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# How Obesity Develops

*Insights from the New Biology*

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**Molecular and genetic studies of animal models have identified numerous genes that may cause or contribute to the development of obesity. They have also provided significant insight into the peripheral and central pathways that control energy intake and expenditure. Genetic studies of families and populations have generated useful information on genes and mutations associated with or linked to obesity, body fat distribution, and other relevant phenotypes. This information, combined with knowledge of the chromosomal location of genes identified from animal studies, has made it possible to identify specific mutations that contribute to the development of obesity in humans.**

**Key Words:** Human genome; transgenic mice; heritability; genetic predisposition; neuropeptides; leptin; melanocortin receptors.

## Introduction

The data from countries of the industrialized world, and even those from the Third World, reveal that a growing proportion of children and adults is overweight or frankly obese *(1)*. The prevalence of overweight and obesity varies according to age, gender, race, and socioeconomic class across the Western and developing world. About 50% of adults have a body mass index (BMI) of 25 kg/m$^2$ or greater in the United States, Canada, and some of the Western European countries. In some population subgroups in the United States and elsewhere, the prevalence of those with a BMI of 25 kg/m$^2$ and greater is more than 70% *(1)*. Moreover, there has been a dramatic increase in the prevalence of obesity in the twentieth century, and all indications are that the problem will get even worse in the coming decades. For instance, it is striking to see that the body mass of men 1.70 m tall recruited in the US military service increased from 66.8 kg in 1863 to 76.3 kg over a century, an increase of about 10 kg for the same stature *(2)*. Based on a recent World Health Organization report, Seidell *(3)* has estimated that there are currently about 250,000,000 obese adults (7% of the population) and at least 500,000,000 overweight (BMI from 25 to 29.9) people worldwide *(3)*. These estimates are thought to be conservative, and the prevalence of both conditions is on the rise worldwide.

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## Etiology of Overweight and Obesity

Body weight is a function of energy and nutrient balance over an extended period of time. Energy balance is determined by macronutrient intake, energy expenditure, and energy or nutrient partitioning. Positive energy balance over weeks and months will result in weight gain whereas negative energy balance will have the opposite effect.

Because positive energy balance is required for weight gain to occur, dietary habits play a key role in the prevalence of overweight and obesity *(4)*. In developed countries, the availability of highly palatable foods in almost unlimited abundance undoubtedly contributes to the epidemic, because some of the affected individuals eat many times a day and consume large portions *(5,6)*. The proportion of calories derived from fats is also potentially involved, particularly in those who consume a high-fat diet while living a sedentary life *(7,8)*, although the exact contribution of a high-fat diet to the current obesity epidemic remains controversial *(9,10)*.

The increase in the prevalence of overweight and obesity cases worldwide is occurring against a background of a progressive reduction in the energy expended for work and occupational activities as well as for the accomplishment of personal chores and daily necessities *(11–13)*. The reduction in energy expenditure associated with physical activity brought about by automation and changing job and professional environmental circumstances has been nothing but dramatic in the second half of the twentieth century. By contrast, the energy expenditure of leisure time physical activity, the most important discretionary component of

total daily energy expenditure, may have increased slightly but not enough to keep pace with the changes brought about by urbanization and automation.

Progress has been slower in the area of energy or nutrient partitioning probably because it was not perceived until recently as an important determinant of long-term energy balance in humans *(2,14)*. However, many physiologic and metabolic studies on the modulation of nutrient storage into fat and protein in various organs and tissues have been conducted in the field of animal husbandry. This research has strong implications for understanding the phenotype of energy partitioning in humans. For instance, these studies indicate that insulin, steroid, thyroid and growth hormones, and various growth factors all influence the fate of ingested energy. Hepatic and skeletal muscle metabolism as well as lipoprotein lipase activity in adipose tissue and skeletal muscle play an important role. The composition of ingested food, including the amino acid composition of the proteins, needs to be considered as well. This line of research suggests that being a "fat storer" as opposed to a "lean tissue storer" is a risk factor for obesity.

Table 1 provides an overview of the commonly recognized correlates of overweight and obesity or of body weight gain over time *(15–17)*. Some of these correlates are true predictors of body fat gain and can be defined as risk factors for overweight or obesity. In most cases, however, the associations are secondary and have arisen as a result of an obese state.

## Evidence for a Genetic Predispositionfor Obesity

It is well established that obesity runs in families. However, except for some rare Mendelian disorders, the vast majority of obese patients do not exhibit a clear pattern of Mendelian inheritance. Despite the large number of studies on the familial aggregation and heritability of the obesity phenotypes, there is no unanimity among researchers regarding the importance of genetic factors. A more complete review of these factors can be found elsewhere *(18–20)*, but a brief summary of the main findings is presented here.

In 1923, Davenport *(21)* described the first comprehensive attempt to understand the role of inheritance in human body mass for stature. Among other findings, he found that normal weight parents sometimes have obese adult offspring. He also observed the converse: obese parents frequently have normal weight adult descendants. In the aggregaate, his study demonstrated quite convincingly that BMI values were more similar among family members than among unrelated persons.

## Heritability Levels

The level of heritability has been considered in a large number of twin, adoption and family studies. The level of heritability is simply the fraction of the population variation in a trait (e.g., BMI) that can be explained by genetic transmission. Results obtained by a good number of investigators indicate that the heritability level estimates depend on how the study was conducted and on the types of relatives on whom they are based (Table 2). For instance, studies conducted with identical twins and fraternal twins or identical twins reared apart have yielded the highest heritability levels, with values clustering around 70% of the variation in BMI.

