Expression of uncoupling protein mRNA in thermogenic or weakly thermogenic brown adipose tissue. J Biol Chem 261: 13905–13910
134. Zhao J, Unelius L, Bengtsson T, Cannon B, Nedergaard J (1994) Coexisting ß-adrenoceptor subtypes: significance for the thermogenic process in brown-fat cells. Am J Physiol 267: C969–C979
135. Puigserver P, Picó C, Stock MJ, Palou A (1996) Effect of selective ß-adrenoceptor stimulation on UCP synthesis in primary cultures of brown adipocytes. Mol Cell Endocrinol 117: 7–16
136. Preitner F, Muzzin P, Revelli JP, Seydoux J, Galitzky J, Berlan M, Lafontan M, Giacobino JP (1998). Metabolic response to various beta-adrenocepor agonists in beta3-adrenoceptor knockout mice: evidence for a new beta-adrenergic receptor in brown adipose tissue. Br J Pharmacol 124: 1684–1688
137. Picó C, Herron D, Palou A, Jacobsson A, Cannon B, Nedergaard J (1994) Stabilization of the mRNA for the uncoupling protein thermogenin by transcriptional/translational blockade and by noradrenaline in brown adipocytes differentiated in culture: a degradation factor induced by cessation of stimulation? Biochem J 302: 81–86
138. Puigserver P, Herron D, Gianotti M, Palou A, Cannon B, Nedergaard J (1992) Induction and degradation of the uncoupling protein thermogenin in brown adipocytes in vitro and in vivo. Evidence for a rapidly degradable pool. Biochem J 284: 393–398
139. Bianco AC, Sheng X, Silva JE (1988) Triiodotironine amplifies norepineprine stimulation of uncoupling gene transcription by a mechanisms not requiring protein synthesis. J Biol Chem 263: 18168–18173
140. Gianotti M, Roca P, Palou A (1988) Body weight and tissue composition in rats made obese by a cafeteria diet; effect of 24 hours of starvation. Horm Metab Res 20: 208–212
141. Proenza A, Lladó I, Serra F, Picó C, Pons A, Palou A (1992) Tissue composition in persistent dietary obesity after early and adulthood overfeeding in the rat. Arch Int Physiol Biochim Biophys 100: 147–154
142. Puigserver P, Lladó I, Palou A, Gianotti M (1991) Evidence for masking of brown adipose tissue mitochondrial GDP-binding sites in response to fasting in rats made obese by dietary manipulation. Biochem J 279: 575–579
143. Puigserver P, Gianotti M, Palou (1992) Impaired starvation-induced loss of mitochondrial protein in the brown adipose tissue of dietary obese rats. Int J Obes 16: 255–261
144. Roca P, Rodriguez AM, Oliver P, Bonet ML, Quevedo S, Picó C, Palou A (1999) Brown adipose tissue response to cafeteria diet-feeding involves induction of the UCP2 gene and is impaired in female rats as compared to males. Pflügers Arch (Eur J Physiol) 438: 628–634
145. Picó C, Pons A, Gianotti M, Palou A (1991) Sustained changes in blood alpha amino nitrogen compartmentation during recovery from cafeteria feeding in rats. Arch Int Physiol Biochim Biophys 99: 345–348
146. Lladó I, Proenza AM, Serra F, Palou A, Pons A (1991) Dietary-induced permanent changes in brown and white adipose tissue composition in rats. Int J Obes 15: 415–419
147. Matamala JC, Gianotti M, Pericás J, Quevedo S, Roca P, Palou A, García-Palmer FJ (1996) Changes induced by fasting and dietetic obesity in thermogenic parameters of rat brown adipose tissue mitochondrial subpopulations. Biochem J 319: 529–534
