Melanocortin-3 receptor regulates the normal fasting response

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AUTHOR SUMMARY

Energy stores in adipose tissues are homeostatically regulated by the central nervous system, and syndromic obesity can result from defects in this process. Neuronal circuits that originate in the hypothalamus and involve a family of peptide hormones known as melanocortins are fundamental to this process (1). Indeed, mutations in the melanocortin-4 receptor (MC4-R) cause a hyperphagic obesity syndrome defined by a greater consumption of calories than is necessary for maintenance of lean body mass. A second receptor for the melanocortin peptides, the melanocortin-3 receptor (MC3-R), also is expressed in the central nervous system, and defects in the gene encoding MC3-R have been shown to cause obesity; however, in these cases, no evidence for overconsumption (hyperphagia) or a decrease in energy expenditure (hypometabolism) has been observed (2, 3). Our analyses of MC3-R-knockout (MC3-R−/−) mice, which lack functional copies of MC3-R, focused on careful measurements of metabolic physiology to elucidate mechanisms underlying the onset of obesity in this strain.

The obesity syndrome in the MC3-R−/− mouse is unusual, particularly compared with diet-induced obesity and MC4-R−/− obesity models, which exhibit overeating, increased lean mass, increased adipose mass throughout the body, high levels of insulin in the blood, and fatty liver disease. In contrast, MC3-R−/− mice develop an obesity syndrome that is characterized by a loss in lean mass and an increase in adipose mass, which occurs in the absence of overeating (2, 3). Although MC3-R deletion does not impact changes in ad libitum feeding or energy expenditure, even in response to high-fat diet (2–4), it does result in obesity. Thus, in our first characterization, we described the MC3-R−/− mouse as a metabolic model of obesity without the behavioral hyperphagia seen in the MC4-R−/− null mouse (2). Mice that lack both the MC3-R and MC4-R receptors are more obese than those lacking either of the two receptors, also suggesting independent roles for these two receptors in the regulation of body adiposity (3).

One method for testing the homeostatic regulation of fat stores involves studying the response to fasting. Fasting normally increases autonomic tone to stimulate lipolysis and the release of nonesterified fatty acids from adipose depots to provide an energy source in the absence of food intake. Fasting also activates the hypothalamic–pituitary–adrenal, or stress, axis to elevate adrenal corticosteroids and maintain blood glucose levels. In this study, we found that, compared with wild-type mice of the same age, sex, and inbred strain, the breakdown of fat in white adipose tissues (important for energy storage) was aberrant in MC3-R−/− mice when fasted. In addition, fasted MC3-R−/− mice did not exhibit appropriate accumulation of liver triglycerides, a normal source of energy for glucose production by liver during a fast. Close examination of the hypothalamic–pituitary–adrenal axis showed that MC3-R−/− mice exhibit elevated nadir corticosterone, the primary glucocorticoid in rodents, as well as blunted fasting-induced activation of the axis. Elevated glucocorticoids also are known to increase adipose stores, although the mechanisms are not well understood. As a result, many of the phenotypes in MC3-R−/− mice associated with body composition—increased visceral adiposity, decreased lean mass, and decreased bone mass—resemble those found in Cushing disease, a disorder resulting from elevated basal glucocorticoids. Fasting also is known to reduce the levels of the adiostatic hormone leptin, a signal known to participate in the mediation of responses to fasting such as those described above. Leptin acts in part by altering the expression of key hypothalamic genes involved in...
energy homeostasis. Our results indicated that in MC3-R−/− mice, fasting did not appropriately regulate the expression of hypothalamic genes critical to the regulation of energy homeostasis, including neuropeptide Y, agouti-related protein, and corticotropin-releasing hormone.

In summary, our findings revealed that MC3-R appears to play a unique role in melanocortin circuitry-associated signaling and communication of the fasted state in central and peripheral tissues involved in energy homeostasis (Fig. P1). Specifically, during nutritional deprivation, MC3-R is essential for mounting an appropriate energy mobilization response in hypothalamus, adrenal, adipose tissue, and liver. The consequence of defective MC3-R function is the gradual accumulation of energy in adipose depots, at the expense of normal muscle and bone development, and these findings help explain how obesity develops in the absence of increased food intake or decreased energy expenditure.

Supporting Information

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SI Materials and Methods

To quantify fat distribution epidydimal, interscapular, dorsal s.c., mesenteric, and retroperitoneal fat pads were removed from animals killed by cervical dislocation following bell jar anesthesia with isoflurane and were weighed. Because collection of all s.c. fat was not possible, a dorsal s.c. fat pad on the hindlimb was collected as a proxy for all s.c. fat.

Fig. S1. (A) Wet adipose tissue weight by depot. (B) Fat depot distribution as a percentage of total body fat in WT (n = 5) and melanocortin 3-receptor-deficient (MC3-R−/−; n = 6) mice. Data are expressed as mean ± SE. *P < 0.05, **P < 0.01, two-tailed t test.

Table S1. Body composition as measured by dual-energy X-ray absorptiometry in 13-wk-old WT and MC3-R−/− mice

<table>
<thead>
<tr>
<th>Property</th>
<th>WT (n = 19)</th>
<th>MC3-R−/− (n = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight</td>
<td>21.5 ± 0.4</td>
<td>21.1 ± 0.4</td>
</tr>
<tr>
<td>Fat mass (g)</td>
<td>3.0 ± 0.09</td>
<td>3.3 ± 0.1*</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>14.3 ± 0.3</td>
<td>16.1 ± 0.3†</td>
</tr>
<tr>
<td>Lean mass (g)</td>
<td>17.9 ± 0.3</td>
<td>17.3 ± 0.3</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SE.

*P < 0.05.
†P < 0.001; two-tailed t test.