

**Themed Issue: Translational Neuropharmacology – Using Appropriate
Animal Models to Guide Clinical Drug Development**

REVIEW

The utility of animal models to evaluate novel anti-obesity agents

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Keywords

obesity; animal models; DIO;
Qnexa; anti-obesity drug

Received

8 November 2010

Revised

20 December 2010

Accepted

28 December 2010

The global incidence of obesity continues to rise and is a major driver of morbidity and mortality through cardiovascular and cerebrovascular diseases. Animal models used in the discovery of novel treatments for obesity range from straightforward measures of food intake in lean rodents to long-term studies in animals exhibiting obesity due to the continuous access to diets high in fat. The utility of these animal models can be extended to determine, for example, that weight loss is due to fat loss and/or assess whether beneficial changes in key plasma parameters (e.g. insulin) are evident. In addition, behavioural models such as the behavioural satiety sequence can be used to confirm that a drug treatment has a selective effect on food intake. Typically, animal models have excellent predictive validity whereby drug-induced weight loss in rodents subsequently translates to weight loss in man. However, despite this, at the time of writing orlistat (Europe; USA) remains the only drug currently marketed for the treatment of obesity, with sibutramine having recently been withdrawn from sale globally due to the increased incidence of serious, non-fatal cardiovascular events. While the utility of rodent models in predicting clinical weight loss is detailed, the review also discusses whether animals can be used to predict adverse events such as those seen with recent anti-obesity drugs in the clinic.

LINKED ARTICLES

This article is part of a themed issue on Translational Neuropharmacology. To view the other articles in this issue visit <http://dx.doi.org/10.1111/bph.2011.164.issue-4>

Abbreviations

5-HT, 5-hydroxytryptamine; CTA, conditioned taste aversion; DIO, dietary-induced obese; EMEA, European Medicines Agency; FDA, Food and Drug Administration; GLP, glucagon-like-peptide; MCH, melanin-concentrating hormone

Introduction

Among healthcare experts around the world, there is agreement that the global epidemic of obesity will be one of the leading causes of morbidity and mortality for current and future generations, unless the rise in the prevalence of this disorder is reversed. Indeed, the metabolic consequences of obesity are drivers of other life-threatening disorders including dyslipidaemia, hypertension, atherogenesis and Type 2 diabetes (for review see Heal *et al.*, 2009). Once considered to be a problem mainly in Western cultures, developing nations have now joined the ranks of countries burdened by obesity. Indeed, a recent report from the World Health Organisation estimated that in 2008 approximately 500 million adults were obese and 1.5 billion were overweight worldwide (World Health Organisation, 2011).

In terms of pharmacotherapy for obesity, only four new drugs have been registered for the treatment of obesity over the last 15 years: dexfenfluramine (Redux), sibutramine (Meridia, Reductil), orlistat (Xenical) and rimonabant (Acomplia). Furthermore, due to the incidence of side effects, orlistat (Europe; USA) remains, at the time of writing, the only marketed drug, with sibutramine having recently been withdrawn in Europe (European Medicines Agency (EMA), 2010) and the USA (voluntarily by Abbott) due to an increased risk of serious, non-fatal cardiovascular events, such as stroke or heart attack in the Sibutramine Cardiovascular OUTcome (SCOUT) trial. This situation appeared unlikely to change in the near future because both Qnexa (a proprietary formulation of topiramate and phentermine) and lorcaserin (a 5-HT_{2C} receptor agonist) suffered setbacks when the Food and Drug Administration (FDA)-appointed expert advisory panel recommended that the products should not be approved for

clinical use in the USA despite significant weight loss in clinical trials (Gadde and Allison, 2009; Smith *et al.*, 2010). Importantly, the principal reason for drug withdrawals has been due to concerns over safety rather than the effect on body weight *per se*. For example, the CB₁ receptor antagonist, rimonabant, significantly reduced body weight in clinical trials (Després *et al.*, 2005; Van Gaal *et al.*, 2005) but was not approved in the USA because treatment had been linked to severe psychiatric adverse events, e.g. anxiety, depression and suicidal ideation. Subsequently, the EMEA suspended the drug (EMA, 2008). Interestingly, although some safety concerns remain, recently Contrave, a fixed dose combination therapy for obesity in a single tablet consisting of sustained release formulations of both naltrexone and bupropion, has been recommended for approval by the FDA advisory panel of experts. If this recommendation is subsequently endorsed by the FDA early in 2011, then this drug will be registered for the treatment of obesity.

Due to the poor track record of approved drugs in this therapeutic indication, there remains an enormous unmet need for the discovery of safer compounds delivering superior efficacy. As a result, the importance of animal models in not only detecting changes in body weight but also providing confidence that these changes are behaviourally specific and not a result of drug-induced side effects, is of critical importance. In addition to the key role in the screening of novel compounds for effects on food intake and/or body weight, animal models have utility in the identification of the physiological and genetic basis of obesity, which can result in the discovery and validation of new therapeutic targets [e.g. through the generation and characterization of transgenic animals (Powell, 2006)].

This review details a number of rodent models, focusing in particular on those with especial relevance to the discovery of new drugs for the treatment of obesity. While no single model is necessarily applicable to all drug research programmes, it is suggested that certain models have greater validity than others and, where possible, should be used in preference in order to achieve the best possible prediction of the outcome in clinical practice.

Animal models used in the discovery of novel ligands for the treatment of obesity

The regulation of body weight is dependent upon the interaction between food intake and energy expenditure. For example, if, over an extended period of time daily food intake increases with an insufficient increase in energy expenditure (the energy imbalance gap) then body weight will increase. This is typically the case in the development of obesity where the main driver is increased total energy intake (Swinburn *et al.*, 2009). Specifically, access to cheap, highly palatable, calorie-dense foods has led to an increase in energy intake, which has been coupled with an increasingly sedentary lifestyle (e.g. increased use of cars, increased use of video games as a leisure activity, etc.). This relationship between food intake, energy expenditure and body weight leads not only to different mechanisms by which a drug may reduce body

weight (i.e. through the reduction of food intake, stimulation of energy expenditure, or both), but is also of relevance in the selection and development of appropriate animal models for evaluating anti-obesity potential. For example, an acute model of food intake may have little utility in detecting compounds that may reduce body weight through the stimulation of energy expenditure.

Acute models of food intake

Rats and mice are the predominant models of human obesity used in drug discovery although phylogenetically, they are not closely related to man. However, like man, they are omnivores and have complex taste and digestive systems for identifying and consuming a variety of different foods. Furthermore, there are similarities neuroanatomically in those brain areas thought to play a role in the control of food intake and it is well established that a number of different neurotransmitters and peptides produce similar effects on food intake and energy homeostasis in laboratory rodents and man. Numerous pharmacological approaches for the treatment of obesity have focused on drug targets that reduce food intake. For example, the clinically effective drugs *d*-fenfluramine, sibutramine, rimonabant and lorcaserin all reduce food intake when given acutely to lean rats or mice (Neill and Cooper, 1989; Jackson *et al.*, 1997; Colombo *et al.*, 1998; Vickers *et al.*, 1999; Verty *et al.*, 2004; Smith *et al.*, 2008; Thomsen *et al.*, 2008). Hence, investigating the acute effect of candidate drugs on the food intake of a lean rat or mouse is not only a rapid screen, but provides information as to both the relative potencies of compounds to inhibit food intake and, also, the duration of action of compounds *in vivo*. Accordingly, superior compounds can be selected and studied further in, for example, chronic studies investigating effects on body weight.

