Causes of Obesity and Consequences of Obesity
Prevention in Non-human Primates and Other Animal Models

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INTRODUCTION

Obesity develops slowly and spontaneously in some rodent strains and in non-human primates, with peak body weight reached in 'middle-age' or in older age. The animal models of obesity may be classified as: (a) spontaneous naturally occurring (of unknown genetic and physiologic cause(s)); (b) specific genetic models of known or unknown single gene mutations occurring spontaneously (and selected for by breeding), or produced by transgenic approaches; (c) dietary or nutritionally induced obesity usually by high fat and/or highly palatable diets in rodents (a form of obesity which is likely to be polygenic), or by high fat diets or forced overfeeding in non-human primates; and (d) neuroendocrine disorders causing obesity, such as via hypothalamic lesions or chemical infusions (e.g. the injection of gold thioglucose into mice), as detailed in Table 14.1. The present review will focus principally upon the spontaneously occurring form of obesity in non-human primates and in rodents, the form(s) of obesity that are highly likely to be directly relevant to most human obesity.

OBESITY AS A DISEASE OF AGING IN PRIMATES: THE NATURAL HISTORY OF CHANGES IN BODY ADIPOSITY

Obesity has been identified in many primate species, including orangutans, gorillas (1), chimpanzees (2), baboons (Papio ursinus) (3), vervet monkeys (Cercopithecus aethiops) (4), cynomolgus monkeys (Macaca fascicularis) (5), bonnet macaques (Macaca radiata) (6), pigtail macaques (Macaca nemistrina) (7), squirrel monkeys (8), and the Celebes ape (Macaca nigra) (9), although the species most studied is the rhesus monkey (Macaca mulatta) (10–12). Several rodent species develop a similar adult-onset obesity, including the Sprague-Dawley rat (13), the gerbil (Psammomys obesus, Israeli desert sand rat) (14), the New Zealand Obese mouse, the
Table 14.1 Animal models of obesity and diabetes

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<th>Classification of animal models of obesity and diabetes</th>
<th>Examples of models/methods</th>
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<td>Spontaneous, naturally occurring of unknown genetic/physiologic causes</td>
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<td>Sprague-Dawley rats</td>
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<td>Specific genetic models of single gene mutations of known or unknown function (spontaneous, bred, or transgenic)</td>
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<td>Others</td>
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<td>Dietary induced obesity</td>
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<td>Neuroendocrine disorders</td>
<td>‘Cafeteria’ (highly palatable) diet fed rodents</td>
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<td>Hypothalamic or related brain area lesions or stimulation (including electrolytic, knife cut, chemical, viral)</td>
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BSB mouse (*Mus spretus* and other strains), and the spiny mouse (*Acomys cahirinus*). Spontaneous obesity also develops in some cats (15) and dogs, as well as in many other species, when the individuals are maintained in an unfettered environment.

Spontaneous adult-onset obesity develops in primates and rodents in an environment that is either permissive of, or facilitative to, weight gain. The usual laboratory setting of *ad libitum* food availability and of protection from predators and disease is sufficient to produce adult-onset obesity in many, but not all, non-human primates (16,17). (Some 20–30% remain lean all of their lives despite a facilitative environment, and further, among the obese, the amount of excess weight and fat varies widely.) The peak body weight in these laboratory housed monkeys is usually not reached until about 15 years of age (‘middle-age’), with the weight gained after age 7 being composed primarily of adipose tissue. Thus, obesity in non-human primates must be considered to be a disease of aging. Although primates are able to reproduce by about the age of 4 years, no spontaneously obese younger monkeys (under the age of 6 years) have ever been described, and thus, primates do not provide a model for childhood-onset obesity in humans.

Does obesity occur in primates living in their natural environment? The best evidence comes from the identification of obese monkeys in the protected environment offered by the island of Cayo Santiago off the coast of Puerto Rico (18). On that island, large colonies of monkeys are positioned with primate chow to provide *ad libitum* intake in a free-ranging, but predator-free, environment. In a reported survey of these monkeys, the obese monkeys ranged from 9 to 16 years of age (19). In some matrilines, the prevalence of obesity ranged as high as 20% in these free-living monkeys (20). Obesity has also been observed in other freely feeding and protective environments, including in some zoological collections. The relationship between obesity, diabetes and aging in monkeys has been reviewed recently (21).

Fat Mass and Distribution: Abdominal or Central Obesity

Obesity in humans and monkeys develops very gradually and progressively in ‘middle-age’. Specifically, percent body fat begins to increase in monkeys after about the age of 7 years, and continues, in some monkeys, to increase into late maturity. This increase in fat mass can be detected widely in subcutaneous tissue and also in intra-abdominal adipose mass. Its distribution is heavily abdominal in both males and females (22,23). The abdominal circumference shows a consistent increase with increasing body weight in middle age (age 7 to 20 in monkeys), as shown in Figure 14.1 for one monkey (D-7), and as previously described for a large group of monkeys (24).

In many monkeys, this change in body fat com-
Longitudinal changes in abdominal circumference and in body weight of a monkey (D-7) followed from age 5 to age 20 years.

