Pro-Opiomelanocortin Modulates the Thermogenic and Physical Activity Responses to High-Fat Feeding and Markedly Influences Dietary Fat Preference

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Complete proopiomelanocortin (POMC) deficiency causes a human syndrome of hypoadrenalism, altered skin and hair pigmentation, and severe hyperphagic obesity. Heterozygote carriers of nonsense mutations are strongly predisposed to obesity. *Pomc*+/- mice have normal body weight on a chow diet but increase food intake and become more obese than wild-type littermates when placed on a high-fat diet. To further explore the mechanisms whereby dietary fat interacts with POMC genotype to produce obesity, we examined *Pomc*-null, *Pomc*+/-, and wild-type mice for changes in the components of energy balance in response to provision of a high-fat diet and macronutrient preference when presented with a selection of dietary choices. In contrast to wild-type mice, *Pomc* null mice did not increase their resting energy expenditure or their spontaneous physical activity when given a high-fat diet.

*Pomc*+/- mice increased resting energy expenditure similarly to wild types, but their increase in physical activity was significantly less than that seen in wild-type mice. In two independent experimental tests of macronutrient preference, *Pomc* genotype was a strong predictor of dietary fat preference with *Pomc* null animals choosing to eat approximately twice as much fat, but similar amounts of carbohydrate and protein, as wild-type animals. *Pomc*+/- mice showed an intermediate response. In summary, POMC-derived peptides have influences on multiple aspects of the organism’s response to the presentation of high-fat diet. This includes a major influence, readily discernible even in heterozygote animals, on the dietary preference for fat. (*Endocrinology* 148: 5331–5338, 2007)
There are also data from human studies potentially linking melanocortins and fat consumption. In a genome-wide scan analysis in Mexican-American families based on data from food questionnaires, Cai et al. (10) suggested that human chromosome 2p22, a region containing \( \text{Pomc} \), might contribute to dietary macronutrient intakes and adiposity phenotypes in Mexican-Americans.

We have previously shown that \( \text{Pomc} \) null mice are prone to develop worsening obesity in the face of a high-fat diet with this being driven by an increase in energy intake (2). In this current study, we again used \( \text{Pomc} \) null mice to further investigate the interaction between melanocortin signaling and diet to determine whether \( \text{Pomc} \) insufficiency not only alters the metabolic response to high-fat feeding but also impacts on ingestive behavior to favor consumption of a high-fat diet.

**Materials and Methods**

\( \text{Pomc}^{−/−} \) mice

\( \text{Pomc} \) null mice were generated on a 129/SvEv background, and genotypes were determined by PCR of DNA from ear tissue using a method previously described (2). All mice were male, aged 11–15 wk. Weight of mice at time of study are given in relevant parts below. All mice were maintained under controlled temperature (22°C) and on a 12-h light, 12-h dark schedule (light from 0700–1900 h). All protocols were in accordance with the United Kingdom Home Office.

**Diet composition**

Standard chow (4.5% fat) was supplied by Special Diet Service [SDS (Witham, Essex, UK)]. In addition, three other commercially available diets with different macronutrient composition were used; high-fat, medium-fat, and low-fat (summarized in Table 1). The high-fat diet (D12492; Research Diets, Inc., New Brunswick, NJ) contains 60% of calories from fat, the medium-fat diet (D12451) contains 45% of calories from fat, and the low-fat diet (D12450B) had 10% fat. All the diets had the same source of fat, and the amounts of protein (20%) and mineral/fiber supplements were also identical. Each diet was a different color to allow determination of intake. Studies of macronutrient selection used diet made fresh daily in the laboratory (Table 2) and presented in a paste form.

**Energy expenditure and activity**

Two matched groups of each genotype were given either a 60% fat or a standard chow from weaning at 3 wk of age. After 12 wk on the specified diet, measures of basal metabolic rate and physical activity were made by placing the mice into a Comprehensive Lab Animal Monitoring System (Columbia Instruments, Columbus, OH). These consisted of individual live-in cages instrumented for automated data collection.

