Appetite regulation: **Shedding new light on obesity**

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Several genes involved in the regulation of appetite and energy metabolism have been cloned and characterized recently. Each seems to form part of the complex regulatory network, centred in the hypothalamus, that is responsible for striking a balance between food intake and energy expenditure.

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New information illuminating the potential causes of obesity has been appearing rapidly, beginning with the cloning, by Friedman's group, of the mouse *obese* (*ob*) gene, as reported in December 1994 [1]. Many reports on related topics and other molecular biological approaches to obesity have followed, particularly in the last several months. These reports help to form a picture that places regulatory elements in the brain — and particularly in the hypothalamus — at the heart of the pathogenesis of obesity. In this report we review these developments and try to show the context in which they are informative.

Recent excitement, scientifically and popularly, began with the sequencing of the *ob* gene [1]. The sequence was found to code for a protein, named leptin, that is expressed uniquely in adipose tissue and secreted into the circulation; mutant copies of the gene were found in mice with the *obese* phenotype. These observations accord with the well-defined physiology of *ob/ob* mice, which have a single-gene defect that causes (among other things) severe obesity, voracious appetite, reduced basal energy expenditure, insulin resistance, and diminished fertility. Coleman had demonstrated in 1973 [2] that parabiotic crossing of circulation between affected and normal mice resulted in diminished food intake and weight loss in the affected mouse. From these and other observations, it had long been supposed that a circulating fat-derived factor was deficient in *ob/ob* mice.

With knowledge of the gene sequence, it became possible to produce significant quantities of wild-type leptin protein. Using such synthetic leptin, workers in three laboratories verified the predictions derived from physiological studies [3–5]. Thus, leptin injections reversed the abnormal phenotype in affected *ob/ob* mice, resulting in diminished appetite, increased energy expenditure, restoration of normal insulin levels, and amelioration of obesity. A recent report indicates that fertility can also be normalized in *ob/ob* mice by leptin administration [6]. More significantly, leptin given to wild-type mice decreased appetite and body weight [3–5], and this effect was particularly potent when the leptin was delivered into the cerebroventricular fluid [5]. This indicates that leptin probably works by signaling fat status to the brain's regulatory pathways. Subsequent reports provided evidence that leptin binds to hypothalamic cell membranes [7], and that leptin administration diminishes the content in the hypothalamus of neuropeptide Y [7,8], one neurochemical that seems to participate in the regulation of appetite and energy metabolism within the brain. Leptin administration appears also to enhance sympathetic nervous activity, and presumably thermogenesis in brown fat [9] — a function that would be predicted from the finding that leptin reduces hypothalamic neuropeptide Y, given that the neuropeptide itself suppresses brown-fat thermogenesis [10].

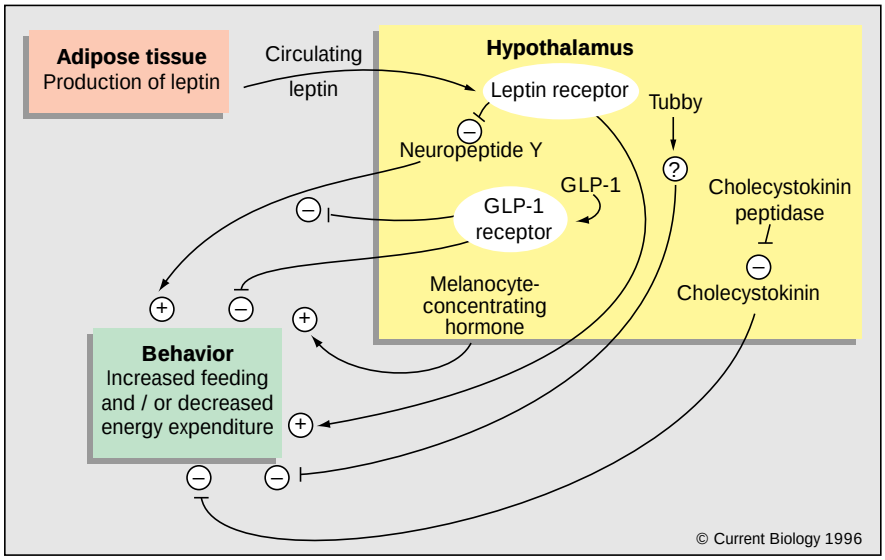
Following the studies of leptin, interest was heightened in a related form of single-gene obesity in mice, that of the *db/db* mutant. Parabiotic crossed-circulation experiments had indicated that the *db/db* mouse is resistant to the effects of the circulating factor presumed to be missing in the *ob/ob* mouse [2,11] — that is, the factor now thought to be leptin. Speculation that the gene defective in the *db/db* mouse codes for the leptin receptor was confirmed by three laboratories this year [12–14]. The leptin receptor seems to be expressed at high concentrations in the hypothalamus [12], and studies of the receptor further support the importance of brain neural networks in the regulation of energy metabolism and appetite.