By contrast, adoption studies have generated the lowest heritability estimates: about 30% or less. Family studies have generally found levels of heritability intermediate between the twin and the adoption study reports. A few investigations have included all or most of these kinds of relatives in the same analysis. By implementing analytical techniques developed to use all the information and maximum likelihood procedures, these studies have concluded that the true heritability estimate for BMI in large sample sizes was between 25 and 40%. Recent surveys undertaken with the collaboration of severely obese and morbidly obese subjects together with information obtained on their parents, siblings, and spouses suggest that the genetic contribution to obesity may indeed be about 25–40% of the individual differences in BMI *(20)*.

## Familial Risk of Obesity

The risk of becoming obese when a first-degree relative is overweight or obese can be quantified using a statistic called the lambda coefficient ($\lambda$), which is defined by the ratio of the risk of being obese when a biological relative is obese compared with the risk in the population at large, i.e., the prevalence of obesity *(22)*. Estimates of $\lambda$ for obesity based on BMI data were recently reported *(23–25)*. Age and gender standardized risk ratios obtained from 2349 first-degree relatives of 840 obese probands and 5851 participants in the National Health and Nutrition Examination Survey III revealed that the prevalence of obesity (BMI $\geq$ 30) is twice as high in families of obese individuals than in the population at large *(24)*. Moreover, the risk increases with the severity of obesity in the proband. Thus, the risk of extreme obesity (BMI $\geq$ 45) is about eight times higher in families of extremely obese subjects. More recently, using data from 15,245 participants ages 7 to 69 yr from the 1981 Canada Fitness Survey, it was shown that the familial risk of obesity was five times higher for relatives in the upper 1% distribution of BMI than in the general Canadian population *(25)*. However, the latter study suggested that the familial risk is not owing entirely to genetic factors.

## Genotype-Environment Interactions

Genotype-environment interaction ($G \times E$) arises when the response of a phenotype to environmental changes depends on the genotype of the individual. Although it is well known that there are interindividual differences in the responses to various dietary interventions, few attempts have been made to test whether these differ-

**Table 1**
Correlates of Overweight and Obesity or of Body Weight and Body Fat Gain Over Time

| Variable | Comment |
|---|---|
| Age | Childhood obesity is a risk factor for adulthood obesity.<br>Body fat content increases during adulthood.<br>Maximal rates of overweight and obesity attained from 55 to 65 yr. |
| Sex | Women have more body fat.<br>Sex differences in prevalence of obesity vary in populations or among ethnic groups. |
| SES | More obese in high SES classes and in poor countries.<br>More obese in low SES classes and in rich countries. |
| Energy intake | Overfeeding causes weight gain and leads to obesity. |
| Dietary fat intake | Dietary fat is related to prevalence of overweight in ecologic studies.<br>High-fat diet causes weight gain.<br>Low-fat diet reduces body weight. |
| RMR | A low body mass and composition adjusted RMR is a risk factor for weight gain, but data are contradictory .<br>Overweight and obese people have a higher absolute RMR. |
| Thermic response to food | Obese people have a depressed response in some studies but contradictory results are abundant. |
| Physical activity level | A low level of PA is a risk factor for weight gain.<br>Level of sedentarism is higher in obese people.<br>Regular PA changes body composition.<br>High levels of PA increase SNS activity and RMR.<br>Regular PA contributes to weight loss and weight maintenance. |
| Lipid oxidation rate | Body fat gains decrease RQ.<br>A high RQ is a risk factor for weight gain but there are contradictory results. |
| - | Ex-obese have a higher RQ than those never obese. |
| Blood leptin level | Low leptin levels are weakly related to weight gain but results are contradictory.<br>Most obese have high leptin levels. |
| SNS activity | Low SNS activity could be a risk factor for weight gain.<br>SNS activity increases with overfeeding and body weight gain. |
| GH level | Low GH is a risk factor for weight gain.<br>Most obese have low GH levels. |
| Insulin sensitivity | Obese are often insulin resistant and hyperinsulinemic.<br>Insulin resistance protects against weight gain but results are contradictory. |
| HPA axis and cortisol levels | Obese generally have a hyperresponsive and hyperactive HPA axis.<br>Obese have elevated cortisol production rates but also accelerated degradation. |
| Sex steroid levels | Obese men often have low androgen levels.<br>Obese women often have high androgen levels with further elevation on ACTH stimulation. |
| Adipose tissue metabolism | Catecholamine-induced lipolysis is reduced in obesity.<br>Lipogenesis from glucose is increased in human fat cells from obese people.<br>Adipose tissue LPL is increased in obesity.<br>Elevated adipose LPL activity remains high in the reduced obese.<br>High adipose tissue LPL is a risk factor for weight gain. |
| Skeletal muscle metabolism | SM type I fiber type proportion is not affected by obesity.<br>SM type IIb fiber type proportion is often elevated in obesity.<br>SM oxidative enzyme markers are inversely related to obesity.<br>SM LPL activity is low in obesity. |
| Energy and nutrient partitioning | Under positive energy balance conditions, some people channel more food carbons into proteins than lipids.<br>High rates of lipid accretion could be a risk for further weight gain. |
| Smoking | Smoking is associated with a lower body weight.<br>Cessation increases body weight in most people. |

SES, socioeconomic status; RMR, resting metabolic rate; PA, physical activity; SNS, sympathetic nervous system; RQ, respiratory quotient; GH, growth hormone; HPA, hypothalamic-pituitary-adrenal; ACTH, adrenocorticotropic hormone; LPL, lipoprotein lipase; SM, skeletal muscle.