Figure 14.1

Figure 14.2

Figure 14.3

OBESITY AS A GENETIC DISORDER

Genetic and metabolic factors in the development of obesity have received increasing attention since the identification of the protein product of the ob gene—leptin, and its receptor. Clearly there are cases among humans, as well as in strains of rodents, in which a single gene mutation has been

identified as the direct and specific cause of obesity. That obesity is a genetic disorder is rarely disputed today. What remains under discussion is the relative magnitude of the contribution of genes to body weight compared to the contributions of excessive ingestion of calories due to environmental considerations. Excessive ingestion of food and reduced energy expenditure, both of which may be exacerbated by an enriched environment, are sometimes considered to be the primary culprits.

Concerning obesity in humans, just as it may be difficult to get a genie back in a bottle, so it may be difficult to ‘demodernize’ the environment of humans, or to otherwise alter it so as to mitigate the ‘New World syndrome’ (25). This is confirmed by the modest success of most weight control programs, and suggests that the forces aligned against weight loss and weight loss maintenance are indeed powerful and poorly susceptible to the combination of environmental manipulations and volitional changes among humans with excess weight.

We have found the same to be true in non-human primates. For example, forced weight reduction by limitation of available calories provided to obese monkeys produces weight loss. However, the recidivism when the monkeys are returned to a non-restrained calorie regimen is 100%. This occurs despite the use of a high fiber, low fat chow diet which is not highly palatable. What might be the nature of these ‘forces’ that mitigate against significant weight loss for most middle-aged persons, and that promote weight regain or recidivism in most of those who have successfully lost weight? Are these ‘forces’ present in animals as well?

The strong familial aggregation of risk for obesity in humans provides evidence for a powerful familial component to the development of obesity. Some suggest that this is a combination of shared environment and shared genetic propensities. Both quantitative trait loci in rodent models and family linkage studies in humans have identified a number of chromosome areas which may carry obesity promoting genes. However, at this time these remain only promising regions, some of which may contain candidate genes for further study.
Figure 14.2 Longitudinal data from a single monkey (A-7), beginning at sexual maturity (young lean adult age 5) through the progressive development of obesity followed through age 17. Panels show sequential changes in body weight, % body fat determined by tritiated water dilution, fasting plasma glucose, fasting plasma insulin (and progressive hyperinsulinemia), M or glucose uptake rate during a euglycemic hyperinsulinemic clamp (a measure of whole body insulin sensitivity which declined over time) and K glucose or glucose disappearance rate during an intravenous glucose tolerance test (which showed a slow declining function).

Single Gene Mutations in Animal Models

Candidate Genes for Human Obesity

Lessons for human obesity can be learned from animal models of genetic obesity. First, single gene mutations can and do cause obesity in both rodent models and in humans—this is indisputable. In rodents such mutations have been identified in at least five genes, including the *ob* gene for the circulating adipose tissue-secreted factor leptin, the *db* gene for the receptor of leptin (and in rats, the *fa* gene), the *agouti yellow (Ay/a)* mutation which controls the production of melanin pigments controlling skin color in mice (with its human equivalent agouti signaling protein gene), the *fat* mutation in the carboxypeptidase E gene which is a prohormone processing enzyme, and the *tub* mutation which is still under study to determine its function, but which may be a protein involved in insulin signaling (26). Other genes which have been implicated in body
weight regulation include the melanocortin-4 receptor (MC4-R) (27), and its other isoforms, melanin-concentrating hormone, receptors mediating leukocyte adhesion (deficiency in intercellular adhesion molecule-1 or in its receptor, leukocyte integrin alpha M beta2 (Mac-1)), and the possible suppressor of obesity, the mahogany protein, which may be a signaling protein or receptor similar to the proteoglycan receptors. Although extensive efforts have been made to identify mutations in these and in other candidate genes for obesity in humans, to date, only a handful of individuals have been identified with mutations in any of the genes which have produced obesity in rodents.

**Mutations of the ob Gene in Rodents and Humans**

Based on the observations of food intake and body weight in parabiotic obese and lean rodents, a circulating product of the adipose tissue had long been suspected of being involved in body weight regulation (28,29). Cross circulation studies in non-human primates, by contrast, did not support the idea that a circulating factor might be involved in feeding regulation in any dominant or major way (30). Interestingly, both studies now appear to be confirmed.

The specific genes and their mutations implicated in the early rat parabiosis studies were identified only a few years ago (31,32). The cloning of these genes, the identification of their mutations in obese rodents, and the identification of the circulating gene product (leptin) and its receptor (32) led to the hope that the genetic basis for human obesity might soon become clear. The ob genes in humans (33) and in monkeys (34) were cloned and sequenced. The deduced amino acid sequence of the human OB protein coding region was found to be 84% identical to that of mice, 83% identical to rats, and 91% identical to that of the rhesus monkey. The genes of many obese persons and monkeys have been searched for defects in the ob or leptin gene and its receptor. As noted, only a handful of patients and no monkeys have been identified with mutations in either of these genes (35–37).

**Variations in the Circulating Product of the ob Gene, leptin**

With the identification of the peptide released from adipose tissue, animal models as well as humans were examined for characteristics associated with variations in plasma leptin levels. Both monkeys and humans have been reported to show strong correlations between body weight and plasma leptin levels, and between body fat and leptin levels, as...
Both body weight (a) and body fat (b) were highly correlated to plasma leptin levels \((P \leq 0.001)\) in a large group of rhesus monkeys (expanded from Hotta et al. (34)).

shown in Figure 14.4 for rhesus monkeys (38). It was immediately noticed that while lean subjects generally have low leptin levels, not only was there an increase in leptin levels with increasing adiposity, but the variability increased greatly, such that obese subjects could be identified with normal to extremely elevated levels, e.g. 5- to 10-fold higher than normal. No explanation for these large variations has been established, since all plasma samples were obtained consistently under overnight fasted conditions. What is clear is that leptin is not released and does not circulate in a simple ratio to fat mass. This is in contrast to the observation that, in rats, the leptin–body fat ratio is a constant for a particular strain (39). Circulating levels are altered significantly by fasting and refeeding, and by many other factors.

