

Thermogenic and metabolic antiobesity drugs: rationale and opportunities

J. C. Clapham¹ and J. R. S. Arch²

¹Department of Molecular Pharmacology, AstraZeneca R & D Mölndal, Mölndal, Sweden

²Clore Laboratory, University of Buckingham, Buckingham, UK

Antiobesity drugs that target peripheral metabolism may avoid some of the problems that have been encountered with centrally acting anorectic drugs. Moreover, if they cause weight loss by increasing fat oxidation, they not only address a cause of obesity but also should promote loss of fat rather than lean tissue and improve insulin sensitivity. Weight loss may be slow but more sustained than with anorectic drugs, and thermogenesis may be insufficient to cause any discomfort. Some thermogenic approaches are the activation of adrenergic, thyroid hormone or growth hormone receptors and the inhibition of glucocorticoid receptors; the modulation of transcription factors [e.g. peroxisome proliferator-activated receptor δ (PPAR δ) activators] or enzymes [e.g. glutamine fructose-6-phosphate amidotransferase (GFAT) inhibitors] that promote mitochondrial biogenesis, and the modulation of transcription factors (PPAR α activators) or enzymes (AMP-activated protein kinase) that promote fatty acid oxidation. More surprisingly, studies on genetically modified animals and with enzyme inhibitors suggest that inhibitors of fatty acid synthesis [e.g. ATP citrate lyase, fatty acid synthase, acetyl-CoA carboxylase (ACC)], fatty acid interconversion [stearoyl-CoA desaturase (SCD)] and triglyceride synthesis (e.g. acyl-CoA : diacylglycerol acyltransferase) may all be thermogenic. Some targets have been validated only by deleting genes in the whole animal. In these cases, it is possible that deletion of the protein in the brain is responsible for the effect on adiposity, and therefore a centrally penetrant drug would be required. Moreover, whilst a genetically modified mouse may display resistance to obesity in response to a high fat diet, it requires a tool compound to demonstrate that a drug might actually cause weight loss. Even then, it is possible that differences between rodents and humans, such as the greater thermogenic capacity of rodents, may give a misleading impression of the potential of a drug.

Keywords: hormone mimetics, hypothalamus, lipid metabolism, mitochondrial biogenesis, obesity drugs, thermogenesis

Received 1 April 2005; returned for revision 1 March 2006; revised version accepted 9 March 2006

Introduction

It is well recognized that the onslaught of obesity is a serious problem for affluent societies. Many reviews over recent years have documented the pandemic, its possible causes and actual and potential treatments, including pharmacotherapy. There are, however, only two widely approved drugs for the treatment of obesity:

sibutramine, which acts centrally to inhibit serotonin and noradrenaline reuptake, and orlistat, which acts in the gut to inhibit fat digestion. Rimonabant, which is an inverse agonist of the CB₁ cannabinoid receptor, may be approved in 2006, having produced good weight loss in phase III clinical trials [1]. It acts centrally to reduce the motivation to eat, especially the consumption of palatable food, and may also act centrally to activate

Correspondence:

Jonathan R. S. Arch, Clore Laboratory, University of Buckingham, Buckingham MK18 1EG, UK.

E-mail:

jon.arch@buckingham.ac.uk

thermogenesis [2,3]. Whether its actions at the CB-1R in adipose tissue [4,5] contribute significantly to its effects on energy balance is unclear. A number of other centrally acting drugs (e.g. fenfluramine and phentermine) have been withdrawn due to either their toxicity or their abuse potential, or their use is heavily regulated. Others, such as ephedrine and its close analogue phenylpropanolamine, may be obtained over the counter in some countries, but adverse effects are a serious concern.

Concern about the safety of centrally acting drugs is one reason why pharmaceutical companies are seeking alternative approaches for the treatment of obesity. Perhaps the fact that orlistat is not even absorbed from the gut is one factor that weighed in favour of a good benefit-to-risk ratio, despite its relatively low antiobesity efficacy [6,7]. Fortunately, studies on genetically modified rodents are revealing many peripheral (or apparently peripheral – see *Hypothalamic Targets and Their Implications for Drug Discovery*) targets for antiobesity drugs [8]. Broadly speaking, these alternative approaches might be termed *metabolic*, meaning that the drug regulates fat or carbohydrate metabolism by a means other than altering the level of a neurotransmitter or the activation of a neuronal receptor, or *thermogenic*, meaning that the drug increases energy expenditure. Clearly, many drugs might be both metabolic and thermogenic, but it is possible for metabolic antiobesity drugs to reduce energy intake and for drugs that target neuronal receptors to be thermogenic. It should also be understood that the initial site of action of both metabolic and thermogenic drugs can be in the brain. Metabolic and thermogenic drugs should preferably act in the periphery, however, if they are to avoid exceptional scrutiny by regulatory authorities.

Rationale for Thermogenic Drugs

Aetiology of Obesity

There are aetiological arguments for thermogenic drugs being a rational approach to the treatment of obesity. The first of these is that reduced energy expenditure due to our modern lifestyles shares, with increased energy intake, responsibility for the modern obesity epidemic. Second, those who are more susceptible to this 'obesogenic' environment have a lower daily energy expenditure [9] and seem to expend less energy on everyday activities that involve muscle movement ('non-exercise activity thermogenesis') [10,11]. Whether drugs other than CNS stimulants can increase muscular activity is questionable, but a recent study found that a low *resting* metabolic rate is also predictive of susceptibility to

obesity [12]. Resting metabolic rate may be more amenable to pharmacotherapy, but its association with obesity is more controversial [13]. Once they have become obese, humans actually tend to have an elevated resting metabolic rate and so must consume more energy than lean people to maintain their obesity. It does not necessarily follow that they became obese by overeating, however. There are examples of genetically modified obese mice in which hyperphagia develops after obesity [14]. Moreover, some, though not all, studies suggest that slimmed down ('post-obese') subjects have a reduced metabolic rate [15].

A third argument, specifically for thermogenic drugs that stimulate fat oxidation, is that fat oxidation is defective in people who are susceptible to obesity, both when they are obese and after they have slimmed [16,17]. The significance of this finding is that fat storage is poorly regulated in all subjects (hence obesity is a problem) compared with carbohydrate (glycogen) and protein storage, which are under much tighter control [18]. Those who resist obesity display increased fat oxidation in response to increased dietary fat, whereas obese subjects seem unable to increase fat oxidation, as assessed by the measurement of respiratory quotient. In other words, those who are susceptible to obesity have even less control of the size of their fat stores than others, because they cannot burn off excess fat.

Irrespective of whether obese subjects have a generally low energy expenditure or a defect specifically in fat oxidation, the phenomenon is likely to reside partly in skeletal muscle, which accounts for 20% of basal metabolic rate [19] and a greater proportion of daily energy expenditure. It is therefore not surprising that differences in respiratory quotient between lean and obese subjects are also seen in muscle biopsy samples [20]. These differences may reflect muscle fibre type. Human skeletal muscle is composed of slow (type I) fibres, which are predominantly oxidative and use fatty acids as their fuel, and fast (type II) twitch fibres. The latter can be predominantly glycolytic (type IIb) or mixed glycolytic and oxidative (type IIa). Increased type I fibre content is associated with a tendency for reduced adiposity and reduced type I fibre content with obesity. As with respiratory quotient, fibre type influences successful maintenance of weight loss. For example, following bariatric surgery weight loss was directly proportional to the proportion of type I oxidative fibres in the rectus abdominus [21].

Therapeutic Benefits

Further arguments for thermogenic drugs are that those that promote fat oxidation – this includes those that in

some way mimic the sympathetic nervous system – cause selective loss of fat and improvements in insulin sensitivity beyond those expected from the reduction in body weight. Thus a variety of evidence demonstrates that sympathomimetic agents, such as β_3 -adrenoceptor agonists, depend on fat oxidation for thermogenesis, and, provided food intake does not decrease, all the weight loss that results is due to fat loss [22,23]. Weight loss in response to moderately reduced energy intake, by contrast, typically includes about 15–25% of fat-free mass, though this proportion can vary widely [24,25]. Insulin sensitivity may be improved, because the stimulation of fat oxidation causes rapid falls in lipid metabolites, such as diacylglycerol, that are known to inhibit insulin signalling [22,26]. By contrast, the triglyceride stores that cause adiposity are so large that it must take longer for increased fat oxidation to deplete them.

Thermogenic drugs that mimic the sympathetic nervous system or depend on the capacity for fat oxidation may not cause as much weight loss as anorectic drugs in the short term. Past and present anorectic drugs initially produce weight loss of about 0.23 kg per week [27,28]. If 15% of this weight loss is lean tissue, which consists of 75% water and contains 1 kcal per gram, and 85% is fat, which contains 9 kcal per gram, then the loss of energy per day is 256 kcal. This is roughly a 10% alteration in energy balance for an obese subject. The uncoupling agent dinitrophenol (see *Miscellaneous Intracellular Targets*) may have increased energy expenditure by much more than 10%, but most thermogenic drugs – at least those that in some way mimic the sympathetic nervous system or depend on the capacity for fat oxidation – are unlikely to have much more effect than anorectic drugs. Thus isoprenaline increases energy expenditure by at most 30% in humans [29], and a drug acting by a similar mechanism would do well to use a third of this capacity.

A disadvantage of selective fat loss, which adds to the possible problem of slow weight loss, is that for the same negative energy balance there is less effect on body weight: the historical value of 0.23 kg per week for anorectic drugs quoted above becomes 0.20 kg for a drug that has the same percentage effect on energy balance but causes only fat loss [22,23]. Nevertheless, if this weight loss was achieved in a 100-kg subject for just 6 months, the resultant weight loss of 5.2 kg would meet the FDA goal of 5% weight loss relative to placebo over a year or the EMEA goal of 10% weight loss, which includes weight loss achieved by lifestyle modification – typically another 5%. It would be more difficult to achieve regulatory goals if a

sympathomimetic thermogenic drug (like exercise) increased the amount of lean tissue [22,23].