At onset of the study, chow-fed mice weighed 27.5 ± 0.5 vs. 27.4 ± 0.8 vs. 27.4 ± 0.8 g [wild type (WT), \( \text{Pomc}^{+/−} \), \( \text{Pomc}^{−/−} \), respectively] and high fat-fed mice weighed 29.3 ± 0.6 g vs. 29.4 ± 0.5 g vs. 24.3 ± 1.9 g (WT, \( \text{Pomc}^{+/−} \), \( \text{Pomc}^{−/−} \), respectively). Before data acquisition, mice were acclimated to the Comprehensive Lab Animal Monitoring System cages (Columbia Instruments) for 72 h. Any loss of body weight during this period was taken as an indication of poor acclimatization, and mice so affected were excluded from subsequent analysis. All data acquisition started at the beginning of the light phase and continued for three consecutive 24-h periods. Body weights were determined at the beginning and end of testing, and again any weight loss during this period removed data from subsequent analysis.

Oxygen consumption (\( \text{VO}_{2} \)) was measured using an eight-chamber open-circuit Oxymax system that is a component of the Comprehensive Lab Animal Monitoring System (Columbia Instruments). Sample air was sequentially passed through oxygen and carbon dioxide sensors for determination of \( \text{O}_{2} \) and \( \text{CO}_{2} \) content.

Physical activity was continuously measured by a dual array of infrared beams surrounding each cage. One array was situated 3.2 cm above the floor of the cage and a second array at 7 cm above the floor. This configuration provided three measures of activity: 1) total x-axis activity defined as movement producing a beam break in the horizontal plane, 2) ambulatory activity, defined as movement producing sequential breaks of different horizontal beams, and 3) rearing, produced by vertical movement when the mouse stands on its hind legs to break the elevated 7-cm beam array. Beam breaks were monitored continuously and activity measurements were expressed in normalized units of beam/breaks per minute.

**Feeding studies**

Buffet with varying fat content. For this feeding study, a three-choice diet paradigm was used in which single-housed mice were allowed selection from three food cups anchored to a raised platform in the cage. The mouse cage was lined with clean white blotting paper. This enabled the easy spotting of spillage from the food containers as the colors (blue, pink, and cream) were clearly discernible without being lost in the usual woodchip bedding. A week before the selection test, mice were acclimated to this feeding setup using standard chow. Placement of food was changed daily to avoid placement preference and intake measurements were corrected for spillage. Body weight and food intake were measured daily (0900–1000 h).

Once the three-choice buffet (60% fat, 45% fat, or 10% fat) was presented to the mice, they were given 48 h to acclimatize to the choice, with a measure of food intake and body weight taken daily for the 5 d following on from this acclimatization period. Weights at onset of period of data acquisition were 25.7 ± 0.5 g vs. 25.9 ± 0.6 g vs. 35.9 ± 0.7 g (WT, \( \text{Pomc}^{+/−} \), \( \text{Pomc}^{−/−} \), respectively).

In the corticosterone-replaced study, an identical protocol was used, but mice of all genotypes had their drinking water replaced by corticosterone-supplemented drinking water for the duration of the study period. Weights at the onset of period of data acquisition were 25.2 ± 0.6 g vs 26 ± 0.7 g vs. 41.8 ± 0.9 g (WT, \( \text{Pomc}^{+/−} \), \( \text{Pomc}^{−/−} \), respectively).