The possibility that these large variations in circulating leptin levels might indicate important differences in the receptors for leptin has also been closely examined in rhesus monkeys. No leptin receptor mutations were identified, and expression levels as determined by polymerase chain reaction (PCR) of the two principal isoforms of the receptor, the long form and the short form, were not associated with body weight as shown in Figure 14.5. There was also no association between expression of the forms of the leptin receptor and fasting plasma insulin, plasma glucose, or circulating plasma leptin levels.

**Insulin and the Insulin Receptor Genes**

Rare genetic defects in either the insulin molecule or the insulin receptor have been identified in humans and associated with insulin resistance and obesity (40). In non-human primates, which have circulating plasma insulin levels five-fold or more higher than in humans, no defects in either have been found to account for the apparent insulin resistance.
The insulin molecule in monkeys is identical to that of humans (41), and sequencing of the insulin receptor has shown it to be remarkably similar to that of humans (42). Molecular examination of the few amino acid substitutions in the monkey insulin receptor compared to that of humans failed to show why monkeys have such elevated insulin levels relative to humans (43).

The insulin receptor is expressed in two isoforms in humans and in monkeys. One form is expressed in higher proportions in obese, hyperinsulinemic pre-diabetic monkeys (and similarly in humans), and this proportion reverts to normal in diabetes (44,45). Whether the relative proportion of these isoforms plays a role in the progression of obesity to diabetes is unknown. The hyperinsulinemia of obese monkeys is not associated with any difference in food intake relative to similarly obese normoinsulinemic monkeys (46).

Many transgenic animal models of obesity are under study at this time. However, they will not be discussed in this review, which focuses upon spontaneous models of the human condition.

**Mendelian Syndromes of Obesity not Identified in Animal Models**

There is a large group of Mendelian syndromes in which obesity is a component, including Prader–Willi, Bardet–Biedl, Alstrom, Carpenter, Cohen, Wilson–Turner and others. However, these lack specific animal models. These genetic disorders are rare in humans, and family studies do not suggest that the genes responsible for these syndromes are involved in the common form(s) of human obesity. For more than 99% of all obese humans, and 100% of all obese non-human primates, the genetic basis of the obesity is unknown.

**Adipose Gene Expression**

In animal models, adipose tissue has been the focus of numerous studies aimed at understanding its physiological and genetic regulation and the differential expression of various genes that might regulate adipose tissue mass. In non-human primates the possibility that the obese may differ in the expression of the genes that regulate adipocyte differentiation has been explored. The peroxisome proliferator-activated receptor γ (PPARγ) has been the specific focus of much study since its identification as the receptor for the insulin sensitizer class of pharmaceutical agents, the thiazolidinediones (see below), and its very early expression during adipose cell differentiation. There are two isoforms of PPARγ, (γ1 and γ2), and both have been cloned and sequenced in the rhesus monkey (47). The latter is highly expressed in adipocytes, while PPARγ1 is expressed widely in many tissues. The ratio of the PPARγ2 mRNA to total PPARγ mRNA was significantly related to body weight and to fasting plasma insulin, while neither total PPAR γ nor its two isoforms individually were related to obesity or to insulin levels (47). This is in contrast to the finding in rats of increased PPARγ mRNA levels in the white adipose tissue of obese rats (48).

Other genes expressed during adipocyte differentiation include: CCAAT/enhancer binding protein α (C/EBP α), lipoprotein lipase (LPL), phosphoenolpyruvate carboxykinase (PEPCK), and the glucose transporter gene (GLUT 4). As shown in Figure 14.6, in a study of normal weight, obese, and diabetic monkeys, none of the best known regulators of adipose differentiation were associated with body weight in any of the three groups, nor in the total group (47). They were, however, found to be associated with aging (49).

A newly identified adipocytokine, adiponectin (an adipose-specific protein abundantly expressed and released into the circulation), is paradoxically decreased in human and in monkey obesity. Its circulating levels are inversely correlated with plasma leptin levels. Adiponectin has been sequenced in both humans and monkeys and found to have 98% identity (50). There was no relationship between body weight or obesity and adiponectin mRNA, suggesting posttranscriptional regulation by adiposity, a regulation which was disturbed when diabetes developed.

**Genetic Susceptibility and Gene–Environment Interactions**

Linkage studies and extensive candidate gene studies give presumptive evidence that multiple genes may be involved in the susceptibility to obesity in both humans and in animals, with each gene contributing in small measure to the propensity or sus-
Figure 14.6 Genes implicated in adipose cell differentiation were examined in normal (open circles), obese (open squares) and diabetic (solid circles) monkeys. The expression levels of PPARγ (a), LPL (b), aP2 (c), CEBPα (d), Glut 4 (e), and PEPCK (f) mRNA measured by slot blot hybridization were not associated with body weight or total body fat, but, with the exception of aP2 and PEPCK, they were highly coordinately regulated with each other. (Redrawn from Hotta et al. (47))
ceptibility to develop obesity. This likelihood may contribute heavily to the difficulty in isolating the specific genetic contributions within a family or group where a single gene mutation is not known to be present.

