

Pharmacotherapy of obesity: targets and perspectives

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The search for anti-obesity agents has become one of the most exciting areas in drug discovery. Subsequent to an enormous increase in the number of possible molecular targets, the focus has shifted from target identification to target validation. Because important biological functions such as the regulation of energy intake and expenditure are controlled by complex systems, an improved understanding of pathophysiology is a prerequisite for the selection of successful development candidates for the treatment of obesity. Although most of the information on the regulation of energy balance has been obtained from rodents, various monogenic forms of human obesity provide clinical proof of concept for some of these mechanisms. However, it is still not known which are the most promising clinical approaches to lowering body weight and subsequently reducing morbidity and mortality.

Obesity results from an imbalance between energy intake and energy expenditure. Environmental factors, such as the general availability of high-calorie food or the limited need for physical exercise, and genetic factors that predispose to weight gain contribute to the development of obesity. Owing to its increasing prevalence in industrialized countries, the associated morbidity and mortality, and the enormous primary and secondary health care costs, obesity has become recognized as a major medical problem. Failure of diet and exercise in the long-term treatment of obesity is common and creates an obvious need for concomitant pharmacotherapy¹. However, only two new anti-obesity agents, sibutramine (Reductil® or Meridia®)², an appetite suppressant, and orlistat (Xenical®)³, an inhibitor of fat absorption, have been successfully introduced into the market during recent years. Both drugs are active in individuals with obesity but their efficacy is limited and their tolerability is far from ideal. Fortunately, recent studies in rodent models and in obese patients have provided numerous possible novel molecular targets, many of which are currently being validated in preclinical and clinical studies^{4,5}. The most important developments in this field and their possible impact on pharmacotherapy will be discussed.

Reduction of energy intake: appetite suppression *Leptin receptors*

For a long time it was poorly understood whether and how the energy reserves of the body (i.e. the size of the fat depots) were regulated. Following the cloning of leptin by Friedman and colleagues, a feedback loop between the peripheral fat depots and the brain became established (Fig. 1), which appeared to be promising for pharmacological interventions⁶.

Leptin is mainly, but not exclusively, expressed in white adipose tissue (WAT) in proportion to adipocyte

size and number. Circulating leptin crosses the blood–brain barrier, most probably by facilitated diffusion, to reach its receptors in the hypothalamus. The binding of leptin to its receptors, which belong to the cytokine receptor family, results in the activation of the intracellular JAK–STAT (Janus protein kinase–signal transducers and activators of transcription) signalling system. Mutant rodent strains with either leptin deficiency (*ob/ob* mice) or leptin receptor defects (*db/db* mice or *fa/fa* rats) share some characteristic symptoms, such as increased food intake, reduced energy expenditure, and various endocrine and metabolic abnormalities. Chronic administration of exogenous leptin to *ob/ob* mice leads to a sustained reversal of these abnormalities^{7,8}.

Soon after the first demonstration of leptin deficiency and leptin receptor dysfunction in mice, comparable mutations were discovered in humans and found to be associated with severe obesity⁹. Some of the symptoms that had been observed in rodents were also present in these patients. Administration of recombinant human (rh) leptin to one of the patients with leptin deficiency restored leptin plasma concentrations to normal values and led to a progressive decrease in body weight¹⁰. By contrast, the results of the first clinical trial with rh leptin in obese patients (without leptin deficiency) were much less promising. Even after high doses, which produced plasma concentrations ~ 20 times above baseline (placebo treatment), weight loss was variable and the average reduction relatively small¹¹.

There are several explanations for the disappointing results with leptin. First, leptin probably plays its physiological role at low plasma concentrations, and very little can be gained from increasing them above normal¹². Second, the therapeutic efficacy of leptin might have been overestimated by extrapolating from rodent studies to the clinical situation⁸. Leptin treatment of *ob/ob* or lean mice resulted not only in a reduction in food intake but also an increase in energy expenditure – the latter effect was not observed in human subjects. Finally, the fact that obese individuals still overeat despite high plasma leptin concentrations suggests that they are resistant to leptin. This resistance could be due to either a rate-limiting transport step for the entry of circulating leptin into the brain or impaired receptor signalling⁷. If leptin resistance occurs at the level of receptor activation, stimulating the same intracellular signalling pathway with another natural agonist might offer a solution.

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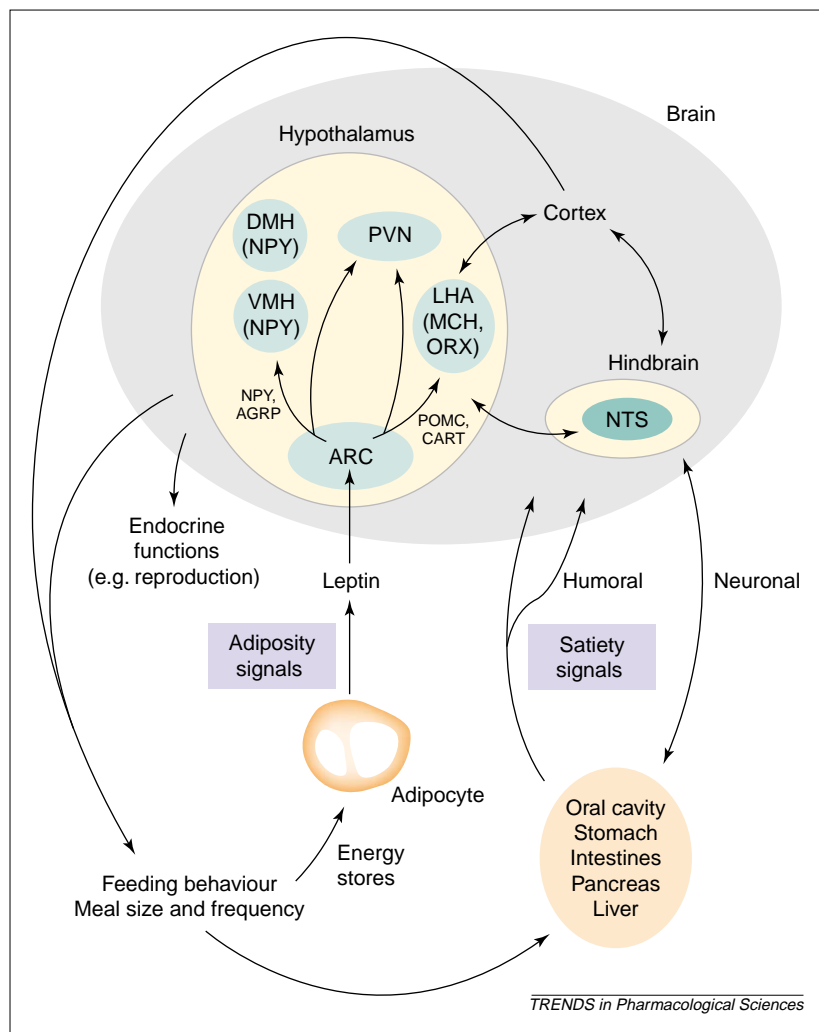


