The contribution of animal models to the study of obesity

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Summary

Obesity results from prolonged imbalance of energy intake and energy expenditure. Animal models have provided a fundamental contribution to the historical development of understanding the basic parameters that regulate the components of our energy balance. Five different types of animal model have been employed in the study of the physiological and genetic basis of obesity. The first models reflect single gene mutations that have arisen spontaneously in rodent colonies and have subsequently been characterized. The second approach is to speed up the random mutation rate artificially by treating rodents with mutagens or exposing them to radiation. The third type of models are mice and rats where a specific gene has been disrupted or over-expressed as a deliberate act. Such genetically-engineered disruptions may be generated through the entire body for the entire life (global transgenic manipulations) or restricted in both time and to certain tissue or cell types. In all these genetically-engineered scenarios, there are two types of situation that lead to insights: where a specific gene hypothesized to play a role in the regulation of energy balance is targeted, and where a gene is disrupted for a different purpose, but the consequence is an unexpected obese or lean phenotype. A fourth group of animal models concern experiments where selective breeding has been utilized to derive strains of rodents that differ in their degree of fatness. Finally, studies have been made of other species including non-human primates and dogs. In addition to studies of the physiological and genetic basis of obesity, studies of animal models have also informed us about the environmental aspects of the condition. Studies in this context include exploring the responses of animals to high fat or high fat/high sugar (Cafeteria) diets, investigations of the effects of dietary restriction on body mass and fat loss, and studies of the impact of candidate pharmaceuticals on components of energy balance. Despite all this work, there are many gaps in our understanding of how body composition and energy storage are regulated, and a continuing need for the development of pharmaceuticals to treat obesity. Accordingly, reductions in the use of animal models, while ethically desirable, will not be feasible in the short to medium term, and indeed an expansion in activity using animal models is anticipated as the epidemic continues and spreads geographically.

Keywords: Obesity; animal models; genetics; genetic modification; dieting; caloric restriction

It is widely agreed that obesity stems from a prolonged imbalance between the level of energy intake and the level of energy expenditure, with the resultant surplus being stored as body lipids, predominantly in adipose tissue. Understanding the factors that regulate both energy intake and expenditure is consequently an important step towards developing obesity treatments, be they environmental/lifestyle manipulations...
or pharmaceuticals. Our understanding of the regulation of food intake and food choice behaviour, and the physiological basis of differences in energy expenditure owes in large part to studies made on animals. Spectacular progress in these areas have been made within the past 15 years, in particular since the discovery of the adipocyte-derived cytokine hormone leptin (Zhang et al. 1994) and its receptor (Tartaglia et al. 1995, Lee et al. 1996), both of which were discovered by characterizing the underlying genetic defect in mutant mice (the ob/ob and db/db mice) that had spontaneously arisen in the Jackson Laboratories in the 1940s and 1950s (Ingall et al. 1950, Hummel et al. 1966). The discovery of leptin heralded an explosion of activity in the field of energy regulation (reviewed in Schwartz et al. 2000, Morton et al. 2006) and this discovery was completely a result of work on animals. The critical importance of using animal models to understand the regulation of food intake and energy balance, however, predates the discovery of leptin by many decades. Key examples are the discovery of the pancreatic regulatory hormone insulin, at the turn of the last century (Banting & Best 1922), which was largely a result of work with dogs, and gut-derived satiety signals such as peptide YY (PYY) (Adrian et al. 1985) and cholecystokinin (CCK) (Gibbs et al. 1973) both discovered long before leptin, by work in animals. Moreover, animal models have not only contributed to our understanding of the physiological and genetic basis of obesity, but have been a cornerstone of studies into environmental effects, such as epigenetics, responses to high-fat and low-calorie diets and the identification and development of several pharmaceutical agents for the treatment of obesity. This review will provide some examples of the animal work that has been performed to understand the physiological/genetic basis of obesity. We will also review environmental factors, focussing on varied approaches that have been taken to using animals, rather than aiming to be a comprehensive summary of all work in the field. A previous review (West & York 1998) has summarized animal approaches on the study of high-fat diets and accordingly we place less emphasis on this area. The review concludes with an assessment of the potential for reducing the use of animals to study obesity.

Physiological and genetic basis of obesity

Spontaneous single gene loss-of-function mutations

The ob/ob (Zhang et al. 1994) and db/db (Bahary et al. 1990) mice and Zucker (fa/fa) obese rat (Zucker & Zucker 1961) are classic cases of spontaneous single gene loss-of-function mutations that generate massive obesity. Characterizing the genetic basis of the mutations revealed that the defect in the ob/ob mouse is a single base pair deletion, and the gene product was called leptin (Zhang et al. 1994), the db/db and fa/fa rat mutations turned out to be mutations in the leptin receptor. There are now at least 10 known single gene loss-of-function defects that cause massive obesity and have been completely genetically characterized. In all the cases where such defects have been discovered, they have initially resulted from spontaneous mutational events in large breeding establishments. Single gene mutants are normally initially detected by sharp-eyed observers and are confirmed later by exhibiting their genetic segregation. The development of high throughput sequencing capability and the completion of the mouse and rat genomes in the early part of the new millennium means that the time between discovery of a new mutant and its characterization have shortened greatly and are likely to get progressively shorter.