**Macronutrient buffet.** An apparatus identical to that used in the fat content buffet above was used in this study. Again, all mice were allowed a period of acclimatization to the novel diet (protein, fat, and carbohydrate as in Table 2) before data acquisition. All mice in the macronutrient study received corticosterone-supplemented drinking water. Weights at the onset of period of data acquisition were 24.6 ± 0.3 g vs. 27 ± 0.6 g vs. 33.5 ± 1 g (WT, \( \text{Pomc}^{+/−} \), \( \text{Pomc}^{−/−} \), respectively). Concentration of plasma corticosterone levels in each genotype were similar in the three genotype

**TABLE 1. Composition of diets used in the study**

<table>
<thead>
<tr>
<th>Diet component (%)</th>
<th>60%F</th>
<th>45%F</th>
<th>10%F</th>
<th>Chow</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat (kcal %)</td>
<td>60.0</td>
<td>45.0</td>
<td>10.0</td>
<td>12.0</td>
</tr>
<tr>
<td>Carbohydrate (kcal %)</td>
<td>20.0</td>
<td>35.0</td>
<td>70.0</td>
<td>61.0</td>
</tr>
<tr>
<td>Protein (kcal %)</td>
<td>20.0</td>
<td>20.0</td>
<td>20.0</td>
<td>27.0</td>
</tr>
<tr>
<td>Total (kcal/g)</td>
<td>5.24</td>
<td>4.73</td>
<td>3.85</td>
<td>3.61</td>
</tr>
</tbody>
</table>

Diets used in the self-selection buffet (10/45/60 buffet). The percent figures refer to percent of total content. 60%F, High-fat diet; 45%F, medium-fat diet; 10%F, low-fat diet.

**TABLE 2. Composition of macronutrient diets**

<table>
<thead>
<tr>
<th>Diet component (%)</th>
<th>Fat</th>
<th>Carbohydrate</th>
<th>Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Safflower oil</td>
<td>6.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lard</td>
<td>93.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flour</td>
<td>59.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dextrin</td>
<td>28.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sucrose</td>
<td>10.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Solka-flock</td>
<td>1.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Casein</td>
<td>99.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin/mineral mix</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Total energy (kcal/g)</td>
<td>7.85</td>
<td>3.3</td>
<td>3.76</td>
</tr>
</tbody>
</table>

Diets used in macronutrient preference study. Composition is expressed as percentage by weight.
groups (WT vs Pomc+/− vs Pomc−/−; 26.1 ± 11 vs. 30.7 ± 13 vs. 51 ± 13 ng/ml, P = n.s. vs. WT).

Corticosterone supplementation

Corticosterone was purchased from Sigma-Aldrich (Poole, UK). Corticosterone replacement was given as supplemented drinking water at final concentration of 25 µg/ml. Plasma corticosterone was determined using commercially available kits according to the manufacturers’ protocols (Immunodiagnostic, Tyne and Wear, UK).

Statistical analysis

Data analysis was performed using Microsoft Excel (Redmond, WA) and MedCalc (Mariakerke, Belgium). All values reported are the mean ± s.e.m for each group. Statistical significance between groups was calculated by ANOVA or by unpaired two-tailed t test. Differences were considered to be significant if P ≤ 0.05.

Results

Pomc−/− mice fail to increase energy expenditure or physical activity when put on a high-fat diet

Using indirect calorimetry to measure oxygen consumption during a short period of the light cycle, we have previously shown that Pomc−/− mice have significantly lower resting VO2 than Pomc+/− and wild-type littermates (2). In this study, measurement of VO2 over a 3-d period in the Comprehensive Lab Animal Monitoring System (Columbia Instruments) also demonstrated that on standard chow Pomc null mice had a lower VO2 than both Pomc+/− and WT mice (WT: 24.5 ± 0.4; Pomc+/−: 23.6 ± 0.4; and Pomc−/−: 19.9 ± 1.9 ml/kg0.75/min; Fig. 1, A and C).

A separate cohort of mice was fed a high-fat diet (60% fat). After 12 wk on this diet, VO2 was measured in each genotype, again over a 3-d period in the Comprehensive Lab Animal Monitoring System (Columbia Instruments). WT mice increased VO2 by 14% from 24.5 ± 0.4 ml/kg0.75-min to 27.8 ± 0.6 ml/kg0.75-min (P < 0.01). Similarly, Pomc+/− mice significantly increased VO2 by 12% from 23.6 ± 0.4 ml/kg0.75-min to 26.4 ± 0.7 ml/kg0.75-min (P < 0.05). In contrast, there was no increase in VO2 seen in Pomc−/− mice on high-fat diet with corrected VO2 unchanged from the value recorded with Pomc−/− mice on standard chow (Fig. 1, B and C).