Inter-laboratory variations in the methods used for detecting drug-induced changes in acute food intake are numerous. However, most if not all study paradigms tend to involve stimulating food intake in rats or mice to some extent and subsequently evaluating the effect of a treatment where baseline food intake is elevated. Male animals are usually used to avoid the influence of the oestrus cycle on food intake (Asarian and Geary, 2006). Perhaps the most straightforward method is to fast an animal before the test, e.g. overnight (Vickers *et al.*, 2001; Hadcock *et al.*, 2010). This method has advantages in that it is straightforward and induces a robust increase in the food intake of vehicle-treated controls. However, it could be argued that this approach is not physiological (e.g. an overnight fast would be a major stressor for a mouse) and there is evidence that the potency and duration of action of drug treatments can differ in such fasting paradigms compared with more refined approaches where baseline food intake may be stimulated either through the use of a palatable diet such as wet mash (e.g. Neill and Cooper, 1989; Vickers *et al.*, 1996, 1999) or the use of reverse-phase lighting so that the initial part of the study is conducted during the dark period when rats in particular exhibit high levels of food intake (Jackson *et al.*, 1997; Hadcock *et al.*, 2010). Typically, in order to reduce food intake by the same amount (e.g. 50% of vehicle), higher doses are often needed

in re-feeding models compared with freely-feeding models. For example, the CB₁ receptor antagonist, CE-178253, more potently reduced food intake in a dark cycle induced feeding paradigm (i.e. spontaneous feeding) compared with an assay where animals were allowed to feed after an overnight fast (Haddock *et al.*, 2010). Such differences may be due to the large drive to eat of animals feeding after a fast, which needs to be overcome by higher doses of the drug.

One consideration when screening drugs in an acute food intake assay is that the test will be insensitive to drugs with a delayed onset of action. Such drugs include 5-HT₆ receptor partial agonists (Heal *et al.*, 2008) and MCH₁ receptor antagonists (Shearman *et al.*, 2003). On such occasions initial screens may require repeated drug administration, or, alternatively, in the case of MCH₁ receptor antagonists, blockade of MCH-induced food intake (Shearman *et al.*, 2003). Another disadvantage of acute food intake tests is that the assay is not relevant to all mechanisms of drug action. For example, an acute food intake model will prove of little value in the identification of either drugs that increase energy expenditure or lipase inhibitors, which may reduce body weight through the reduced absorption of fat from the gut.

In general, acute food intake studies do not need to be undertaken in obese animals. Furthermore, such studies can be run in rats or mice, because both species are sensitive to the acute effects of clinically effective compound classes such as serotonin and noradrenaline reuptake inhibitors (e.g. sibutramine), CB₁ receptor antagonists (e.g. rimonabant) and 5-HT_{2C} receptor agonists (e.g. lorcaserin) (Hewitt *et al.*, 2002; Matsumoto and Iijima, 2003; Poncelet *et al.*, 2003; Halford *et al.*, 2010). At this stage of the discovery process the use of mice may prove advantageous because the amount of each compound required is much less. Accordingly, resource can be focused on the synthesis of novel ligands rather than scaling up existing candidates with suitable *in vitro* profiles for rat studies. That said, in some instances species selection is of critical importance. For example, in rats and humans 5-HT₆ receptors are widely expressed and focused particularly in the basal ganglia (Hirst *et al.*, 2003). In contrast, not only is the 5-HT₆ receptor less widely expressed in the mouse CNS, but it has a different pharmacological profile to the other two species despite similar sequence homology (Hirst *et al.*, 2003). Accordingly, where 5-HT₆ receptor ligands are to be developed for the treatment of obesity (or any other disorder), then rat models should be used in preference to mouse models.

Long-term (chronic) models of food intake and body weight

Acute studies are typically undertaken in lean male animals in order to profile compounds rapidly and obtain information in regard to the potency, efficacy, duration of action, and potentially the side effect profile of a compound, *in vivo*. Such models can be used to select compounds that have a suitable profile for chronic testing (e.g. 28 days) because effects of a drug over a longer dosing period are usually required in order to demonstrate a maintained reduction of body weight. Sub-chronic or chronic feeding studies are sometimes performed

in normal, lean rats and mice (e.g. Vickers *et al.*, 2000; 2003a; Smith *et al.*, 2008) or in animal models of 'overweight' such as rats maintained on a high-fat diet for a relatively short period (e.g. 2 weeks) before the start of the study (Thomas *et al.*, 2006). These studies can be used as a screen to bridge the gap between acute feeding studies and chronic feeding studies in obese animals that are expensive.

The choice of sex in animal models of obesity is important. In humans, adipose tissue is distributed subcutaneously and in the abdomen (visceral fat). Females have relatively more subcutaneous fat than males whereas, in contrast, males have more visceral fat, which is associated with the complications of obesity (Wajchenberg, 2000). Recent studies have shown similar differences in the amount and distribution of adipose tissue in rats and in the circulating levels of the hormones related to adiposity. Thus, female rats have more fat and especially more subcutaneous fat than male rats and male rats have more visceral fat (Clegg *et al.*, 2003a,b). In addition, although the levels of the adipose hormones, leptin and insulin, generally correlate with the amount of adipose tissue in both males and females, females are more sensitive to the inhibitory effects of leptin on food intake whereas male rats are more responsive to insulin (Clegg *et al.*, 2003b). These differences argue that the effects of potential anti-obesity agents on body weight should be evaluated in both male and female animals.

Although rats and mice are the predominant models of human obesity, there are some important differences in physiology between rodents and man (e.g. rats do not have a vomit reflex or gall bladder). Furthermore, the standard environment for most rodent feeding studies is not analogous to the human situation as animals are typically individually housed (which restricts social interaction and therefore physical activity), in relatively small cages (which restrict physical activity) and are normally housed at temperatures of 20–23°C, which are several degrees lower than their thermoneutral temperature (29–32°C) such that animals will be expending energy to keep warm. In addition, food is readily available at all times, which does not always happen in man. Another limitation of these models is that they do not take into account the complex psychological factors that control food intake and can lead to overeating in man (Halford *et al.*, 2010). That said, despite these weaknesses there are a number of strengths to obese animal models and these are discussed later.

Broadly speaking, obese animal models can be subdivided into two types: dietary-induced models of obesity and genetic models of obesity. An animal model of obesity will ideally mimic as closely as possible the human condition in terms of the causes of the disorder (construct validity) and the phenomenological similarity between the characteristics exhibited by the animal model and the specific symptoms of the human condition (face validity). Furthermore, the effects of pharmacological manipulation in the model should be identical to the clinical outcome in man (predictive validity). Although single gene mutations observed in some animal models have been linked to human obesity in the case of leptin (Montague *et al.*, 1997), the leptin receptor (Clément *et al.*, 1998) and others, such instances are rare as mentioned below and it is generally considered that an individual's susceptibility to obesity in the current environment of high

calorie foods and reduced activity is determined by a polygenic background (Bell *et al.*, 2005; Mutch and Clément, 2006). Accordingly, polygenetic models such as the dietary-induced obese rat and mouse are of especial importance.