Satisfying regulatory authorities is different from satisfying patients, who would value much greater weight loss. Because exercise seems to be important in maintaining long-term weight loss [30], perhaps thermogenic drugs would be especially beneficial in those subjects who could achieve rapid initial weight loss by dieting or with the aid of an anorectic drug. Indeed, it is possible that weight loss in response to a thermogenic drug would last longer than the 6 months generally seen with anorectic drugs. In rodents at least, the effect of sympathomimetic agents increases in the early stages of treatment, provided animals are obese and can therefore provide fuel for thermogenesis [22]. Obese animals do not normally compensate by eating more, just as moderate exercise in obese humans does not in the short-term increase food intake [31]. By contrast, various centrally acting agents, such as sibutramine, phentermine and bupropion, reduce food intake for a few days at most in rodents, and it is thermogenesis that accounts for their antiobesity activity over a period of weeks [32–34]. This does not mean that anorectic agents are no longer influencing food intake: body weight stays below the control level, and if the anorectic agent is withdrawn, then there is a rebound hyperphagia. It seems therefore that the effect of the anorectic agent meets opposition from counter-regulatory forces. Where such forces (e.g. a low plasma leptin level) are due to loss of fat, they will oppose both thermogenic and anorectic agents [35,36]. However, signals that indicate that the gut is empty or that an individual is hedonically or socially deprived will only oppose anorectic drugs.

There is a common concern that taking a thermogenic drug will make people feel permanently hot, as happened with dinitrophenol (see *Miscellaneous Intracellular Targets*). It is important to note, however, that if a thermogenic drug increases energy expenditure by 10 or even 20%, then it is unlikely to produce discomfort. In one study, ‘sitting and fidgeting’ increased energy expenditure by 54%, and walking at only 1.6 km/h increased it by 154% compared with lying down [10]. Of course, this means that those who fidget or walk for much of the day are unlikely to be obese.

Biochemistry and Endocrinology of Thermogenesis

How does the energy generated by thermogenic drugs end up as heat? The great majority of the chemical energy in the macronutrients is funnelled through NADH or (in the mitochondrion) FADH. Most of the

energy carried by these coenzymes is then used to produce ATP, mainly by oxidative phosphorylation in the mitochondrion. A small amount of energy is funnelled through NADPH, which is used in various anabolic reactions. Together with NADH, NADPH can also be consumed by non-mitochondrial oxidases that do not conserve chemical energy by producing ATP (figure 1).

Thermogenic agents must increase either the utilization of ATP or the oxidation of the reduced coenzymes by pathways that are not coupled to ATP synthesis, for example by uncoupling oxidative phosphorylation. Excluding growth, in which some of the energy in ATP is retained, ATP utilization may involve ion transport, simple substrate cycles or more complex cycles. An example of a simple substrate cycle is the phosphorylation of fructose-6-phosphate to fructose-1,6-bisphosphate and hydrolysis of the bisphosphate back to the monophosphate. More complex cycles include triglyceride and protein turnover, and the Cori cycle, in which glucose and lactate are transferred between muscle and liver.

In the resting state, about 90% of oxygen consumption takes place in the mitochondrion, and 80% of this is coupled to ATP synthesis. Perhaps 30% of the ATP is used for Na^+/K^+ and Ca^{2+} pumps, 30% for protein synthesis and 20% for gluconeogenesis, ureagenesis and in turning over carbohydrate and lipid stores [19].

Resting metabolic rate in humans can be increased by about 30% by the sympathetic nervous system [29] and by about 15% by growth hormone [37]. Thyroid hormones can increase metabolic rate by about 15%, and hypothyroidism reduces it by 30% [38]. Glucocorticoids suppress energy expenditure in rodents [39], but in humans cortisol and methylprednisolone have been shown to increase resting energy expenditure by up to 15% and about 7%, respectively [40,41].

Uncoupling of oxidative phosphorylation in brown adipose tissue is a major thermogenic mechanism in

response to sympathetic activation in rodents, but in humans, the stimulation of ATP utilization is more important. Thyroid hormones sensitize tissues to the sympathetic nervous system and also have a number of independent effects [42], including the stimulation of protein turnover. The net effect on protein turnover involves more loss of lean tissue than does a low energy diet [23]. Growth hormone, by contrast, stimulates protein synthesis and conserves protein. It also promotes lipolysis and fatty acid oxidation [43]. Glucocorticoids suppress sympathetic activity and the responsiveness of brown adipose tissue to sympathetic activity in rodents [44]. In humans, however, the stimulation of protein turnover and catabolism may have the predominant influence on energy expenditure.

Hormone Mimetics and Antagonists

Various approaches have been employed to mimic or (in the case of glucocorticoids) block these hormone systems to develop thermogenic drugs (figure 2).

Thyroid Hormones

Thyroid hormones were used to treat obesity in the 1890s. Weight loss was, however, accompanied by increased protein catabolism and cardiac stimulation. Selective stimulants of the thyroid hormone receptor- β may have less effect on the heart than thyroid hormones [45]. One such compound, GC-1, reduced body weight in cynomolgus monkeys by 4% over 7 days without any evidence of muscle wasting. There was also, however, no evidence of muscle wasting over 7 days in response to triiodothyronine at a dose level that caused similar weight loss [46]. Other potentially deleterious effects, such as bone loss, fatigue and CNS effects, were not evaluated [47].

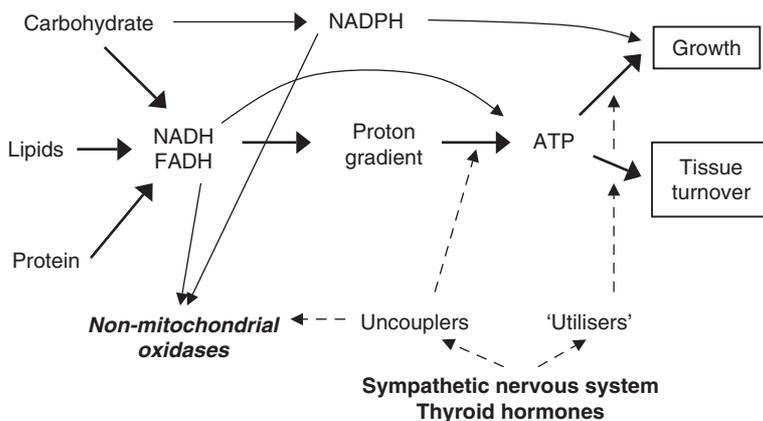
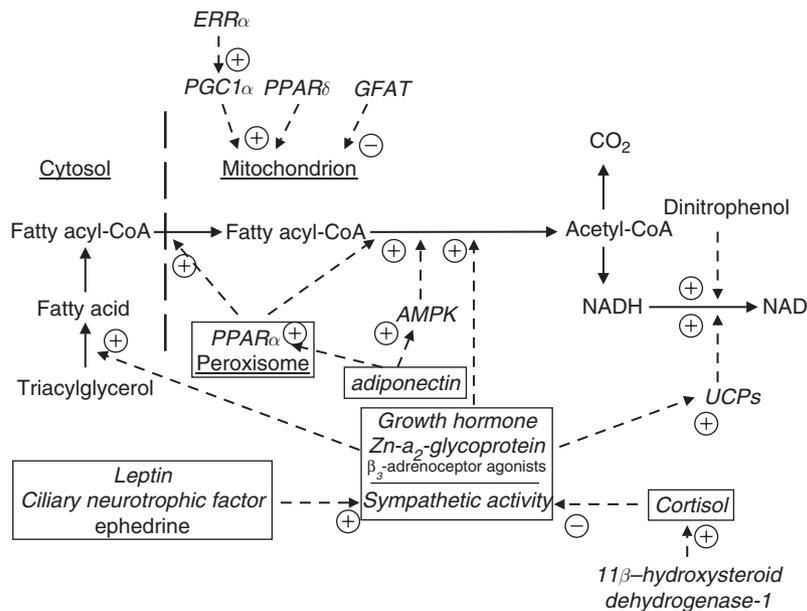


Fig. 1 Production and utilization of reduced adenine dinucleotides and ATP. Hormones and drugs that increase energy expenditure must increase either the utilization of ATP or the oxidation of reduced coenzymes by pathways that are not coupled to ATP synthesis.

Fig. 2 Targets in triglyceride catabolism. Enzymes, transcription factors and hormones are shown in italics. The dashed arrows pointing to 'mitochondrion' indicate factors that affect mitochondrial biogenesis. Peroxisome proliferator-activated receptor α (PPAR α) promotes peroxisomal proliferation in rodents. ERR, oestrogen-related receptor; PGC, PPAR γ coactivator; GFAT, glutamine fructose-6-phosphate amidotransferase; AMPK, AMP-activated protein kinase; UCP, uncoupling protein.



Sympathetic Nervous System

Most anorectic drugs also increase energy expenditure [48]. Thermogenesis may, like the anorectic effect, be initiated centrally, or it may be a direct peripheral action. For example, ephedrine acts centrally to raise sympathetic activity and peripherally to stimulate the release of noradrenaline from nerve endings, as well as to directly activate β -adrenoceptors in non-neuronal tissues. Sibutramine, by contrast, seems only to increase sympathetic activity, though whether this contributes to weight loss in humans is unclear [49].

Unfortunately, sibutramine not only increases sympathetic outflow to tissues that are important for thermogenesis but also raises heart rate and blood pressure. Ephedrine has the same problems. Indeed, it is advisable to be cautious when any novel, centrally acting agent raises energy expenditure, because this may be due to a generalized increase in sympathetic activity. There is some evidence that it might be possible to activate the sympathetic nervous system selectively with a centrally acting agent [50], but this has not been demonstrated with a drug candidate.

β_3 -Adrenoceptor agonists stimulate fat oxidation in rodents without producing the cardiovascular side effects associated with β_1 - and β_2 -adrenoceptor stimulation. Unfortunately, the human β_3 -adrenoceptor differs from the rodent receptor, and those compounds that selectively activate the human receptor have mostly displayed poor oral bioavailability or have been rapidly excreted. Moreover, β_3 -adrenoceptor agonists have less

effect on energy expenditure in humans than in rodents. Weight loss following 28 days' treatment with the β_3 -adrenoceptor agonist L-796568 was correlated with the blood level of the compound [51], but the results were not deemed sufficiently exciting to attempt to improve bioavailability further or conduct a longer trial. A longer (12 week) study with ephedrine produced significant weight loss [52].

Lipid-mobilizing factor/Zn- α_2 -glycoprotein is produced by tumours and adipose tissue [53] and has been claimed to stimulate β_3 -adrenoceptors [54,55], though perhaps it promotes responses mediated by any G_{α_s} -coupled receptor. Its effects on energy balance and body composition are similar to those of β_3 -adrenoceptor agonists, raising the possibility that, as an injectable drug, it could be successful where β_3 -adrenoceptor agonists have so far failed.

Growth Hormone

AOD 9401 is a synthetic analogue of growth hormone in phase II clinical trials that is reported not to affect blood glucose or growth [56]. Weight loss of 2 kg over 12 weeks relative to placebo was achieved with a dose of 1 mg per day in a phase IIa study, but higher doses were less effective [57]. The obvious suggestion that this was because higher doses cause a gain in lean body mass that equalled the loss in fat mass seems to have been ruled out. A further study is being conducted at doses of 1 mg and below.