Nevertheless, there is an inherent randomness in the discovery of single gene defects meaning that genes with no or only minor effects on the heterozygote are unlikely to be discovered (Crawley 2003). Moreover, the detection of only the major genetic defects means that almost by definition these defects will only affect a very minor proportion of the population, as was the case with the ob/ob mouse and the leptin gene. Although this discovery was a...
major leap forward, genetic screens of the human population have revealed trivially small numbers of individuals who have loss-of-function mutations in this gene (Farooqi & O’Rahilly 2005). The same is true for all other genes that have been discovered as spontaneous single gene defects and characterized genetically and functionally (Rankinen et al. 2006).

The real progress that the study of these genes allows is to further our understanding of how the energy regulation system works. In fact, many of the genes that appear important in single gene mutation events seem to be involved in a common pathway that includes leptin and insulin as signalling molecules. Our knowledge of this pathway has been crucially informed by characterization studies of these spontaneous ‘single gene loss-of-function’ defects.

Artificially generated single gene loss-of-function mutations

The arbitrary nature of relying on spontaneous mutational events resulting in major loss-of-function mutations of critical genes has led to attempts to accelerate the process by increasing the mutation rate artificially. This is performed by treating the animals with mutagenic chemicals or exposing them to radiation (Russell & Russell 1992, Dhar et al. 2000, 2004, Gailus-Durner et al. 2005). Because such mutagenesis is not targeted, the results are random disruptions spread across the genome that are passed to the offspring. The animals generated from these experiments not only illustrate energy regulation, but many other aspects of animal function. A mutagenesis programme of work in Germany has resulted in the identification of a mouse with disruption of the growth hormone receptor [SMA-1]. These animals have some relevance to the work on obesity because of their phenotype which involves a distinctive small body due to growth retardation along with elevated adiposity (Meyer et al. 2004).

The major problem with this approach is that while it is relatively easy to induce mutations in animals artificially and at random, it is extremely costly to phenotype the resultant offspring of these animals on any reasonable scale. Given that the mouse genome consists of around 25,000–30,000 genes and that a given mutation may only produce a loss-of-function effect in say 3–5% of cases (the other mutations being benign – third codon effects or modifications that do not affect amino acid sequence, or even if they do affect sequence are not functional), then to discover the effect of loss of function in a given gene might require phenotyping of over half a million individuals. Just measuring the body weight of animals on a single occasion in these sorts of numbers would be prohibitively expensive. Little progress has, therefore, been made from these studies in the context of understanding energy regulation, which is a much more complex phenotype than body weight to characterize.

Genetic modifications

An enormous number of transgenic and knockout (KO) models with obese or lean phenotypes have been created since the characterization of the first obesity genes (Inui 2000, Salton et al. 2000). The 2005 update of the human obesity gene map cited 248 genes that, when mutated or expressed as transgenes in the mouse, result in phenotypes affecting body weight and adiposity (Rankinen et al. 2006). The ability to introduce genes into or eliminate genes from the germline of animals has facilitated the development of complex genetic models of disease, as well as the in vivo study of gene function. Investigations into obesity have been challenging due to its complex aetiology involving genetic, metabolic, behavioural and environmental factors. Research using genetically-modified animal models of obesity and leanness has led to a significant expansion in our knowledge of the physiological and molecular mechanisms affecting energy balance. This is beneficial for the identification of potential targets for developing therapeutics to treat human obesity. With traditional transgenic technologies developed during the late 1980s and 1990s there was little control over where or how many copies of genes were introduced into the genome. However, there is now an
abundance of sophisticated gene-targeting strategies permitting investigators to manipulate the genome in ways that essentially allow the introduction of virtually any desired change into the genome. Furthermore, advanced techniques allow for the control of alterations to the genome that act only at specific times, or have constructs that are expressed only in specific tissues [Davey & MacLean 2006].

Overexpression of target genes was the first widely used technique. The full-length coding sequence of the gene is cloned downstream of a promoter that may provide global, or tissue-specific expression, resulting in a transgenic offspring overexpressing the target gene. Although relatively straightforward and inexpensive, the level of gene and protein expression does not always demonstrate a physiological effect. More predictable and reproducible than the overexpression models are the global KO mouse models. Here, the phenotype is created through total ablation of the target gene in all the tissues, and these global gene KO mice can be used in the identification of many factors involved in the development. These KO models often result in unpredicted actions of the target genes, which although sometimes disadvantageous, for instance resulting in embryonic abnormalities, can in some cases allow an unexpected insight into the action of the target gene.

An example of an unexpected insight developed from a transgenic KO is the axl mouse, originally developed to determine whether the tyrosine kinase receptor, axl, played a role in leukaemia. As anticipated, this mouse was not found to exhibit haematopoietic malignancies, but by chance the phenotypic characteristics exhibited were associated with non-insulin-dependent diabetes mellitus. The axl mouse has hyperglycaemia and hyperinsulinaemia, severe insulin resistance and progressive obesity, but is not hyperphagic [Augustine et al. 1999]. Further analysis of these axl animals revealed systemic elevations of tumour necrosis factor (TNF)-alpha, also shown to be elevated in both rodent and human models of obesity [Yamakawa et al. 1995, Katsuki et al. 1998] suggesting that expression of axl affects endogenous modulation of TNF-alpha production, which indirectly contributes to the onset of obesity.