Dietary-induced obesity models

The first use of a 'high-fat diet' to induce obesity in rats was by Masek and Fabry (1959). Since then numerous methods have been published using the general approach whereby normal, lean rats or mice are provided free access to diets high in fat over a period of 3–4 months. With time animals exhibit increased weight gain characterized principally by a marked increase in body fat (e.g. Harrold *et al.*, 2000; Jones *et al.*, 2001; Naderali *et al.*, 2001; Ravinet-Trillou *et al.*, 2003; Li *et al.*, 2008; Madsen *et al.*, 2010). In addition, although the animals do not typically develop diabetes (hyperglycaemia) they exhibit insulin resistance, glucose intolerance, elevated plasma leptin and a mild dyslipidaemia with plasma cholesterol and triglyceride often elevated compared with appropriate controls on a standard diet (Dickinson *et al.*, 1998; Harrold *et al.*, 2000; Naderali *et al.*, 2001; Ravinet-Trillou *et al.*, 2003; Li *et al.*, 2008; Madsen *et al.*, 2010). Furthermore, there are some reports that blood pressure is altered in dietary-induced obese (DIO) rats (e.g. Lobbey *et al.*, 2007). Indeed, some workers have developed models of dietary-induced obesity using spontaneously hypertensive rats (Miesel *et al.*, 2010). Importantly, such changes in DIO mice and rats mimic the changes observed in obese patients (See Table 1).

There are numerous differences in the methodology used throughout the literature, with some laboratories using commercially available high fat diets (e.g. Pierroz *et al.*, 2002; Thornton-Jones *et al.*, 2006) and others using cafeteria diets where animals have a choice of various palatable foods such as chocolate, peanuts, condensed milk, etc., which encourages overeating and is closer to the human situation than maintaining rats on a single food source (e.g. Naderali *et al.*, 2001; Fisas *et al.*, 2006). Although some diet-dependent

differences in the phenotype of obese animals have been reported (Buettner *et al.*, 2006), in general, despite across laboratory methodological variations, the metabolic profiles of rodents allowed long-term access to high-fat diets are reasonably uniform. Other differences across laboratories include the use only of animals that 'respond' to the high fat diet in terms of weight gain. Perhaps the most well-known example is the DIO and diet-resistant out-bred rat model of Levin and colleagues (Levin *et al.*, 1997; Madsen *et al.*, 2010).

Irrespective of the precise nature of the model used, the changes seen in DIO animals are remarkably consistent with those seen in obese patients in the clinic (for summary see Table 1). Indeed, functional genomic studies comparing gene expression changes in DIO rats and obese humans have demonstrated significant commonalities for various tissues and biological pathways affected in obesity (Li *et al.*, 2008). For example, not only was the gene expression profile of adipose tissue similar in the DIO rat to that of obese Pima Indians, but genes in immune response and angiogenesis pathways were significantly up-regulated in obese rats and humans in comparison with non-obese controls (Li *et al.*, 2008). Such data reinforce both the face and construct validity of the DIO rat as a model of human obesity. Using the functional genomics approach at least one key difference between the obese rat and human was identified. Hence, genes involved in lipid metabolism tended to be down-regulated in obese humans but not in the DIO rat model used in the publication (Li *et al.*, 2008). While this finding illustrates that the DIO rat model is not a perfect match to the human situation, it also appears to be consistent with data suggesting that in the study of lipid metabolism, rats and mice are inappropriate models of the human situation and other rodent species such as the hamster are to be preferred (Kris-Etherton and Dietschy, 1997).

The ability of DIO animals to predict clinically meaningful weight loss is good. Hence, drugs such as *d*-fenfluramine (Bradbury *et al.*, 2005), sibutramine (Jackson *et al.*, 2004;

Table 1

Comparison of genetically-obese and DIO rodent models to human obesity

Characteristic Sex	<i>ob/ob</i> mouse Male or female	<i>db/db</i> mouse Male or female	<i>fa/fa</i> Zucker rat Male or female	DIO rat/mouse Male or female	Common human obesity Male or female ¹
Polygenic basis	X	X	X	✓	✓
Access to calorie-dense high-fat diet	X	X	X	✓	✓
Marked visceral adiposity	✓	✓	✓	✓	✓
Hyperleptinaemia	X	✓ ²	✓ ²	✓	✓
Hyperinsulinaemia	✓	✓	✓	✓	✓
Hyperglycaemic	✓	✓	X	X/✓	X/✓
Hypertensive	?/✓	?/✓	?/✓	?/✓	✓
Sensitive to clinically effective weight loss agents	?/✓	?/✓	?/✓	✓	✓

The table illustrates the similarities between the obesity and cardio-metabolic risk factors observed in DIO rats and mice, with humans.

¹However, greater than 70% of patients recruited into trials of novel anti-obesity drugs are female.

²Increased plasma leptin levels are evident compared with age-matched controls. However, unlike DIO animals or most human patients with obesity, *db/db* mice and *fa/fa* Zucker rats have mutations of the leptin receptor such that leptin receptor function is impaired.