Reproduced from ref. *17*.

**Table 2**
Overview of Genetic Epidemiology of Human Body Fat/Obesity[a]

| | Heritability/ transmission | Maternal/ paternal | Familial environment |
|---|---|---|---|
| Nuclear families | 30–50 | No | Minor |
| Adoption studies | 10–30 | Mixed results | Minor |
| Twin studies | 50–80 | No | No |
| Combined strategies | 25–40 | No | Minor |

[a]Data are based on the trends in about 50 different studies. In most of these studies, the BMI was the phenotype considered. In some cases, skinfolds or estimates of percentage body fat or fat mass were used. From ref. *18*.

ences are genotype dependent, particularly for obesity-related phenotypes.

Some individuals are prone to excessive accumulation of fat, whereas others seem relatively well protected against such a menace. The results from a series of experiments performed with monozygotic twins revealed that the response to a positive or negative energy balance treatment is very heterogeneous among twin pairs but quite homogeneous within members of the same pair.

Twelve pairs of male monozygotic twins ate a 4.2 MJ (1000 kcal) per day caloric surplus, 6 d a week, over a period of 100 d *(26)* for a total caloric surplus of 353 MJ (84,000 kcal) over the energy cost of weight maintenance. Significant increases in body weight and fat mass were observed after the period of overfeeding. Data showed that there were considerable interindividual differences in the adaptation to excess calories and that the variation observed was not randomly distributed, as indicated by the significant within-pair resemblance in response. For instance, there was at least three times more variance in response between pairs than in response within pairs for the gains in body weight, sum of skinfolds, fat mass, and fat-free mass (Fig. 1, left). These data demonstrate that some individuals are more at risk than others to gain fat when energy intake surplus is clamped at the same level for everyone and when all subjects are confined to a sedentary lifestyle. The within–identical twin pair response to the standardized caloric surplus suggests that the amount of fat stored is likely influenced by the genotype.

Seven pairs of young adult male identical twins completed a negative energy balance protocol during which they exercised on cycle ergometers twice a day, 9 of 10 d, over a period of 93 d, while being kept on a constant daily energy and nutrient intake *(27)*. The mean total energy deficit caused by exercise above the estimated energy cost of body weight maintenance reached 244 MJ (58,000 kcal). Baseline energy intake was estimated over a period of 17 d preceding the negative energy balance protocol. Mean body weight loss was 5.0 kg, and it was entirely accounted for by the loss of fat mass. Intrapair resemblance was observed for changes in body weight (Fig. 1, right), fat mass, percentage of fat, and sum of skinfolds. Even though there were large individual differences in response to the negative energy balance and exercise protocol, subjects with the same genotype were more alike in responses than subjects with different genotypes. These results are remarkably similar to those for body mass and body fat gains in 12 pairs of twins subjected to the 100-d overfeeding protocol.

## Human Single Gene Defects

This section focuses on single gene mutations known to cause obesity. In such cases, and there are 30 cases reported to date in the literature, obesity is the dominant feature of the ensuing syndrome. In addition, there are other syndromes for which obesity is a clinical manifestation but not a dominant feature. The genes responsible for the latter syndromes have not been identified yet. This section focuses on the cases for which the specific genetic defects have been identified. These human single gene obesity cases reported to date are listed in Table 3 *(28,29)* and are briefly described here. These cases are quite informative because they reveal pathways and mechanisms that may lead to positive energy balance and body fat accretion when disrupted.

In the LEPR gene, a G to A base substitution in exon 16 was found in three severely obese individuals who also showed pituitary dysfunction *(30)*. Mutations in the proopiomelanocortin (POMC) gene were found in two patients with severe early onset obesity, adrenal insufficiency, and red hair pigmentation *(31)*. One was a compound heterozygote for two mutations in exon 3, a G to T change at nucleotide (nt) 7013, causing a premature termination at codon 79 and a 1-bp deletion at nt 7133, which also caused a premature termination, but at codon 131. The other subject was homozygous for a C to A transversion at nt 3804 in exon 2. Mild obesity along with combined hypothyroid and hyperthyroid symptoms was reported in a heterozygous patient with a point mutation in exon 10 of the THRB gene, which produced a stop codon with a 28 amino acid deletion and abolished T3 binding *(32)*.