Fig. 1. Central and peripheral pathways involved in the regulation of food intake and energy stores. Leptin is secreted by adipose tissue and circulates to the brain, where it crosses the blood-brain barrier to reach the arcuate nucleus (ARC) within the hypothalamus. Here, a cascade is initiated that ultimately regulates feeding behaviour, various endocrine systems and other functions. Leptin directly affects neurones (so-called first-order neurones⁷) in which either the anorexigenic peptides pro-opiomelanocortin (POMC) and cocaine- and amphetamine-regulated transcript (CART) or the orexigenic peptides neuropeptide Y (NPY) and agouti-related protein (AGRP) are colocalized. The POMC-CART- and NPY-AGRP-containing neurones, which are regulated in an opposing manner by leptin, project further to other brain centres. These include the ventro- and dorsomedial hypothalamus (VMH and DMH), which also express NPY, the paraventricular nucleus (PVN) and the lateral hypothalamic area (LHA), which express the neuropeptides orexin (ORX) and melanin-concentrating hormone (MCH). The LHA and other brain areas communicate with the cerebral cortex, where feeding behaviour is finally coordinated. During and after a meal, various signals are generated in the periphery, which include taste signals from the oral cavity, gastric distension and humoral signals (e.g. cholecystokinin) from secretory cells of the gastrointestinal tract. These afferent signals are transmitted mainly by the vagus nerve, but also by the sympathetic nervous system to the hindbrain, particularly the nucleus of the solitary tract (NTS). This brain region communicates with higher brain areas such as the hypothalamus and the cerebral cortex.

Ciliary neurotrophic factor (CNT) might be such a candidate and the current trials with axokine, a peptide CNTF analogue, could soon determine whether this agent is of benefit¹³. If the increased expression of an intracellular agent such as suppressor of cytokine signalling 3 (SOCS 3) is involved¹⁴, inhibition of this protein might lead to receptor sensitization. However, the best option to circumvent leptin resistance could be to focus on targets downstream of leptin receptor activation such as neuropeptide Y (NPY)

or α -melanocyte stimulating hormone (α -MSH) (Fig. 1) because these peptides act at G-protein-coupled receptors (GPCRs), a receptor family proven to be amenable to pharmacological interventions.

NPY receptors

The potency and efficacy of exogenous NPY as an appetite stimulant after its acute administration and its persistent effects during chronic administration have led to the assumption that endogenous NPY could play an essential role in the short- and long-term regulation of energy balance¹⁵. For a long time it was unclear which receptor subtype mediated the effects of NPY on food intake. Initially the NPY Y_1 receptor or a related receptor subtype seemed to be the correct molecular target for antagonists. Subsequently, an additional receptor subtype, Y_5 , appeared to be an even better candidate because peptide agonists showed a similar order of potency for Y_5 receptor activation *in vitro* as for appetite stimulation *in vivo*¹⁶.

The first studies with antisense oligonucleotides¹⁷ and a selective Y_5 receptor non-peptide antagonist¹⁸ supported an important function of the Y_5 subtype. However, because Y_5 -receptor-deficient mice showed no phenotype¹⁹, and another group reported that selective Y_5 receptor antagonists had no effect on spontaneous feeding²⁰, it is possible that nonspecific actions contributed to the reduction in food intake observed in the earlier experiments.

Melanocortin receptors

The importance of melanocortin-mediated pathways in the regulation of body weight has been demonstrated using genetic studies in obese mice and obese humans. The melanocortin MC_4 receptor, which is expressed in various brain regions (including the hypothalamus), integrates an agonist signal provided by α -MSH, a melanocortin produced by cleavage of pro-opiomelanocortin (POMC), with an antagonist signal provided by agouti-related protein (AGRP)⁷. Evidence for a central function of α -MSH in the regulation of food intake comes from studies in MC_4 receptor or POMC knockout mice, both of which show an obese phenotype. Overexpression of AGRP or ectopic expression of the agouti protein in mice also results in obesity⁷. The genetic findings have been confirmed by pharmacological studies demonstrating that treatment with a selective peptide agonist of MC_4 receptors reduces appetite in rats and mice, whereas an antagonist had the opposite effect²¹.

Moreover, clinical observations provide proof that the melanocortin-mediated pathway also plays an important role in the regulation of body weight in humans. Human POMC mutations and various mutations in the gene encoding the MC_4 receptor^{7,22,23} have been identified as being associated with an obese phenotype. In fact, such mutations appear to be the most common monogenic form of human obesity described so far.

Recently, MC₃ receptor knockout mice have also been reported to be obese despite their hypophagia, which strongly suggests that this receptor subtype also has an important role in the regulation of energy homeostasis²⁴. Taken together, these results strongly suggest that agonists of MC₄ receptors and possibly also of MC₃ receptors could be valuable novel anorectic drugs. Agents that interfere with the endogenous antagonist AGRP should have comparable effects by preventing the inhibition of α -MSH binding at the MC₃ or MC₄ receptors.