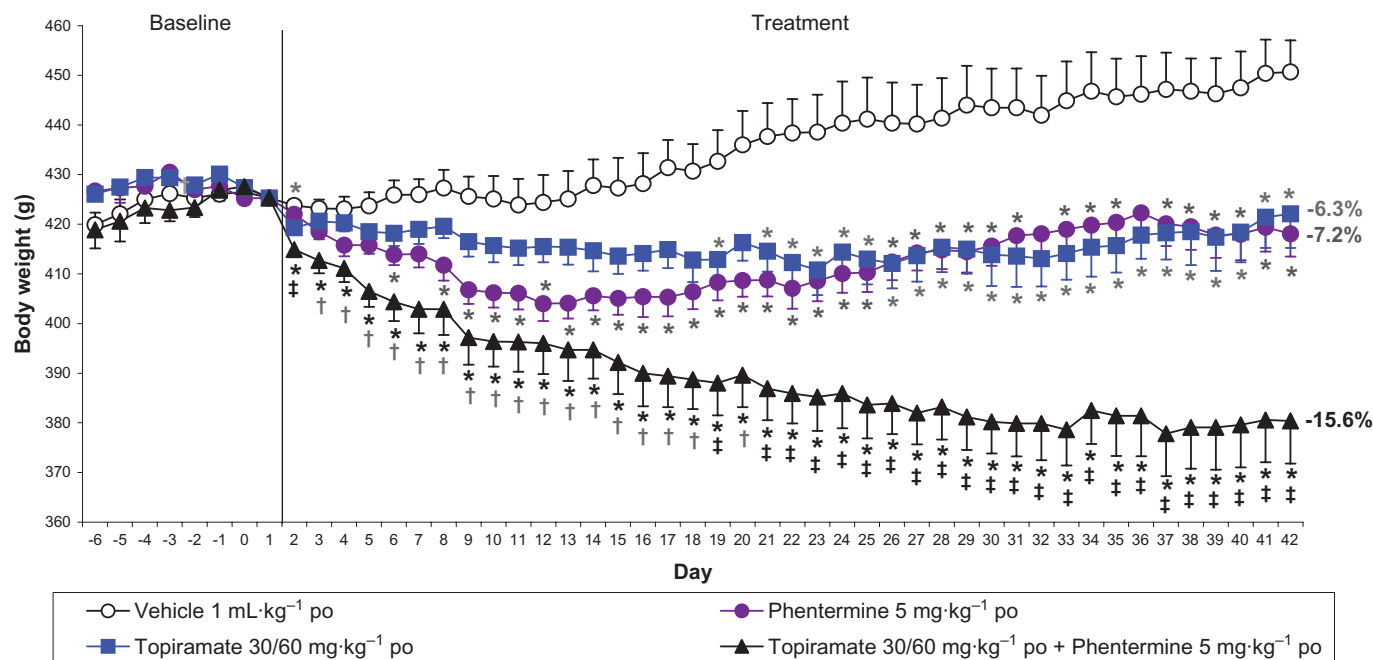


Figure 1

The effect of phentermine and topiramate alone and in combination on body weight in rats with dietary-induced obesity. Female Wistar rats were maintained on reverse-phase lighting (lights out 09.30–17.30 h) with free access to powdered high-fat chow, chocolate, peanuts and tap water during the induction of obesity (14 weeks) and throughout the feeding study. After a 7 day run-in period, during which rats were dosed orally with vehicle once daily, rats were dosed orally with vehicle, topiramate, phentermine or topiramate plus phentermine once daily for 41 days. Topiramate was given in a dose of 30 mg·kg⁻¹ (increased to 60 mg·kg⁻¹ on Day 15). Phentermine was given in a dose of 5 mg·kg⁻¹. Rats, feeding jars and water bottles were weighed every day at the time of dosing, which was at the onset of the dark period. Topiramate and phentermine were dissolved in 1% Tylose MH50/0.1% poloxamer (in deionized water; dose volume 1 mL·kg⁻¹). All doses are for the free base. Results are adjusted means $\pm$ SEM; $n = 10$. * $P < 0.05$ (vs. controls), † $P < 0.05$ (topiramate plus phentermine vs. topiramate alone), ‡ $P < 0.05$ (topiramate plus phentermine vs. both topiramate and phentermine alone). Numbers represent % reduction in body weight compared with the control group on Day 42.

2005; Fisas *et al.*, 2006; Madsen *et al.*, 2010), orlistat (Hogan *et al.*, 1987; Hauptman *et al.*, 2000; Jackson *et al.*, 2004) and rimonabant (Ravinet-Trillou *et al.*, 2003; Jackson *et al.*, 2005) all significantly reduce body weight in DIO rats or mice and are often used as reference agents in studies with novel ligands (e.g. Bradbury *et al.*, 2005; Fisas *et al.*, 2006; Hansen *et al.*, 2010; Madsen *et al.*, 2010). Accordingly, the model is widely used to gain insight into the potential weight loss a drug will deliver in the clinic. Because a placebo-subtracted weight loss of >5% maintained over 1 year is the primary efficacy end point for approval of anti-obesity drugs and sibutramine (for example) delivers a 5–10% reduction in the body weight of obese patients (Bray, 2001) then a dose of sibutramine that delivers approximately 10% weight loss in DIO animals would appear to be a sensible positive control to use in these studies (e.g. Fisas *et al.*, 2006).

It could be argued that in rodent studies, reference compounds should be administered at doses reflecting their clinical steady-state exposure rather than the clinical % body weight loss. However, this is not practical. Many of the drugs that are identified using animal models have not been tested in man. Indeed, relatively little may be known about their pharmacokinetics in animals at the time of testing and they could not be given in comparable doses. Instead reference compounds are given at doses producing a clear endpoint,

that is, a consistent degree of weight loss that matches their clinical efficacy as mentioned above.

Assessing the predictive validity of the DIO rat and mouse is a continuous process because it is only when compounds that are efficacious in an animal model are tested in the clinic, that the utility of the model is understood. Two novel drug applications recently reviewed by the FDA are the 5-HT_{2C} receptor agonist, lorcaserin, and the proprietary combination of topiramate and phentermine, Qnexa. As one would expect, lorcaserin reduced body weight in DIO animals (e.g. the Levin rat model, Thomsen *et al.*, 2008). Similarly, topiramate, phentermine and the combination were all observed to reduce body weight in the DIO rat (Jackson *et al.*, 2007). In agreement with the clinical studies, the combination of phentermine and topiramate delivered greater effects on body weight compared with either treatment when dosed alone. Indeed, in the DIO rat the body weight loss with the combinations was substantial (15% cf. vehicle-treated controls) and greater than that typically observed with sibutramine in the model. The body weight and daily food intake data are shown in Figures 1 and 2 and illustrate that DIO animals are also useful in detecting the body weight efficacy of drug combinations and their overall effect on food intake. Such combination strategies are currently a popular approach in the development of novel anti-obesity agents. Indeed,