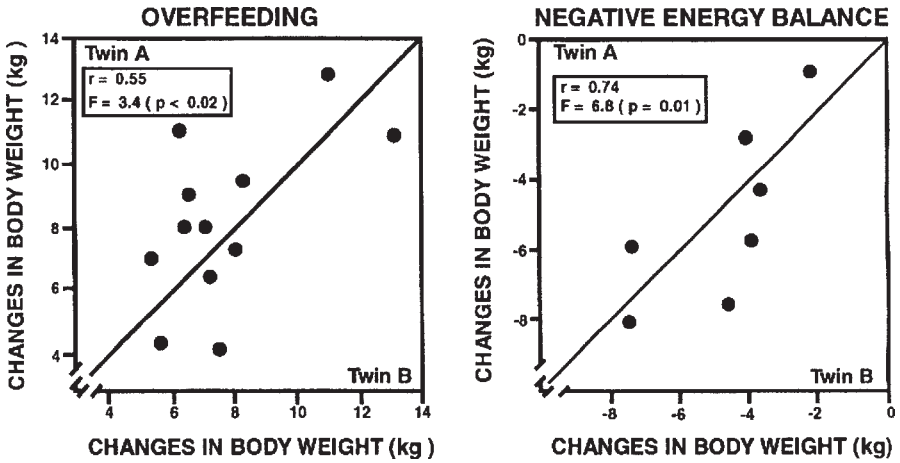


**Fig. 1.** Intrapair resemblance in the response of identical twins to long-term changes in energy balance. **(Left)** Twelve pairs of identical twins were submitted to an 84,000-kcal energy intake surplus over 100 d. **(Right)** Seven pairs were subjected to a negative energy balance protocol caused by exercise. The energy deficit was 58,000 kcal over 93 d. Reproduced from refs. *26* and *27*.

Four markedly obese individuals have been shown to be carriers of a missense mutation in the PPARG2 gene *(33)*. This Pro to Gln conversion at position 115 was associated with a higher BMI compared with an obese control population without the mutation. One case of an impaired prohormone processing associated with a G to A substitution at codon 483 in the PCSK1 gene was also reported *(34)*. One of the earliest identified mutations causing obesity was reported by Montague et al. *(35)* in the leptin gene. Two severely obese children with a mutation in codon 133 (G398Δ) were then identified. Subsequently, three individuals with a mutation (C105T) in exon 3 of the LEP gene were reported *(36)*. Further investigation of the same family revealed an additional female member carrying the same mutation *(37)*.

Two different groups reported on nine patients who were found to have mutations in the MC4R gene associated with a dominant form of obesity *(38,39)*. Two heterozygous males shared a CTCT deletion at codon 211 that resulted in a missing leucine and a premature stop codon. Seven other subjects had a GATT insertion at nt 732 resulting in the expression of a nonfunctional truncated receptor. More recently, Hinney et al. *(40)* described six female obese subjects with mutations in the MC4R gene, two with the already identified CTCT deletion at codon 211, and the others (two probands and their respective mothers) with a novel mutation at position 35 leading to a premature stop codon generating a truncated protein product. Seven missense mutations in MC4R of unknown significance in seven other extremely obese subjects (BMI > 99th percentile) were also described *(40)*.

In summary, 11 mutations have been identified in seven different genes. They account for 30 obesity cases. This is admittedly only a very small fraction of the obese population. However, it can be predicted that thousands, and perhaps more, of these cases will be proven to be caused by the same mutations or others in the same or different genes.

## Scanning the Whole Human Genome

Several other strategies have been used in the efforts to identify the genes and mutations responsible for the predisposition to obesity. Among these is a whole series of studies on candidate genes. These studies have been reviewed elsewhere *(20,29)* and are not discussed here. In general, however, the results of these observational studies have been disappointing, with small sample size, small effect size, and lack of replication studies or failure to replicate being the most common problems.

Another approach has been to perform genomewide scans with a view to identifying chromosomal regions of interest. These studies have typically been performed with pairs of siblings, sometimes whole nuclear families or pedigrees, and are based on about 300 microsatellite markers or more. The results of four such genomic scan studies are briefly summarized here.

In a genomic scan with 600 markers obtained on 277 Pima Indian siblings, linkages were observed on chromosomes 3 and 11 with percentage of body fat *(41)*. The Pima Indian cohort was further investigated with a more extensive panel of genetic markers and of obesity-related phenotypes. Additional linkages were then observed on 11q with BMI *(42)* and at 11q23-q24 with 24-h energy expenditure *(43)*. The same study provided evidence for linkage at 18q21 with percentage of body fat and at 20q13 with 24-h respiratory quotient. In a second scan, 169 markers were analyzed among 458 subjects from 10 Mexican-American pedigrees for fat mass and leptin levels. Positive linkages were found with markers on chromosome 2p *(44)*.

Another study was conducted on a French cohort of 514 subjects (264 sib-pairs) from 158 nuclear families. Multipoint linkage analysis using affected sib-pairs revealed significant evidence of linkage with obesity on chromosome 10p12 *(45)*. Finally, a fourth study was based on a mixed sample of Caucasian and African-American subjects from 124 nuclear families *(46)*. The investigators