Other receptors

In addition to NPY and AGRP, neuropeptides such as galanin, melanin-concentrating hormone (MCH) and the orexins (also termed hypocretins) have been reported to exert orexigenic effects^{5,7}. Targeted deletion of the gene encoding MCH results in hypophagia and decreased body weight²⁵. The receptor for MCH, a G_i-coupled receptor previously known as SLC-1, has recently been identified^{26,27}. Orexin A and B are derived from a common precursor and bind with different affinities to their receptors termed OX₁ receptor and OX₂ receptor²⁸. Although the orexins have been shown to stimulate food intake, their suitability for pharmacotherapy might be challenged by the findings that knocking out the orexin precursor or the gene encoding the OX₂ receptor causes narcolepsy^{29,30}. Information about the phenotype of OX₁-receptor-deficient mice will clarify whether the effects on food intake are predominantly mediated by this receptor.

Central administration of cocaine- and amphetamine-regulated transcript (CART) has been shown to suppress food intake³¹. This neuropeptide also attenuates NPY-induced feeding, which suggests that CART acts downstream from NPY (Ref. 32) (Fig. 1). However, the CART receptor has yet to be identified. A recent study describes the anorectic effects of neuromedin U (NMU) and the identification of its receptor, NMU₂, in the brain³³.

Other peptides that have attracted attention include corticotrophin-releasing factor (CRF), which acts predominantly at the CRF₁ receptor, and the CRF-like neuropeptide urocortin, which acts predominantly at CRF₂ receptors *in vivo*¹. Both peptides have been shown to inhibit food intake³⁴. The potential of CRF mimetics for obesity treatment still needs to be determined because these could also affect behaviour and interfere with the hypothalamo-pituitary-adrenal axis³⁴.

The pancreatic hormone insulin has previously been suggested to act centrally as an adiposity signal that is similar to leptin and thus to play a major role in regulating food intake and body weight⁷. Recent findings demonstrate that the disruption of insulin receptors in the brain results in hyperphagia in female mice and causes obesity in both sexes³⁵, which thus provides new support for this hypothesis.

A recent study that describes brain fatty acid synthase (FAS) as a potential link between anabolic

energy metabolism and appetite control has raised much interest³⁶. The studies show that FAS inhibition by C75, a compound originally developed for the treatment of breast cancer, reduces food intake and body weight in mice. This anorectic effect is presumably mediated via accumulation of the FAS substrate malonyl-CoA because this effect could be prevented by lowering malonyl-CoA concentrations by inhibition of acetyl-CoA carboxylase.

Peripheral signals

Meal size appears to be regulated by a feedback loop in which afferent signals that originate in the oral cavity and the gastrointestinal (GI) tract are transmitted mainly by the vagus nerve to the nucleus of the solitary tract (NTS) in the brainstem. This brain region communicates with higher hypothalamic centres involved in feeding⁷ (Fig. 1). Specific attention has been paid to afferent humoral signals such as cholecystokinin (CCK), which are released post-ingestively from secretory cells of the GI tract and act mostly as satiety factors³⁷. The observation that the anorectic response to peripherally administered CCK is enhanced by leptin and insulin reflects the crosstalk between systems involved in the acute and the long-term regulation of energy homeostasis⁷.

Another peripheral signal is glucagon-like peptide 1 (GLP-1), which is processed from proglucagon in the pancreas, intestinal cells and the CNS (Ref. 38). The peptide presumably regulates feeding by both peripheral and central mechanisms because it delays gastric emptying and suppresses food intake. However, administration of GLP-1 to certain brain areas caused gastric malaise^{38,39}, which makes it a controversial target for pharmacotherapy. A recent study describes the anorectic effects of a related peptide, GLP-2 (Ref. 40), for which taste aversion was not observed. The authors identified a GLP-2-containing neuronal pathway that connects the NTS with a hypothalamic feeding centre that expresses the GLP-2 receptors.

A selection of central and peripheral signals involved in feeding is listed in Table 1. Many of these signals have been validated using elegant transgenic techniques⁴¹. New genes such as that encoding beacon⁴² or new findings with known peptides such as ghrelin⁴³ are constantly being added to the list of anorectic or orexigenic pathways. Their importance is as yet unclear but could change the current view of the complex pathways involved in the regulation of food intake and energy stores.

Reduction of energy intake: inhibition of absorption

Inhibition of the absorption of fat represents the most efficient approach for the reduction of caloric intake. Several steps are required for the absorption of dietary fat, which involves proteins that might represent promising drug targets. In the intestine, triglycerides are split into free fatty acids (FFAs) and monoglycerides by lipases, the targets for orlistat³. After hydrolysis, FFAs cross the membrane of the

Table 1. Selected central and peripheral signals affecting food intake and energy homostasis

Target system	Pharmacological approach	Validation ^{a,b}	Refs
Neuropeptides, hormones and monoamines			
Pro-opiomelanocortin (POMC)	Melanocortin type 4 (and 3?) receptor agonists	Preclinical (KO mice) and clinical (human mutations)	7,22,23
	Agouti-related protein (AGRP) blockers	Preclinical (transgenic mice)	7,41
Cocaine- and amphetamine-related transcript (CART)	Receptor agonists	Receptor not yet known	
Neuromedin U (NMU)	NMU ₂ receptor agonists	No validation	
Neuropeptide Y (NPY)	Y ₁ or Y ₅ receptor antagonists	Preclinical data controversial	17–20
Melanin-concentrating hormone (MCH)	MCH receptor antagonists	Preclinical (MCH KO mice)	25
Orexin (OX)	OX ₁ receptor antagonists	Preclinical evidence incomplete	
Corticotropin-releasing factor (CRF) or urocortin	CRF ₂ receptor agonists, CRF-binding protein blockers	Preclinical evidence incomplete	34
Cannabinoids	CB ₁ receptor antagonists	No validation	
Galanin	Galanin receptor antagonists	No validation	
Opioids	μ, κ and/or δ receptor antagonists	No validation	
Noradrenaline	α-Adrenoceptor agonists	No validation	
	Stimulators of noradrenaline release	No validation	
	Blockers of noradrenaline reuptake	Sibutramine, phentermine	1,2
5-HT	5-HT _{2C} receptor agonists	5-HT _{2C} KO mice unresponsive to D-fenfluramine	1
	Stimulators of 5-HT release	No validation	
	Blockers of 5-HT reuptake	Sibutramine	1,2
Peripheral peptides^c			
Leptin (adipocyte hormone)	Leptin receptor agonists, leptin sensitizers, leptin mimics: ciliary neurotrophic factor (CNTF) and axokine	Preclinical (mutant mice, leptin treatment in rodents) Clinical (human mutations, leptin trial)	7,41 9–11
Cholecystokinin (CCK)	CCK analogues, CCK ₁ and CCK ₂ receptor agonists	CCK ₁ receptor KO mice and mutant rats controversial	37,41
	Inhibitors of degradation	In clinical studies	
Enterostatin	Enterostatin analogues	Clinical evidence (i.v. study) negative	1,37
	Enterostatin receptor agonists	Receptor not yet known	
Glucagon-like peptides GLP-1 and GLP-2	GLP analogues or receptor agonists	Clinical	37
	Inhibitors of degradation	No validation	
Amylin	Amylin and analogues	No validation	
	Amylin receptor agonists	Receptor not yet cloned	
Bombesin	Bombesin analogues	Preclinical (BRS ₃ KO mice)	41
	Bombesin-related receptor subtype 3 (BRS ₃) agonists		