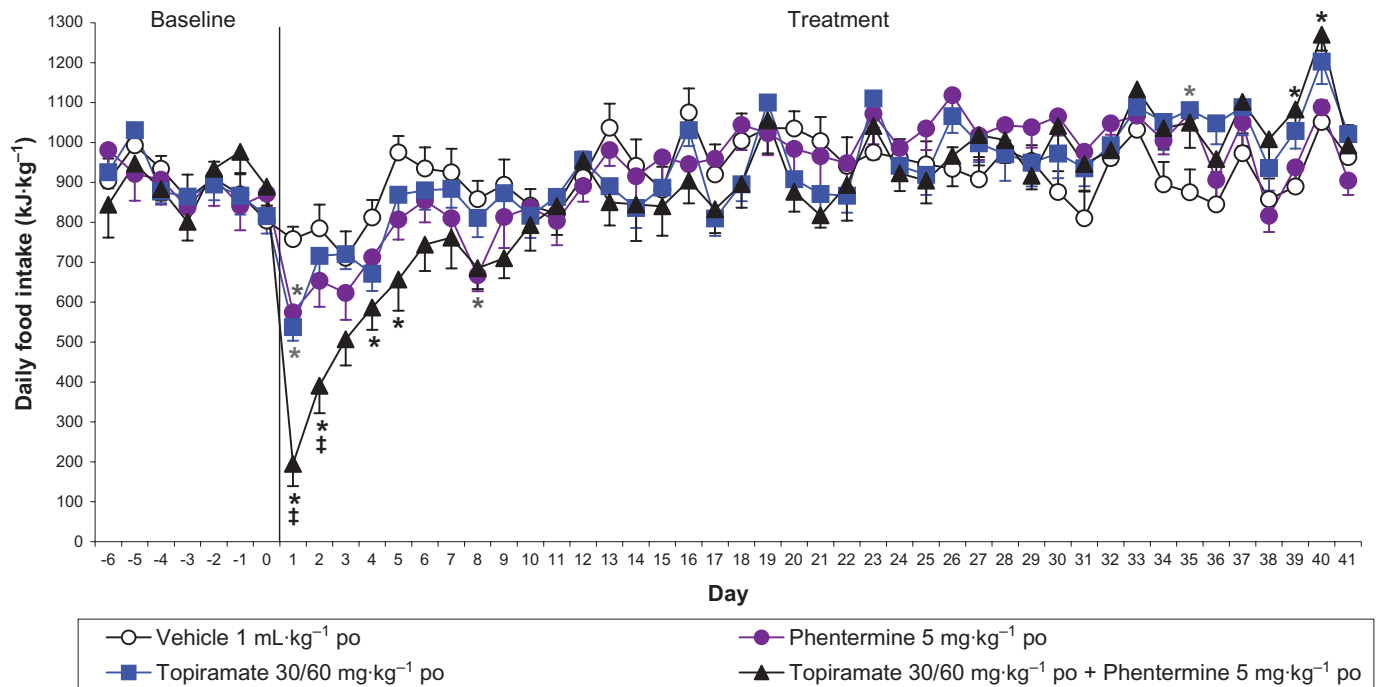


Figure 2

The effect of phentermine and topiramate alone and in combination on food intake in rats with dietary-induced obesity. Food intake data are expressed as $\text{kJ}\cdot\text{kg}^{-1}$ to take account of the different caloric values of the diet components (high fat chow, peanuts and chocolate). Results are adjusted means $\pm$ SEM; $n = 9\text{--}10$. * $P < 0.05$ (vs. controls), ‡ $P < 0.05$ (topiramate plus phentermine vs. both topiramate and phentermine alone).

the FDA advisory panel recently recommended that the bupropion/naltrexone fixed dose combination therapy, Contrave, be approved for the treatment of obesity. Accordingly, with the FDA expected to make a decision in January 2011, this proprietary combination may be the next anti-obesity drug registration. In preclinical studies, this combination reduces the acute food intake of DIO mice at doses of naltrexone and bupropion that do not significantly affect food intake when given alone (Greenway *et al.*, 2009). To our knowledge this combination has not been tested long-term in a DIO rat model, and such data would be of especial interest. Further combination strategies being assessed in the clinic include pramlintide (synthetic amylin) and metreleptin (a human leptin analogue). This approach is not only effective in preclinical DIO rat studies using amylin and leptin (Roth *et al.*, 2008) but the pramlintide/metreleptin combination also significantly reduced body weight in a 24 week study in obese or overweight patients (Ravussin *et al.*, 2009).

Statistical analysis can reveal whether the effects of two drugs on body weight, food intake and other factors are additive or synergistic in nature, that is, whether the response to the combination treatment is greater than predicted from the sum of the effects of the two drugs given alone. Such studies have shown that the reductions in body weight and food intake produced by the combination of topiramate and phentermine in DIO rats (Figures 1 and 2) are simply additive (unpublished data) whereas the reductions in body weight and food intake produced by the combination of amylin and leptin in DIO rats are synergistic as amylin restores leptin responsiveness in these animals (Trevaskis *et al.*, 2008).

As discussed, the DIO rat model appears to have excellent predictive validity, even in regard to the modern trend towards the development of drug combinations for obesity. This is substantiated by Figure 3 where the weight loss induced by a number of drugs in the clinic is compared with the weight loss seen in the DIO rat.

Genetically modified models of obesity

Genetically obese animals that have been characterized following spontaneously occurring single gene mutations and are widely used as animal models of obesity include the *ob/ob* mouse, *db/db* mouse and the Zucker *fa/fa* rat (Ingalls *et al.*, 1950; Zucker and Zucker, 1961; Coleman, 1978). The obesity in these animals becomes visible within several weeks of birth and they continue to put on weight and eventually become several times heavier than their lean counterparts with 50% of their body weight accounted for by fat. The phenotype of all these animals has now been well characterized (for review see Bailey and Flatt, 2003) and can depend on the background strain. All three models display hyperphagia, hyperinsulinaemia and hyperlipidaemia but differ in the degree of hyperglycaemia. Thus, *db/db* mice have markedly elevated plasma glucose levels and are often used as a model of diabetes whereas *ob/ob* mice display mild to moderate hyperglycaemia and Zucker *fa/fa* rats are not hyperglycaemic but are insulin-resistant (for review see Bailey and Flatt, 2003). As in obese patients, there are reports that genetically-obese animals such as the *db/db* mouse are hypertensive (e.g. Su *et al.*, 2008), although such findings are not consistent across the literature and, unlike in obese humans (Kotsis *et al.*,

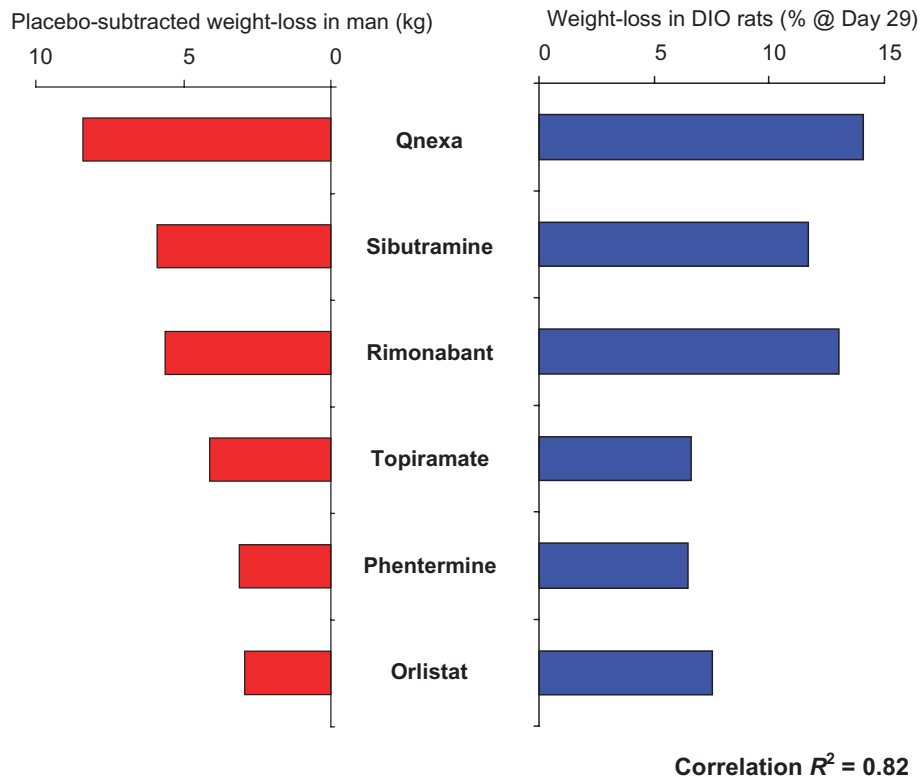


Figure 3

A comparison of the weight-loss produced by various anti-obesity drugs in man versus the body weight loss over a 28 day dosing period observed in the DIO rat. Correlation $r^2 = 0.82$. Figure adapted from Heal *et al.*, 2011. DIO rat data taken from Jackson *et al.*, 2004, Jackson *et al.*, 2005, Jackson *et al.*, 2007. Human data taken from Bray *et al.*, 2003, <http://www.vivus.com/pipeline/qnexa-obesity>, Pi-Sunyer *et al.*, 2006, Després *et al.*, 2005, Apfelbaum *et al.*, 1999, Hauptman *et al.*, 2000, James *et al.*, 2000.