**Table 3**
Cases of Human Obesity Caused by Single-Gene Mutations[a]

| Gene | Location | Mutation | Case no. | Sex | Age (yr) | Weight (kg) | BMI (kg/m$^2$) | References |
|---|---|---|---|---|---|---|---|---|
| LEPR | 1p31 | G→A (exon 16) | 1 | F | 19 | 166 | 65.5 | *30* |
| | | | 2 | F | 13 | 159 | 71.5 | |
| | | | 3 | F | 19 | 133 | 52.5 | |
| POMC | 2p23 | G7013T and C deletion at nt 7133 exon 3 | 4 | F | 3 | 30 | NA | *31* |
| | | C3804A exon 2 | 5 | M | 7 | 50 | NA | |
| THRB | 3p24.1-p22 | Cys434Stop C→A exon 10 | 6 | F | 15 | 46 | 26.3 | *32* |
| PPARG | 3p25 | Pro115Gln | 7 | M | 65 | NA | 47.3 | *33* |
| | | | 8 | F | 32 | NA | 38.5 | |
| | | | 9 | M | 54 | NA | 43.8 | |
| | | | 10 | M | 74 | NA | 37.9 | |
| PCSK1 | 5q15-q21 | Gly483Arg | 11 | F | 3 | 36 | NA | *34* |
| LEP | 7q31 | G398Δ (codon 133) | 12 | F | 8 | 86 | 45.8 | *35* |
| | | | 13 | M | 2 | 29 | 36.6 | |
| | | C→T (codon 105) (exon 3) | 14 | F | 6 | NA | 32.5 | *36* |
| | | | 15 | M | 22 | NA | 55.8 | |
| | | | 16 | F | 34 | NA | 46.9 | |
| | | | 17 | F | 30 | 130 | 54.9 | *37* |
| MC4R | 18q21.3 | ΔCTCT nt 631–634 (codon 211) | 18 | M | 4 | 32 | 28 | *38* |
| | | | 19 | M | 30 | 139 | 41 | |
| | | | 20 | F | 20 | NA | 42.1 | *40* |
| | | | 21 | F | 43 | NA | 37.6 | |
| | | GATT insertion at nt 732 (codon 246) | 22 | F | 58 | NA | 51 | *39* |
| | | | 23 | F | 35 | NA | 57 | |
| | | | 24 | F | 34 | NA | 50 | |
| | | | 25 | M | 24 | NA | 33 | |
| | | | 26 | F | 11 | NA | 30 | |
| | | C105A (Tyr35X) | 27 | F | 10 | NA | 31.3 | *40* |
| | | | 28 | F | 17 | NA | 45.9 | |
| | | | 29 | F | 31 | NA | 48.2 | |
| | | | 30 | F | 36 | NA | 38.6 | |

[a]NA, not available.
Adapted from ref. *29*.

found significant evidence of linkage between obesity (BMI ≥ 30 kg/m$^2$) and markers located on chromosome 20q13.

## Central Mechanisms Regulating Energy Balance

The application of molecular approaches and transgenic technologies to rodent models has provided considerable new insight into the central mechanisms through which food intake and energy expenditure are regulated. The importance of the hypothalamus, in particular the ventromedial hypothalamus, the lateral hypothalamus, and the paraventricular nucleus, has been recognized for many years, but only recently have we begun to understand the neurochemical mechanisms through which these and other nuclei regulate food intake, autonomic activity, and energy expenditure *(47,48)*.

The identification of neuropeptide Y (NPY) as a potent orexigenic agent that will produce an obesity identical to the ventromedial hypothalamus lesion obesity provided the first insight into the neurochemical basis of obesity. NPY is synthesized in neurons in the arcuate and released from nerve terminals in the para- and periventricular regions to increase food intake. Peptide and mRNA levels peak before normal feeding; are reduced after feeding; are elevated by

conditions (e.g., diabetes), that increase food intake; and are suppressed in response to intracerebroventricular insulin, which attenuates feeding. Although such data support a role for NPY in feeding behavior, the absence of any effects of an NPY gene knockout on the feeding behavior of mice was a surprise *(49)*.

At least five receptor subtypes for NPY have now been cloned *(50)*. By the use of selective antagonists and agonists and antisense pharmacology, it is now evident that the NPY feeding effect is modulated through NPY5 receptors, at least in part, although other evidence suggests a role for Y1 receptors also *(51,52)*. This again contrasts with the absence of any phenotype associated with knockout of the NPY Y5 receptor *(53)*. Indeed, in contrast to expectations, the NPY Y1 receptor knockout mouse develops obesity and insulin resistance *(54)*. However, it seems likely that congenital knockouts that affect systems essential to life may, in some cases, be overcome by the plasticity of neural systems. Nevertheless, this plasticity is not evident for other neuropeptide systems, e.g., MC4 receptors, and melanin-concentrating hormone (MCH).

A major impetus to increase knowledge of the neuropeptidergic control of feeding came from the identification of the agouti protein *(55)*. When secreted ubiquitously in the congenital obesity of yellow $A^y$/a mice, it promotes hyperphagia and obesity through central actions. Transgenic overexpression of agouti linked to the β-actin promoter was used for proof of function *(56)*. These developments were important for two reasons: first, they led to the identification of the melanocortin receptor family and proved the importance of α-MSH as an antiobesity peptide; second, they led to identification of an agouti-analog (agouti-related protein [AGRP]) that is normally synthesized and secreted in the brain. This information has led to our current understanding of the role of α-MSH and the melanocortin MC4 and MC3 receptors as a major inhibitory pathway for feeding *(57,58)*. Both agouti protein and AGRP promote feeding through inhibition of the actions of α-MSH at MC4 receptors *(58,59)*. Agonists of the MC4 receptor inhibit feeding. Mice lacking the POMC gene, which codes for α-MSH and a number of other peptides, become obese *(60)*. This obesity was reversed by treatment with a stable α-MSH agonist. Transgenic mice ectopically expressing the agouti gene *(56)* or with a knockout of the MC4 receptor *(61)* have a similar body weight phenotype to that of the yellow $A^y$/a mouse. A search of an expressed sequence transcript database led to the cloning of AGRP gene *(62)*, which has subsequently been shown to be expressed in the arcuate nucleus of mouse hypothalami and to act as an inhibitor of α-MSH at the MC4 receptor *(57,59)*.