In addition to the global and tissue-specific KOs, genetic modification also involves ‘knock-in’ models replacing the endogenous gene with a mutation. Knock-in mice have the ability to address more specific roles and can be used to determine the effects of subtle changes in protein structure or function. They have been used to model human diseases or to determine the functional significance of particular receptor signalling pathways. For example, the s/s mice created by Bates et al. (2003) were developed to investigate the role of individual leptin signals, by introducing a knock-in mutation that disrupted the dominant intracellular signalling pathway through which leptin was believed to have its major action (the STAT3 pathway). The absence of the long form of the leptin receptor (ObRb), as in the case of db/db mice, results in obesity and diabetes. The s/s mice were found to share similarities with the db/db, displaying early development of obesity, characterized by hyperphagia, elevated leptin and insulin levels, suggesting leptin resistance. However, caloric restriction normalized the glycaemic control in the s/s mice, which showed an improvement in insulin resistance and glucose intolerance. Consequently, the transgenic s/s mouse has contributed to further research into obesity and diabetes [Bates et al. 2005].

A key problem with conventional KOs is the potential that the gene may result in early embryonic death preventing the study of its effects in adults. A less obvious problem is that a genetic manipulation that acts over the whole life may force animals to make compensatory changes during the period of development. Hence, what is normally a key gene in the process of energy regulation may appear to have little importance when it is knocked out because its action has been taken over by other compensatory mechanisms. A case in point is the neuropeptide Y (NPY) gene. When introduced directly into the brain, this neuropeptide is one of the most potent stimulators of feeding behaviour. Moreover, when animals
are deprived of food, natural levels of NPY increase, while in satiated animals they decline (Lin et al. 2004). When NPY was knocked out, however, the resultant mouse had no obvious abnormal phenotype (Erickson et al. 1996a,b, Palmiter et al. 1998). The Cre/loxP system is a tool for tissue-specific and time-specific KO of target genes, which allows for the investigation of such genes. This system involves two separate transgenic lines, one expressing Cre recombinase (Cre) and the other in which Cre recombinase recognition (loxP) sites are strategically positioned on either side of the target gene. When Cre is expressed in mice harbouring a loxP-containing target gene, the desired gene is excised. Depending on the tissue specificity and the timing of recombinase expression, these modifications can be restricted to certain cell types or developmental stages (Kuhn & Torres 2002).

Examples of use of the Cre/loxP system include its use to create mice with tissue-specific disruptions of the insulin receptor, muscle-specific (MIRKO) (Bruning et al. 1998), β-cell-specific (BIRKO) (Kulkarni et al. 1999), liver-specific (LIRKO) (Michael et al. 2000) and fat-specific (FIRKO) (Bluher et al. 2002). The FIRKO mouse has a low fat mass, loss of the normal relationship between plasma leptin and body weight and appears protected against age-related and hypothalamic lesion-induced obesity. Interestingly, while having normal levels of food intake these mice also demonstrate many features similar to calorie-restricted mice; including the fact that they live longer than wild types.

Studies of genetically-modified animal models have not only informed our understanding of the regulation of food intake and energy balance, but have also played a key role in advancing our understanding of the links between obesity and disorders such as diabetes and the metabolic syndrome. For example, the late-onset obesity rat (termed LOB) was discovered as a result of transgenic manipulations originally created to investigate the growth hormone expression in the vasopressin system (Wells et al. 2003). When compared with most classic spontaneous mutations, several differences were found in this transgenic model. First, the mutation was autosomal dominant, developing obesity late, as opposed to most mutations, which are recessive with early-onset obesity. In almost all the models, obesity is apparent in all fat depots, whereas in the male-specific LOB rat, fat accumulated selectively in visceral, but not peripheral, depots, as is often the case in humans. In addition, unlike most other models that are hyperphagic, obesity occurred despite a normal intake of a low-fat diet, which interestingly, could be initiated in young LOB rats by feeding a high-fat diet. Again, in contrast, with most other models displaying insulin resistance, LOB maintained insulin sensitivity despite massive visceral obesity (Hummel et al. 1966, Coleman & Hummel 1967) providing a valuable model for investigations into obesity-related diseases.

Several studies have suggested that while obesity predisposes to several disease conditions, it is not the total fat mass but excessive abdominal fat that is the best predictor of adverse metabolic consequences such as insulin resistance, glucose intolerance and dyslipidaemia, associated with the metabolic syndrome X (Montague & O’Rahilly 2000). To study this linkage more closely, genetic modifications of the two forms of 11β-hydroxysteroid dehydrogenase (11β-HSD) have been studied. In contrast to 11β-HSD type 2, which deactivates glucocorticoids, 11β-HSD type 1 regenerates this action. Mice overexpressing 11β-HSD-1 (Masuzaki et al. 2001, Masuzaki & Flier 2003) displayed similar symptoms of the metabolic syndrome as seen in humans, i.e. visceral obesity, insulin and leptin resistance, dyslipidaemia and hypertension (Rask 2001), whereas mice with a null mutation in the 11β-HSD-1 gene displayed a diabetes-resistant phenotype, resisting visceral fat accumulation and insulin resistance (Kotelevtsev 1997, Holmes 2001).

Polygenic models
It is clear that in most circumstances obesity, and its related energetic precursors, are normally multifactorial or polygenic traits, resulting from the combined actions of many genes and environmental interactions.
In humans, single gene mutations are of limited importance when considering the increasing obesity epidemic, as they account for only a few cases in the obese population. In light of this, several research programmes have turned to locating the polygenic basis of obesity, and many of these have employed animal models. Although an animal’s phenotype is often easily measured, identifying the genes underlying this trait can be a laborious process. Compared with human studies, however, using animal models is often faster as enhanced techniques and some destructive methods (i.e. full body dissection) allow accurate description of the phenotype. In addition, the power to detect quantitative trait loci (QTL) using model animal species is improved because of the larger family size and formalized pedigree structure. Although several animal models have been used such as rats (Watanabe et al. 1999), chickens (Jennen et al. 2005) and pigs (Andersson et al. 1994), the mouse is the most common genetic model species and recent advances in determining the mouse genetic map has driven their increased use. Currently, the molecular marker map consists of over 6500 polymerase chain reaction-based microsatellite markers (Dietrich et al. 1996). As the mouse is homologous with other mammalian species, its use in locating homologous human genetic loci is invaluable.