2005), may be related to the incidence of sleeping behaviour and the light/dark cycle (Silvani *et al.*, 2009). All three models also display a number of other abnormalities, notably in reproductive biology. Specifically, female *ob/ob* mice are infertile (Kennedy *et al.*, 2010).

Each of these monogenetic models of obesity exhibits deficits in the signalling pathways involving the pleiotropic adipokine, leptin. In 1994, the *obese* gene and its protein, leptin, were discovered (Zhang *et al.*, 1994). The mutation in *ob/ob* mice prevents the production of leptin whereas *db/db* mice and Zucker *fa/fa* rats have marked elevations in circulating leptin but have deficient leptin receptors (see Bailey and Flatt, 2003 and Kennedy *et al.*, 2010 for reviews). The fact that the *ob/ob* mice are not hyperleptinaemic is a major discrepancy in the aetiology of the disease between *ob/ob* mice and common obesity in man where leptin levels are substantially increased (Correia and Haynes, 2007). Thus, in most mammals, including man, circulating leptin levels, correlate closely with the degree of adiposity (Maffei *et al.*, 1995).

The discovery of leptin led to an extensive search for leptin or leptin receptor-deficient humans but it soon became apparent that only very small numbers of obese individuals had a loss-of-function mutation in this gene (12 people up to 2008; Farooqi and O'Rahilly, 2008). Furthermore, despite the finding that leptin-replacement therapy produced dramatic weight loss in leptin-deficient *ob/ob* mice and humans with

congenital leptin deficiency (Farooqi and O'Rahilly, 2009), the results of clinical trials in normal obese patients using recombinant leptin were disappointing (Heymsfield *et al.*, 1999). This was thought to be because polygenic common human obesity is associated with high circulating levels of leptin and leptin-resistance (Correia and Haynes, 2007). Polygenic DIO animals display hyperleptinaemia and are leptin-resistant and therefore mirror the normal obese state (Halaas *et al.*, 1997; Dickinson *et al.*, 1998; Roth *et al.*, 2008; Madsen *et al.*, 2010). It is clear that in contrast, monogenetic models such as *ob/ob* mice do not have as good construct or face validity and are an inferior model of common human obesity. While such models are routinely used in order to evaluate the effect of novel test compounds on body weight, caution should be taken when translating potential effects to man. For example, although the clinically effective CB₁ receptor antagonist, rimonabant, reduces body weight in the *fa/fa* Zucker rat (Vickers *et al.*, 2003b) and *ob/ob* mouse (Mohapatra *et al.*, 2009), the effects of sibutramine in both these models, and others, appears to be modest (Day and Bailey, 1998; Matsumoto and Iijima, 2003) compared with the effects exhibited clinically. Indeed, the hypophagic effect of sibutramine is reported to be reduced in *db/db* mice, *fa/fa* Zucker rats and KK-A^y mice compared with wild-type animals (Matsumoto and Iijima, 2003). Furthermore, in our laboratory, doses of rimonabant that reduced body weight markedly in the DIO

mouse did not affect the body weight of *db/db* mice (S. Vickers, unpubl. obs.). This contrasts with the clinical situation where, broadly speaking, sibutramine and rimonabant induce a similar weight loss in obese patients (for review see Vickers and Kennett, 2005).

Such examples indicate limitations in the predictive validity of these genetic models for assessing the effects of a drug on body weight loss and suggest that the use of DIO animals with their polygenetic basis and intact leptin system may provide a more robust approach for the screening of potential anti-obesity drugs. However, the relative sensitivity of genetically or dietary-induced obese animals (and appropriate wild-type or chow-fed controls) to reference anti-obesity drugs would ideally be tested more rigorously by the same laboratory using standardized conditions and matching drug exposure levels so that formal statistical analysis could be performed to confirm this hypothesis.

In addition to animals exhibiting an obese phenotype due to spontaneous gene mutations, molecular biology techniques have been used to produce obese animals by targeting specific genes. For example, mice with a targeted deletion of the 5-HT_{2C} receptor exhibit mild obesity and Type 2 diabetes (Tecott *et al.*, 1995). These models can provide useful information about the receptors and hormones that regulate the control of food intake and energy balance and can be used to provide proof of concept for novel anti-obesity targets. For example, the phenotype of the 5-HT_{2C} receptor knockout mouse suggests that the 5-HT_{2C} receptor is involved in the control of body weight and, in addition, suggests that 5-HT_{2C} receptor agonists (such as the subsequently developed drug, lorcaserin) could be useful treatments for obesity. While such models may have value in further understanding the mechanisms involved in the control of food intake and body weight, and may provide useful tools in drug screening strategies for specific molecular targets, they do not reflect the human situation and, in general, are not used as models of obesity.

Long-term models of food intake and body weight: additional measurements

The evaluation of a test compound in an animal model over an extended dosing period such as 28 days is time-consuming and can be resource-intensive. For example, the animals are not only dosed and require husbandry on each day of the feeding study, but, in the case of dietary-induced obesity, require husbandry and handling during the induction of obesity, which can take several months. As a result, typically it is not only body weight and food intake that are measured during such studies because they also provide excellent opportunities to assess (for example) whether drug treatments have led to changes in water intake, improvements in glucose control or insulin sensitivity (e.g. by the performance of a glucose tolerance test), or to confirm that changes in body weight are attributable to fat loss (body composition analysis). In addition, blood samples can be taken to measure changes in key hormones and markers (e.g. leptin) or to assess drug exposure after (and during) chronic dosing.

In some instances (e.g. to investigate the potential role of a drug on energy expenditure) it is important to understand

the contribution made by reduced food intake to the overall weight loss. Such studies usually involve pair-fed controls. Specifically, vehicle-treated animals are given the same amount of food as the drug treatment group and would be expected to lose the same amount of weight unless the reduction in body weight is due to factors other than, or in addition to, food intake. For example, if a compound acts to increase energy expenditure then the reduction in body weight of these animals will be greater than pair-fed controls. While such studies are routinely used (e.g. Vickers *et al.*, 2003a; Thornton-Jones *et al.*, 2006), one disadvantage of the approach is that the pair-fed control is likely to consume its daily food ration over a different time period. Specifically, an animal treated with an effective drug treatment may consume 10 g over a 24 h period whereas a pair-fed control is likely to be hungry and may consume this quantity over a much shorter time period (i.e. shortly after presentation of the food).