^aValidation refers to confirming data from knockout, transgenic or mutant animals, or from human studies, including clinical trials and reported mutations.

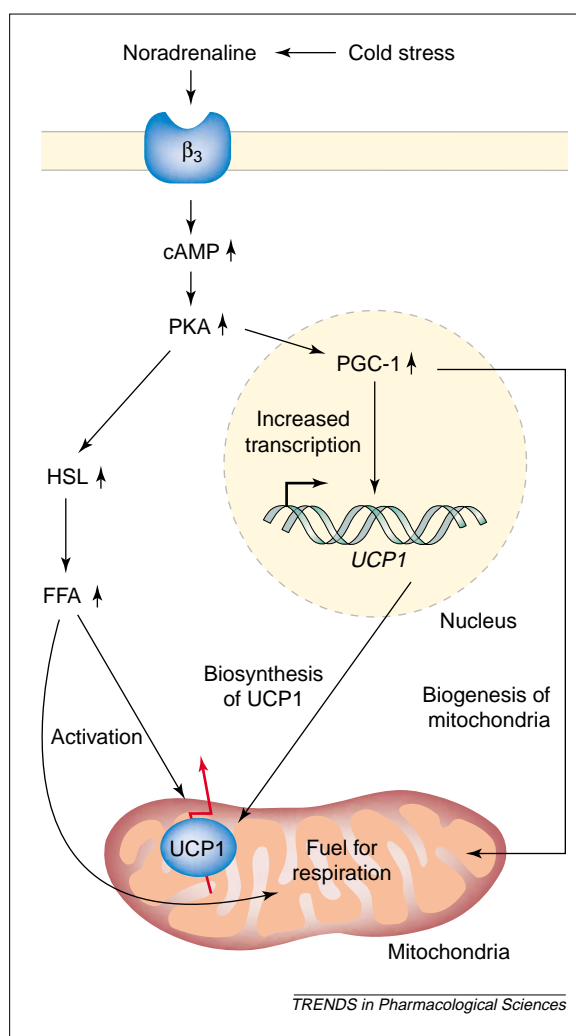
^bAbbreviations: i.v., intravenous injection; KO, knockout.

^cMost of the peripheral peptides are released from the gastrointestinal (GI) tract in response to a meal and regulate food intake, mainly as satiety factors. Some peptides can, in addition, modulate nutrient absorption and passage through the GI tract. Many peripheral peptides were shown to reduce food intake when administered centrally.

epithelial cells lining the intestinal wall. A newly discovered FFA transporter, FATP4, which is mainly expressed in the intestine, could have a major role in this process⁴⁴. The value of FATP4 as a therapeutic target, however, might be limited if passive diffusion represents a major route of uptake or if other FFA transport systems compensate for its inhibition. Once inside the epithelial cell, FFAs are donated to

acyl-CoA synthetase in the endoplasmic reticulum by fatty-acid-binding proteins (FABPs). Inhibiting FABPs could in principle reduce the absorption of fatty acids but the feasibility of this approach is questionable, owing to the very high concentration of FABPs in the enterocyte cytosol. Acyl-CoA is then transferred to 2-monoacylglycerol to resynthesize triglycerides. Acyl CoA:diacylglycerol acyltransferase

Fig. 2. Regulation of thermogenesis in brown adipose tissue (BAT). BAT has the specific function of thermogenesis. Adrenoceptor agonists (noradrenaline) acting at β_3 -adrenoceptors induce the activation of a mitochondrial protein called uncoupling protein 1 (UCP1). UCP1 is unique to brown adipocytes and resides in the inner mitochondrial membrane where it dissipates the proton gradient coupled to the oxidation of metabolites and thus generates heat (red arrow)⁵⁴. Acute activation of UCP1 occurs via interaction of the protein with free fatty acids (FFAs) derived from an increased activity of the hormone-sensitive lipase (HSL). In addition, there is a chronic increase in transcription of the gene encoding UCP1, which is mediated by the cAMP-dependent upregulation of the expression of peroxisome proliferator activated receptor γ (PPAR- γ) co-activator-1 (PGC-1), a transcriptional co-activator of the nuclear PPAR- γ that also induces the biogenesis of mitochondria.



(DGAT) is a key enzyme in triglyceride synthesis and its inhibition could represent a valid new strategy. Recently, mice that lack DGAT have been shown to be resistant to obesity. However, these mice exhibited normal fat absorption, which suggests the existence of other pathways for triglyceride synthesis⁴⁵.