In the search to locate regions of the genome responsible for polygenic obese phenotypes, QTL mapping is a valuable tool that screens an organism’s genome for statistical associations between phenotypic and marker information. The mapped QTL may be a result of either individual genes or a number of linked loci. To date, over 200 obesity QTL have been located in the mouse, although many of these are at the same chromosomal region and may, therefore, reflect the same gene(s) (Snyder et al. 2004). QTL are most commonly detected from F2 generation intercrosses, which are best achieved by using two baseline populations of animals that show large significant differences in the trait in question. Many obesity-related traits are highly heritable and so within-line selection for the two extreme phenotypes (high and low) leads to divergent characteristics (Sharp et al. 1984). The divergent lines are subsequently crossed to produce an F1 population and then crossed with siblings to produce an inbred F2 population, which typically exhibit wide phenotypic variation between individuals (Pomp 1997). Molecular marker genotypic and phenotypic information is used to determine statistical associations between the phenotype and areas of the genome, localizing QTL. A key advantage of this approach is that the technique requires no prior knowledge concerning the biological nature of the trait under examination (Fisler & Warden 1997).

Obesity-related traits that have been divergently selected over many generations include body mass (White et al. 1968, Hastings et al. 1993, Bünger & Hill 1999), body composition (Sharp et al. 1984, Hastings & Hill 1989), food intake (Sharp et al. 1984), heat loss (Nielsen et al. 1997a,b) and spontaneous activity (Swallow et al. 2001). A detailed list of those where QTL mapping has occurred is available in Brockmann and Bevova (2002). Most QTL have only a small effect, however, some contribute more significantly. For example, Fob2 accounts for 19.5% of the variability in mouse lines selected for body fat percentage (Horvat et al. 2000).

In some cases, it requires environmental intervention by placing mice on a high-fat diet, to reveal obesity prone or obesity resistance in selected lines or inbred strains. For example, the selected lines for both high- and low-food intake developed by Sharp et al. (1984) show resistance to obesity when fed a diet high in fat by lowering food intake to maintain the same energy intake as when fed low-fat chow (Hambly et al. 2005). In contrast, there are certain inbred strains of mouse that are highly obesity prone, i.e. C57BL/6J (York et al. 1996, Johnston et al. 2006). By crossing strains that are prone and resistant to obesity, QTL can be located that underlie dietary-induced obesity and are strongly linked to the human condition.

Although the use of QTL mapping techniques frequently locate new candidate genes for obesity traits that become targets in
human candidate gene studies, the search is nowhere near being exhausted. Many new selected lines and inbred strains of mouse have still to be utilized and their possible association with dietary-induced obesity explored.

Studies of other species

Non-human primates Although rodents are the most common animal model for research in obesity (above), the separation of the primate and rodent lineages is a relatively ancient divergence in the eutherian mammals (65–85 million years ago; Eizirik et al. 2001). In contrast, the separation of the Hominoidea [humans and the other great apes] from the Cercopithecoidae [the Old World monkeys] occurred more recently [approximately 25 million years ago; Page & Goodman 2001]. Given this closer phylogenetic relationship to humans, the Old World monkeys (such as macaques, rhesus monkeys and baboons) may provide a more appropriate model for studying human obesity and its related co-morbidities (West & York 1998, Wagner et al. 2006). Several monkey species show a prevalence of age-related obesity around 10–15% even when maintained on a relatively low-fat diet (10–15% of energy) [Kemnitz 1984]. This does not appear to be a consequence of reduced physical activity because of caging, as a similar pattern has been observed in free-ranging monkeys on an island supplied with extra food [Kemnitz 1984]. Most of the interest in the study of non-human primates and obesity has been with respect to their responses to high-fat diets and epigenetic effects (see below under environmental effects). Studies of brain mechanisms of body weight and food intake control, however, have been relatively limited (but see e.g. Koutcherov et al. 2003, Grove et al. 2005).

Dogs Domestic dogs have experienced their own epidemic of obesity that is perhaps even more extreme than that observed in humans. By the mid-1980s, already 25–45% of domestic dogs presented at veterinary clinics were classed as obese [Hand et al. 1989]. This epidemic has become a serious veterinary problem. Studies of dogs so far (e.g. Romosos et al. 1976, 1978, Rocchini et al. 1989, Diez et al. 2002, Martin et al. 2006) have mainly concentrated on the aetiology of obesity in them as a problem in their own right. However, they have clear potential as a study model for human obesity, principally because there is a vast amount of background literature on the physiology of the dog and the dog genome has already been mapped [Lindblad-Toh et al. 2005]. We have perhaps the best pathological data available for any species apart from man, and different breeds appear to present with obesity at clinics with highly different probabilities [Edney & Smith 1986], providing an opportunity to dissect the genetic contribution to the problem.

This potential for the use of dogs to inform us about obesity in humans should be realized during the coming decade.