Leptin

Leptin is released primarily from adipocytes and signals to the brain whether fat stores are adequate. In rodents, leptin not only decreases food intake but also increases energy expenditure, the latter effect being mainly due to increased sympathetic outflow. Presumably, leptin might have similar effects in humans if they are thin enough to have low leptin levels, but marked effects of leptin on energy balance have been demonstrated only in rare obese individuals that totally lack leptin. Leptin and modified leptins have not proved of benefit in most obese subjects [58]. Low molecular weight leptin mimetics that bypass the transporter that takes leptin into the brain may have greater potential [59].

Axokine is a modified version of ciliary neurotrophic factor, which shares some signalling mechanisms with leptin. It is therefore no surprise that Axokine increases energy expenditure and produces weight loss that is not all due to reduced food intake [60,61]. Because leptin raises energy expenditure by increasing sympathetic outflow, it is likely that Axokine acts similarly. Axokine performed well in phase II trials, but less well in phase III, possibly due to the generation of neutralizing antibodies.

Adiponectin

Adiponectin is a protein that is secreted by adipose tissue, especially by small adipocytes. It reduces weight gain and adipose tissue mass by stimulating fatty acid oxidation in muscle and liver. It seems to play an important role in the insulin sensitizing effects of thiazolidinedione antidiabetic drugs [62,63] (though these cause weight gain through other mechanisms). The discovery of two receptors for adiponectin provides possible new targets, though whether these receptors are amenable to small molecular weight activators remains to be seen [64].

Glucocorticoids

The glucocorticoid system is usually seen as offering targets for antidiabetic rather than antiobesity drugs. It clearly has a profound effect on energy balance, however, as evidenced by visceral obesity in Cushing's syndrome and the ability of adrenalectomy to prevent most forms of rodent obesity. Simply inhibiting cortisol production or blocking the glucocorticoid receptor activates counter-regulatory mechanisms. One approach to this problem has been to make compounds that, because of their pharmacokinetics, inhibit only the liver enzyme. However, while this gave a compound with antidiabetic

activity, its only antiobesity activity was that of preventing rosiglitazone-induced weight gain [65].

A more subtle approach is to reduce the conversion of cortisone (inactive) to cortisol (active) in target tissues (11-dehydrocorticosterone to corticosterone in rodents) by inhibiting 11 β -hydroxysteroid dehydrogenase-1 (figure 2). Inhibitors may reduce glucocorticoid receptor stimulation within tissues but have little effect on plasma glucocorticoid concentrations or on feedback inhibition of the hypothalamic-pituitary-adrenal axis. They improve insulin sensitivity in mice, partly by reducing food intake [66,67], but possibly also by increasing energy expenditure in mice [68]. Moreover, mice that lack 11 β -hydroxysteroid dehydrogenase have raised energy expenditure and are protected from diet-induced obesity [69]. Whether these effects are peripherally or centrally mediated and whether they will translate to humans remain to be established. Recent evidence suggests that reduced levels of corticosterone specifically in adipose tissue protect mice from diet-induced obesity, but, in contrast to the knockout mouse, this appears to be due to decreased food intake rather than increased energy expenditure. Thus energy expenditure was increased relative to body weight, which is not surprising [70,71], but not in absolute terms [72].

Miscellaneous Intracellular Targets

Uncoupling Agents

Dinitrophenol, which uncouples the oxidation of NADH and FADH from ATP synthesis in the mitochondrion, was used as an antiobesity agent in the 1930s. Weight loss was as much as 3 kg per week but side effects included sweating and, more seriously, hypoxia at higher doses [16]. Perhaps lower doses of dinitrophenol or a similar uncoupling agent might retain adequate efficacy and also be safe.

In recent years, there has been greater interest in the uncoupling proteins (UCPs). The identification of at least five paralogs of the brown fat UCP-1 and their putative roles has been reviewed elsewhere [73]. UCP-1, -2 and -3 have been studied most. UCP-1 clearly plays a role in the regulation of energy expenditure and body weight in rodents but is less important in humans. UCP-2 and -3 can uncouple oxidative phosphorylation, but whether they are normally involved in the regulation of energy expenditure is questionable [73]. Irrespective of their normal roles, can any UCP be exploited as a target for antiobesity drugs? UCP-3 is of interest because of its predominant expression in skeletal muscle and because transgenic overexpression of UCP-3 in skeletal muscle produced mice with reduced adiposity, insulin, glucose

and cholesterol levels, despite their eating more than their wild-type littermates [74]. Mitochondria from the transgenic mice were more uncoupled, but there is debate as to whether this is an artefact. There appears to be little current activity in the area of UCP activators, although a series of 2-(1-pyrazolyl) thiazole derivatives have been described by Tularik as increasing UCP-3 mRNA more than 10-fold in rat-derived L6 muscle cells [75].

AMP-Activated Protein Kinase

AMP-activated protein kinase (AMPK) is a multi-subunit enzyme that plays a key role in the regulation of energy provision within cells, initiating cascades that increase fat oxidation, decrease fat synthesis and enhance glucose uptake to maintain cellular energy stores [76]. AMPK appears to have a central role in orchestrating the flux of fatty acid away from triacylglycerol (TAG) synthesis and into β -oxidation. Downstream targets of AMPK include acetyl-CoA carboxylase-2 (ACC2), and perhaps glycerol-3-phosphate acyltransferase (GPAT) (see *Inhibition of Lipid Synthesis*), which are inhibited by phosphorylation, and hexokinase II and UCP-3, which are transcriptionally upregulated by AMPK. AMPK may also play a role in mitochondrial biogenesis in response to endurance training [77].

Recent studies implicate AMPK in the mechanism of action of the antidiabetic agent metformin, which also has a small antiobesity effect, and the adipokines leptin and adiponectin [78]. Leptin and adiponectin exert many of their beneficial effects on metabolism via the activation of AMPK, including stimulation of fatty acid oxidation, uptake of glucose and prevention of tissue accumulation of TAG.

Metformin, leptin and adiponectin do not activate AMPK directly, but they may activate AMPK by raising the cellular AMP concentration [79–81]. In addition, it has been known for many years that 5-aminoimidazole-4-carboxamide 1- β -D-ribofuranoside (AICAR) is phosphorylated within cells to a mimic of AMP that is a potent activator of AMPK. Chronic treatment of obese Zucker *fa/fa* rats with AICAR produces effects similar to those of metformin [82,83]. AICAR probably activates a number of enzymes, however, and so it must not be assumed that its metabolic effects are all mediated by AMPK. Indeed, if they are, AMPK activation may not be a viable approach for the treatment of obesity, because AICAR acutely simulates glucose output from the liver in dogs [84,85]. Studies in which AMPK has been overexpressed in liver have been more encouraging, but liver triglyceride content was raised [86], contrasting with a lowering of liver triglycerides elicited by AICAR [83].

AMPK is activated by various upstream kinases, including the LKB1 tumour suppressor protein kinase [87,88]. Searching for AMPK activators using a whole cell screen is difficult because any agent that raises the AMP : ATP ratio of the cell will cause the activation of AMPK, as has been found for example in the UCP-3 overexpressing mouse [89]. The activation of AMPK in the stearoyl-CoA desaturase-1 (SCD-1) knockout mouse (see *Inhibition of Lipid Synthesis*) might also be due to elevation of the cytosolic AMP concentration [90].

Mitochondrial Biogenesis

Various proteins might be targeted to promote mitochondrial biogenesis or the proportion of type I fibres in skeletal muscle. One of these is peroxisome proliferator-activated receptor γ (PPAR γ) coactivator-1 α (PGC-1 α). This is a transcriptional activator that interacts with a number of nuclear hormone receptors. It is found mainly in type I rather than type II skeletal muscle fibres, and its expression is increased by cold and exercise, which increase mitochondrial biogenesis. Overexpression of PGC-1 α in mouse C2C12 myotubes increased oxygen consumption and mitochondrial biogenesis, and overexpression of in type II fibres resulted in their conversion to a more type I-like phenotype [91].

PGC-1 α itself may not be amenable to small molecular weight drugs because it has no natural small ligands. However, both PGC-1 α and the related PGC-1 β enhance the activity of oestrogen-related receptor α (ERR α), which has a similar tissue distribution to PGC-1 α and promotes fatty acid oxidation [92,93]. An ERR inverse agonist or siRNA directed against ERR α blocked the induction of oxidative phosphorylation genes by PGC-1 [94], supporting the case for ERR α agonists, although surprisingly, mice that lack ERR α are resistant to high fat diet-induced obesity [95].

PGC-1 α coactivates PPAR δ , which may be an important mediator of its effects on fatty acid oxidation. Overexpression of PPAR δ in skeletal muscle increased the proportion of type I fibres [96]. This manipulation or treatment of mice with selective PPAR δ agonists also protected mice from diet-induced obesity [97].

A number of companies are attempting to develop PPAR δ agonists, possibly incorporating activity at other PPARs to improve activity against diabetes and dyslipidaemia. Two notes of caution must be mentioned. First regulatory authorities are concerned about the potential carcinogenicity of novel PPAR agonists of all types. Second, bezafibrate is claimed to be an agonist of both PPAR α and PPAR δ [98], but despite being used in humans for many years, it has not been reported to have an antiobesity

effect. It is possible, however, that bezafibrate is used clinically at a dose that allows it to act as a PPAR α agonist (see below, in this section) but not as a PPAR δ agonist.

PGC-1 α and PPAR δ promote mitochondrial biogenesis in adipose tissue as well as skeletal muscle, in effect converting white into brown adipose tissue, whose main role is lipid oxidation. Overexpression of PPAR δ in adipose tissue alone is sufficient to provide protection from diet-induced obesity [99]. Various other transcription factors and their coactivators and corepressors also affect mitochondrial biogenesis in skeletal muscle and adipose tissue, and may offer, or point to, targets for antiobesity drugs [100].

The enzyme glutamine fructose-6-phosphate amidotransferase (GFAT) may provide a relatively tractable target for drugs that promote mitochondrial biogenesis in skeletal muscle. It has previously been recognized that inhibitors of GFAT have potential as insulin sensitizing drugs [101] but overexpression of GFAT in liver resulted in obesity as well as insulin resistance [102,103]. GFAT regulates the rate of hexosamine synthesis. The terminal metabolite of this pathway is UDP-*N*-acetyl glucosamine, which glycosylates various proteins involved in nutrient sensing. Infusion of glucosamine decreased the expression of a number of mitochondrial genes involved in oxidative phosphorylation in rat skeletal muscle, and whole body

energy expenditure was reduced [103]. Therefore inhibitors of GFAT might increase energy expenditure.