Water intake is often (although not always) measured during chronic feeding studies. Reductions in water intake are often secondary to reduced food intake. However, some drugs increase water intake while reducing food intake or having no effect on feeding behaviour at all demonstrating that these effects can be dissociated. Increased water consumption can be secondary to hyperactivity or diuresis. If changes in water intake are observed, body composition analysis can be used to examine the effects of the drugs on water content of the carcass.

Models used to assess the behavioural specificity of a drug treatment on food intake

Food intake in rats and mice can be decreased by a variety of factors including stress, sickness or drug-induced toxicity and not just by the enhancement of satiety or other specific mechanisms. For obvious reasons it is important to determine at an early stage of a research programme, that the role of a drug class in the regulation of food intake and body weight is specific in nature and not due to unwanted side effects. Ideally, a drug found to reduce gross food intake would be evaluated in other tests to examine whether the decrease in food intake was a specific effect on the mechanisms regulating food intake or due to non-specific disruption of normal feeding patterns (e.g. by hyperactivity or sedation) or drug-induced malaise.

The behavioural satiety sequence

In all feeding studies, animals should be observed every time the food is weighed. These gross behavioural observations should detect any overt behavioural effects such as increased or decreased locomotor activity or the induction of any other motor behaviours, which could interfere with the ability of the animal to eat. If required, the effects of the test drug on locomotor activity can be quantified using standard automated activity boxes. More rigorous methods couple behavioural observations with measurement of food intake (usually stimulated by mild food deprivation or the

presentation of a palatable wet mash diet) to examine the effects of drugs on the behavioural satiety sequence (Antin *et al.*, 1975). This characteristic sequence of behaviours (feeding followed by activity, grooming and resting) normally occurs when a rat or mouse eats until it is full (Antin *et al.*, 1975; Kitchener and Dourish, 1994; Vickers *et al.*, 1999). These studies, which can be performed in either rats or mice, have been used to show that anti-obesity agents such as *d*-fenfluramine and sibutramine inhibit food intake in a physiological manner by advancing the natural satiety sequence rather than by disrupting normal feeding behaviour (Halford *et al.*, 1998; Vickers *et al.*, 1999; Jackson *et al.*, 2000; Tallett *et al.*, 2009). Importantly, these drugs have also been reported to enhance satiety in man (Blundell and Hill, 1988; Hansen *et al.*, 1999). On the other hand, comprehensive behavioural profiling revealed that the reduction in food intake produced by the CB₁ antagonist, rimonabant, in rats may be due, at least in part, to response competition from compulsive scratching and grooming (Tallett *et al.*, 2007), analogous to the well-documented 'side effect' of pruritis (itching) produced by this compound in man. For a comprehensive review of the value of behavioural satiety sequence analysis in the screening strategy for novel anti-obesity agents see Rodgers *et al.*, 2010.

Models of drug-induced malaise and aversion

Some drugs may decrease food intake by producing gastrointestinal malaise, which can be difficult to detect as the animals may look behaviourally normal. Rats and mice lack the emetic response, which is a clear difference from man. However, the persistent eating of inert substances in rodents can be used to measure illness-response behaviour analogous to vomiting in other species (Takeda *et al.*, 1993; 1995a,b; Yamamoto *et al.*, 2002). This behaviour is called pica (after the magpie that is known for its voracious and indiscriminate appetite). The substance widely used to measure pica is kaolin or china clay. The hypothesis is that the animals eat kaolin, which is highly adsorbant. Toxins attach to the clay and are not absorbed into the circulation. Accordingly, this provides relief from the gastrointestinal distress. The model has been validated using motion sickness and a variety of agents known to produce sickness in man such as cisplatin, copper sulphate and apomorphine (Takeda *et al.*, 1993). Furthermore, the increases in cisplatin-induced kaolin intake in rats and mice can be reduced by the 5-HT₃ receptor antagonist, ondansetron, which prevents emesis in man (Takeda *et al.*, 1993, 1995b; Yamamoto *et al.*, 2002).

The technique has been used to show that the potential anti-obesity drugs, E-6837, exenatide (Byetta) and davalintide (AC2307), do not induce kaolin intake at doses reducing food intake (Fisas *et al.*, 2006; Mack *et al.*, 2006; 2010). Interestingly, despite these findings there is evidence in man that exenatide (an approved medicine for Type 2 diabetes that leads to some weight loss in patients) is associated with an increased incidence of nausea (Ellero *et al.*, 2010). Hence, data from the assay should be treated with some caution and used in conjunction with other tests such as the behavioural satiety sequence and/or the conditioned taste aversion (CTA) assay.

The CTA assay is a paradigm where animals are allowed exposure (e.g. 3 h) to a novel palatable substance (typically a

saccharin solution) and are then dosed with a test compound or vehicle. Subsequent to this conditioning (e.g. 72 h later), animals are re-exposed to the saccharin solution (often in the presence of an additional water bottle). Saccharin intake (or the preference for the saccharin solution over normal tap water) is recorded. A reduction in saccharin intake or saccharin preference ratio (i.e. the amount of saccharin solution drunk as a proportion of total fluid intake) compared with vehicle-treated controls is regarded as being indicative of a test compound, or dose, having aversive properties (Benoit *et al.*, 2003; Fisas *et al.*, 2006). In contrast to other paradigms this assay is not widely validated with anti-obesity agents and care should be taken in the interpretation of data because a number of drugs that are self-administered in humans (e.g. cocaine) are reported to support the development of a CTA in rodents (for review see Hunt and Amit, 1987). Hence, the test may be particularly sensitive to false positives. It does not identify the origin of the aversion that may be wholly unrelated to nausea or malaise and, accordingly, potential anti-obesity drugs should ideally be assessed in additional specificity tests in order to confirm the outcome of the CTA. Interestingly, not only does the peptide, GLP-1, support the development of a CTA (Thiele *et al.*, 1997) but the CTA induced by lithium chloride is blocked by a GLP-1 receptor antagonist (Seeley *et al.*, 2000). Such a finding is of relevance because the GLP-1 receptor agonist, exenatide, is associated with nausea in man as mentioned above (Ellero *et al.*, 2010).

Recently, conditioned gaping in rats has been described as a new preclinical tool to examine whether drugs are likely to produce nausea in man. The test is based on observations that rats display a characteristic gaping reaction when intraorally infused with a flavoured solution that has previously been paired with an emetic drug (Parker and Limebeer, 2006). The advantage of this model is that it appears to be more selective than the CTA test as only emetic drugs produce conditioned gaping in rats. Furthermore, known antiemetic drugs prevent the development of conditioned gaping but not CTA (Parker and Limebeer, 2006).

Determining effects on energy expenditure

As discussed previously, pharmacological strategies to increase energy expenditure have implications in body weight control and may lead to the development of novel treatments for obesity. Increased energy output may be due to increased physical activity, basal metabolic rate and/or thermogenesis. Increased activity is normally detected by observation or the use of activity boxes as described earlier whereas increased basal metabolic rate and thermogenesis can typically be measured in animals using closed circuit calorimeters (indirect calorimetry). Indirect calorimetry is so-called because the expenditure of calories is calculated from a measurement of oxygen uptake and relies on the fact that burning 1 calorie (Kilocalorie) requires 208.06 mL of oxygen. Typically animals are placed in sealed chambers at a thermoneutral temperature (29°C in the case of rats so that they do not need to expend any energy to maintain their core temperature) and their resting oxygen consumption is

recorded (Stock, 1975). Accordingly, this is used as a measure of energy expenditure.

