The proof of the pudding is in the eating: Editorial Focus on “Hyperphagia, not hypometabolism, causes early onset obesity in melanocortin-4-receptor knockout mice”

D. Euan MacIntyre1 and Susan B. Glueck2

1Department of Pharmacology, Merck Research Laboratories, 2Deputy Editor, Physiological Genomics

FOR HIGHER ORGANISMS TO SURVIVE, they must efficiently procure, utilize, and conserve energy. Accordingly, mammalian species have developed complex mechanisms to ensure a constant supply of energy for cellular functions during fluctuations in their environment. Despite imbalances between day-to-day food intake and energy expenditure, adiposity (body fat content) remains remarkably constant over time in normal adult individuals. Such energy homeostasis requires the coordinated regulation of appetite and adiposity and involves a complex neuroendocrine system in which circulating hormones and neural signals convey information about energy balance to brain pathways that control eating and energy expenditure. Our understanding of the genetic and molecular basis of energy balance regulation has increased markedly over the last few years, driven initially by positional cloning and characterization of the molecular defects underlying certain mouse obesity mutations, then galvanized by the emergence of obesity as a preeminent public health problem (11). Indeed, the increasing prevalence of obesity suggests that the systems controlling energy homeostasis defend more effectively against weight loss than weight gain (12). It also follows that a detailed understanding of the mediators and mechanisms involved in energy homeostasis should assist in the identification of suitable and/or novel molecular targets for the pharmacotherapy of obesity.

Short-term aspects of feeding such as taste perception, meal size, and satiety are regulated by nutrient, neural, and peptide signals (“satiety signals”) originating from the gut, whereas neural signals from the liver report meal composition. Longer term regulation of body weight and adiposity is mediated by “adiposity signals” in the form of hormones that circulate at concentrations proportional to body fat content, such as leptin, secreted by adipocytes, or insulin, secreted by pancreatic β-cells. Leptin deficiency produces behavioral and neuroendocrine profiles analogous to those evoked by chronic starvation. Reception and integration of the adiposity and satiety signals occurs within various brain regions: the brain stem for satiety signals, and the hypothalamus for adiposity signals. Diverse neuronal circuits then coordinate the resultant neuroendocrine, autonomic, and/or behavioral responses, which directly or indirectly influence food intake or energy expenditure (1, 10).

A variety of studies have identified multiple hypothalamic neurotransmitters and peptides implicated in the modulation of food intake and energy expenditure and their functional interrelationships. Neuropeptide Y (NPY) and agouti-related protein (AgRP) are coexpressed within a subset of neurons in the hypothalamic arcuate nucleus (ARC), and an adjacent subset of ARC neurons coexpress pro-opiomelanocortin (POMC) and the cocaine- and amphetamine-related transcript (CART). Neurons expressing POMC can synthesize the POMC product α-melanocyte stimulating hormone (α-MSH). These NPY/AgRP and POMC/CART neurons project to the lateral hypothalamic area where there exist distinct neuronal subsets expressing melanin-concentrating hormone or orexins, and to the paraventricular nucleus where different neurons express thyrotropin-releasing hormone or corticotropin-releasing hormone.

Based upon the pharmacological effects of their peptide constituents, and the neuronal response to the adiposity signals from leptin and insulin, the ARC neuronal pathways can be characterized as anabolic or catabolic (11). ARC NPY/AgRP neurons are considered to be anabolic, i.e., they are inhibited by leptin or insulin, but when activated they stimulate food intake, inhibit energy expenditure, and promote weight gain. Interestingly, genetic knockout of NPY does not alter feeding responses in (otherwise) normal mice, but reduces the degree of hyperphagia and obesity in ob/ob mice. Npy−/− mice display enhanced responsiveness to the anorectic effects of leptin. AgRP is an inverse agonist at neuronal melanocortin receptors, MC3R and MC4R, and when injected intracerebroventricularly in rodents produces marked stimulation of food intake and weight gain. Agrp−/− mice are phenotypically normal and respond normally to leptin.