Increase of energy expenditure

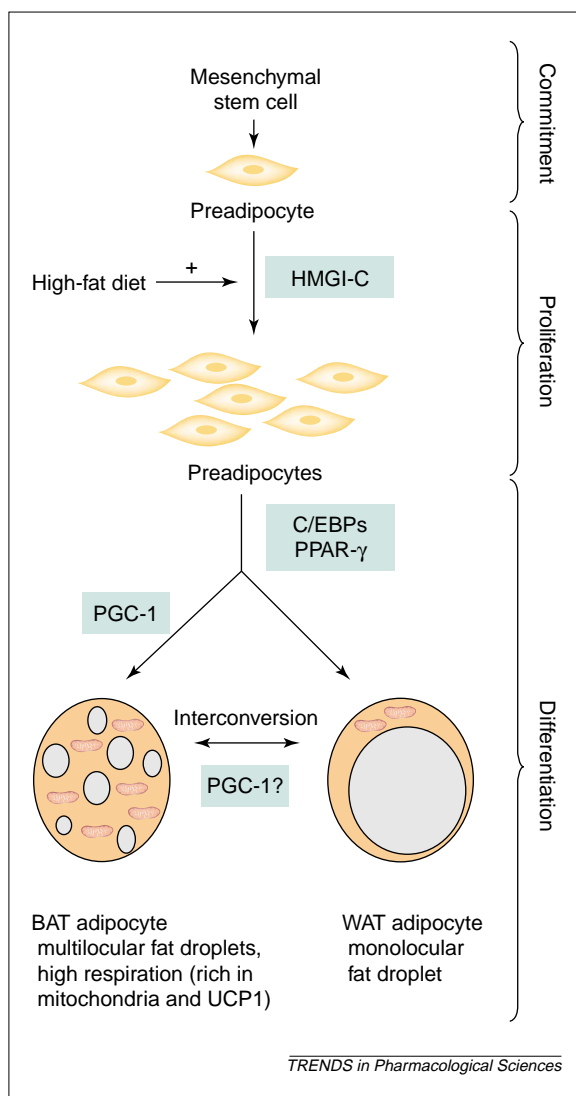
Total energy expenditure is the sum of basal metabolism, the constant obligatory energy expenditure required for cell and organ survival, and a variable portion needed for physical activity and adaptive thermogenesis. The latter component confers the ability to adapt to prolonged exposure to cold (non-shivering thermogenesis) or overfeeding (diet-induced thermogenesis). The biogenesis of mitochondria and the induction of specific mitochondrial proteins that control the efficiency of oxidative phosphorylation are the key cellular processes of adaptive thermogenesis⁴⁶ (Fig. 2). Thyroid hormone and noradrenaline released from sympathetic nerve endings have a profound impact on adaptive thermogenesis, in particular in fat tissue and in skeletal muscle. Thyroid hormone, however, also causes loss of lean body mass and mobilizes Ca^{2+}

from bone, so that pharmacological stimulation of the hypothalamic–pituitary–thyroid axis is not a viable anti-obesity approach.

Rodents have brown adipose tissue (BAT), which is rich in mitochondria. A particular fat-selective adrenoceptor subtype (β_3) is involved in the coordination of both fat mobilization and its subsequent burning in the BAT, which is mediated by the mitochondrial uncoupling protein UCP1 (Ref. 47) (Fig. 2). β_3 -Adrenoceptor-selective agonists have been shown in several studies to have anti-obesity and anti-diabetic effects in rodents⁴⁸. However, whether β_3 -adrenoceptor agonists will have a relevant impact on energy expenditure in humans is still controversial. Although BAT is well developed in newborn babies, where it contributes to maintaining body temperature, it atrophies in adults. Recently, evidence has been provided that chronic stimulation with selective agonists causes upregulation of β_3 -adrenoceptors and induces BAT hypertrophy not only in rodents but also in dogs and monkeys⁴⁸, which suggests recruitment of BAT could also occur in humans. Results of clinical trials over the past decade with poorly bioavailable and marginally selective β_3 -adrenoceptor active compounds have been disappointing, but a novel generation of highly selective, orally bioavailable β_3 -adrenoceptor agonists is currently in clinical trials.

In adult humans, the major thermogenic tissue is skeletal muscle, which, in non-obese subjects, comprises ~40% of body weight and accounts for 20–30% of the total oxygen consumption at rest⁴⁹. In the past few years, a few proteins with high sequence homology to UCP1 have been identified that are also expressed in non-BAT tissues, and their involvement in thermogenesis has been postulated. Most relevant are UCP2, which is widely distributed in the body, and UCP3, which, in humans, is expressed primarily in skeletal muscle⁵⁰. Owing to the ubiquitous localization of UCP2, the likelihood of undesired side-effects makes this protein a less promising target for anti-obesity drugs. By contrast, stimulation of the skeletal-muscle-specific UCP3 activity could provide a safer mechanism to increase whole body thermogenesis in humans. Skeletal muscle UCP3 is correlated with energy expenditure in Pima Indians⁵¹, and mutations in the gene encoding UCP3 have been identified in some individuals suffering from severe obesity and non-insulin dependent diabetes mellitus (NIDDM)⁵². Transgenic mice with muscle-directed overexpression of UCP3 were shown to be hyperphagic and lean when fed a palatable diet, and they had an improved glucose tolerance⁵³. UCP3-deficient mice do not develop obesity but have more efficiently coupled skeletal muscle mitochondria⁵⁴, which demonstrates that UCP3 is indeed a major contributor to the mitochondrial proton leak in this tissue. Thus, a pharmacological stimulation of UCP3 activity could result in beneficial effects against obesity and NIDDM.

Fig. 3. Modulation of fat storage. Preadipocytes derive from pluripotent mesenchymal stem cells and are committed to the adipocyte lineage. After a proliferative phase that can be promoted by a high-fat diet and by factors such as HMGI-C (high mobility group I protein), the preadipocytes can terminally differentiate into either white adipocytes whose primary function is to store lipids or brown adipocytes whose primary function is to dissipate energy by burning lipids. Both differentiations require the activation of a specific set of nuclear factors of which the C/EBPs (CAAT/enhancing binding proteins) and peroxisome proliferator activated receptor γ (PPAR- γ) play a crucial role. Even though very little is known about the molecular basis of the differentiation between white and brown adipocytes, a novel nuclear factor, known as PPAR- γ co-activator-1 (PGC-1), has been identified that can promote the development of the brown adipocyte phenotype. Whether PGC-1 could also promote interconversion of pre-existing fully differentiated white adipocytes into brown adipocytes is not known. Abbreviations: BAT, brown adipose tissue; UCP1, uncoupling protein 1; WAT, white adipose tissue.