Studies both in animals and in humans support the contention that individuals differ in their susceptibility to weight gain and to overt obesity under conditions in which the environment is facilitative or non-constraining. For example, a high fat diet fed to some strains of mice results in significantly more excess body fat than when fed to other strains; indeed there are strains that are resistant to the obesifying effects of a high fat diet (51). A high fat diet also induces obesity in adult rabbits (52). Similarly, in humans differences in susceptibility to weight gain have been identified (53,54).

Efforts are continuing to identify the genetic and molecular basis of obesity or of the ‘obesities’. The likely outcome in the future is the identification of many genes, and, within those gene coding regions or in nearby regions that affect gene expression, many different mutations or variants which are responsible for the heavy genetic burden of obesity in humans and in non-human primates. Combinations of these genes are likely to increase susceptibility to weight gain and obesity when the environment is permissive or culpatory. The direct effects of these interacting genes may be to alter metabolic rate or nutrient partitioning, to alter lipid metabolism or adipose tissue function, to alter lean mass, to alter the hormonal milieu, and/or to regulate feeding behavior and appetite. Polygenic as well as major gene effects may be acting to produce the complex phenotype of obesity and its associated disorders in primates. The current state of this understanding for human obesity has been reviewed extensively (55). In addition, many of these genes are likely to interact with each other and with the environment for both humans and animals, thus further increasing the challenge for the future understanding of the mechanisms underlying the physiological basis of obesity.

Obesity as a Nutritionally Induced Disorder

It is clear that obesity results from an imbalance of energy input and energy output, and that in some animal models it can be induced by dietary methods. For example, the *Psammomys obesus* or Israeli desert gerbil or sand rat exhibits obesity only under nutritional conditions that this species does not see in the wild (14). Susceptibility of various animal models to nutritionally induced obesity appears to differ across strains and even within a single strain of rodents, and is a characteristic which some investigators have used in selective breeding (51). The mechanism which underlies this susceptibility to dietary obesity is unclear, but in the sand rat is suspected to relate to impaired activation of the insulin receptor and compromised tyrosine kinase activation, which interestingly, is reversible with calorie restriction (see below for further discussion of calorie restriction in obesity). High fat feeding produces many changes in metabolism, as illustrated by the recent finding of increased uncoupling protein 3 (UCP3) levels in brown adipose tissue and reduced skeletal muscle UCP3 in dietary obese rats (56). High fat diets in monkeys have produced significant weight gain, increased body fat, and increases in triglycerides and low density lipoprotein (LDL) and high density lipoprotein (HDL) cholesterol (57).

Viral Models

Viruses have been suspected of being involved in obesity in humans (58), and have been shown to be capable of producing obesity in rodents (59). Whether this is an important mechanism for the induction of obesity is, as yet, unclear.

Adipose Tissue Metabolism During the Development of Obesity

Although insulin sensitivity at the whole body level generally declines as obesity develops, as shown above for monkey A-7, it is seldom appreciated that this longitudinal change at the whole body level is not associated with a similar decline in the sensitivity of isolated adipocytes to insulin actions. Generally, it is believed that whole body insulin action, as measured by a euglycemic hyperinsulinemic clamp, is principally determined by glucose uptake into muscle, with a small contribution of adipose tissue and other organs. At the level of the
Figure 14.7  Insulin action on adipocytes obtained from the abdominal subcutaneous tissue of four groups of monkeys whose characteristics are differentiated in the top four panels: lean normal monkeys, obese normoinsulinemic monkeys, obese hyperinsulinemic monkeys, and obese with overt type 2 diabetes. Differences in body weights, fasting plasma glucose, intravenous glucose tolerance, and fasting plasma insulin are shown in the top four panels. Panel 5 is the effect of insulin to increase glucose oxidation in isolated adipocytes and panel 6 is the effect of insulin to increase lipid synthesis, both in isolated subcutaneous abdominal adipocytes. (Redrawn with permission from Hansen et al. (60))

Adipocyte, the action of insulin, as measured in biopsies obtained both cross-sectionally and longitudinally from rhesus monkeys during the development of obesity and progression to diabetes, shows increased ability of insulin to stimulate glucose oxidation and to stimulate lipid synthesis in obese animals compared to normals (60). The deterioration in insulin action at the adipocyte is a late event accompanying the development of diabetes, as shown in Figure 14.7.

RELATIONSHIP BETWEEN OBESITY AND TYPE 2 DIABETES MELLITUS

Glucose Tolerance in Obesity

Animal models of obesity are highly associated with observations of reduced glucose tolerance (61), and in the non-human primate this glucose intolerance precedes overt type 2 diabetes, as recently
reviewed (62). Glucose tolerance to an intravenous glucose infusion ranged in monkeys from a glucose disappearance rate of 5%/min to 2%/min without any change in fasting plasma glucose. When tolerance dropped below 2%, fasting plasma glucose began to be elevated (61). Thus, there is a powerful compensatory response which, despite declining glucose uptake rates, maintains plasma glucose at normal levels in the obese non-human primate.

Hepatic Glucose Production

Longitudinal in vivo studies in rhesus monkeys have shown that as obese monkeys begin to make the final transition from impaired insulin sensitivity and impaired glucose tolerance at the whole body to overt type 2 diabetes, increasing fasting plasma glucose very closely parallels increasing basal hepatic glucose production (63). In a related event, as hyperinsulinemia progresses to high levels, hepatic extraction of insulin (the proportion of the insulin presented to the liver which is removed by the liver) declines (64).