In addition to measuring VO2, we assessed whether changes in dietary fat content altered physical activity levels in Pomc−/− mice. Of note, when fed standard chow, the physical activity (measured by x-axis movement, ambulation, and rearing) was identical across all three genotypes. However, on the 60% fat diet, wild-type mice significantly increased their activity levels in all three measured parameters. They increased total x-axis activity by 29%, ambulatory activity by 44%, and rearing behavior by 4-fold. This was in sharp contrast to Pomc−/− mice whose activity levels remained entirely unchanged from those seen on standard chow. Similarly, although there was a tendency for Pomc−/− mice to increase ambulatory and rearing activity when fed the 60% fat, no measure of physical activity was significantly different from the level seen on standard chow (Fig. 2, A–C).

Fig. 1. Pomc null mice fail to increase VO2 consumption after 12 wk on 60% high-fat diet (HFD). A, VO2 of Pomc+/− (black triangle), Pomc−/− (gray triangle), and Pomc−/− (white triangle) mice on chow. Data were recorded every 18 min over a 72-h period. B, VO2 on normal chow (white circle) or high-fat (60%) diet (HFD, black circle) for each of the three genotypes. C, Average VO2 over the 72-h period illustrated in B, Pomc−/− (black), Pomc−/− (gray), and Pomc−/− (white) mice on chow or 60% HFD. * P < 0.05; ** P < 0.01; *** P < 0.001 (n = 6–8/genotype). Light and dark cycle indicated by white and black bars, respectively.
Pomc-insufficient mice fail to increase physical activity after 12 wk on 60% high-fat diet (HFD). Locomotor activity of wild-type, \( \text{Pomc}^{+/+} \), and \( \text{Pomc}^{-/-} \) mice on chow (white bar) or 60% HFD (black bar) over a 72-h period. Activity was assessed by movement in the x-axis (total beam break in the horizontal plane) (A), ambulatory activity (movement producing sequential horizontal beam breaks of different beams) (B), and rearing (vertical movement) (C). Bars, means ± SEM; *, \( P < 0.05 \); **, \( P < 0.01 \) (n = 6–8/genotype).

\( \text{Pomc}^{-/-} \) and \( \text{Pomc}^{+/+} \) mice preferentially consume diet with a higher fat content

To determine whether \( \text{Pomc} \) insufficiency causes an increase preference for fat consumption, we undertook a number of feeding studies in which mice had \textit{ad libitum} access to a three-choice buffet of food pellets containing 10, 45, or 60% fat (referred to as 10/45/60 buffet). When given such a dietary choice, wild-type mice significantly reduced their total intake, compared with the amount consumed when offered standard chow alone (standard vs. buffet, 17 ± 0.3 vs. 14.4 ± 0.4 kcal/d, \( P < 0.001 \); Fig. 3A). In contrast, \( \text{Pomc}^{-/-} \) mice significantly increased their total intake when feeding from the 10/45/60 buffet (standard vs. buffet, 17.7 ± 0.6 vs. 21.4 ± 1.2 kcal/d, \( P < 0.05 \); Fig. 3A). These data remained significant when corrected for total body mass (Fig. 3B).

The food choices made by each genotype were further analyzed. Although 60% fat made up the major part of the calorific intake of each genotype (WT vs. \( \text{Pomc}^{+/+} \) vs. \( \text{Pomc}^{-/-} \), 52 vs. 54 vs. 65%), \( \text{Pomc}^{+/+} \) mice ate 83% more of the 60% fat than wild-type mice. Indeed, when corrected for total body weight, both \( \text{Pomc}^{+/+} \) and \( \text{Pomc}^{-/-} \) ate significantly more 60% fat than wild-type mice. Interestingly, heterozygous mice ate more 45% fat than either wild-type or homozygous null mice.