Blood levels of leptin, which seem to form a signal from adipose tissue to the brain, are increased in nearly every form of obesity in animals [15,16] and humans [16–18], the exception being the *ob/ob* mouse itself; no human has yet been identified who has no leptin, reduced leptin levels or ineffective leptin. There is strong evidence that the degree of obesity (or the fat mass) is directly correlated with serum leptin levels [16–18]. This correlation is now widely interpreted as meaning that obesity is a condition associated with leptin resistance.

High levels of leptin in obese humans might mean that the fatter a person gets, the less sensitive they become to the effects of their own leptin. Some experimental support for this concept has been reported by Schwartz and colleagues [8], who found that leptin transport across the blood–brain barrier between the circulation and the hypothalamus was diminished in fat animals with high leptin levels. Even so, it is important to approach this issue with care, because

Figure 1

A simplified scheme of the relationships between adipose tissue, hypothalamic centres and behavioral changes in feeding and/or energy expenditure, which depend on circulating factors and neurochemicals as discussed in the text. It is not yet clear to what extent the effects of the hypothalamic neurochemicals on behavior are direct or inter-related. There are many other neurochemicals and brain sites that participate in the regulation of feeding and energy metabolism that are not shown in the figure.



other explanations are possible. A serum leptin-binding protein might reduce the free (and therefore effective) leptin level. More importantly, leptin may be only one of many signals received and integrated by the brain's regulatory mechanisms. If this were the case, what appears to be leptin resistance could instead be due to a predominance of non-leptin signals, or a primary abnormality in the regulatory mechanisms themselves. Leptin may thus prove to be a potent tool for examining the central regulatory mechanisms governing appetite and energy metabolism.

Another single-gene defect, *tubby*, which confers sensitivity to diet-induced obesity in the mouse, has recently been characterized at the molecular level [19,20]. Abnormal splicing of the *tubby* transcript, so that it retains an intron, appears to characterize the abnormal gene. The identity and function of the normal tubby protein have yet to be revealed, but it is known that the normal gene is abundantly expressed in the hypothalamus [20]. It should now be possible to produce the normal tubby protein synthetically and to determine its physiological effects. Expanding on the neural localization and function of the tubby protein may be useful in defining neural pathways that participate in energy metabolism regulation. Drawing conclusions about human obesity from single-gene animal conditions must be done with great care, however, because nearly all human obesity is polygenic. Although knowledge of the biological mechanisms underlying obesity has been, and will continue to be, expanded tremendously by recent discoveries using mutant mice, the direct application of this knowledge to humans is not possible as yet.

The insights that have come from the genetic obesities so far appear to be squarely centred on the hypothalamus (see Fig. 1). In studies of the hypothalamus itself, other discoveries have put forward new candidate neurochemicals for potential roles in the control of appetite and energy metabolism. Many of these neurochemicals are already known, and gathering evidence supporting and defining their role in the neural network regulating energy balance remains a work in progress. The predominant facts are often that the neurochemical either increases or decreases feeding, and that the neurochemical is found somewhere in the hypothalamus [21]. How the neurochemicals interact, and in some cases where they act, is more than ever a focus of great interest. New neurochemicals for which there is strong evidence of involvement in the energy-regulatory neural network include glucagon-like peptide 1 (GLP-1) and melanocyte-concentrating hormone, while additional evidence verifying the satiating role of cholecystokinin has also appeared (Fig. 1).