Two additional genes associated with changes in coat color and obesity have been identified: *mahogany* (*mg*) and *mahogonoid* (*md*). Both mutations suppress the effects of agouti ($A^Y$) on coat color and obesity and appear to act on the agouti signaling pathway. Mahogony encodes a transmembrane form of attractin *(63)*, a circulating molecule produced by activated T-cells that is implicated in immune function but that is also widely distributed in the brain. It appears to act downstream of agouti expression but upstream of the melanocortin receptors because mahogony fails to suppress the obese phenotype of MC4 receptor knockout mice or those associated with leptin signaling deficiency, tub, or $Cpe^{fat}$ genes *(64)*. It may also have effects independent of agouti *(65)*.

Cocaine and amphetamine-regulated transcript (CART) is a powerful inhibitor of feeding behavior *(66,67)* and one of the few peptides that will overcome the orexigenic effects of NPY. Immunohistochemical and neuronal tracing techniques have shown that AGRP and NPY are co-expressed in the same neurons within the arcuate nucleus, whereas CART and POMC are co-expressed in a separate population *(68)*. Both sets of neurons have been shown to express mRNA for the long form of the leptin receptor, implying regulation by leptin.

The identification of neuropeptides that modulate the effects of the lateral hypothalamus, a region known to enhance feeding after stimulation and cause anorexia after destructive lesions, was unclear until recently. Using a differential display technique, Qu et al. *(69)* identified MCH and showed that this would induce feeding when administered centrally. Its physiologic role in feeding is suggested by observations that MCH mRNA is increased by fasting as well as by leptin deficiency and by the lean phenotype of MCH knockout mice *(70)*. A second family of peptides, the orexins *(71)* or hypocretins *(72)*, were identified independently by two groups. Orexin A stimulates feeding, and expression of prepro-orexin mRNA is also enhanced in the fasted mouse. However, it is not clear at this stage whether orexin has a primary role in regulating feeding. Rather, its effects on this behavior, which are not as robust as the other neuropeptides, might be mediated by its enhancement of arousal. The recent demonstration that mutations in the Orexin 2 receptor, the subtype associated with feeding, resulted in narcolepsy and sleep would seem to support this argument *(73,74)*.

Neuronal tracing techniques combined with *in situ* hybridization and immunohistochemical approaches have allowed considerable new insight into the local networks that prompt feeding. These studies have shown that both subtypes of neurons in the arcuate not only innervate the paraventricular nucleus (PVN) but also send axonal connections to the lateral hypothalamus (LH) to synapse on MCH containing neuronal cell bodies. Conversely, MCH neurons send axonal projections not only to the PVN but also to the brain stem regions known to affect feeding *(75)*.

## Peripheral Mechanisms Regulating Energy Balance

The results of numerous physiologic experiments suggest that there are systems that defend body weight or body fat in the long term and that much of this defense is through compensatory changes in food intake. These data have been reviewed recently by Caterson et al. *(76)*. However, it was the classical parabiotic studies *(77,78)* that provided the strongest evidence for a circulating factor that regulated food intake in relation to body energy stores. Schwartz et al. *(79)* suggested that insulin had this role, because serum insulin increased with body fat and insulin given centrally does inhibit food intake through a downregulation of the NPY system. Zhang et al. *(80)* used a positional cloning technique to identify leptin as the gene mutated in the ob/ob mouse. When these mice were injected with recombinant leptin either peripherally or centrally, the obesity, hyperphagia, diabetes, and infertility were all reversed *(81–83)*.

The similarity of the phenotypes of obese *ob/ob* and diabetes *db/db* mice when placed on the same background strain suggested that the mutation in the *db/db* mouse was associated with the leptin signaling system. This gene codes for the long form of the leptin receptor; the mutation in the *db/db* mouse results in a receptor that lacks the major part of the intracellular domain responsible for the signaling properties *(84)*. Not surprisingly, exogenous leptin does not reverse the obesity of the *db/db* mouse. Subsequently, a mutation in the extracellular ligand-binding domain of the leptin receptor was shown to be responsible for the hyperphagia and obesity of the *fa/fa rat (85)*. This rat remains partially sensitive to leptin.

There have been considerable advances in our understanding of the pathways through which leptin modulates feeding behavior since its identification and availability for physiologic experiments. Long-form leptin receptors (Rb) are localized in the hypothalamus in regions associated with feeding, including the arcuate and paraventricular nuclei *(86)*. Leptin reduces NPY gene expression *(87)* and the response to NPY *(88)*, but this is not its sole mode of action because NPY gene knockout only attenuates but does not abolish the effects of the *ob* mutation *(89)*. Indeed, it now appears that leptin may affect several of the major neuropeptides that affect feeding, including activation of corticotropin-releasing hormone, CART, and POMC expression, and inhibition of AGRP expression *(62,90,91)* (Fig. 2). All these effects would lead to a reduction in food intake.