Modulation of fat storage

Pharmacological interference with the storage of fat has only recently been recognized as a possible approach to anti-obesity treatment. Experimental and clinical studies have shed light on the mechanisms that regulate adipocyte differentiation and on the crucial role of some nuclear receptors such as peroxisome proliferator activated receptor γ (PPAR- γ)⁵⁵ (Fig. 3). Activation of PPAR- γ leads to a decrease in circulating FFAs and improves insulin sensitivity in the periphery. Various PPAR- γ ligands, all of them thiazolidinediones, are on the market for the treatment of NIDDM (Ref. 55). However, these compounds are not effective against obesity and, on the contrary, might promote weight gain. Inhibitors of the PPAR- γ pathway can be considered for the treatment of obesity because they are expected to inhibit the recruitment of new adipocytes. Heterozygous PPAR- γ -deficient mice, in fact, are resistant to high-fat diet-induced obesity⁵⁶. Unexpectedly, these mice do not develop insulin resistance but instead are much more insulin sensitive than wild-type mice, which is difficult to

reconcile with the studies that used PPAR- γ stimulation.

A high mobility group I protein (HMGI-C) was recently reported to regulate the size of fat pads⁵⁷. HMGI-C is induced in mice WAT by a high-fat diet and favours preadipocyte proliferation, which thus leads to increased numbers of adipocytes. Accordingly, HMGI-C-deficient mice have a reduced propensity to develop obesity. A reduction of WAT can also be obtained by a stimulation of lipolysis. The adipocyte protein perilipin was recently shown to play a crucial role in the inhibition of basal hormone-sensitive lipase (HSL) activity, and mice with perilipin deficiency are lean and resistant to obesity⁵⁸. Clearly, pharmacological inhibition of WAT formation will be safe only if associated with an increase in fat oxidation or with a reduction of fat absorption. Otherwise, the consequence of the high concentrations of circulating lipids would remain a crucial issue. These risks have been dramatically illustrated by the phenotype of the A-ZIP/F-1 fatless mice, which develop a fatty liver and are diabetic⁵⁹.

A safer anti-obesity approach could be the stimulation of BAT formation, either by *de novo* recruitment from preadipocytes or by interconversion of white adipocytes. PPAR- γ ligands are very effective in inducing UCP1 expression in brown but not in white adipocytes, which indicates the existence of a BAT-specific cofactor. Recently, such a cofactor, PPAR- γ co-activator-1 (PGC-1), was identified, which is strongly induced by cold⁶⁰. PGC-1 allows PPAR- γ to function in the specific context of thermogenesis [i.e. by allowing the expression of UCP1 and by favouring multiplication of mitochondria (Fig. 3)]. Ectopic expression of PGC-1 in white adipocytes converts them to a more BAT-like phenotype. Therefore, it seems that PGC-1 plays a key role in the transcriptional programme of adaptive thermogenesis occurring in BAT and, possibly, other tissues. These findings could stimulate the development of promising novel concepts of anti-obesity therapy.

Issues and trends

The remarkable progress in the identification of novel targets for anti-obesity agents has prepared the ground for intensive efforts in drug discovery. Although it is questionable whether all of the mechanisms found in obese rodents also apply to humans, several rare monogenic forms of human obesity have confirmed the importance of some of these regulatory systems. In all other cases, early clinical concept validation might help to prevent failure in later stages of development. In the future, human genomic studies might even identify subgroups of obese patients who would respond particularly well to drugs with a mechanism of action that is tailored to their pathophysiology. However, a limiting factor in all drug discovery efforts is the requirement that a target must be suitable for

pharmacological intervention (i.e. be 'drugable'). In this respect, GPCRs are preferable because it is known that their function can be influenced by low molecular weight ligands, whereas other targets, such as cytokine receptors, are not readily affected by small molecules.

The high number of approaches in preclinical research contrasts sharply with the small number of compounds in clinical development, which suggests that the search for anti-obesity agents is inherently difficult. Indeed, it is a formidable task to find centrally acting agents that reduce meal size and frequency, while not curtailing the pleasure of eating. Similarly, it is difficult to conceive that new drugs could inhibit fat absorption to a higher degree than lipase inhibitors and be better tolerated. There is probably also a limit to the extent to which thermogenesis can be increased without serious side-effects or the stimulation of compensatory mechanisms.

From a clinical point of view it can be assumed that different drugs will be needed for the initial phase of weight loss and for the subsequent phase of weight maintenance, when counter-regulatory mechanisms are activated and lead to relapse or rebound. In a majority of individuals, it will be necessary to prescribe

a combination of drugs to prevent compensatory phenomena and to achieve the maximum therapeutic benefit (e.g. combinations of centrally acting agents with different mechanisms of action or of a centrally and a peripherally acting agent). For the clinician's risk-benefit analysis it will also be important to consider whether a new anti-obesity agent is to be targeted to morbidly obese patients or is intended for use as a so-called lifestyle drug.

In any case, anti-obesity drugs should only be prescribed as an adjunct to diet and exercise, and there will never be a miracle drug that allows patients to slim despite an increased food intake or reduced physical activity. Furthermore, with any drug, weight loss will remain a slow and time-consuming process, owing to the high caloric content of triglycerides. Finally, it should be emphasized that the ultimate therapeutic goal in the treatment of obesity is not weight loss, but rather a reduction in morbidity and mortality from associated diseases. Such considerations would favour new anti-obesity agents that not only affect weight control but also improve metabolic and cardiovascular function. For the time being it is not known whether this can be more easily achieved with centrally or peripherally acting compounds.