Activation of PPAR α in liver promotes the oxidation of fatty acids [104]. PPAR α knockout mice are mildly obese [105–107], and various PPAR α agonists (nafeno-pin, MEDICA-16, GW-9578, bezafibrate) have anti-obesity activity in rodents, largely, though not entirely, due to increased energy expenditure [108–111]. Some PPAR α agonists, notably oleoylethanolamide, also reduce food intake [112]. Adiponectin increases the expression of PPAR α , and this may contribute to its antiobesity effect in rodents [64]. However, drugs of the fibrate class are PPAR α agonists, and there are no reports that they cause substantial weight loss in humans.

Inhibition of Lipid Synthesis

Paradoxically, inhibitors of fatty acid and triglyceride synthesis (figure 3) usually alter energy balance in rodents by promoting fat oxidation, though some also inhibit food intake. With the exception of ACC inhibition, the primary molecular mechanisms that link inhibition of lipid synthesis with stimulation of lipid oxidation are unclear.

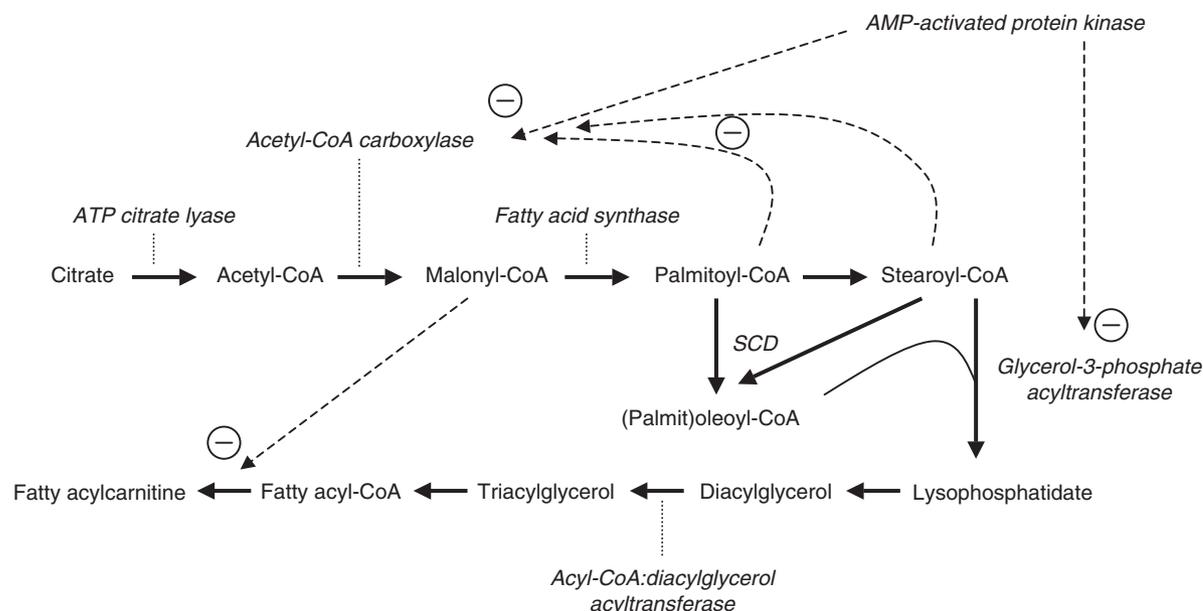


Fig. 3 Target enzymes in lipid synthesis. Acetyl-CoA is produced in the mitochondrion and condenses with oxaloacetate to form citrate. Citrate is transported out of the mitochondrion by the tricarboxylate carrier, so that fatty acid and triglyceride synthesis can take place in the cytosol. Cytosolic citrate is the starting point of the scheme. The scheme also shows that malonyl-CoA inhibits the formation of fatty acyl-carnitine, thereby preventing the entry of fatty acids into the mitochondrion for oxidation. Potential antiobesity drugs must inhibit each of the enzymes, except for AMP-activated protein kinase (AMPK), which inhibits the activity of other enzymes. SCD, stearoyl-CoA desaturase.

Fatty Acid Synthesis

(-)-Hydroxycitric acid is an example of a compound that appears to both increase fat oxidation and reduce food intake [113,114]. It is an inhibitor of ATP citrate lyase, a key enzyme of fatty acid synthesis, and is marketed as a natural supplement for weight management [115]. Like some other targets in lipid synthetic pathways, it is unclear whether peripheral (adipose tissue or liver) or hypothalamic ATP citrate lyase plays the primary role in its anorexic and thermogenic effects [116].

ACC produces malonyl-CoA. Malonyl-CoA is used to initiate fatty acid chain building by fatty acid synthase. ACC1, a cytosolic enzyme found mainly in lipogenic tissues, provides malonyl-CoA to initiate fatty acid chain building by fatty acid synthase. Malonyl-CoA produced by ACC2, an enzyme that is associated with mitochondria and found mainly in oxidative tissues, appears, by contrast, to play the role of a 'fuel sensor'. The malonyl-CoA does this by inhibiting carnitine palmitoyl transferase 1 (CPT1), the role of which is to transfer fatty acids into the mitochondrion for oxidation. Inhibition of ACC2 promotes fatty acid oxidation by reducing the malonyl-CoA concentration and disinhibiting CPT1.

The main evidence for this concept is that ACC2 knockout mouse are lean, despite consuming more food than wild-type mice [117]. Encouragingly, the phenotype was observed in heterozygous mice, which is, arguably, a better indicator of the potential of an enzyme inhibitor. Malonyl-CoA levels were markedly reduced in heart and skeletal muscle, but not in liver, where ACC1 predominates. Nevertheless, hepatic as well as muscle fatty acid oxidation appeared to be increased, as judged by 80–90% lower hepatic lipids in the ACC2 null mouse. Adipocytes from these mice also display increased fatty acid oxidation [118].

Non-selective inhibition of ACC may also be an option. CP-610431 inhibited both ACC1 and ACC2 with IC₅₀ values of around 50 nM and stimulated fatty acid oxidation in human liver and rodent muscle cell lines. A metabolically stable analogue increased fatty acid oxidation *in vivo* [119]. Whether an inhibitor is selective or not, it may be important that it does not penetrate the brain, because hypothalamic malonyl-CoA appears to oppose weight gain (see *Hypothalamic Targets and Their Implications for Drug Discovery*).

The fatty acid synthase inhibitors cerulenin and C75 were originally claimed to inhibit feeding by acting within the brain. The mechanism seemed to involve elevation of hypothalamic malonyl-CoA [120]. (Contrast the benefit of lowering malonyl-CoA in the periphery by

inhibiting ACC2.) Subsequent studies have shown that C75 also promotes fat oxidation, perhaps in part due to a peripheral action [121–123], and disconnected the anorectic effect from hypothalamic fatty acid synthase inhibition [124]. Whatever, the mechanism, inhibition of fatty acid synthase seems more likely to produce toxicity than approaches that involve selective inhibition of one isozyme of an enzyme that regulates the concentration of a regulatory molecule, as in the case of ACC2.

SCD-1 is another target involved in lipid metabolism that affects energy expenditure. There are at least four isoforms of SCD-1 in mice, and two have been described in humans [125]. Mice that carry a mutant SCD-1 are resistant to obesity. Moreover, antisense oligonucleotide inhibitors of SCD-1, apparently acting in the liver, prevented diet-induced obesity in mice [126]. One suggestion is that saturated but not monounsaturated fatty acyl-CoAs potently inhibit ACC, so that knockout of SCD-1 might resemble knockout of ACC2. Other proposed mechanisms involve activation of AMP-activated protein kinase and downregulation of protein tyrosine phosphatase-1B (PTP1B) [127].

Triglyceride Synthesis

Synthesis of triglyceride (TAG) starts with esterification of glycerol-3-phosphate by fatty acyl-CoA, catalysed by GPAT. There are two forms of GPAT: one is located in the outer mitochondrial membrane (mtGPAT) and the other in the endoplasmic reticulum (erGPAT). In most tissues, mtGPAT comprises only 10% of total GPAT activity, and its role is unclear [128]. One possibility is that it diverts fatty acyl-CoA away from oxidation in the mitochondrion and sends it in the form of lysophosphatidate to the endoplasmic reticulum to be used for TAG synthesis. Thus the expression of mtGPAT mRNA is regulated by nutritional and hormonal status; its overexpression in rat primary hepatocytes both increased TAG synthesis and decreased fatty acid oxidation, and overexpression in mouse liver increased TAG synthesis, reduced fatty acid oxidation and caused marked hepatic steatosis [129]. By contrast, in mice fed on a high sucrose and fat diet, the absence of mtGPAT diverted fatty acids in liver away from TAG synthesis and towards oxidation and ketogenesis [130].

The mtGPAT knockout mouse weighs less than its wild-type counterpart and has a lower adipose tissue weight and liver fat content [128]. Energy expenditure has not been reported, although the reduction in hepatic and plasma TAG suggests that fatty acid oxidation is increased. The amount of the mitochondrial phospholipid cardiolipin

was reduced, and it would be interesting to determine whether this affects the mitochondrial proton leak.

The last enzyme of the TAG biosynthetic pathway is acyl-CoA : diacylglycerol acyltransferase (DGAT). Two isoforms are known. DGAT1 knockout mice survive and are lean and resistant to obesity. DGAT1 knockout mice have increased energy expenditure due in part to increased physical activity but also to increased fatty acid oxidation in brown adipose and other tissues. Increased fatty acid oxidation would explain why the concentration of diacylglycerol, the substrate of DGAT, paradoxically tended to be low in white adipose tissue, skeletal muscle, and especially in liver of DGAT1 knockout mice, and not high as would be expected. Presumably the absence of DGAT1 in some other tissue promotes diacylglycerol utilization or inhibits its formation. Because the knockout mice have increased locomotor activity, this other tissue could be the brain [131,132]. White adipose tissue transplantation studies suggested that the trigger for increased energy expenditure resides in this tissue. White adipose tissue was proposed to affect energy expenditure in other tissues through an increased ratio of adiponectin to tumour necrosis factor α release, but there is little evidence to support this proposal. DGAT2 knockout mice do not survive long after birth, but studies using DGAT2 antisense suggest that inhibitors might reduce hepatic steatosis and hyperlipidaemia. In contrast to DGAT1 antisense, however, DGAT2 antisense does not have an antiobesity effect [133].