Although the leptin signaling system has the potential for preventing obesity, it clearly lacks effectiveness in all forms of obesity, because leptin secretion increases as the adipose mass expands. This implies some form of leptin resistance. There may be multiple sites for expression of this resistance. We have shown that OM rats that become obese on a high-fat diet enhance their leptin secretion and remain sensitive to leptin administered centrally, suggesting that resistance is associated with an impaired access of leptin to the site of action *(92)*. Similar results have been reported in mice *(93)*. Recent experimental data have been provided to suggest that attenuated blood-brain barrier transport of leptin is associated with obesity *(94)*. Alternatively, leptin resistance might arise from inhibition of the signaling systems responsive to the activated leptin receptor, and the report that leptin induces the expression of SOCS-3 gene, a cytokine inhibitor protein, supports this suggestion *(95)*. Leptin resistance does not appear to be in the cytokine JAK-STAT signaling pathway, because activation of this pathway with ciliary neurotropic factor corrects the obesity of *ob/ob*, *db/db*, and dietary obese mice *(96)*.

Leptin may be an important peripheral feedback signal of energy stores on feeding behavior and energy expenditure, but other peripheral signals may also be important. The gastrointestinal tissues are important endocrine and paracrine organs. Communication from the gut to the brain is either neural, particularly through the afferent vagus, or endocrine in nature. Alterations in the activity of these systems may also have effects on energy balance. Cholecystokinin (CCK) released in response to dietary intake of protein and fat is well recognized for its acute effects to slow stomach emptying and inhibit food intake of a single meal. These responses are modulated through CCK-A subtype receptors in the afferent vagal system. The absence of CCK-A receptors, as in the OLETF rat, results in a moderate increase in food intake and moderate obesity *(97)* and their failure to compensate for fat but not carbohydrate calories ingested *(98)*. Null mutations of the GLP-1 receptor gene have been less informative. While GLP-1 will inhibit food intake in both animals and humans *(99)*, knockout mice have normal feeding behavior and do not become obese although they are glucose intolerant *(100)*.

Bombesin (BBS) is a satiety-inducing peptide found in amphibians that also causes hypothermia and changes in locomotor activity when injected into rodents. Its counterpart in mammalian systems appears to be related to a family of peptides and receptors that include gastrin-releasing peptide (GRP) acting on the GRP-preferring receptor, neuromedin B acting on the NMB receptor, and the bombesin receptor subtype 3 for which no ligand has yet been identified. Studies with transgenic knockout models suggest that the NMB-R has a role in thermoregulation *(101)* and that the GRPR might modulate activity and feeding *(102,103)*. The BBS-3 null mutation mouse also shows an enhanced response to palatable and aversive taste stimuli *(104)*. These data imply that both the GRPR and BBS-3 receptors are important signaling systems for the maintenance of normal energy balance. However, although neither of these knockouts has an obesity phenotype, the BBS-3 receptor knockout mouse does develop mild obesity associated with a decrease in metabolic rate, late onset hyperphagia, hypertension, and impaired glucose metabolism *(105)*. These studies strongly emphasize the importance of

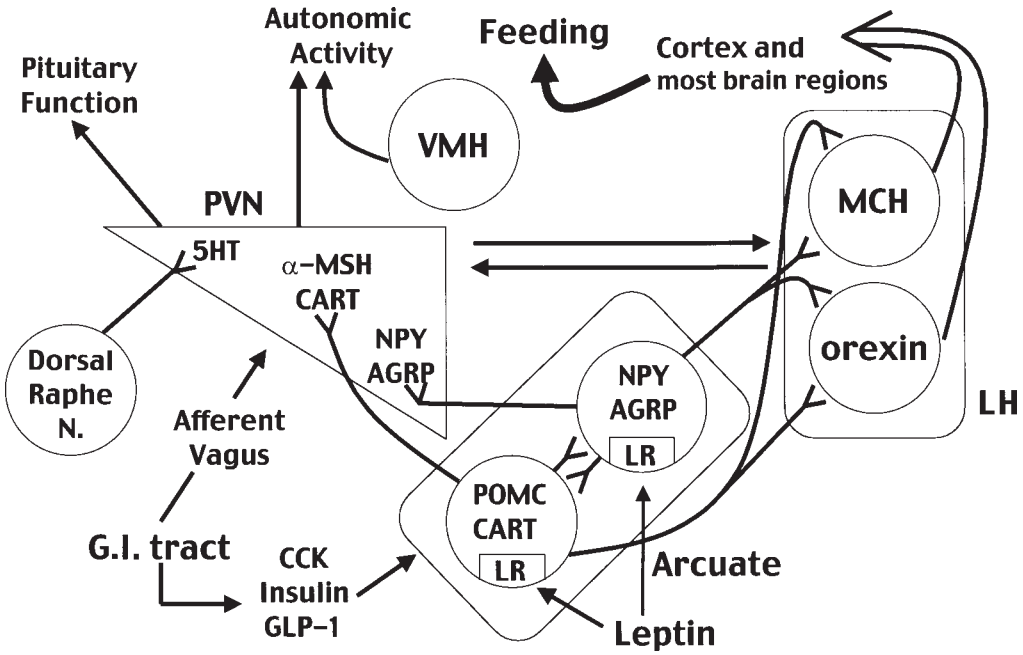


**Fig. 2.** Central pathways contributing toward the regulation of energy intake and energy expenditure. Molecular and genetic techniques have enabled the identification of many of the neuropeptides and receptor systems involved.

peripheral signals in modulating feeding behavior and energy balance (Table 4).