Seasonal models of obesity Many small mammals exhibit annual cycles of body mass and adiposity [e.g. Heldmaier & Steinlechner 1981, Stebbins 1984, Bartness & Wade 1985, Klingenspor et al. 1996, Bartness et al. 2002, Drazen 2002]. Most rodents rely on changing day length to trigger these responses [Dark et al. 1983, Mrosovsky 1983, Bartness et al. 2002], which can be readily mimicked in the laboratory by transferring animals between long day (LD) and short day (SD) photoperiods. This makes seasonal mammals attractive for investigations of mechanisms underlying the regulation of body mass [Mercer & Speakman 2001, Bartness et al. 2002, Morgan et al. 2003b].

Seasonal fat cycles have been studied most extensively in the Siberian or Dzungarian hamster (Phodopus sungorus), Syrian or golden hamster (Mesocricetus auratus) and the collared lemming (Dicrostonyx groenlandicus). In the Siberian hamster, both sexes defend a maximal body mass [and adiposity] in the summer [Steinlechner & Heldmaier 1982, Bartness & Goldman 1988]. The transfer of adult male hamsters [housed at room temperature] from LD [16 h light cycle] to SD [8 h light] results in a gradual weight loss accompanied by reduced food intake.

In contrast, Syrian hamsters and collared lemmings have their maximum body masses in the winter (Bartness & Wade 1985). In response to SD, adult female hamsters housed at room temperature increase their body mass by approximately 50–60% over an eight-week period, with no concomitant increase in food intake (Campbell & Tabor 1983). In collared lemmings, males weaned into SD grow to an adult body mass of 75 g, 88% heavier than males weaned into LD (Reynolds & Lavigne 1989, Hunter & Nagy 2002), the majority of the difference being fat (Nagy & Negus 1993, Nagy 1993).

Considerable progress has been made in understanding the brain mechanisms that regulate the seasonal cycle of body mass in both species that become fatter under LD or SD manipulations. However, this work has largely been built on previous work in mice. To date, studies of seasonal rodents have not provided any new insights that have not been already apparent from the extensive mouse studies.

Animal studies of environmental effects

Exposure to high-fat and palatable diets

One hypothesis for the rapidity of the obesity epidemic in humans is the possibility that our regulatory systems become overwhelmed by high-fat high-density palatable foods, which have become increasingly available over the past 20–30 years. In rodents, there have been many studies that have attempted to characterize the responses of animals exposed to high-fat and high-sucrose diets. The initial work in this area was based on feeding rats with ‘cafeteria’ type diets, which promote hyperphagia (e.g. Rothwell & Stock 1982, Stock & Rothwell 1982, Rothwell et al. 1983). One problem with these approaches is that the actual macronutrient composition of the diet may be very variable preventing the isolation of key dietary factors that may be important. In the light of this, these ‘human’ type diets have been replaced by more homogeneous commercial pelleted ‘high fat’ and ‘western’ diets, which allow nutrient composition to be more closely monitored. This work has been previously comprehensively reviewed (West & York 1998), hence we will spend less time on these studies here.

The most extensive studies have been made in rodents and non-human primates, although other studies have been made in other species such as hamsters (Wade 1982), squirrels (Faust & Mrosovsky 1987, Dark et al. 1992), pigs (Pond et al. 1985), dogs (Romosos et al. 1976, 1978) and sheep (Tolkamp et al. 2007). Two types of response have been observed when animals are given high-fat or high-fat high-sucrose diets: some species and strains gain weight, but others do not. This pattern has been observed in both non-human primates and in rodents. In non-human primates, for example, Ausman et al. (1981) fed squirrel monkeys with either high-fat high-sucrose diets (21–31% of energy from fat) or a low-fat and sucrose diet containing 13% fat, from weaning until they were four years. At this age, those fed with the high-fat high-sucrose diets had approximately 30% body fat compared with only 7% body fat in those fed with the low-fat low-sucrose diet. In contrast, cebus monkeys did not become obese when consuming the high-fat high-sucrose diet (Ausman et al. 1981). Similarly, in rodents some species and strains show profound increases in their body fatness in response to high-fat and high-fat high-sucrose diets (e.g. C57BL/6 mouse; Surwit et al. 1988) and are termed diet-induced obese (DIO) rodents. Many studies have used this DIO paradigm to examine the regulation of food intake under...
conditions of high-fat intake as a model of human obesity, in an attempt to understand why some humans exposed to these diets also become obese. On the other hand, some mouse strains (e.g. Hambly et al. 2005) and many wild rodents in captivity (e.g. Siberian hamster, Phodopus sungorus McElroy et al. 1986, Shaw’s jird, Meriones shawi El-Bakry et al. 1999, Bank voles Clethrionomys glareolus Peacock & Speakman 2001), seem to be resistant to weight gain when fed with high-fat diets. In mice and rats, these have been termed as dietary-resistant (DR) strains.

In the outbred Sprague-Dawley rat many studies have been performed examining the responses to a high-fat high-palatability diet (Levin & Dunn-Meynell 2002). These outbred animals show diverse responses that include some animals (DIO) with excessive gain and others showing a DR profile (Levin & Keesey 1998, Levin & Dunn-Meynell 2000). Levin et al. (1997) have derived inbred lines from the most resistant and most susceptible to developing obesity and have directed considerable effort to identifying those aspects of physiology (particularly in the brain) (Ricci & Levin 2003) and genetics (via QTL mapping) that may underlie the difference between these lines. This may give us insights into why some individuals develop obesity while others do not.