This methodology has been used to demonstrate the thermogenic effects of thyroid hormones, β_3 -adrenoceptor agonists and the 5-HT and noradrenaline reuptake inhibitor, sibutramine, in rats (Silva, 1995; Connolly *et al.*, 1999; Skill *et al.*, 2000). Furthermore, chronic administration of sibutramine increased basal metabolic rate in these animals (Skill *et al.*, 2000). A number of other thermogenic approaches have been explored as a strategy for the treatment of obesity including activation of growth hormone receptors; inhibition of glucocorticoid receptors and the modulation of transcription factors or enzymes that promote mitochondrial biogenesis and fatty acid oxidation (Clapham and Arch, 2007). However, most of these approaches have not been developed beyond the preclinical stage. This has been largely due to concerns that differences between the thermogenic capacity of rodents and man may give a misleading impression of the potential of a drug in the clinic. This notion was because it was believed for a long time that adult humans did not possess brown adipose tissue. Furthermore, although the weight loss induced by the uncoupling agent, dinitrophenol, demonstrates that pharmacological manipulation of energy expenditure could be used to a strategy to treat obesity, the compound led to fatal hyperthermia (see Elangbam, 2009) and it was unclear whether thermogenesis could be controlled to the extent that it produced weight loss but not discomfort or toxicity. Over recent years, however, important progress has been made in our understanding of brown adipose tissue biology and several independent research teams using a combination of positron-emission tomography and computed tomography (PET/CT) imaging, immunohistochemistry and gene and protein expression assays have shown that functional brown adipose tissue is present in man (Cypess and Kahn, 2010). These findings have renewed interest in the manipulation of energy output as a mechanism to produce weight loss. The presence of functional brown adipose tissue in man, which can be regulated by adrenergic stimulation, accords with reports that sibutramine induces thermogenesis in lean and obese humans and that it can decrease the decline in energy expenditure that accompanies weight loss (Hansen *et al.*, 1998; 1999; Walsh *et al.*, 1999; Saraç *et al.*, 2006). These findings support the use of animals to investigate the effects of novel anti-obesity drugs on the energy output side of the energy balance equation, an aspect that is often ignored.

Side effects and safety of anti-obesity drugs

Animal models can be particularly useful to investigate the anti-obesity potential of both novel compounds and combinations of different compounds (which may or may not be novel). This later, drug combination, approach has become increasingly attractive as a possible method of overcoming some of the compensatory homeostatic mechanisms, which are called into play when weight loss occurs, and has the potential to produce more efficacious anti-obesity drugs (Kennett and Clifton, 2010). Existing animal models can be

used to explore such combinations, and in the case of Qnexa and pramlintide/metreleptin for example, the DIO rat model in particular appears to have good predictive validity for therapeutic potential. However, despite animal models used in obesity drug research having, in some cases such as the DIO rat and mouse, excellent face, construct and predictive validity for efficacy to produce weight loss, the field has been beset by failures in the clinic in regard to unacceptable drug safety profiles. Anti-obesity drugs are likely to be taken for a long period, perhaps the lifetime of the patient. Accordingly, it is perhaps unsurprising that routine toxicologic screens did not detect the cardiovascular safety issues that emerged on repeated administration of anti-obesity drugs such as Amilorax and fenfluramine. Indeed, there are still no reliable, validated animal models, which can accurately predict whether drugs will produce pulmonary arterial hypertension and valvulopathy in man (Elangbam, 2009). This is of importance because fenfluramine was implicated in the induction of primary pulmonary hypertension (Brenot *et al.*, 1993) and cardiac valvulopathy (Connolly *et al.*, 1997). In addition, while animals can be used to assess the abuse liability of novel drugs (Ator and Griffiths, 2003) and should detect the abuse/dependence problems associated with the amphetamines, some psychiatric side effects are more difficult to evaluate in an animal model. For example, the CB₁ receptor antagonist rimonabant was not recommended for clinical use in the USA and was suspended in Europe due to associations of the drug with severe psychiatric adverse events such as depression and suicidal ideation in patients. The results of animal experiments have been conflicting. Some studies clearly failed to predict the psychiatric side effects of rimonabant as CB₁ receptor blockade was found to produce similar behavioural and neurochemical effects to established anxiolytics and antidepressants in rats and mice (Griebel *et al.*, 2005; Witkin *et al.*, 2005) whereas other workers reported that rimonabant was anxiogenic in rodent models (Navarro *et al.*, 1997; Arévalo *et al.*, 2001; Patel and Hillard, 2006) and produced a depression-like phenotype when given chronically to rats (Beyer *et al.*, 2010), findings that are more in line with the clinical reports.

Rare and possibly human-specific adverse effects of novel drugs are by their very nature difficult to model in preclinical studies and such events will only emerge following extensive post-marketing surveillance. Accordingly, the successful development of anti-obesity drugs is likely to remain high risk because the bar of the regulatory hurdle has been raised with particularly onerous safety criteria in light of past compound failures (Heal *et al.*, 2009). Indeed, psychiatric adverse events, abuse, dependence and withdrawal side effects are explicitly noted by the FDA and EMEA as potential safety issues to be investigated for centrally acting drug candidates.

Summary

This review has detailed the relative strengths and weaknesses of various animal models typically used during the research and development of novel anti-obesity drugs. The models available need to be selected according to the nature of the drug target and the mechanism of action, but in general they possess excellent utility in predicting weight loss in man,

especially in the case of the DIO rat. Chronic studies can also examine whether the reductions in body weight are due to a specific loss of fat and accompanied by improvements in associated comorbidities and risk factors. Nevertheless, it should always be remembered that rodents are not humans and that species differences in pharmacokinetic disposition, efficacy and tolerability can occur. Furthermore, the doses used in rodent efficacy studies are likely to range beyond those tolerated in the clinic because of difficulties in assessing anything other than gross tolerability issues in rats and mice. Therefore, animal studies will always only have limited value in assessing the therapeutic index of novel anti-obesity agents in the clinic. Thus, over recent years novel anti-obesity drugs have significantly reduced body weight in clinical studies; however, the field has been beset with safety issues and limited efficacy and drugs have been withdrawn or have not been approved by the regulatory authorities because of negative risk/benefit profiles. Particular concerns are that anti-obesity drugs would have to be given chronically and that the expanding size of the anti-obesity market means that any approved drugs would be given to extremely large numbers of patients in a relatively short space of time. Accordingly, the development of anti-obesity drugs remains high-risk. That said, there remains an enormous unmet clinical need for safe and more efficacious treatments for obesity, and for any drug companies that succeed in developing such a product, the rewards are considerable.

Acknowledgements

None.

Conflict of interest

The authors are employees of RenaSci Consultancy: a fee-for-service Contract Research Organisation.

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