References

- 1 Van der Ploeg, L.H.T. (2000) Obesity: an epidemic in need of therapeutics. *Curr. Opin. Chem. Biol.* 4, 452–460
- 2 McNeely, W. and Goa, K.L. (1998) Sibutramine. A review of its contribution to the management of obesity. *Drugs* 56, 1093–1124
- 3 Hvizdos, K.M. and Markham, A. (1999) Orlistat. A review of its use in the management of obesity. *Drugs* 58, 743–760
- 4 Campfield, L.A. *et al.* (1998) Strategies and potential molecular targets for obesity treatment. *Science* 280, 1383–1387
- 5 Bray, G.A. and Tartaglia, L.A. (2000) Medicinal strategies in the treatment of obesity. *Nature* 404, 672–677
- 6 Friedman, J.M. and Halaas, J.L. (1998) Leptin and the regulation of body weight in mammals. *Nature* 395, 763–770
- 7 Schwartz, M.W. *et al.* (2000) Central nervous system control of food intake. *Nature* 404, 661–671
- 8 Himms-Hagen, J. (1999) Physiological roles of the leptin endocrine system: differences between mice and humans. *Crit. Rev. Clin. Lab. Sci.* 36, 575–655
- 9 Strosberg, A.D. and Issad, T. (1999) The involvement of leptin in humans revealed by mutations in leptin and leptin receptor genes. *Trends Pharmacol. Sci.* 20, 227–230
- 10 Farooqi, I.S. *et al.* (1999) Effects of recombinant leptin therapy in a child with congenital leptin deficiency. *New Engl. J. Med.* 341, 879–884
- 11 Heymsfield, S.B. *et al.* (1999) Recombinant leptin for weight loss in obese and lean adults. *J. Am. Med. Assoc.* 282, 1568–1575
- 12 Ahima, R.S. *et al.* (1996) Role of leptin in the neuroendocrine response to fasting. *Nature* 382, 250–252
- 13 Gloaguan, I. *et al.* (1997) Ciliary neurotrophic factor corrects obesity and diabetes associated with leptin deficiency and resistance. *Proc. Natl. Acad. Sci. U. S. A.* 94, 6456–6461
- 14 Bjorbaek, C. *et al.* (1998) Identification of SOCS-3 as a potential mediator of central leptin resistance. *Mol. Cell* 1, 619–625
- 15 Sahu, A. and Kalra, S.P. (1993) Neuropeptidergic regulation of feeding behaviour. Neuropeptide Y. *Trends Endocrinol. Metab.* 4, 217–224
- 16 Gerald, C. *et al.* (1996) A receptor subtype involved in neuropeptide-Y-induced food intake. *Nature* 382, 168–171
- 17 Schaffhauser, A.O. *et al.* (1997) Inhibition of food intake by neuropeptide YY₅ receptor antisense oligodeoxynucleotides. *Diabetes* 46, 1792–1798
- 18 Criscione, L. *et al.* (1998) Food intake in free-feeding and energy-deprived lean rats is mediated by the neuropeptide Y₅ receptor. *J. Clin. Invest.* 15, 2136–2145
- 19 Marsh, D.J. *et al.* (1998) Role of the Y₅ neuropeptide Y receptor in feeding and obesity. *Nat. Med.* 4, 718–721
- 20 Kanatani, A. *et al.* (2000) L-152,804: orally active and selective neuropeptide YY₅ receptor antagonist. *Biochem. Biophys. Res. Commun.* 272, 169–173
- 21 Benoit, S.C. *et al.* (2000) A novel selective melanocortin-4 receptor agonist reduces food intake in rats and mice without producing aversive consequences. *J. Neurosci.* 20, 3442–3448
- 22 Vaisse, C. *et al.* (2000) Melanocortin-4 receptor mutations are a frequent and heterogeneous cause of morbid obesity. *J. Clin. Invest.* 106, 253–262
- 23 Farooqi, I.S. *et al.* (2000) Dominant and recessive inheritance of morbid obesity associated with melanocortin 4 receptor deficiency. *J. Clin. Invest.* 106, 271–279
- 24 Chen, A.S. *et al.* (2000) Inactivation of the mouse melanocortin-3 receptor results in increased fat mass and reduced lean body mass. *Nat. Genet.* 26, 97–102
- 25 Shimada, M. *et al.* (1998) Mice lacking melanin-concentrating hormone are hypophagic and lean. *Nature* 39, 670–674
- 26 Saito, Y. *et al.* (1999) Molecular characterization of the melanin-concentrating hormone-receptor. *Nature* 400, 265–269
- 27 Chambers, J. *et al.* (1999) Melanin-concentrating hormone is the cognate ligand for the orphan G-protein coupled receptor SLC-1. *Nature* 400, 261–265
- 28 Sakurai, T. *et al.* (1998) Orexins and orexin receptors: a family of hypothalamic neuropeptides and G-protein coupled receptors that regulate feeding behaviour. *Cell* 92, 573–585
- 29 Chemelli, R. (1999) Narcolepsy in orexin knockout mice: molecular genetics of sleep regulation. *Cell* 98, 437–451
- 30 Siegel, J. (1999) Narcolepsy: a key role for hypocretins (orexins). *Cell* 98, 409–412
- 31 Kristensen, P. *et al.* (1998) Hypothalamic CART is a new anorectic peptide regulated by leptin. *Nature* 393, 72–76
- 32 Lambert, P.D. *et al.* (1998) CART peptides in the central control of feeding and interactions with neuropeptide Y. *Synapse* 29, 293–298
- 33 Howard, A.D. *et al.* (2000) Identification of receptors for neuromedin U and its role in feeding. *Nature* 406, 70–74
- 34 Cone, R.D. (2000) Editorial: the corticotropin-releasing hormone system and feeding behaviour – a complex web begins to unravel. *Endocrinology* 141, 2713–2714
- 35 Brünig, J.C. *et al.* (2000) Role of brain insulin receptor in control of body weight and reproduction. *Science* 289, 2122–2125
- 36 Loftus, T.M. *et al.* (2000) Reduced food intake and body weight in mice treated with fatty acid synthase inhibitors. *Science* 288, 2379–2381
- 37 Bray, G.A. (2000) Afferent signals regulating food intake. *Proc. Nutr. Sci.* 59, 373–384
- 38 van Dijk, G. and Thiele, T.E. (1999) Glucagon-like peptide-1 (7–36) amide: a central regulator of satiety and interoceptive stress. *Neuropeptides* 33, 406–414
- 39 Seeley, R.J. *et al.* (2000) The role of CNS glucagon-like peptide 1 (7–36) amide receptors in mediating the visceral illness effects of lithium chloride. *J. Neurosci.* 20, 1616–1621