A major difference between these experimental designs and the situation in humans is that a large aspect of human susceptibility to obesity may not depend on the ability to resist weight gain when force-fed with a high-fat diet, but rather may hinge on individual differences in the propensity to choose high-fat foods in the first place. Relatively few studies have explored this aspect of choice behaviour in animal models (Smith et al. 1998, 2000). Two different types of study have been made in this respect. These studies focus on the role of brain neuropeptides in macronutrient choice, while other studies have focused on the peripheral aspects of the taste and olfaction system. The neuropeptide work has highlighted the fact that expression levels of certain neuropeptides may be linked to dietary intake (Schwartz et al. 2000). One of these neuropeptides is galanin (GAL), which may be involved in fat preference (Crawley 1999, Odorizzi et al. 2002), although its role has been hotly contested with some studies showing no changes in the macronutrient choice of GAL-treated animals (Smith et al. 1994, 1997). A strong positive correlation also exists between the daily intake of carbohydrate and the level of NPY in the arcuate and paraventricular nuclei (Jhanwar-Uniyal et al. 1993). In the field of studies of taste preference, inbred strains show very large differences in their preference for sweet tasting water, suggesting that there may be significant differences in individuals in their propensity to select different foods based on polymorphic variation in their taste receptors. Ultimately, this may be a much more rewarding avenue for investigation compared with the models where animals are force-fed with high-fat diets without choice, since humans rarely face this latter scenario.

Epigenetic effects
A popular recent hypothesis concerning not only obesity, but also many aspects of our adult health is that our susceptibility to disease may be programmed during the period of our lives that we spend in utero. This hypothesis has often been called the ‘Barker’ hypothesis after the scientist who first characterized potential linkages between late life health patterns and birth weight in humans (Barker 1992, 1994). By characterizing the health of adults in The Netherlands whose mothers had endured an enforced period of famine during the Second World War, compared with children born immediately prior to and following the famine, strong experimental support for the hypothesis has been gathered in humans (Ravelli et al. 1998, Roseboom et al. 2001, Painter et al. 2005). This is one area where an obesity-related phenomenon has been first discovered in humans rather than in animal models. The in utero effects are also often called epigenetic effects, because they can be difficult to separate from genetic effects.

Although the phenomenon was first discovered in humans, animal models provide a
A highly valuable tool for the study of the mechanisms by which such epigenetic effects arise. Animals provide two very clear advantages. First, it would be ethically unacceptable to impose deliberate restriction on human fetuses, and the prospects for any interventional monitoring in this context would also be very limited. Moreover, the timescale of the effects, that span half a lifetime, would be impossible to study. Animal studies have been made in both non-human primates and rodents. In baboons, exposure to high-density, high-fat diets during the preweaning period by manipulating the bottle feeding formulas led to a significant increase in body fat content at the age of five years (Lewis et al. 1989). However, this was sex-specific, with only the female baboons showing an effect of early overfeeding. Many intervention studies have been made in rats and mice that involve changes in the intrauterine number of fetuses (Nagasawa & Yanai 1971) or manipulations of fetal nutrition by ligating the placenta (reviewed in Holemans et al. 2003). The bi-fold nature of the rodent uterine horns provides a very amenable paradigm to selectively restrict the nutrient supply to only half the litter and hence have control individuals that have no restriction generated within the same individual. In lactation, there may also be neonatal programming effects, and here manipulating the litter size is a convenient method for regulating the supply of nutrients to the offspring affecting pup growth. If rodents give birth to many offsprings, the mother is incapable of upregulating the nutrient supply in the milk in direct proportion and hence pups in large litters are relatively undernourished (Fuchs 1982, Johnson et al. 2001). Similar effects can be caused by lactation in hot conditions (Król & Speakman 2003).

A difference between rodents and non-human primates is the pattern of early development of arcuate nucleus (ARC) and other hypothalamic circuits that modulate feeding and energy expenditure. Specifically, the ARC projections in primates develop during the third trimester of pregnancy, whereas in rodents this does not occur till the third week of postnatal development (e.g. Koutcherov et al. 2003, Grove et al. 2005). This species difference suggests that maternal diet and health are likely key factors for the development of ARC projections in the primate, whereas the postnatal environment would be more important in the rodent. Thus, the non-human primate model of obesity may provide more useful insights into the epigenetic contribution of adult obesity, type 2 diabetes, coronary heart disease and hypertension. Nevertheless, the consequences of these fetal and neonatal restrictions in rodents appear to mimic the situation found in humans, in that offspring that are undernourished early in their lives seem to be those that later develop health problems as adults. Beyond documenting that this phenomenon exists in animals and that they may be a good model for understanding the mechanism that produces the same effects in humans, progress has been relatively slow in this field. We can expect considerable progress over the next decade in this area.

Responses to low calorie and other weight loss diets
Caloric restriction is the most frequent self- and clinician-prescribed treatment for obesity. Despite its popularity, the long-term success of caloric restriction is limited. This is primarily because of two factors. First, weight loss becomes progressively harder to achieve as the diet continues, and second, weight loss is mostly regained when the diet ends. In humans, this lack of continued weight loss may be a primary factor leading to non-compliance with the regime. Direct studies of human responses to dieting are compromised because of difficulties in measuring human energy intake (Poppitt et al. 1998). Animals provide an ideal alternative because of the ease of characterizing their energy budgets.

Two potential mechanisms may explain why weight loss does not continue under caloric restriction. First, there may be an increase in digestive efficiency to extract more energy from the ingested food. Alternatively, aspects of energy expenditure may be reduced, for example, by suppressing
resting energy expenditure, body temperature or activity. The contribution of these different mechanisms has been an issue of considerable debate. It seems that the compensatory mechanisms adopted and their extent may depend on the levels of restriction enforced (Hill et al. 1984, Even & Nicolaidis 1993, Hambly & Speakman 2005). However, a common pattern is for reductions to be observed in both the resting and activity levels of energy expenditure.