148. Bonet ML, Serra F, Matamala, JC, García-Palmer FJ, Palou A (1995) Selective loss of the uncoupling protein from light versus heavy mitochondria of brown adipocytes after a decrease in noradrenergic stimulation in vivo and in vitro. Biochem J 311: 327–331
149. Gianotti M, Clapés J, Lladó I, Palou A (1998) Effect of 12, 24 and 72 hours

fasting in thermogenic parameters of rat brown adipose tissue mitochondrial subpopulations. Life Sci 62: 1889–1899
150. Moreno M, Puigserver P, Llull J, Gianotti M, Lanni A, Goglia F, Palou A (1994) Cold exposure induces different uncoupling-protein thermogenin masking/unmasking processes in brown adipose tissue depending on mitochondrial subtypes. Biochem J 300: 463–468
151. Smas CM, Sul HS (1995) Control of adipocyte differentiation. Biochem J 309: 697–710
152. Spiegelman BM, Flier JS (1996) Adipogenesis and obesity: rounding out the picture. Cell 87: 377–389
153. Tontonoz P, Hu E, Spiegelman BM (1994) Stimulation of adipogenesis in fibroblasts by PPAR gamma 2, a lipid-activated transcription factor. Cell. 79: 1147–1156
154. Hu E, Tontonoz P, Spiegelman BM (1995) Transdifferentiation of myoblasts by the adipogenic transcription factors PPAR gamma and C/EBP alpha. Proc Natl Acad Sci USA 92: 9856–9860
155. Kersten S, Desvergne B, Wahli W (2000) Roles of PPARs in health and disease. Nature 405: 421–424
156. Forman BM, Tontonoz P, Chen J, Brun RP, Spiegelman BM, Evans RM (1995) 15-Deoxy-Δ12,14-prostaglandin J2 is a ligand for the adipocyte determination factor PPARγ. Cell 83: 803–812
157. Kliewer SA, Lenhard JM, Willson TM, Patel I, Morris DC, Lehmann JM (1995) A prostaglandin J2 metabolite binds peroxisome proliferator-activated receptor gamma and promotes adipocyte differentiation. Cell 83: 813–819
158. Kliewer SA, Sundseth SS, Jones SA, Brown PJ, Wisely GB, Koble CS, Devchand P, Wahli W, Willson TM, Lenhard JM, Lehmann JM (1998) Fatty acids and eicosanoids regulate gene expression through direct interactions with perosisome proliferator-activated receptors alpha and gamma. Proc Natl Acad Sci USA 94: 4318–4323
159. Kim JB, Wright HM, Wright M, Spiegelman BM (1998) ADD1/SREBP1 activates PPAR gamma through the production of endogenous ligand. Proc Natl Acad Sci USA 95: 4333–4337
160. Puigserver P, Wu Z, Park CW, Graves R, Wright M, Spiegelman BM (1998) A cold-inducible coactivator of nuclear receptors linked to adaptive thermogenesis. Cell 92: 829–839
161. Wu Z, Puigserver P, Andersson U, Zhang C, Adelmant G, Mootha V, Troy A, Cinti S, Lowell B, Scarpulla RC, Spiegelman BM (1999) Mechanisms controlling mitochondrial biogenesis and respiration through the thermogenic coaactivator PGC-1. Cell 98: 115–124
162. Freytag SO, Paielli DL, Gilbert JD (1994) Ectopic expression of the CCAT/enhancer-binding protein a promotes the adipogenic program in a variety of mouse fibroblastic cells. Genes Dev 8: 1654–1663
163. Puigserver P, Nadal-Ginard B, Palou A (1994) Expression and interaction of C/EBP alpha adipogenic transcription factor and retinoblastoma protein in adipocytes during differentiation. Int J Obes 18(S2): 113
164. Puigserver P, Gianotti M, Nadal-Ginard B, Palou A (1996) Involvement of the retinoblastoma protein (pRB) in adipocyte cell differentiation and thermogenesis. In vitro and in vivo interaction of pRB with the adipogenic transcription factor C/EBPα. Int J Obes 20(S4): 132