Validation of these various targets involved in lipid synthesis has mostly been from the phenotypes of genetically modified mice. In some cases, the phenotype is resistance to dietary obesity rather than leanness on a chow diet, and there are no examples where the genetic modification has been activated in an obese animal to show that pre-existing obesity can be reversed. Nevertheless, it is expected that full papers showing effects of tool compounds *in vivo* will soon be published for more of the targets.

Hypothalamic Targets and Their Implications for Drug Discovery

Initial interest in many of the new intracellular antiobesity targets has been driven by the phenotypes of mice in which a gene has been knocked out in the whole animal. It might seem that if the gene regulates lipid or carbohydrate metabolism, then drugs targeted towards the gene product must act peripherally. However, the hypothalamus responds to fuels of various kinds – lipids, glucose and amino acids – as well as to insulin [134], and we have suggested that resistance to obesity in the DGAT1 knockout mouse might be due to the absence of the

enzyme in the brain. Resistance to obesity in the 11 β -hydroxysteroid dehydrogenase-1 knockout mouse might also be due to the absence of the enzyme in the brain [135].

Consider also PTP1B. This enzyme deactivates the insulin receptor and possibly insulin receptor substrates. Inhibitors might be expected to be useful in the treatment of type 2 diabetes, but the PTP1B mouse is also resistant to obesity [136]. Because insulin promotes anabolism when it acts in the periphery, but catabolism when it acts centrally, it seems likely that the lean phenotype of the PTP1B mouse is due to the absence of the enzyme in the brain. Consistent with this view, the neuronal insulin receptor knockout mouse is obese [137], and insulin mimetics act centrally to reduce food intake [138]. Another consideration is that PTP1B also deactivates the leptin receptor and Janus-activated kinase-1, which plays a role in leptin signalling. These are also central targets. PTP1B inhibitors have not so far been shown to have antiobesity activity [136].

The existence of these central targets raises issues for drug discovery. First, the phenotypes of animals in which a gene has been knocked out globally must be interpreted with caution. Even when the gene is involved in a major pathway of fuel metabolism (e.g. fatty acid synthase), it is possible that its effects on energy balance are primarily centrally mediated. This would mean that drugs for the target would have to penetrate the brain.

Second, opposite phenotypes may result from peripheral or central actions of the drug. Inhibition of ACC2 in the periphery may be beneficial because it reduces malonyl-CoA levels. The antiobesity activity of fatty acid synthase inhibition has, by contrast, been attributed to *elevation* of malonyl-CoA levels in the hypothalamus [139]. Similarly, activation of AMPK in the periphery is beneficial, but in the brain activation of AMPK stimulates food intake, whilst inhibition reduces intake. There is logic in these opposing peripheral and central actions of malonyl-CoA and AMPK on energy balance. They both play a role in sensing fuel availability. In times of energy need, not only must oxidative metabolism increase but food intake must increase to replace the oxidized fuel. On the contrary, leptin, which has the role of regulating body weight, activates AMPK in the periphery to burn off fuel, whilst inhibiting AMPK in the hypothalamus to prevent the fuel being replaced [140]. (Surprisingly, intracerebroventricular infusion of insulin, which also reduces food intake, activates AMPK [141].) It may therefore be important with some drugs to avoid central penetration and with others to maximize it.

Limitations of Rodent Models

The use of rodents as models for humans presents particular issues when validating targets for thermogenic compounds. One difference is that effects of compounds are usually studied in rodents when they are growing, but we are not aware of any compound that reduced fat accretion in growing rodents but could not elicit fat loss in weight stable rodents. Other issues seem more important.

First, it can be difficult to determine whether obesity or resistance to obesity in genetically modified animals is because their energy expenditure or their energy intake differs from wild-type animals. If the genetically modified animal is obese but eats less than the wild-type animal, or is lean but eats more, then altered energy expenditure is clearly important. But what if, for example, the animal is obese and both eats more and expends more energy? In the past, some clinical researchers have interpreted such data for obese humans as evidence that obesity was due to overeating. Many animal researchers, by contrast, take fundamentally similar data divide it by body weight; find that the obese animals have a lower energy expenditure per gram body weight and come to the opposite conclusion, that is, that obesity is due to *reduced* energy expenditure.

Both approaches are flawed: the former fails to recognize that increased energy expenditure may be a consequence rather than a cause of obesity [14,142] and the latter that a gram of fat makes a smaller contribution to energy expenditure than a gram of lean tissue. Nor does expressing energy expenditure relative to a power of body weight (such as body weight^{0.75}) solve the problem, because such exponents were not derived to compare lean and obese animals. The best solution is to conduct an analysis of covariance of energy expenditure against body weight as discussed elsewhere [71,143]. When investigating the acute thermogenic response to a compound, variation in body composition is not an issue, but if chronic effects on energy expenditure are determined, body weight and composition may have been altered by the treatment.

A second issue is that rodents have a greater capacity for non-shivering thermogenesis than humans. They have far more brown adipose tissue than humans, and consequently more β_3 -adrenoceptors and UCP-1. Therefore compounds that raise sympathetic activity may affect energy balance more profoundly in rodents than humans. Moreover, targets expressed in brown adipose tissue or those that promote the conversion of white to brown adipose tissue (or mitochondrial biogenesis in

white adipocytes) may be overvalued by studies in rodents.

A third issue is that humans tend to live near thermoneutrality. They wear clothes and, if necessary, they heat their buildings. Rodents are usually studied below thermoneutrality. This raises their sympathetic activity and their energy expenditure. Although this reduces the 'window' available for drugs to increase sympathetic activity and fat oxidation [144] and mitigates the problem of a greater thermogenic capacity in rodents, it introduces further complexity in the prediction of efficacy in humans from rodent data.

The evaluation of compounds in genetically obese rodents presents further difficulties. Rodents that have a dysfunctional leptin system, such as *lep^{ob}/lep^{ob}* mice and *lepR^{fa}/lepR^{fa}* rats, are very different from obese humans who have raised leptin levels. It is argued (somewhat illogically [145]) that obese humans are therefore leptin resistant, but leptin clearly has some effect in most obese humans because their obesity is far less severe than in those rare obese humans whose obesity is due to a defective leptin system [146]. The absence of a functional leptin system in some rodents results, for example, in raised hypothalamic neuropeptide Y levels, making them especially sensitive to the anorectic and, presumably, the thermogenic effects of neuropeptide Y receptor antagonists [147]. The absence of leptin also lowers sympathetic activity, though whether there is a greater or smaller response to β -adrenoceptor agonists in *lep^{ob}/lep^{ob}* mice may vary with background strain and age [144].

The answer would appear to be to evaluate compounds in diet-induced obese rather than genetically obese rodents. Diet-induced obesity takes time to develop, however, and there is greater body weight and fat variability than in the mutant strains. Some workers prefer to reserve diet-induced obesity for key studies.

Other types of mutant obese rodent, such as the agouti *A^y* mouse, in which the mutation is less fundamental to body weight control than leptin, or polygenic obesity, may provide results that are more predictive of efficacy in humans than *lep^{ob}/lep^{ob}* mice do. It may be especially interesting to conduct studies in mice that lack brown adipose tissue. Not only are they obese, but also they lack the tissue that makes thermogenic capacity in rodents so different from that in humans [148].

Conclusion

There are many possible targets for antiobesity drugs that work by stimulating energy expenditure or altering

substrate utilization. Some of these are suggested by physiological mechanisms that are known to influence energy expenditure and may be supported by effects of compounds in rodents and humans. Others are more speculative and supported only by the phenotypes of genetically modified mice.

Ultimately, antiobesity drugs must reduce energy intake (or absorption) or increase expenditure (or excretion). Increased energy expenditure involves either increased ATP utilization or oxidation of reduced coenzymes by enzymes or pathways that are not coupled to ATP. Thermogenesis is a logical approach to the treatment of obesity, because low energy expenditure, especially low oxidation of fat, is a factor in its aetiology. Moreover, peripherally acting thermogenic drugs seem less likely to have side effects than centrally acting drugs, and those that promote fat oxidation are especially likely to have metabolic benefits. Surprisingly, knockout or inhibition of enzymes of fatty acid and triglyceride synthesis may increase energy expenditure as well as decrease intake, but in some cases this effect may be initiated centrally rather than peripherally. This caution is important if the attraction of influencing energy expenditure and substrate utilization is that the target appears to be 'below the neck'. There is no shortage of targets. The difficulty is in deciding which, if any, is likely to deliver a drug.

Acknowledgements

We thank Mike Cawthorne for his comments on the manuscript, Julie Cakebread for typing it and John Wilding, who provided the stimulus for writing it.