Insulin Secretion and Insulin Sensitivity

Beta cell insulin secretion is minimally affected during the early stages of obesity, as shown by the comparison of changes in body fat and in fasting plasma insulin for monkey A-7 illustrated in Figure 14.2. Insulin sensitivity, measured at the whole body by the euglycemic hyperinsulinemic clamp, remained normal during that period as well, while fat mass more than doubled. The increase in insulin secretion and the decline in insulin sensitivity that took place in this monkey after age 12 were closely related, and have been observed in a larger group of monkeys (65). Thus, changes in adiposity per se were not directly associated with changes in insulin secretion or insulin sensitivity. In most monkeys and in most humans, increased adiposity appears play a role as an apparent facilitator or permissive factor for the declines in whole body insulin action and the increase in insulin output. Both basal fasting levels of insulin and β cell responsiveness to a glucose stimulus increase in the early stages of the transition from ‘simple’ obesity to insulin-resistant prediabetic obesity (66). Insulin secretion subsequently generally declines as fasting plasma glucose begins to rise in the early stages of overt diabetes (notably shown in Figure 14.4) (67). Nevertheless, at the time of diagnosis of diabetes, insulin levels are usually still elevated above normal, and β cell responsiveness to glucose is completely absent, as recently reviewed (62).

Under basal fasting conditions in both monkeys and humans, insulin is secreted with a 10 to 14 minute oscillating periodicity (68, 69), and the amplitude of this periodic secretory output increases with obesity and hyperinsulinemia. This amplification of the secretory output in obesity may play a protective role, or alternatively, may signal disturbed β cell functioning and regulation in the prodrome to diabetes. Further, this periodic secretory pattern is completely disrupted by 48 hours of fasting or by the development of diabetes, even when basal insulin levels are above or within the normal range (70). Oscillations are also lost by an exogenous infusion of glucose raising glucose levels to about 6 mmol (71). The presence of oscillations in plasma levels of insulin may thus be viewed as a reflection of a ‘contented’ beta cell (71).

Insulin Resistance

Tissue Specificity in the Sequence of Appearance of Defective Insulin Action

There is a wide range of insulin sensitivity in monkeys with normal glucose tolerance (24,72), including obese monkeys with or without insulin resistance.

We tend to think of insulin sensitivity, or its converse, insulin resistance, as a single entity, regardless of how or where it is measured. This is unfortunate and has resulted from the paucity of studies in which whole body insulin-resistance and resistance at each of the major insulin sensitive tissues have been measured simultaneously. Most commonly, so-called ‘normals’ (usually age and/or weight matched) are compared to so-called diabetics (individuals with significant hyperglycemia). Under these groupings, normals are normal in both whole body and tissue determinations and diabetics are ‘resistant’ at both the whole body and at various tissues. More detailed analysis has shown in rhesus monkeys that whole body insulin resistance, probably principally reflective of skeletal muscle insulin
resistance, develops in obesity in parallel with hyperinsulinemia (72), and well before the appearance of insulin resistance at adipose tissue and at liver (63). Resistance in the latter two tissues seems to be directly associated with the progression of individuals from obese with hyperinsulinemia to overtly diabetic.

**Insulin Action on Glycogen Metabolism in Skeletal Muscle, Adipose Tissue, and Liver**

Obese hyperinsulinemic monkeys had a significant decline in insulin-mediated change in glycogen synthase activity in skeletal muscle (73). This defect appeared early at about the same time as the increasing insulin secretion noted above, that is, at the same time as β cell hyper-responsiveness developed. Obese and insulin resistant monkeys also had significantly higher insulin stimulated glucose 6-phosphate concentrations compared to normal monkeys, suggesting that a step distal to glucose 6-phosphate is a major contributor to reduced insulin-mediated glucose disposal and reduced insulin action on glycogen synthase activity (74).

In adipose tissue, by contrast, both basal and insulin-stimulated total activities of both glycogen synthase and glycogen phosphorylase were increased above normal in obese hyperinsulinemic monkeys (75). Insulin action to increase glycogen synthase independent activity was reduced in obese hyperinsulinemic monkeys compared to the normal monkeys. Specifically, in normal monkeys insulin stimulation induced a 100% increase in glycogen synthase independent activity over basal levels compared to a 50% increase in obese hyperinsulinemic monkeys (and no increase in diabetic monkeys) (75).

At the liver of monkeys, a different picture of defects in insulin action with obesity has emerged. Glycogen synthase activation and glycogen phosphorylase inactivation by insulin (in a reciprocal fashion) were significant in the liver of both normal lean monkeys (76) and obese hyperinsulinemic monkeys under the condition of a euglycemic hyperinsulinemic clamp (76). In obese insulin-resistant monkeys, under the same conditions, glycogen synthase (GS) total activity was lower under basal conditions compared to the lean young animals. Nevertheless, total GS activity was significantly increased by insulin stimulation in the liver of insulin-resistant monkeys. Both the basal GS independent activity and the insulin-stimulated independent activity of the insulin-resistant monkeys were higher in the latter group compared to the lean animals (77). Thus, insulin action at the liver was found to be strong in monkeys that were otherwise determined to be insulin resistant at muscle and adipose tissue, as well as resistant at the whole body level. This would accord with the normal hepatic glucose production of insulin-resistant obese monkeys (increased only in diabetics where insulin’s suppression of hepatic glucose production is significantly impaired (63)).