By contrast, POMC/CART neurons are considered to be catabolic, i.e., they are stimulated by leptin or insulin, and, when activated, serve to inhibit food intake, enhance energy expenditure, and promote fat loss. α-MSH is an agonist at both MC4R and MC3R. Block-

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Address for reprint requests and other correspondence: D. E. MacIntyre, Dept. of Pharmacology, Merck Research Laboratories, R80Y-135, PO Box 2000, Rahway, NJ 07065.
ade of MC4R (e.g., in mice ectopically expressing agouti protein or overexpressing AgRP) results in obesity. Moreover, Pomp1+/− mice and Mc4r+/− mice are obese, and humans with MC4R or POMC mutations are insulin resistant and predispose to obesity that may be of early onset (15). Activation of MC4R by agonists evokes inhibition of food intake and stimulation of thermogenesis. Current research suggests that MC4R-expressing neurons are downstream targets for some, but not all, of the effects of leptin. For example, MC4R antagonism blocks leptin-induced sympatho-excitation, and Mc4r+/− mice are resistant to leptin-induced thermogenesis in brown adipose tissue, as well as to the anorectic effects of leptin. MC4Rs are widely expressed in the central nervous system and are present in the key feeding, endocrine, and autonomic control sites within the hypothalamus and brain stem. In addition, MC4R are present in sympathetic and parasympathetic preganglionic neurons, consistent with their potential involvement in autonomic regulation of energy expenditure and pancreatic β-cell function (7). Thus the central melanocortin system, and in particular the MC4R, plays a pivotal role in mammalian energy homeostasis (3, 8).

In this release of Physiological Genomics, Weide et al. (Ref. 16; see page 47 in this release), explore the early phenotypic manifestations of mice either heterozygous or homozygous for the MC4R knockout, which was originally generated by homologous recombination. They sought to determine whether the onset of obesity in Mc4r−/− mice, which has been previously characterized in adults (6), was due to lower energy expenditure or to hyperphagia. In addition, they wished to identify the time point when the affected individuals first evinced a higher body fat content than their wild-type littermates. Their survey consisted of a battery of tests of physiological function, body composition, and genetic expression upon sets of littermates fed an identical diet and ranging in age from 10–56 days: either homozygous null vs. heterozygotes, or homozygous wild-type vs. heterozygotes.

Mice were euthanized at 36 and 56 days. The body composition (percent water, fat, and fat-free dry mass) was determined, in addition to the plasma leptin concentration and mRNA levels of the orexigenic peptide NPY and the anorexigenic molecule POMC in the ARC. In addition, the levels of oxygen consumption and food intake were measured, either directly or indirectly via estimates, to determine any differences in energy intake between genotypes.

Weide et al. employed a variety of careful statistical analyses, taking into account the effects of genotype, litter, and sex, to evaluate differences in body composition; plasma leptin concentration; and energy balance. Their main findings were that both food intake and energy expenditure, measured over postnatal days 21 to 35, were higher in the homozygous null mice than in the other genotypes. Consequently, the excessive body fat in homozygous null mice, which first became significant at 35 days, could not be attributed to hypo-

metabolism, but to a higher net energy intake. In other words, the Mc4r−/− mice were eating more and consuming more oxygen than either their heterozygous or wild-type littermates. Relative to their wild-type littermates, the heterozygotes also showed increased food intake and energy expenditure with higher net energy intake, leading to a higher body fat content.

That hyperphagia is the mechanism that initiates fat deposition in weanling Mc4r−/− mice contrasts with published observations in adult (10–12 wk old) mice, where pair-feeding studies clearly indicate that hypometabolism is the primary cause of obesity (13). Moreover, that the onset of fat deposition occurs under conditions of hyperphagia and modest hypermetabolism is an unusual finding that potentially differentiates Mc4r−/− mice from the vast majority of other knockout models of obesity where hyperphagia and/or hypometabolism are listed as causative (2). These differences could be attributed to the chronology of the measurements relative to obesity development in this vs. other studies and/or to the precision and sensitivity of the measurements of energy balance used in the present studies. However, as there is controversy regarding the quantification of energy expenditure in animals differing markedly in body mass and composition, the observed modest hypermetabolism may merely reflect methodological differences among laboratories. Indeed, previous reports indicate increases in metabolic rate (i.e., hypermetabolism) of Mc4r−/− mice relative to wild-type littermates when the data are expressed on a per animal basis or in terms of lean body mass, whereas modest hypometabolism is evident if the data are expressed in terms of total body mass. Furthermore, by measuring both oxygen consumption and carbon dioxide production, one can estimate respiratory exchange ratio (RER; VCO2/VO2), which reports indices of energy substrate utilization. The RER of Mc4r−/− mice reportedly is higher than that of wild-type littermates, indicative of reduced fat metabolism in the former (4). It would be of interest to assess the chronology and genotype-dependence of changes in RER in Mc4r−/− mice.