References

- 1 Van Gaal LF, Rissanen AM, Scheen AJ, Ziegler O, Rossner S. Effects of the cannabinoid-1 receptor blocker rimonabant on weight reduction and cardiovascular risk factors in overweight patients: 1-year experience from the RIO-Europe study. *Lancet* 2005; **365**: 1389–1397.
- 2 Ravinet Trillou C, Arnone M, Delgorge C *et al.* Anti-obesity effect of SR141716, a CB1 receptor antagonist, in diet-induced obese mice. *Am J Physiol Regul Integr Comp Physiol* 2003; **284**: R345–R353.
- 3 Liu YL, Connoley IP, Wilson CA, Stock MJ. Effects of the cannabinoid CB1 receptor antagonist SR141716 on oxygen consumption and soleus muscle glucose uptake in *Lep (ob)/Lep (ob)* mice. *Int J Obes Relat Metab Disord* 2005; **29**: 183–187.
- 4 Osei-Hyiaman D, DePetrillo M, Pacher P *et al.* Endocannabinoid activation at hepatic CB1 receptors stimulates fatty acid synthesis and contributes to diet-induced obesity. *J Clin Invest* 2005; **115**: 1298–1305.
- 5 Bensaïd M, Gary-Bobo M, Esclançon A *et al.* The cannabinoid CB1 receptor antagonist SR141716 increases Acp30 mRNA expression in adipose tissue of obese *fa/fa* rats and in cultured adipocyte cells. *Mol Pharmacol* 2003; **63**: 908–914.
- 6 Norris SL, Zhang X, Avenell A *et al.* Efficacy of pharmacotherapy for weight loss in adults with type 2 diabetes mellitus: a meta-analysis. *Arch Intern Med* 2004; **164**: 1395–1404.
- 7 Li Z, Maglione M, Tu W *et al.* Meta-analysis: pharmacologic treatment of obesity. *Ann Intern Med* 2005; **142**: 532–546.
- 8 Fruhbeck G, Gomez-Ambrosi J. Control of body weight: a physiologic and transgenic perspective. *Diabetologia* 2003; **46**: 143–172.
- 9 Ravussin E, Lillioja S, Knowler WC *et al.* Reduced rate of energy expenditure as a risk factor for body-weight gain. *N Engl J Med* 1988; **318**: 467–472.
- 10 Levine JA, Eberhardt NL, Jensen MD. Role of nonexercise activity thermogenesis in resistance to fat gain in humans. *Science* 1999; **283**: 212–214.
- 11 Levine JA, Lanningham-Foster LM, McCrady SK *et al.* Interindividual variation in posture allocation: possible role in human obesity. *Science* 2005; **307**: 584–586.
- 12 Buscemi S, Verga S, Caimi G, Cerasola G. Low relative resting metabolic rate and body weight gain in adult Caucasian Italians. *Int J Obes Relat Metab Disord* 2005; **29**: 287–291.
- 13 Hunter GR, Byrne NM. Physical activity and muscle function but not resting energy expenditure impact on weight gain. *J Strength Cond Res* 2005; **19**: 225–230.
- 14 Arch JR. Lessons in obesity from transgenic animals. *J Endocrinol Invest* 2002; **25**: 867–875.
- 15 Astrup A, Gotzsche PC, van de Werken K *et al.* Meta-analysis of resting metabolic rate in formerly obese subjects. *Am J Clin Nutr* 1999; **69**: 1117–1122.
- 16 Clapham JC. Treating obesity: pharmacology of energy expenditure. *Curr Drug Targets* 2004; **5**: 309–323.
- 17 Blaak EE, Wolfenbutter BH, Saris WH, Pelsers MM, Wagenmakers AJ. Weight reduction and the impaired plasma-derived free fatty acid oxidation in type 2 diabetic subjects. *J Clin Endocrinol Metab* 2001; **86**: 1638–1644.
- 18 Prentice AM. Manipulation of dietary fat and energy density and subsequent effects on substrate flux and food intake. *Am J Clin Nutr* 1998; **67**: 535S–541S.
- 19 Rolfe DF, Brown GC. Cellular energy utilization and molecular origin of standard metabolic rate in mammals. *Physiol Rev* 1997; **77**: 731–758.
- 20 Kim JY, Hickner RC, Cortright RL, Dohm GL, Houmard JA. Lipid oxidation is reduced in obese human skeletal muscle. *Am J Physiol Endocrinol Metab* 2000; **279**: E1039–E1044.

- 21 Tanner CJ, Barakat HA, Dohm GL *et al.* Muscle fiber type is associated with obesity and weight loss. *Am J Physiol Endocrinol Metab* 2002; **282**: E1191–E1196.
- 22 Arch JR. β_3 -Adrenoceptor agonists: potential, pitfalls and progress. *Eur J Pharmacol* 2002; **440**: 99–107.
- 23 Arch JR. Thermogenesis and related metabolic targets for anti-obesity drugs. In: Antel J, Heal D, Finer N, Krause G eds. *Obesity and Metabolic Disorders*. Amsterdam: IOS Press, 2005; 143–160.
- 24 Yang MU, Van Itallie TB. Composition of weight lost during short-term weight reduction. Metabolic responses of obese subjects to starvation and low-calorie ketogenic and nonketogenic diets. *J Clin Invest* 1976; **58**: 722–730.
- 25 Durrant ML, Garrow JS, Royston P *et al.* Factors influencing the composition of the weight lost by obese patients on a reducing diet. *Br J Nutr* 1980; **44**: 275–285.
- 26 Darimont C, Turini M, Epitoux M *et al.* β_3 -adrenoceptor agonist prevents alterations of muscle diacylglycerol and adipose tissue phospholipids induced by a cafeteria diet. *Nutr Metab (Lond)* 2004; **1**: 4.
- 27 Galloway SM, Farquhar DL, Munro JF. The current status of antiobesity drugs. *Postgrad Med J* 1984; **60** (Suppl. 3): 19–26.
- 28 Padwal R, Li SK, Lau DC. Long-term pharmacotherapy for overweight and obesity: a systematic review and meta-analysis of randomized controlled trials. *Int J Obes Relat Metab Disord* 2003; **27**: 1437–1446.
- 29 Schifflers SLH, Blaak EE, Saris WHM, van Baak MA. In vivo β_3 -adrenergic stimulation of human thermogenesis and lipid use. *Clin Pharmacol Ther* 2000; **67**: 558–566.
- 30 Wing RR, Phelan S. Long-term weight loss maintenance. *Am J Clin Nutr* 2005; **82**: 222S–225S.
- 31 Blundell JE, Stubbs RJ, Hughes DA, Whybrow S, King NA. Cross talk between physical activity and appetite control: does physical activity stimulate appetite? *Proc Nutr Soc* 2003; **62**: 651–661.
- 32 Arch JRS. The contribution of increased thermogenesis to the effect of anorectic drugs on body composition in mice. *Am J Clin Nutr* 1981; **34**: 2763–2769.
- 33 Day C, Bailey CJ. Effect of the anti-obesity agent sibutramine in obese-diabetic ob/ob mice. *Int J Obes Relat Metab Disord* 1998; **22**: 619–623.
- 34 Liu YL, Connoley IP, Heal DJ, Stock MJ. Pharmacological characterisation of the thermogenic effect of bupropion. *Eur J Pharmacol* 2004; **498**: 219–225.
- 35 Morton GJ, Blevins JE, Williams DL *et al.* Leptin action in the forebrain regulates the hindbrain response to satiety signals. *J Clin Invest* 2005; **115**: 703–710.
- 36 Figlewicz DP. Adiposity signals and food reward: expanding the CNS roles of insulin and leptin. *Am J Physiol Regul Integr Comp Physiol* 2003; **284**: R882–R892.
- 37 Bray GA. Calorigenic effect of human growth hormone in obesity. *J Clin Endocrinol Metab* 1969; **29**: 119–122.
- 38 Silva JE. The thermogenic effect of thyroid hormone and its clinical implications. *Ann Intern Med* 2003; **139**: 205–213.
- 39 Moriscot A, Rabelo R, Bianco AC. Corticosterone inhibits uncoupling protein gene expression in brown adipose tissue. *Am J Physiol* 1993; **265**: E81–E87.
- 40 Brillon DJ, Zheng B, Campbell RG, Matthews DE. Effect of cortisol on energy expenditure and amino acid metabolism in humans. *Am J Physiol* 1995; **268**: E501–E513.
- 41 Tataranni PA, Larson DE, Snitker S *et al.* Effects of glucocorticoids on energy metabolism and food intake in humans. *Am J Physiol* 1996; **271**: E317–E325.
- 42 Silvestri E, Schiavo L, Lombardi A, Goglia F. Thyroid hormones as molecular determinants of thermogenesis. *Acta Physiol Scand* 2005; **184**: 265–283.
- 43 Hansen M, Morthorst R, Larsson B *et al.* Effects of 2 wk of GH administration on 24-h indirect calorimetry in young, healthy, lean men. *Am J Physiol Endocrinol Metab* 2005; **289**: E1030–E1038.
- 44 Soumano K, Desbiens S, Rabelo R *et al.* Glucocorticoids inhibit the transcriptional response of the uncoupling protein-1 gene to adrenergic stimulation in a brown adipose cell line. *Mol Cell Endocrinol* 2000; **165**: 7–15.
- 45 Grover GJ, Mellstrom K, Ye L *et al.* Selective thyroid hormone receptor- β activation: a strategy for reduction of weight, cholesterol, and lipoprotein (a) with reduced cardiovascular liability. *Proc Natl Acad Sci USA* 2003; **100**: 10067–10072.
- 46 Grover GJ, Egan DM, Sleph PG *et al.* Effects of the thyroid hormone receptor agonist GC-1 on metabolic rate and cholesterol in rats and primates: selective actions relative to 3,5,3'-triiodo-L-thyronine. *Endocrinology* 2004; **145**: 1656–1661.
- 47 Baxter JD, Webb P, Grover G, Scanlan TS. Selective activation of thyroid hormone signaling pathways by GC-1: a new approach to controlling cholesterol and body weight. *Trends Endocrinol Metab* 2004; **15**: 154–157.
- 48 Clapham JC, Arch JRS, Tadayyon M. Anti-obesity drugs: a critical review of current therapies and future opportunities. *Pharmacol Ther* 2001; **89**: 81–121.
- 49 Luque CA, Rey JA. The discovery and status of sibutramine as an anti-obesity drug. *Eur J Pharmacol* 2002; **440**: 119–128.
- 50 Rahmouni K, Morgan DA, Morgan GM *et al.* Hypothalamic PI3K and MAPK differentially mediate regional sympathetic activation to insulin. *J Clin Invest* 2004; **114**: 652–658.
- 51 Larsen TM, Toubro S, van Baak MA *et al.* Effect of a 28-d treatment with L-796568, a novel β_3 -adrenergic receptor agonist, on energy expenditure and body composition in obese men. *Am J Clin Nutr* 2002; **76**: 780–788.
- 52 Greenway FL, De Jonge L, Blanchard D, Frisard M, Smith SR. Effect of a dietary herbal supplement