**PREVENTION OF OBESITY: LESSONS FROM ANIMAL MODELS**

**The Need for Obesity Prevention**

Although the causes of obesity in the vast majority of humans (99%) and in all obese non-human primates are unknown, studies in rodents and in non-human primates have unequivocally demonstrated that calorie restraint, sufficient to prevent significant increase in total body fat, prevents obesity (by definition), but more importantly, prevents most obesity-associated diseases. Excess body fat or altered energy balance clearly plays a facilitative role in many of the comorbidities of obesity. While we continue the search for the underlying causes of obesity, animal models show that even without knowing the causes, interventions to reduce the degree of obesity can have very strong positive consequences for many obesity-associated diseases, as well as for overall reduction in mortality and extension of life span.

Trowell and Burkitt’s studies of epidemiological changes in modernizing societies showed that obesity is the first of the ‘diseases of civilization’ to emerge in the longitudinal picture (78). As such obesity is clearly the earliest target for intervention to halt a wide range of non-communicable diseases of modern and modernizing societies. Gracey has termed this defined cluster of diseases the New World syndrome (79), and has included within its sphere obesity, type 2 diabetes, hypertension, dyslipidemia, and cardiovascular disease (also termed the metabolic syndrome X (80)) (with the addition of cigarette smoking and alcohol abuse).

The World Health Organization has commented...
extensively on the societal factors which have accompanied or induced the changes leading to the New World syndrome, and which have led to identification of obesity as a global epidemic (25). The WHO Report cited such components of modernization as the development of market economies, reliance on imported non-traditional foods, increasing urbanization, changing occupational structures, increasing socioeconomic status, increases in animal fat and animal protein intake, decreases in vegetable fat and vegetable protein intake, reduction in total and specifically complex carbohydrates, and increases in sugar intake. The net effect of these factors might be viewed as providing an unrestrained environment in which genetic potential becomes fully expressed. Alternatively, the environment may be interacting in such a way as to be detrimental to ‘normal’ gene expression.

As noted below, evidence from animal models strongly indicates that these diseases are not independent. As in diseases of human civilization, the sequence of the appearance in animals starts with obesity. The prevention of obesity can prevent or greatly reduce all of the others, and as a further consequence, greatly reduce morbidity and mortality. Because the benefits of obesity prevention have to date been difficult to attain in humans, examination of the data from animal models of obesity prevention can be informative for the human condition and the potential importance of obesity mitigation.

Primary prevention includes all measures aimed at reducing the incidence or preventing the occurrence of a disease and its complications or of reducing the risk of disease. Secondary prevention includes the measures introduced to mitigate the consequences of a disease, slow its progression, and reduce its associated morbidities following the early diagnosis of the disease. For example, primary prevention of obesity in animals is achieved when the development of increased body fat is completely prevented, usually by calorie restriction, while secondary prevention is introduced when weight reduction or body fat ablation of an already obese animal has been instituted.

Successful prevention interventions rely upon an understanding of the natural history of a disease, together with the identification of sufficiently powerful and successful methods for preventing the disease. In the case of obesity prevention in humans a significant number of risk factors for obesity have been identified, and a modest amount is known about the natural history of obesity (as established in this volume). In humans, however, only a few methods of limited applicability (primarily surgical approaches) have been identified which truly modify the course of the disease, generally mitigating its consequences after obesity has reached severe stages (81). In rodents and in non-human primates, by contrast, deeper understanding of the aging-related changes in body composition and of the factors increasing risk for obesity have been developed. In addition, the usual laboratory environment, with its readily available constraints and manipulability, has enabled the introduction of powerful non-surgical approaches to obesity prevention, thus allowing assessment of its consequences and its likely implications for humans. Current efforts in humans are principally limited to identification of high-risk individuals for ‘lifestyle’ changes, and environmental manipulations. The power of these interventions is extremely limited at this time. Nevertheless, studies in non-human primates demonstrate that the successful prevention of obesity has far-reaching consequences and extraordinarily high potential for positive impact on human health.

**Efficacy of Primary Prevention of Obesity in Rodents and Non-human Primates**

**Extension of Average Life Expectancy**

The longest ongoing study of calorie restriction in non-human primates, initiated when the monkeys were fully adult at about age 10, continues at this time and has already shown the powerful effects of obesity prevention in preventing or greatly postponing morbidity and increasing average lifespan. Further, simply instituting primary prevention of obesity (by calorie titration on an individual animal basis) has provided the most powerful means known to prevent the development of overt diabetes (82). In the restricted animals, there is no diabetes, while in the ad libitum fed animals diabetes rate exceeds 30%, and the obesity rate exceeds 50% (82). Calorie restriction also results in the prevention or significant postponement of many features that normally contribute to cardiovascular risk, including many diabetes-associated metabolic dysfunctions, such as reduced dyslipidemia, and improved blood
pressure profile (83). As a result of this disease prevention, there appears to be excellent potential to extend maximal lifespan as well. This, however, must await further extension of the study as the monkeys are now in their late 20s and the maximal reported lifespan in captivity for the rhesus monkey is 40 years (83).