The overall aim when humans undergo energy restriction is to lower their fat content, although along with fat loss, some lean tissue is also lost. Rodent models can be used to assess the proportions of fat to lean tissue loss, which may depend strongly on their initial proportions or the genetic make-up of the model strain. Differences in fat tissue are the main cause of increased body mass in DIO compared with DR Sprague-Dawley rats (Levin et al. 1997). When both are restricted by 50% of ad libitum food intake, both phenotypes lose similar proportions of weight, but DIO rats reduce their fat mass while DR rats primarily lose lean tissue (Levin & Dunn-Meynell 2000). QTL mapping of these rats, as previously described, may locate the loci responsible for determining the tissue loss on dietary restriction.

In addition to caloric restriction, there are many other dietary manipulations that have been suggested to cause weight loss. The most common of these are diets involving restricted intake of carbohydrates, while simultaneously elevating the intake of dietary protein. These diets do seem to promote rapid weight loss. Although short-term benefits are easy to measure in the human population, the long-term risks (or benefits) are harder to evaluate because of the long human lifespan. Here, animal models are also of interest because they are short-lived and can be used to locate the mechanisms that lie behind successful weight loss using these different diets and their potential pitfalls. For example, concern has been expressed that low-carbohydrate high-protein diets might cause problems with hepatic and renal functions because of their role in nitrogen metabolism. Obesity-prone Wistar rats fed a diet with 50% of the energy as protein for six months (equivalent to one-quarter of their lifespan) had significantly reduced adiposity and showed no detrimental health problems on renal and hepatic function, oxidative stress or calcium balance (Lacroix et al. 2004). This animal model has suggested that prolonged intake of high levels of protein is unlikely to be detrimental to human health. These examples show that although, ultimately, diet-based research has to focus on humans, additional useful information can be gained from experiments using short-lived rodents.

**Development of pharmaceuticals for the treatment of obesity**

Powell (2006) highlighted the important role that KO mice may play in the future prospective identification of putative pharmaceutical targets for drug development. He reviewed the phenotypes of 21 different types of KO mice where the gene knocked out was a potential therapeutic target for obesity. These were compared with the phenotypes of mice treated with therapeutics designed for the same targets. Of the 21 obesity gene targets considered, 16 showed a close correspondence between the KO phenotype and drug effect in mice and/or rats. This suggests that, as far as the evaluation of drug targets for obesity is concerned, compensatory developmental changes that are precipitated by the whole-life KO do not normally prevent the detection of the relevant phenotype. Importantly, it was also found that where data were available, the KO phenotypes not only mimicked the effects of therapeutics in rodents, but also the effects when relevant therapeutics were delivered to human targeting the same genes. Transgenic mouse technology may, therefore, be a valuable tool to prospectively identify genes that regulate body fat in vivo, and then to develop anti-obesity therapeutics, by targeting the human protein products of these genes.

An important message from the review by Powell (2006) is that not only does the transgenic mouse represent a valuable tool for therapeutic development, but also that the therapeutics that are designed to interfere
with levels of fat storage in rodents, also generally have the same effects on fat storage in humans. As an identification and testing ground for potential obesity pharmaceuticals, rodents will remain a crucial model. An example of a drug target that was identified in animals, leading to the development of a class of potential obesity therapeutics, which ultimately generated a useable drug that has just finished clinical trials, was the development of the Cannabinoid receptor type 1 (CB1) antagonist Rimonabant.

The involvement of the CB1 in the central regulation of food intake was discovered in animals by the targeted transgenic disruption of this receptor (Cota et al. 2003, Ravinet Trillou et al. 2004). At 16 weeks of age animals with KO of CB1 fed on standard rodent chow had a 9% lower body mass and 20% lower fat mass than their wild-type littermates (Cota et al. 2003). In a second study, CB1 KOs at 20 weeks of age were 23% lighter and had 60% less body fat than their wild-type littermates. The details of how this effect was generated seemed to implicate not only a reduction in food intake, but also an increase in energy expenditure (Ravinet Trillou et al. 2004). The involvement of the CB1 receptor in the regulation of energy balance, therefore, suggested that an antagonist of this receptor might be a valuable therapeutic for obesity treatment.

Rimonabant was developed as a specific CB1 antagonist. When administered to diet-induced obese rodents on a daily basis for five weeks, the animals decreased body mass by 25% and their body fat 60% (Ravinet Trillou et al. 2003). By pair-feeding a group of animals to the same intake as the treated mice, it was shown that the lost mass was primarily because of suppressed appetite, but with a small additional effect on energy expenditure. In another study using DIO mice, Rimonabant decreased body mass and fat pad mass via an effect on food intake, but critically had no impact on transgenic mice with the CB1 receptor knocked out (Ravinet Trillou et al. 2004). This study demonstrated that Rimonabant acts by specifically inhibiting the CB1 receptor. Rimonabant also decreases food intake and body weight gain in both the obese Zucker rats and lean Zucker controls (Vickers et al. 2003). In the rats, the effects are consistent with the findings in DIO mice, except that in rats weight loss was entirely the result of decreased food intake.