As in wild-type animals, the plasma concentration of leptin was found to be strongly correlated with body fat content, regardless of genotype. However, mRNA levels of POMC and NPY in the brains of Mc4r knockout mice varied depending upon genotype. There was a nonstatistically significant tendency for a lower expression level of NPY in the ARC of Mc4r−/− individuals vs. either +/+ or +/- mice, and this level decreased with age. The opposite was observed for POMC levels, which increased significantly both with age and with more copies of the defective gene. The directional changes in NPY- and POMC-mRNA accord with the known effects of adiposity on expression of these neuropeptides and are consistent with the concept that coordinate regulation of NPY/AgRP and POMC/CART neurons defends body weight and maintains energy homeostasis.

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Identifying cause and effect relationships between genotype and changes in neuropeptide expression is complicated by accompanying changes in adiposity and adiposity signals. Weide et al. believe that this variability of expression levels is influenced more strongly by body fat composition and by plasma leptin concentrations than by genotype. Interestingly, when the impact of change in body fat content was taken into consideration, analysis of covariance revealed that both NPY- and POMC-mRNA were increased in Mc4r−/− individuals compared with +/+ mice. The observed upregulation of POMC contrasts with previous studies using adult Mc4r−/− mice which revealed no changes in ARC expression of POMC but is consistent with reports that chronic MC4R blockade in rats upregulates hypothalamic POMC mRNA. As Weide et al. suggest, upregulation of agonist (POMC) expression may be an adaptive response to the absence of the MC4R. As Mc4r−/− mice respond normally to the hyperphagic effects of NPY, suggesting that NPY acts independently or downstream of MC4R (9), upregulation of NPY mRNA in Mc4r−/− mice seems paradoxical: why would an anabolic/orexigenic pathway be activated under conditions of hyperphagia and fat deposition?

The interplay between NPY and POMC pathways is complex and only now is beginning to be elucidated (5). It is known that a subset of NPY neurons, which also contain γ-aminobutyric acid (GABA), innervate POMC neurons and serve to inhibit neuronal activation. Leptin activation of POMC neurons reportedly is mediated directly by depolarization of POMC neurons and indirectly by hyperpolarizing NPY/GABA neurons. This inhibits the release of GABA from presynaptic nerve terminals onto POMC neurons. POMC products activate MC3R, and MC3R agonists are known to modulate NPY/AgRP and NPY/GABA neurons. Whether MC3R modulation of NPY/AgRP neuronal activity and NPY gene expression is altered in Mc4r−/− mice remains to be determined.

Although the studies of Weide et al. document the time course of obesity development associated with MC4R deficiency in weaning mice and identify the greater importance of perturbation of appetitive vs. thermogenic mechanisms in the ensuing fat deposition, the relevance of these observations to the human condition remain to be determined. Of course, the fidelity with which experimental findings in animals accurately reflect the pathoetiology of human disease frequently is problematic, and the authors acknowledge the limitations of their experimental approaches in this regard, with temperature and diet being major considerations. As reported by the extent of fat deposition relative to lean littersmates, obesity in ob/ob and db/db mice is accentuated at temperatures below thermoneutrality, and the increased metabolic efficiency is attributed to reduced energy expenditure on thermoregulatory thermogenesis (14). Such observations led to the concept that development of obesity in leptin-resistant mice stems from hyperphagia and hypometabolism. As Weide et al. imply, it would be of interest to evaluate the kinetics and the mechanisms underlying the development of obesity in Mc4r−/− mice under thermoneutral conditions and in animals exposed to a moderately high-fat diet. In addition, studies where Mc4r−/− mice are pair fed to age-matched wild-type or heterozygous littersmates would be useful to substantiate the conclusions regarding the relative importance of hyperphagia vs. hypometabolism in the genesis of the obese state in these animals. As this study identifies in Mc4r−/− mice potential differences between the mechanisms underlying the development of obesity and those maintaining the obese state in adulthood, careful evaluation of the chronology and etiology of obesity development in other knockout models would be insightful.

Overall, the data from Weide et al. are consistent with the concept that MC4R and the melanocortin pathway are important determinants of obesity. That this pathway can be modulated for therapeutic benefit by using MC4R agonists to treat obesity in humans is attractive, but remains to be proven, and testing of this hypothesis awaits the development of potent, selective human MC4R agonists (8).

REFERENCES