**Anti-aging Properties of Obesity Prevention**

The extension of average life expectancy and the potential for extension of maximal lifespan raise the possibility that, as in rodents, calorie restriction in non-human primates exerts an anti-aging effect which is separate from and in parallel with the anti-disease effects so clearly already demonstrated (83). The mechanisms by which such anti-aging effects of obesity prevention are achieved are, as yet, unknown. However, several such potential mechanisms are under study in rodents as well as in non-human primates. In rodents, a reduction in mitochondrial oxidative damage during aging has been found with calorie restriction (84). Examination of differential gene expression in rodents with and without calorie restriction has indicated a marked reduction in the stress response and a lower expression of a number of metabolic genes in the calorie-restricted group (85).

**Body Fatness Required to Reduce Disease**

Improvement in glucose tolerance, dyslipidemia and blood pressure, and prevention of diabetes do not appear to require maximal leanness. In one reported study, body fatness in calorie-restricted monkeys has been maintained at levels ranging from 10 to 22%, a normal range for non obese adult monkeys (83). Whether levels below this (excessive leanness) will further improve health or will in fact be detrimental to health will await the reports of other studies of calorie restriction in non-human primates (86).

Fat distribution may also be playing a role, as calorie restriction in non-human primates results in improved body fat distribution (reduced fat in the abdominal region) which is directly associated with the reduced overall body fat content (87).

**Prevention of Hyperleptinemia**

The hyperleptinemia associated generally with obesity in humans and monkeys as noted above (38) is prevented by calorie restriction with the prevention of obesity, and the normalization of leptin levels could potentially be advantageous, although this is speculative at this point.

**Prevention of Obesity: Effects on Glucoregulation, Insulin Secretion, and Insulin Action**

**Regulation of Plasma Glucose and Glucose Tolerance**

In rodents as well as in rhesus monkeys, calorie restriction results in maintenance of normal fasting plasma glucose levels, and normal glucose tolerance (82). Both fasting plasma glucose and fasting plasma insulin are lower relative to control *ad libitum* fed monkeys, but are not reduced relative to normal lean young adult monkeys (88,89).

**Insulin Sensitivity**

In monkeys, dietary restriction produces significantly higher *in vivo* insulin action compared to *ad libitum* fed monkeys of the same age (90). Aging and obesity-associated insulin resistance appear to be mitigated by long-term restraint on calories in monkeys, and the same is likely to be true in humans. The development of type 2 diabetes that is prevented by calorie restriction and obesity prevention may be accomplished by this sustaining of insulin action (82), presumably particularly in skeletal muscle.

**Glycogen Synthesis and Glycogen Synthase Activity**

In non-human primates, glycogen synthase activity in skeletal muscle is increased by calorie restriction (91). The mechanism by which this effect is achieved is unknown. In some of those monkeys, however, there was an unexpected decrease in glycogen synthase fractional activity with insulin stimulation, a greater increase in skeletal muscle glucose 6-phosphate, and the greatest increase in glycogen phosphorylase activity with insulin. These unusual responses were associated with a relatively lower whole body glucose uptake rate compared to the other calorie-restricted monkeys. There was an unexpected increase in the glucose 6-phosphate $K_a$ of
skeletal muscle glycogen synthase, indicating phosphorylation (rather than dephosphorylation) of glycogen synthase in response to insulin (91). These changes may be involved in the anti-diabetogenic properties of caloric restriction.

**Obesity, Dyslipidemia, and its Prevention by Calorie Restriction**

**Dyslipidemia: Hypertriglyceridemia and Low HDL Cholesterol**

Monkeys, like humans, frequently develop dyslipidemia in middle age, including hypertriglyceridemia and reduced HDL cholesterol levels (92), and this dyslipidemia is highly associated with the presence of obesity, with or without diabetes. Pharmaceutical agents which alter dyslipidemia in humans do so in monkeys as well (93,94).

**Prevention of Dyslipidemia by Calorie Restriction to Prevent Obesity**

Abnormalities in plasma lipid levels are virtually entirely prevented by calorie restriction in non-human primates (95,96). Both the reduction in plasma triglyceride levels relative to ad libitum fed controls, and the increase in the HDL2b subfraction, which is associated with reduction in atherosclerotic risk, were principally accounted for by the reduction in body mass and the associated improvement in glucoregulation noted above (96). Calorie restriction in rhesus monkeys, while producing no change in plasma LDL cholesterol concentrations, reduced the molecular weight of the LDL particles, and reduced their triglyceride and phospholipid content, together with reduced proteoglycan binding (97).

**Restraining Calories to Prevent Obesity: Effects on Energy Expenditure**

**Energy Expenditure**

When rhesus monkeys were studied after 10 years of adult onset calorie restriction with long-term stabilized body weight, total daily energy expenditure was reduced compared to ad libitum fed monkeys, as shown in Figure 14.8. Note that the regression line shown is for the ad libitum monkeys only. This reduced energy expenditure was present even when the energy expenditure rate was adjusted for differences in body weight, body surface area, or lean body mass (98).

**Thyroid Hormone**

In the same study of long-term calorie restriction in adult rhesus monkeys, thyroxine (T4) was reduced and the free thyroxine index tended to be lower, without change in triiodothyronine (T3).

**Body Temperature**

Calorie restraint in adult monkeys has been reported to result in lower body temperature (99). However, this was not observed in a group of long-term older calorie-restricted monkeys (98).

**Physical Activity**

Physical activity of the calorie-restricted monkeys was greater than of the similar aged ad libitum monkeys (who were considerably fatter), but activity did not differ between calorie-restricted older monkeys and similar body weight younger adult animals (98).
The reduced energy expenditure during caloric restriction is therefore not due to a reduced activity level as might have been suspected.