Because the sequence of GLP-1 is highly conserved phylogenetically and the peptide is found near its receptor in the hypothalamus, Turton and colleagues [22] tested GLP-1 for appetite-modulatory effects. They found that, when given into the cerebral ventricles, GLP-1 suppresses feeding and a GLP-1 antagonist increases feeding. The feeding stimulation caused by neuropeptide Y, but not that caused by fasting, was enhanced by co-administration of the GLP-1 antagonist. In studies aimed at defining the neural substrate for these responses, Turton and colleagues [22] found that GLP-1 induced neural activation, as exemplified by c-*fos* gene expression, in the paraventricular nucleus of the hypothalamus and in the central nucleus of the amygdala. Considerable evidence implicates both of these neural sites in the appetite/energy metabolism neuroregulatory network.

Using differential display coupled to the polymerase chain reaction, Qu and colleagues [23] compared the genes expressed in the hypothalamus of homozygous *ob/ob* mice with that in heterozygous *ob/*$^{+}$ mice. One mRNA overexpressed in the *ob/ob* mice was a neuropeptide, melanocyte-concentrating hormone. Although this peptide had not been implicated in appetite regulation previously, the investigators were able to show that central injection of the peptide stimulated food intake. This study therefore provides new physiological information about appetite regulation, but also illustrates the use of a valuable tool for investigating hypothalamic gene expression and function. A recent report [24] has extended the already well-developed data supporting the role of the hormone cholecystokinin as a mediator of satiety. In this report, a cholecystokinin-inactivating peptidase was purified and found in cholecystokinin-sensitive neurons. When the action of the peptidase was inhibited, cholecystokinin was protected and satiation was detectable in the treated animals.

The complicated nature of the neuroregulation of appetite and energy metabolism is well illustrated by the essentially normal phenotype of the neuropeptide Y 'gene knockout' mouse [25]. Neuropeptide Y is a neurochemical for which there is extensive evidence supporting a role in these controls [26], including robust feeding increases and thermogenesis decreases after neuropeptide Y is administered into specific brain sites (see Fig. 1), and appropriate changes in endogenous neuropeptide Y at arcuate and paraventricular hypothalamic nuclei after changes in energy balance. The absence of an abnormality of energy metabolism in the neuropeptide Y knockout mice may reflect the lack of brain-site specificity in gene knockouts, as neuropeptide Y is widely distributed in the neuroaxis and participates in many functions (few of which are abnormal in the knockout). A more intriguing possibility is that the regulatory machinery is plastic and redundant, allowing other mechanisms to compensate for the absence of neuropeptide Y in the knockout mice.

The apparent significance of neuropeptide Y in energy metabolism regulation is heightened by a newly described neuropeptide Y receptor [27]. This new receptor, named Y5 was found by expression-cloning from rat hypothalamus. The Y5 receptor sequence is quite different from that of other neuropeptide Y receptors, and the pattern of Y5 receptor activation by peptides related to neuropeptide Y correlates well with the potency of these peptides in stimulating feeding. The Y5 receptor is found principally in the brain, and particularly in the paraventricular nucleus of the hypothalamus, a location that is consistent with the known actions of neuropeptide Y on food intake and energy metabolism [10].

Many of the recent advances point to a role for the hypothalamus in regulating appetite and energy metabolism. We know, however, that it is not the whole hypothalamus that serves this function. The hypothalamus is made up of many cell populations, traditionally grouped into nuclei. These nuclei can be thought of as nodes in networks which regulate not only energy metabolism, but water metabolism, sex hormone levels, breathing, blood pressure and many other basic autonomic functions. Some of the hypothalamic nuclei appear more relevant in energy metabolism regulation than others (although many appear to have a role): the arcuate, paraventricular, suprachiasmatic and dorsomedial, in particular. Furthermore, we know that there are functional connections in the energy-metabolism regulatory network that link the hypothalamus to other key brain structures involved in autonomic regulation, such as the nucleus of the solitary tract in the medulla [28]. The amygdala appears to participate in the response to more than one neurochemical as well [22,29]. Given this perspective, we can expect further advances as we apply some of the powerful new knowledge and techniques to more specific portions of the hypothalamus and to other brain sites. The great challenge facing us — made easier by recent developments — is to shine a light on the components of the regulatory neural network and discern how the components functionally interact to regulate appetite and energy metabolism.

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