- 40 Tang-Christensen, M.T. *et al.* (2000) The proglucagon-derived peptide, glucagon-like peptide-2, is a neurotransmitter involved in the regulation of food intake. *Nat. Med.* 6, 802–807
- 41 Inui, A. (2000) Transgenic approach to the study of body weight regulation. *Pharmacol. Rev.* 52, 35–61
- 42 Collier, G.R. *et al.* (2000) Beacon: a novel gene involved in the regulation of energy balance. *Diabetes* 49, 1766–1771
- 43 Tschöp, M. *et al.* (2000) Ghrelin induces adiposity in rodents. *Nature* 407, 908–913
- 44 Stahl, A. *et al.* (1999) Identification of the major intestinal fatty acid transport protein. *Mol. Cell* 4, 299–308
- 45 Smith, S.J. *et al.* (2000) Obesity resistance and multiple mechanisms of triglyceride synthesis in mice lacking Dgat. *Nat. Genet.* 25, 87–90
- 46 Lowell, B.B. (1998) Adaptive thermogenesis: turning on the heat. *Curr. Biol.* 8, R517–R520
- 47 Klingenberg, M. and Huang, S.G. (1999) Structure and function of the uncoupling protein from brown adipose tissue. *Biochim. Biophys. Acta* 1415, 271–296
- 48 Weyer, C. *et al.* (1999) Development of β -3 adrenoceptor agonists for the treatment of obesity – an update. *Diabetes Metab.* 25, 11–21
- 49 Rolfe, D.F.S. and Brown, G.C. (1997) Cellular energy utilization and molecular origin of standard metabolic rate in mammals. *Physiol. Rev.* 77, 731–758
- 50 Boss, O. *et al.* (2000) Uncoupling protein 2 and 3: potential regulators of mitochondrial energy metabolism. *Diabetes* 49, 143–156
- 51 Schrauwen, P. *et al.* (1999) Skeletal muscle uncoupling protein 3 expression is a determinant of energy expenditure in Pima Indians. *Diabetes* 48, 146–149
- 52 Agropoulos, G. *et al.* (1998) Effects of mutations in the human uncoupling protein 3 gene on the respiratory quotient and fat oxidation in severe obesity and type 2 diabetes. *J. Clin. Invest.* 102, 1345–1351
- 53 Clapham, J.C. *et al.* (2000) Mice overexpressing human uncoupling protein-3 in skeletal muscle are hyperphagic and lean. *Nature* 406, 415–418
- 54 Gong, D.W. *et al.* (2000) Lack of obesity and normal response to fasting and thyroid hormone in mice lacking uncoupling protein-3. *J. Biol. Chem.* 275, 16251–16257
- 55 Spiegelman, B.M. (1998) PPAR- γ : adipogenic regulator and thiazolidinedione receptor. *Diabetes* 47, 507–514
- 56 Kubota, N. *et al.* (1999) PPAR- γ mediates high-fat diet-induced adipocyte hypertrophy and insulin resistance. *Mol. Cell* 4, 597–609
- 57 Anand, A. and Chada, K. (2000) *In vivo* modulation of Hmgic reduces obesity. *Nat. Genet.* 24, 377–380
- 58 Martinez-Botas, J. *et al.* (2000) Absence of perilipin results in leanness and reverses obesity in *Lepr^{db/db}* mice. *Nat. Genet.* 26, 474–479
- 59 Kim, J.K. *et al.* (2000) Mechanism of insulin resistance in A-ZIP/F-1 fatless mice. *J. Biol. Chem.* 275, 8456–8460
- 60 Wu, Z. *et al.* (1999) Transcriptional activation of adipogenesis. *Curr. Opin. Cell Biol.* 11, 689–694

Chemical name

C75: *trans*-tetrahydro-3-methylene-2-oxo-5-*n*-octyl-4-furancarboxylic acid

Cardiac mechanotransduction: from sensing to disease and treatment

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In heart muscle a mechanical stimulus is sensed and transformed into adaptive changes in cardiac function by a process called mechanotransduction. Adaptation of heart muscle to mechanical load consists of neurohumoral activation and growth, both of which decrease the initial load. Under prolonged overload this process becomes maladaptive, leading to the development of left ventricular hypertrophy and ultimately to heart failure. Widespread synergism and crosstalk among a variety of molecules and signals involved in hypertrophic signaling pathways make the prevention or treatment of left ventricular hypertrophy and heart failure a challenging task. Therapeutic strategies should include either a complete and continuous reduction of load or normalization of left ventricular mass by interventions aimed at specific targets involved in mechanotransduction.

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The process of sensing mechanical load and the consequent physiological responses is called mechanotransduction. In heart muscle, a mechanical stimulus is transformed into altered contraction force, altered ion balance, exocytosis or gene expression. In cardiac myocytes, mechanotransduction can be divided into a series of events, from the coding of the mechanical stimuli to second messengers and decoding the information into changes in heart function (Fig. 1). The same mechanosensors, signals, kinases and transcription factors are involved in both normal and pathological hypertrophy. Therefore, any given pharmacological intervention aimed at treating or preventing hypertrophy might influence not only pathological

developments but also normal adaptation and mechanotransduction itself. In this article, present and future pharmacological approaches will be discussed in the context of mechanotransduction.

Stretch-sensitive molecular elements in cardiac myocytes

In cardiac myocytes, Ca^{2+} defines contractile function but also serves as a second messenger that is able to control many other cellular functions (Figs 1,2). Therefore, it is not surprising that early events induced by mechanical stretch of cardiac muscle include an increase of contraction force, partly caused by an increase of the systolic Ca^{2+} transients¹. The nonselective cation channels that can be activated by longitudinal stretch of the cells² could be the possible stretch transducers. When opened, these channels cause an influx of Na^{+} and Ca^{2+} , both of which are able to augment the Ca^{2+} transients via sarcoplasmic reticulum (SR) Ca^{2+} loading. Several agents such as aminoglycoside antibiotics and Gd^{3+} are known to inhibit these stretch-activated (SA)-channels but are relatively nonspecific. Indeed, the early events in mechanotransduction are inaccessible to pharmacological tools. For example, in isolated cardiac trabeculae even almost total inhibition of SR function with ryanodine and cyclopiazonic acid does