On the basis of the positive outcomes of these animal studies and the absence of any obvious side-effects a one-year randomized, double-blind clinical trial was initiated, which evaluated the effects of Rimonabant on weight loss in over 1500 overweight humans (Van Gaal et al. 2005). Consistent with the data from the animal models, Rimonabant induced a dose-dependent weight loss, which significantly exceeded that in a placebo group. Patients treated with 20 mg of Rimonabant daily lost 5 kg more than those receiving placebo. This example provides a classic case of the use of fundamental knowledge derived from animal models, utilization of animals as a test-bed for pharmaceutical development, and the ultimate clinical trial of a successful drug. Such developments would have been impossible without the use of experimental animals.

On the other hand for each success in terms of pharmaceutical development, there are notable failures to translate findings in fundamental biology based on animal studies into useable drugs for humans. Perhaps, the most obvious case in recent years has been the hope that leptin itself, and downstream targets in the leptin system, might be amenable for development as drugs. Shortly, after its first description (Zhang et al. 1994), several studies were performed in animals which showed that not only was leptin treatment effective at reversal of the obesity in ob/ob mutant mice that lack leptin, but also it reduced (modestly) the fatness of normal mice that were not leptin-deficient (Pelleymounter et al. 1995, Halaas et al. 1997). Placebo-controlled randomized clinical trials were started shortly afterwards, involving a major dose escalation trial [Heymsfield et al. 2002]. These studies showed a significant weight loss in relation to the dose of leptin administered. However, the magnitude of the loss was relatively modest [7 kg in the highest dose group – 0.3 mg/kg] and there were mild to moderate
immune responses at the sites of injection. Moreover, the dose required to generate this effect was prohibitively expensive. In contrast, when leptin was administered to leptin-deficient children, the response was spectacular and immediate, reversing the excessive weight gain in a relatively short period (Farooqi et al. 1999, Gibson et al. 2004). These contrasting responses led to the notion that part of the pathology of obesity is resistance to the leptin signal. If obese people do not respond to exogenous leptin because of resistance to the leptin signal, it was argued that intervening further downstream in the pathway might overcome this problem. An obvious target in this respect was the melanocortin 4 receptor [MC4R], which animal studies had established is a downstream focus of neurons that initially pick up the leptin signal in the ARC (Schwartz et al. 2000, Morton et al. 2006). Studies of mice with KO of the MC4R supported the view that this is a critical component of the body weight regulation system (Butler & Cone 2002, 2003) and polymorphisms in this receptor are known to underpin about 3–5% of all cases of morbid obesity in humans (Hinney et al. 1999). Treatment of rodents with an antagonist of the MC4R called MTII produced weight loss as anticipated (Murphy et al. 2000, Wirth et al. 2001, Hamilton & Dodds 2002). However, in humans as well as weight loss the same compound generated some unforeseen adverse effects – in particular it generated penile erection in male subjects and sexual arousal in females (Hadley 2005). On the brighter side, this has led to the development of a novel compound PT-141 and treatment for erectile dysfunction (Hadley & Dorr 2006). These studies highlighted the fact that while animal studies may lead us in the right direction in terms of the development of obesity therapies, the differences in physiology between mice and humans mean the translation from animal studies to drug development is never straightforward. The route is littered with stories of both success and failure.

Discussion

The use of animal models to study the phenomena that underlie obesity (genetic, physiological, epigenetic and environmental) as well as investigations of potential treatments in animals has provided an enormous amount of information that has had both direct and indirect impacts on our understanding of the condition. Unfortunately, the welfare of any animal which undergoes a manipulation that leads to the development of morbid obesity is potentially compromised. Ideal housing, animal numbers and husbandry practices for obese mice have been reviewed by Good (2005). Animal health can be affected as obesity leads to a reduction in mobility and increased pressure on the joints. As a result, the fur on the ventral surface may be worn down as animals shuffle around their cages and exceptionally they may develop pressure sores on their feet (personal observation). Even the most simple dietary interventions often require animals to be held in isolation for protracted times (weeks or months) to accurately measure energy intake. Although this isolation has often been presumed to be stressful, the impact of stress on isolation has been recently questioned (Hunt & Hambly 2006). In addition, obesity predisposes these animals to associated problems such as diabetes (e.g. the db/db mouse; Bahary et al. 1990), cancer (Hakkak et al. 2005) or the metabolic syndrome (e.g. 11β-HSD-1 overexpressers have insulin and leptin resistance, dyslipidaemia and hypertension; Rask 2001). Where pharmaceutical interventions require animal testing the procedures performed on the animal often require surgery to administer the compound over prolonged periods using mini-pumps or ICV cannulae (e.g. Kask et al. 1999). As studies on obesity are actively selecting strains that are prone to these conditions or require surgical interventions, the likelihood of animals suffering during this type of research is high.

Although progress using animal models to study energy regulation has been considerable, as is often the case, as knowledge expands in an area it generally serves mostly to document the extent of our ignorance. This is certainly true in the study of energy regulation and the resultant modulations of energy storage. Hence, while we have made some spectacular leaps forward in our
understanding, models of energy regulation remain relatively simple and there are considerable gaps in our understanding. It remains very plausible that there are additional hormonal signals that are derived in the periphery [like leptin, insulin, PYY and CCK] about which we are currently completely unaware. Indeed, several studies have directly hinted at such signals [e.g. Bünger et al. 2001]. While reducing the numbers of animals that are used in experimental work is therefore a laudable goal, we are not yet at a stage where replacing animals with computer-based models of energy regulation, or focusing on cell culture-based work could realistically overtake the use of live animals in experimental investigations of energy balance and obesity. Consequently, it is very unlikely that significant reductions in the numbers of animals involved in obesity research could be envisaged in the near future. Indeed, it seems likely that an increase in such work will be desirable as our understanding starts to improve and the boundaries of our ignorance become more defined.

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