- containing caffeine and ephedra on weight, metabolic rate, and body composition. *Obes Res* 2004; **12**: 1152–1157.
- 53 Bao Y, Bing C, Hunter L *et al*. Zinc-alpha2-glycoprotein, a lipid mobilizing factor, is expressed and secreted by human (SGBS) adipocytes. *FEBS Lett* 2005; **579**: 41–47.
- 54 Russell ST, Hirai K, Tisdale MJ. Role of β_3 -adrenergic receptors in the action of a tumour lipid mobilizing factor. *Br J Cancer* 2002; **86**: 424–428.
- 55 Sanders PM, Tisdale MJ. Effect of zinc-alpha2-glycoprotein (ZAG) on expression of uncoupling proteins in skeletal muscle and adipose tissue. *Cancer Lett* 2004; **212**: 71–81.
- 56 Heffernan MA, Thorburn AW, Fam B *et al*. Increase of fat oxidation and weight loss in obese mice caused by chronic treatment with human growth hormone or a modified C-terminal fragment. *Int J Obes Relat Metab Disord* 2001; **25**: 1442–1449.
- 57 Herd C, Wittert G, Caterson I *et al*. The effect of AOD9604 on weight loss in obese adults: results of a randomized, double-blind, placebo-controlled, multicenter study. *Obes Res* 2005; **13** (Suppl.): A27.
- 58 Proietto J, Thorburn AW. The therapeutic potential of leptin. *Expert Opin Investig Drugs* 2003; **12**: 373–378.
- 59 Maneuf Y, Higginbottom M, Pritchard M *et al*. Small molecule leptin mimetics overcome leptin resistance in obese rats. *Fundam Clin Pharmacol* 2004; **18** (Suppl. 1): 83.
- 60 Lambert PD, Anderson KD, Sleeman MW *et al*. Ciliary neurotrophic factor activates leptin-like pathways and reduces body fat, without cachexia or rebound weight gain, even in leptin-resistant obesity. *Proc Natl Acad Sci USA* 2001; **98**: 4652–4657.
- 61 Bluher S, Moschos S, Bullen J Jr. *et al*. Ciliary neurotrophic factor Ax15 alters energy homeostasis, decreases body weight, and improves metabolic control in diet-induced obese and UCP1-DTA mice. *Diabetes* 2004; **53**: 2787–2796.
- 62 Kang ES, Park SY, Kim HJ *et al*. The influence of adiponectin gene polymorphism on the rosiglitazone response in patients with type 2 diabetes. *Diabetes Care* 2005; **28**: 1139–1144.
- 63 Nawrocki AR, Rajala MW, Tomas E *et al*. Mice lacking adiponectin show decreased hepatic insulin sensitivity and reduced responsiveness to PPAR γ -agonists. *J Biol Chem* 2006; **281**: 2654–2660.
- 64 Kadowaki T, Yamauchi T. Adiponectin and adiponectin receptors. *Endocr Rev* 2005; **26**: 439–451.
- 65 Jacobson PB, von Geldern TW, Ohman L *et al*. Hepatic glucocorticoid receptor antagonism is sufficient to reduce elevated hepatic glucose output and improve glucose control in animal models of type 2 diabetes. *J Pharmacol Exp Ther* 2005; **314**: 191–200.
- 66 Alberts P, Nilsson C, Selen G *et al*. Selective inhibition of 11 β -hydroxysteroid dehydrogenase type 1 improves hepatic insulin sensitivity in hyperglycemic mice strains. *Endocrinology* 2003; **144**: 4755–4762.
- 67 Hermanowski-Vosatka A, Balkovec JM, Cheng K *et al*. 11 β -HSD1 inhibition ameliorates metabolic syndrome and prevents progression of atherosclerosis in mice. *J Exp Med* 2005; **202**: 517–527.
- 68 Wang SJ, Birtles S, de Schoolmeester J, Swales J, Moody G, Hislop D, O'dowd J, Smith DM, Tumbull AV, Arch JR. Effects of an inhibitor of 11 β hydroxysteroid dehydrogenase type 1 inhibitor on energy balance and glucose homeostasis in diet induced obesity. *Diabetologia* 2006; Epub ahead of print.
- 69 Morton NM, Paterson JM, Masuzaki H *et al*. Novel adipose tissue-mediated resistance to diet-induced visceral obesity in 11 β -hydroxysteroid dehydrogenase type 1-deficient mice. *Diabetes* 2004; **53**: 931–938.
- 70 Himms-Hagen J. On raising energy expenditure in ob/ob mice. *Science* 1997; **276**: 1132–1133.
- 71 Arch JR, Hislop D, Wang SJY, Speakman JR. Some mathematical and technical issues in the measurement and interpretation of open-circuit indirect calorimetry in small animals. *Int J Obes* 2006. In press.
- 72 Kershaw EE, Morton NM, Dhillion H *et al*. Adipocyte-specific glucocorticoid inactivation protects against diet-induced obesity. *Diabetes* 2005; **54**: 1023–1031.
- 73 Nedergaard J, Cannon B. The 'novel' 'uncoupling' proteins UCP2 and UCP3: what do they really do? Pros and cons for suggested functions. *Exp Physiol* 2003; **88**: 65–84.
- 74 Clapham JC, Arch JRS, Chapman H *et al*. Mice overexpressing human uncoupling protein-3 in skeletal muscle are hyperphagic and lean. *Nature* 2000; **406**: 415–418.
- 75 Amaral CM, Chen J-L, Jaen J, Johnston DN, Rafferty P. Bis-aryl thiazole derivatives. PCT patent, WO/2003/002062.
- 76 Ruderman N, Prentki M. AMP kinase and malonyl-CoA: targets for therapy of the metabolic syndrome. *Nat Rev Drug Discov* 2004; **3**: 340–351.
- 77 Taylor EB, Lamb JD, Hurst RW *et al*. Endurance training increases skeletal muscle LKB1 and PGC-1(α) protein abundance: effects of time and intensity. *Am J Physiol Endocrinol Metab* 2005; **289**: E960–E968.
- 78 Hardie DG. Minireview: the AMP-activated protein kinase cascade: the key sensor of cellular energy status. *Endocrinology* 2003; **144**: 5179–5183.
- 79 Owen MR, Doran E, Halestrap AP. Evidence that metformin exerts its anti-diabetic effects through inhibition of complex 1 of the mitochondrial respiratory chain. *Biochem J* 2000; **348**: 607–614.
- 80 Minokoshi Y, Kim YB, Peroni OD *et al*. Leptin stimulates fatty-acid oxidation by activating AMP-activated protein kinase. *Nature* 2002; **415**: 339–343.
- 81 Yamauchi T, Kamon J, Minokoshi Y *et al*. Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. *Nat Med* 2002; **8**: 1288–1295.

- 82 Buhl ES, Jessen N, Pold R *et al.* Long-term AICAR administration reduces metabolic disturbances and lowers blood pressure in rats displaying features of the insulin resistance syndrome. *Diabetes* 2002; **51**: 2199–2206.
- 83 Yu X, McCorkle S, Wang M *et al.* Leptinomimetic effects of the AMP kinase activator AICAR in leptin-resistant rats: prevention of diabetes and ectopic lipid deposition. *Diabetologia* 2004; **47**: 2012–2021.
- 84 Camacho RC, Pencek RR, Lacy DB *et al.* Portal venous 5-aminoimidazole-4-carboxamide-1-beta-D-ribofuranoside infusion overcomes hyperinsulinemic suppression of endogenous glucose output. *Diabetes* 2005; **54**: 373–382.
- 85 Pencek RR, Shearer J, Camacho RC *et al.* 5-Aminoimidazole-4-carboxamide-1-beta-D-ribofuranoside causes acute hepatic insulin resistance in vivo. *Diabetes* 2005; **54**: 355–360.
- 86 Foretz M, Ancellin N, Andreelli F *et al.* Short-term overexpression of a constitutively active form of AMP-activated protein kinase in the liver leads to mild hypoglycemia and fatty liver. *Diabetes* 2005; **54**: 1331–1339.
- 87 Carling D. The AMP-activated protein kinase cascade – a unifying system for energy control. *Trends Biochem Sci* 2004; **29**: 18–24.
- 88 Sakamoto K, Goransson O, Hardie DG, Alessi DR. Activity of LKB1 and AMPK-related kinases in skeletal muscle: effects of contraction, phenformin, and AICAR. *Am J Physiol Endocrinol Metab* 2004; **287**: E310–E317.
- 89 Schrauwen P, Hardie DG, Roorda B *et al.* Improved glucose homeostasis in mice overexpressing human UCP3: a role for AMP-kinase? *Int J Obes Relat Metab Disord* 2004; **28**: 824–828.
- 90 Dobrzyn P, Dobrzyn A, Miyazaki M *et al.* Stearoyl-CoA desaturase 1 deficiency increases fatty acid oxidation by activating AMP-activated protein kinase in liver. *Proc Natl Acad Sci USA* 2004; **101**: 6409–6414.
- 91 Puigserver P, Spiegelman BM. Peroxisome proliferator-activated receptor-gamma coactivator 1 alpha (PGC-1 alpha): transcriptional coactivator and metabolic regulator. *Endocr Rev* 2003; **24**: 78–90.
- 92 Kamei Y, Ohizumi H, Fujitani Y *et al.* PPARgamma coactivator 1beta/ERR ligand 1 is an ERR protein ligand, whose expression induces a high-energy expenditure and antagonizes obesity. *Proc Natl Acad Sci USA* 2003; **100**: 12378–12383.
- 93 Wende AR, Huss JM, Schaeffer PJ, Giguere V, Kelly DP. PGC-1alpha coactivates PDK4 gene expression via the orphan nuclear receptor ERRalpha: a mechanism for transcriptional control of muscle glucose metabolism. *Mol Cell Biol* 2005; **25**: 10684–10694.
- 94 Willy PJ, Murray IR, Qian J *et al.* Regulation of PPARgamma coactivator 1alpha (PGC-1alpha) signaling by an estrogen-related receptor alpha (ERRalpha) ligand. *Proc Natl Acad Sci USA* 2004; **101**: 8912–8917.
- 95 Luo J, Sladek R, Carrier J *et al.* Reduced fat mass in mice lacking orphan nuclear receptor estrogen-related receptor alpha. *Mol Cell Biol* 2003; **23**: 7947–7956.
- 96 Wang YX, Zhang CL, Yu RT *et al.* Regulation of muscle fiber type and running endurance by PPARdelta. *PLoS Biol* 2004; **2**: e294.
- 97 Tanaka T, Yamamoto J, Iwasaki S *et al.* Activation of peroxisome proliferator-activated receptor delta induces fatty acid beta-oxidation in skeletal muscle and attenuates metabolic syndrome. *Proc Natl Acad Sci USA* 2003; **100**: 15924–15929.
- 98 Tenenbaum A, Motro M, Fisman EZ. Dual and pan-peroxisome proliferator-activated receptors (PPAR) co-agonism: the bezafibrate lessons. *Cardiovasc Diabetol* 2005; **4**: 14.
- 99 Wang YX, Lee CH, Tjep S *et al.* Peroxisome-proliferator-activated receptor delta activates fat metabolism to prevent obesity. *Cell* 2003; **113**: 159–170.
- 100 Tiraby C, Langin D. Conversion from white to brown adipocytes: a strategy for the control of fat mass? *Trends Endocrinol Metab* 2003; **14**: 439–441.
- 101 Chou KC. Molecular therapeutic target for type-2 diabetes. *J Proteome Res* 2004; **3**: 1284–1288.
- 102 Veerababu G, Tang J, Hoffman RT *et al.* Overexpression of glutamine: fructose-6-phosphate amidotransferase in the liver of transgenic mice results in enhanced glycogen storage, hyperlipidemia, obesity, and impaired glucose tolerance. *Diabetes* 2000; **49**: 2070–2078.
- 103 Obici S, Rossetti L. Minireview: nutrient sensing and the regulation of insulin action and energy balance. *Endocrinology* 2003; **144**: 5172–5178.
- 104 Mandard S, Muller M, Kersten S. Peroxisome proliferator-activated receptor alpha target genes. *Cell Mol Life Sci* 2004; **61**: 393–416.
- 105 Akiyama TE, Nicol CJ, Fievet C *et al.* Peroxisome proliferator-activated receptor alpha regulates lipid homeostasis, but is not associated with obesity: studies with congenic mouse lines. *J Biol Chem* 2001; **276**: 39088–39093.
- 106 Haluzik M, Gavriloova O, LeRoith D. Peroxisome proliferator-activated receptor-alpha deficiency does not alter insulin sensitivity in mice maintained on regular or high-fat diet: hyperinsulinemic-euglycemic clamp studies. *Endocrinology* 2004; **145**: 1662–1667.
- 107 Costet P, Legendre C, More J *et al.* Peroxisome proliferator-activated receptor alpha-isoform deficiency leads to progressive dyslipidemia with sexually dimorphic obesity and steatosis. *J Biol Chem* 1998; **273**: 29577–29585.
- 108 Assimakopoulos-Jeannet F, Moinat M, Muzzin P *et al.* Effects of a peroxisome proliferator on beta-oxidation and overall energy balance in obese (fa/fa) rats. *Am J Physiol* 1991; **260**: R278–R283.
- 109 Brown PJ, Winegar DA, Plunket KD *et al.* A ureidothioisobutyric acid (GW9578) is a subtype-selective PPARalpha agonist with potent lipid-lowering activity. *J Med Chem* 1999; **42**: 3785–3788.