165. Puigserver P, Ribot J, Serra F, Gianotti M, Bonet ML, Nadal-Ginard B, Palou A (1998) Involvement of the retinoblastoma protein in brown and white adipocyte cell differentiation: functional and physical association with the adipogenic transcription factor c/EBPα. Eur J Cell Biol 77: 117–123
166. Comuzzie AG, Allison DB (1998). The search for human obesity genes. Science 280: 1374–1377
167. Chagnon YC, Perusse L, Weisnagel J, Rankinen T, Bouchard C (2000) The human obesity gene map: the 1999 update. Obes Res 8: 89–117
168. Barsh GS, Farooqi IS, O'Rahilly S (2000) Genetics of body-weight regulation. Nature 404: 644–651
169. Jackson RS, Creemers JW, Ohagi S, Raffin-Sanson ML, Sanders L, Montague CT, Hutton JC, O'Rahilly S (1997) Obesity and impaired prohormone processing associated with mutations in the human prohormone convertase 1 gene. Nature Genet 16: 303–306
170. Krude H, Biebermann H, Luck W, Horn R, Brabant G, Gruters A (1998) Severe early-onset obesity, adrenal insufficiency and red hair pigmentation caused by POMC mutations in humans. Nature Genet 19: 155–157
171. Duggirala R, Stern MP, Mitchell BD, Reinhart LJ, Shipman PA, Uresandi OC, Chung WK, Leibel RL, Hales CN, O'Connell P, Blangero J (1996) Quantitative variation in obesity-related traits and insulin precursors linked to the OB gene region on human chromosome 7. Am J Hum Genet 59: 694–703
172. Allison D, Heo M (1998) Meta-analysis of linkage data under worst-case conditions: a demonstration using the human OB region. Genetics 148: 859–865
173. Rotimi CN, Comuzzie AG, Lowe WL, Luke A, Blangero J, Cooper RS (1999) The quantitative trait locus on chromosome 2 for serum leptin levels is confirmed in African-Americans. Diabetes 48: 643–644
174. Walston J, Silver K, Bogardus C, Knowler W, Celi FS, Austin S, Manning B, Strosberg AD, Stern MP, Raben N, Sorkin JD, Roth J, Shuldiner AR (1995) Time of onset of non-insulin-dependent diabetes mellitus and genetic variation in the ß3-adrenergic-receptor gene. N Engl J Med 333: 343–347
175. Clément K, Vaisse C, Manning BST, Basdevant A, Guy-Grand B, Ruiz J, Silver KD, Shuldiner AR, Froguel P, Strosberg AD (1995) Genetic variation in the ß3-adrenergic receptor and an increased capacity to gain weight in patients with morbid obesity. N Engl J Med 333: 352–354
176. Widen E, Letho M, Kanninen T, Walston J, Shuldiner AR, Groop LC (1995) Association of a polymorphism in the ß3-adrenergic receptor gene with features of the insulin resistance in Finns. N Engl J Med 333: 348–351
177. Gagnon J, Mauriege P, Roy S, Sjöström D, Chagnon YC, Dionne FT, Oppert JM, Perusse L, Sjöström L, Bouchard C (1996) The Trp64Arg mutation of the beta3 adrenergic receptor gene has no effect on obesity phenotypes in the Quebec Family Study and Swedish Obese Subjects cohorts. J Clin Invest 98: 2086–2093
178. Large V, Hellström L, Reynisdottir S, Lönnqvist F, Eriksson P, Lannfelt L, Arner P (1997) Human beta–2 adrenoceptor gene polymorphisms are highly frequent in obesity and associate with altered adipocyte beta2 adrenoceptor function. J Clin Invest 100: 3005–3013