**Secondary Prevention or Weight Reduction in Obesity**

Secondary prevention, or mitigation of already existing obesity for the purpose of reducing the negative health consequences of obesity, is also important. Behavioral and environmental manipulations are widely applied but only modestly successful. Surgical treatments are of limited application. Pharmacological approaches are expanding and currently animal models are contributing to the examination of new anti-obesity agents.

**New Pharmacological Studies in Animal Models of Obesity**

**Beta 3 Adrenergic Receptor Agonists to Increase Energy Output from Adipose Tissue and Reduce Fat Mass**

Although β3 adrenergic agonists have been under study in rodents for more than 20 years, only in the past several years has the uniqueness of the primate (human and non-human) β3 receptor been identified. Currently, β3 agents under study are specific for the human receptor and are being extensively tested in non-human primates whose β3 receptor is very similar the human sequence (100). The β3 receptor sequence in the rhesus monkey is shown in Figure 14.9. Studies of β3 agonists in monkeys have been reviewed recently (101). Such agonists have been shown to be active at the non-human primate receptor (102), acutely producing lipolysis and metabolic rate elevation and increased UCP1 expression in brown adipose tissue. To date, however, none has been reported to produce a reduction in body weight. This may be due to an insufficient number of β3 receptors on the adipose tissue of humans. Recent studies have shown an increase in the expression of the mitochondrial uncoupling proteins (UCP2 and 3), and possible increase in the number of brown adipocytes (103). These agonists also seem to have lipid-lowering and insulin-sensitizing effects. In young rats a β3 agent has led to reduced body mass and adiposity which was blunted in older rats (104).

**Glucose-lowering and Insulin-sensitizing Agents**

**GLP-1 and exendin-4.** GLP-1 (glucagon-like polypeptide-1) has been studied in non-human primates (101), but its short half-life has precluded it from being considered for clinical use. Another amino acid peptide with 53% sequence similarity to GLP-1, exendin-4, has been shown to have prolonged glucose lowering action *in vivo* in obese non-human primates and in rodent models of obesity and diabetes (105).

**Thiazolidinediones.** Thiazolidinediones, a class of insulin sensitizers, have been examined in animal models of obesity and of diabetes, including application to non-human primates (101). In general they improve insulin sensitivity and lower plasma glucose levels in some, but not all prediabetic and diabetic subjects (106). They also reduce hypertriglyceridemia. Recently a new mechanism of action of this class has been reported, the enhancement of glycogen synthase activity in skeletal muscle, possibly accounting for some of the apparent insulin-sensitizing effects observed in the whole body (107). Figure 14.10 shows the results of a study in which the thiazolidinedione R-102380 was administered for 6 weeks with measurements made before and at the end of the dosing period. Glycogen synthase activity was measured in skeletal muscle biopsies obtained under basal and insulin-stimulated conditions (before and during a euglycemic clamp). All four monkeys studied showed an increase in insulin action to increase glycogen synthase independent activity as well as fractional activity (independent divided by total activity). Insulin-sensitizers are likely to continue to be a focus of expanded efforts to mitigate the health consequences of obesity and to slow or prevent the development of diabetes.

**Products Secreted from Adipose Tissue**

**Leptin administration.** The administration of leptin to either rodents or to humans with leptin deficiency has been shown to reverse this form of obesity (108). The leptin-deficient obese subjects lost significant adipose tissue mass, and reproductive function was restored (108). When administered
to non-human primates without any abnormality in the leptin axis, peripheral leptin had no effect on food intake or body weight (109). However, when leptin was administered to the normal monkeys intracerebroventricularly there appeared to be a delayed reduction in food intake the next day. Leptin administered to the obese *Psammomys obesus* sand rat failed to affect body weight, body fat, or adipose gene expression (110), although there were some gene expression changes induced by leptin in lean control animals.

Further studies of possible central mechanisms of leptin action are needed, but the hope for a quick ‘obesity fix’ is unlikely to be realized by the administration of leptin to most obese persons.

**CONCLUSIONS**

**Is It Time to Enhance Investigation of the Means to Prevent Obesity?**

Sufficient data currently exist from studies of animal models to demonstrate unequivocally the extraordinary power of obesity prevention to permanently alter the trajectory of a wide range of diseases, including but not limited to heart disease, type 2 diabetes, hypertension, cancer, and other aging-associated chronic diseases.

Public health approaches have to date proven to be too weak in efficacy to provide for strong positive cost–benefit conclusions related to widespread obesity treatment. Behavioral modification has had a small measure of success in a very limited number of humans, and the likelihood of strengthening these behavioral or environmental manipulations to a level sufficient to impact overall incidence of obesity is low. Biomedical approaches may, in the future, offer the potential power to alter the obesity trajectory and to change the negative health consequences of this extraordinarily prevalent disease.

Non-human primates in the study of obesity have offered numerous pointers to the human condition, some of which have been reviewed here. Together these findings offer an optimistic view of the future benefits of obesity mitigating efforts.
Figure 14.10 An insulin sensitizer of the thiazolidinedione class (R-102380) administered to four monkeys for more than 6 weeks induced a significant increase in the change in glycogen synthase activity induced by insulin (before and during a euglycemic hyperinsulinemic clamp). Both the glycogen synthase independent activity and the fractional activity of glycogen synthase were significantly increased (107)

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