- 110 McCarty MF. Peroxisome proliferators as adjuvants for the reverse-electron-transport therapy of obesity: an explanation for the large increase in metabolic rate of MEDICA 16-treated rats. *Med Hypotheses* 1999; **53**: 272–276.
- 111 Peters JM, Aoyama T, Burns AM, Gonzalez FJ. Bezafibrate is a dual ligand for PPAR α and PPAR β : studies using null mice. *Biochim Biophys Acta* 2003; **1632**: 80–89.
- 112 Oveisi F, Gaetani S, Eng KT, Piomelli D. Oleoylethanolamide inhibits food intake in free-feeding rats after oral administration. *Pharmacol Res* 2004; **49**: 461–466.
- 113 Lim K, Ryu S, Nho HS *et al.* (-)-Hydroxycitric acid ingestion increases fat utilization during exercise in untrained women. *J Nutr Sci Vitaminol (Tokyo)* 2003; **49**: 163–167.
- 114 Tomita K, Okuhara Y, Shigematsu N, Suh H, Lim K. (-)-hydroxycitrate ingestion increases fat oxidation during moderate intensity exercise in untrained men. *Biosci Biotechnol Biochem* 2003; **67**: 1999–2001.
- 115 Westerterp-Plantenga MS, Kovacs EM. The effect of (-)-hydroxycitrate on energy intake and satiety in overweight humans. *Int J Obes Relat Metab Disord* 2002; **26**: 870–872.
- 116 Ohia SE, Opere CA, LeDay AM *et al.* Safety and mechanism of appetite suppression by a novel hydroxycitric acid extract (HCA-SX). *Mol Cell Biochem* 2002; **238**: 89–103.
- 117 Abu-Elheiga L, Matzuk MM, Abo-Hashema KA, Wakil SJ. Continuous fatty acid oxidation and reduced fat storage in mice lacking acetyl-CoA carboxylase 2. *Science* 2001; **291**: 2613–2616.
- 118 Oh W, Abu-Elheiga L, Kordari P *et al.* Glucose and fat metabolism in adipose tissue of acetyl-CoA carboxylase 2 knockout mice. *Proc Natl Acad Sci USA* 2005; **102**: 1384–1389.
- 119 Harwood HJ Jr. Acetyl-CoA carboxylase inhibition for the treatment of metabolic syndrome. *Curr Opin Investig Drugs* 2004; **5**: 283–289.
- 120 Loftus TM, Jaworsky DE, Frehywot GL *et al.* Reduced food intake and body weight in mice treated with fatty acid synthase inhibitors. *Science* 2000; **288**: 2379–2381.
- 121 Tu Y, Thupari JN, Kim EK *et al.* C75 alters central and peripheral gene expression to reduce food intake and increase energy expenditure. *Endocrinology* 2005; **146**: 486–493.
- 122 Thupari JN, Landree LE, Ronnett GV, Kuhajda FP. C75 increases peripheral energy utilization and fatty acid oxidation in diet-induced obesity. *Proc Natl Acad Sci USA* 2002; **99**: 9498–9502.
- 123 Kuhajda FP, Landree LE, Ronnett GV. The connections between C75 and obesity drug-target pathways. *Trends Pharmacol Sci* 2005; **26**: 541–544.
- 124 Rohrbach KW, Han S, Gan J *et al.* Disconnection between the early onset anorectic effects by C75 and hypothalamic fatty acid synthase inhibition in rodents. *Eur J Pharmacol* 2005; **511**: 31–41.
- 125 Wang J, Yu L, Schmidt RE *et al.* Characterization of HSCD5, a novel human stearoyl-CoA desaturase unique to primates. *Biochem Biophys Res Commun* 2005; **332**: 735–742.
- 126 Jiang G, Li Z, Liu F *et al.* Prevention of obesity in mice by antisense oligonucleotide inhibitors of stearoyl-CoA desaturase-1. *J Clin Invest* 2005; **115**: 1030–1038.
- 127 Rahman SM, Dobrzyn A, Dobrzyn P *et al.* Stearoyl-CoA desaturase 1 deficiency elevates insulin-signaling components and down-regulates protein-tyrosine phosphatase 1B in muscle. *Proc Natl Acad Sci USA* 2003; **100**: 11110–11115.
- 128 Thuresson ER. Inhibition of glycerol-3-phosphate acyltransferase as a potential treatment for insulin resistance and type 2 diabetes. *Curr Opin Investig Drugs* 2004; **5**: 411–418.
- 129 Lindén D, William-Olsson L, Ahnmark A *et al.* Liver directed overexpression of mitochondrial glycerol-3-phosphate acyltransferase results in hepatic steatosis, increased triglyceride secretion and reduced fatty acid oxidation. *FASEB J* 2006; **20**: 434–443.
- 130 Hammond LE, Neschen S, Romanelli AJ *et al.* Mitochondrial glycerol-3-phosphate acyltransferase-1 is essential in liver for the metabolism of excess acyl-CoAs. *J Biol Chem* 2005; **280**: 25629–25636.
- 131 Chen HC, Farese RV Jr. Inhibition of triglyceride synthesis as a treatment strategy for obesity: lessons from DGAT1-deficient mice. *Arterioscler Thromb Vasc Biol* 2005; **25**: 482–486.
- 132 Yu YH, Ginsberg HN. The role of acyl-CoA: diacylglycerol acyltransferase (DGAT) in energy metabolism. *Ann Med* 2004; **36**: 252–261.
- 133 Yu XX, Murray SF, Pandey SK *et al.* Antisense oligonucleotide reduction of DGAT2 expression improves hepatic steatosis and hyperlipidemia in obese mice. *Hepatology* 2005; **42**: 362–371.
- 134 Lam TK, Schwartz GJ, Rossetti L. Hypothalamic sensing of fatty acids. *Nat Neurosci* 2005; **8**: 579–584.
- 135 Stulnig TM, Waldhausl W. 11 β -Hydroxysteroid dehydrogenase type 1 in obesity and type 2 diabetes. *Diabetologia* 2004; **47**: 1–11.
- 136 Taylor SD, Hill B. Recent advances in protein tyrosine phosphatase 1B inhibitors. *Expert Opin Investig Drugs* 2004; **13**: 199–214.
- 137 Niswender KD, Baskin DG, Schwartz MW. Insulin and its evolving partnership with leptin in the hypothalamic control of energy homeostasis. *Trends Endocrinol Metab* 2004; **15**: 362–369.
- 138 Air EL, Strowski MZ, Benoit SC *et al.* Small molecule insulin mimetics reduce food intake and body weight and prevent development of obesity. *Nat Med* 2002; **8**: 179–183.

- 139 Hu Z, Cha SH, Chohann S, Lane MD. Hypothalamic malonyl-CoA as a mediator of feeding behavior. *Proc Natl Acad Sci USA* 2003; **100**: 12624–12639.
- 140 Kim MS, Lee KU. Role of hypothalamic 5'-AMP-activated protein kinase in the regulation of food intake and energy homeostasis. *J Mol Med* 2005; **83**: 514–520.
- 141 Perrin C, Knauf C, Burcelin R. Intracerebroventricular infusion of glucose, insulin, and the adenosine monophosphate-activated kinase activator, 5-aminoimidazole-4-carboxamide-1-beta-D-ribofuranoside, controls muscle glycogen synthesis. *Endocrinology* 2004; **145**: 4025–4033.
- 142 Trevaskis JL, Butler AA. Double leptin and melanocortin-4 receptor gene mutations have an additive effect on fat mass and are associated with reduced effects of leptin on weight loss and food intake. *Endocrinology* 2005; **146**: 4257–4265.
- 143 Packard GC, Boardman TJ. The use of percentages and size-specific indices to normalise physiological data for variation in body size: wasted time wasted effort? *Comp Biochem Physiol A* 1999; **122**: 37–44.
- 144 Wilson S, Arch JRS, Thurlby PL. Genetically obese C57BL/6 ob/ob mice respond normally to sympathomimetic compounds. *Life Sci* 1984; **35**: 1301–1309.
- 145 Arch JRS, Stock MJ, Trayhurn P. Leptin resistance in obese humans: does it exist and what does it mean. *Int J Obes Relat Metab Disord* 1998; **22**: 1159–1163.
- 146 Montague CT, Farooqi IS, Whitehead JP *et al.* Congenital leptin deficiency is associated with severe early-onset obesity in humans. *Nature* 1997; **387**: 903–908.
- 147 Ishihara A, Kanatani A, Okada M *et al.* Blockade of body weight gain and plasma corticosterone levels in Zucker fatty rats using an orally active neuropeptide Y Y1 antagonist. *Br J Pharmacol* 2002; **136**: 341–346.
- 148 Lowell BB, Susulic VS, Hamann A *et al.* Development of obesity in transgenic mice after genetic ablation of brown adipose tissue. *Nature* 1993; **366**: 740–742.