179. Mori Y, Kim-Motoyama H, Ito Y, Katakura T, Yasuda K, Ishiyama-Shigemoto S, Yamada K, Akanuma Y, Ohashi Y, Kimura S, Yazaki Y, Kadowaki T (1999) The Gln27Glu beta2-adrenergic receptor variant is associated with obesity due to subcutaneous fat accumulation in Japanese men. Biochem Biophys Res Commun 258: 138–140
180. Sakane N, Yoshida T, Umekawa T, Kogure A, Kondo M (1999) Beta2-adrenoceptor gene polymorphism and obesity. Lancet 353: 1976
181. Meirhaeghe A, Helbecque N, Cottel D, Amouyel P (1999) Beta2-adrenoceptor gene polymorphism, body weight, and physical activity. Lancet 353: 896
182. Oppert JM, Vohl MC, Chagnon M, Cassard-Doulcier AM, Ricquier D, Pérusse L, Bouchard C (1994) DNA polymorphism in the uncoupling protein (UCP) gene and human body fat. Int J Obesity 18: 526–531
183. Fumeron F, Durack-Bown I, Betoulle D, Cassard-Doulcier AM, Tuzet S, Bouillard F, Melchior JC, Ricquier D, Apfelbaum M (1996) Polymorphisms of uncoupling protein (UCP) and ß3 adrenoreceptor genes in obese people

submitted to a low calorie diet. Int J Obes 20: 1051–1054
184. Proenza AM, Poissonnet CM, Ozata M, Ozen S, Guran S, Gundogan A, Palou A, Strosberg AD (2000) Association of sets of alleles of genes encoding ß3-adrenoreceptor, uncoupling protein 1 and lipoprotein lipase with increased risk of metabolic complications in obesity. Int J Obes 21: 93–100
185. Bouchard C, Pérusse L, Chagnon C, Warden C, Ricquier D (1997) Linkage between markers in the vicinity of the uncoupling protein 2 gene and resting metabolic rate in humans. Hum Mol Genet 6: 1887–1889
186. Comuzzie AG, Hixson JE, Almasy L, Mitchell, BD, Mahaney MC, Dyer TD, Stern MP, McCluer JW, Blangero J (1997) A major quantitative trait locus determining serum leptin levels and fat mass is located on human chromosome 2. Nature Genet 15: 273–276
187. Silver K, Stern MP, MacCluer JW, Hixson JE (1998) A paired sibling analysis of the beta-3 adrenergic receptor and obesity in Mexican Americans. J Clin Invest 101: 584–587
188. Bray GA, Tartaglia LA (2000) Medicinal strategies in the treatment of obesity. Nature 404: 672–677
189. Drent ML, Larsson I, William-Olsson T, Quaade F, Czubayko F, von Bergmann K, Strobel W, Sjöström L, van der Veen EA (1995) Orlistat (Ro 18-0647), a lipase inhibitor, in the treatment of human obesity: a multiple dose study. Int J Obes 19: 221–226
190. Drent ML, van der Veen EA (1995) First clinical studies with Orlistat: a short review. Obes Res 3: 623S–625S
191. Guerciolini R (1997) Mode of action of orlistat. Int J Obes 21(S3): S12–S23
192. Puigserver P, Vazquez F, Bonet ML, Picó C, Palou A (1996) In vitro and in vivo induction of brown adipocyte uncoupling protein (thermogenin) by retinoic acid. Biochem J 317: 827–833
193. Bonet ML, Serra F, Puigserver P, Vazquez F, Picó C, Ribot J, Palou A (1997) Retinoic acid modulates retinoic X receptor a levels of cultured brown adipocytes. FEBS Letters 406: 196–200
194. Serra F, Bonet ML, Puigserver P, Palou A (1999) Stimulation of uncoupling protein 1 expression in brown adipocytes by naturally occurring carotenoids. Int J Obes 23: 650–655
195. Portillo MP, Serra F, Simon E, del Barrio AS, Palou A (1998) Energy restriction with high-fat diet enriched with coconut oil gives higher UCP1 and lower white fat in rats. Int J Obes 22: 974–979