Molecular and transgenic approaches have also been used to provide insight into the peripheral $\beta_3$-adrenergic system that modulates feeding. The reciprocal regulation of the sympathetic nervous drive to brown adipose tissue (BAT) and food intake has been well documented. By selectively reexpressing $\beta_3$-adrenergic receptors in $\beta_3$-adrenergic receptor knockout mice, Grujic et al. *(106)* were able to show that this $\beta_3$-adrenergic inhibition of food intake was modulated through white adipose tissue (WAT) rather than BAT.

The ability to regulate body composition through modulation of energy expenditure has been demonstrated in numerous animal models. These studies received their impetus from the understanding of the thermogenic role of the uncoupling protein UCP1 in BAT mitochondria. Overexpression of UCP1 produced a lean phenotype and a mouse resistant to obesity *(107)*. Destruction of BAT with a diphtheria toxin-A construct led to obesity *(108)* but knockout of the UCP1 gene did not, although it made the mice cold sensitive *(109)*. The explanation for this is still unclear. However, targeted disruption of the R11β subunit of protein kinase A (PKA) resulted in a lean phenotype *(110)*. This unexpected observation results from the compensatory increase in the R1α subunit that leads to increased basal PKA activity despite decreased total PKA activity. These changes were associated with a 35% increase in basal lipolysis in WAT and increased UCP1 levels in BAT.

A family of uncoupling proteins showing significant homology to UCP1 that are expressed in a variety of tissues has led to speculation of alternative pathways for adaptive thermogenesis. This still remains a controversial area, but the observations that UCP2 and UCP3 are regulated quite differently from UCP1 and that their expression may increase with food restriction *(111,112)* does not support a role in adaptive thermogenesis that would regulate weight gain. However, UCP2 expression may be related to fat metabolism in WAT *(113)* and UCP3 may, in part, modulate the thermogenic response to thyroid hormones *(114)*. By contrast, the glycerophosphate cycle, a cytosolic-mitochondrial cycle that transfers reducing equivalents from NADH to FADH to waste energy, does appear to be a very effective substrate cycle that limits weight gain and restricts the development of obesity in response to dietary or hypothalamic perturbations *(115)*.

Much of our understanding of adipocyte proliferation, differentiation, and maturation has come from studies using preadipocyte cell lines in culture. Information from this research has facilitated the use of targeted transgenic technology to inhibit the activity of essential transcription factors in adipose tissue. The mice that develop are either completely devoid of adipose tissue *(116)* or severely lipodystrophic *(117)*. Moitra et al. *(116)* used the aP2 promoter to overexpress a gene (A-ZIP/F-1) that prevents binding of C/EBP and Jun family transcription factors, whereas Shimomura et al. *(117)* overexpressed the sterol regulatory element binding protein gene (nSREBP-1c). In both situations, diabetes and severe insulin resistance were apparent, indicating the importance of WAT to glucose homeostasis, and providing an excellent model for human lipodystrophies.

**Table 4**
Examples of Manipulations That Target Genes Affecting Energy Balance[a]

| Gene | Transgenic expression | Phenotype | References |
|---|---|---|---|
| Centrally expressed genes | | | |
| NPY | KO | Attenuates obesity of ob/ob | *49, 88* |
| NPY Y5 receptor | KO | None | *53* |
| NPY Y1 receptor | KO | Moderate obesity/no hyperphagia | *54* |
| Agouti | Overexpression | Obesity | *56* |
| AGRP | Overexpression | Obesity/diabetes | *58* |
| POMC | KO | Obesity | *60* |
| MC4 receptor | KO | Obesity | *61* |
| MCH | KO | Lean | *70* |
| GLP-1 | KO | Glucose intolerance | *100* |
| Neuromedin B | KO | Reduced hypothermic response to NMB | *101* |
| GRP receptor | KO | No feeding response to bombesin/increased activity | *102, 103* |
| BBS-3 receptor | KO | Increased taste responses/mild obesity | *104, 105* |
| CRH binding protein | KO | Reduced body weight and food intake | *119* |
| Peripherally expressed genes | | | |
| CCK-A | Null mutation | Mild obesity, increased food intake | *97* |
| UCP1 | Overexpression | Lean/resistant to obesity | *107* |
| UCP1 | KO | Cold sensitivity, not obese | *108* |
| R11β PKA subunit | KO | Lean | *109* |
| aP2 | KO | Dietary obesity without insulin resistance | *118* |
| A-ZIP/F-1 | Overexpression | No white adipose tissue/diabetes | *116* |
| nSREBP-1c | Overexpression | Lipodystrophy/diabetes | *117* |

[a]Abbreviations: NPY, neuropeptide Y; AGRP, agouti-related protein; POMC, proopiomelanocortin; MC, Melanocortin; MCH, melanin concentrating hormone; CRH corticotropin releasing hormone; CCK, cholecystokinin; GRP, gastrin releasing peptide; BBS, bombesin; UCP, uncoupling protein; PKA, protein kinase A; SREBP, sterol response element binding protein; KO, knock out.

## Conclusion

The application of molecular and genetic techniques in animal studies and human populations has provided the technical means to rapidly advance our knowledge of the genes that contribute to the control of feeding, energy expenditure, and energy partitioning and the contribution that such genes may make to the development of obesity. Understanding of the genetic basis for the predisposition of individuals toward developing obesity will provide new targets for pharmacotherapy of this chronic disease.

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