An important component of \( \text{Pomc} \) deficiency is concomitant glucocorticoid deficiency, and thus any interpretation of food preference data must be tempered by the fact that \( \text{Pomc}^{-/-} \) have undetectable circulating corticosterone. We previously demonstrated that restoration of plasma corticosterone to within the physiological range significantly worsens the hyperphagia seen in \( \text{Pomc} \) null mice (11). We therefore repeated the 10/45/60 buffet test in a cohort of mice receiving corticosterone (CORT) supplemented drinking water. Whereas CORT-treated wild-type mice decreased energy intake when offered a high-fat food (Fig. 4A), once again there was a clear gene-dose effect on ingestive behavior with both CORT-treated \( \text{Pomc}^{+/+} \) and \( \text{Pomc}^{-/-} \) increasing energy intake by 13 and 48%, respectively, on the 10/45/60 buffet diet (Fig. 4, A and B).

Upon analyzing which components of the buffet were consumed, with CORT treatment all mice preferred 60% fat. However, the magnitude of the change between genotypes varied considerably. CORT treatment caused a 42% rise in 60% fat consumption in wild-type mice (from 7.6 to 10.8 kcal/d) but an 88% increase (from 13.9 to 26.2 kcal/d) in \( \text{Pomc}^{+/+} \) mice. Once again, \( \text{Pomc}^{+/+} \) showed the strongest preference for 45% fat chow (Fig. 4, C and D).

Pomc insufficiency influences macronutrient selection

The data from the 10/45/60 buffet study indicate that \( \text{Pomc} \) deficiency results in a specific preference for fat. However, the constraints imposed by the physical make-up of the pellets used in the study dictate that an increase in fat content is at the cost of carbohydrate content. Thus, as fat content increases from 10 to 45 and 60%, so carbohydrate content falls from 70 to 35 and 20%, respectively (Table 1). This reduction in carbohydrate content may have influenced pellet consumption patterns. Therefore, to further determine whether fat is indeed the preferred macronutrient in \( \text{Pomc} \) deficiency we carried out a further buffet test, but this time using three foodstuffs of near-pure macronutrient content (fat, protein, and carbohydrate; see Table 2). All mice in the study were again given CORT-supplemented water.

Given a free choice of three different macronutrients, total kilocalorie per mice was significantly increased in \( \text{Pomc} \)-insufficient mice, whereas \( \text{Pomc}^{+/+} \) mice attained an energy intake intermediate to wild-type and \( \text{Pomc}^{-/-} \) (Fig. 5A). When this total energy intake was analyzed in terms of constituent components, each genotype ate identical amounts of carbohydrate and protein. However, there was a clear gene dosage effect on fat consumption, with \( \text{Pomc}^{+/+} \) and \( \text{Pomc}^{-/-} \) mice eating 45 and 98% more fat than wild-type
mice, respectively. When normalized for total body mass, Pomc⁻/⁻ mice showed a significant preference for fat consumption at the expense of protein and carbohydrate (Fig. 5B).

**Discussion**

In this study we extended our previous observations on the interaction between Pomc insufficiency and high-fat feeding. We demonstrated that loss of only one copy of Pomc results in the inability to increase activity levels when challenged with a high-fat diet. Furthermore, data in this study show a gene-dosage effect of Pomc insufficiency on macronutrient preference, with increasing loss of Pomc resulting in increased fat consumption. Taken together, these data indicate that the increased weight gain seen with high-fat feeding of Pomc-insufficient mice is driven by a specific preference for fat coupled with dysfunctional mechanisms of energy expenditure.

Several other studies have indicated that differences in hypothalamic Pomc expression may be important in determining susceptibility to develop obesity when fed a high-fat diet. Bergen et al. (12) studied neuropeptide expression in A/J mice, recognized as being resistant to diet-induced obesity. After 14 wk on a high-fat diet, Pomc expression was increased, whereas hypothalamic levels of neuropeptide Y mRNA were decreased (12). In a separate study with only 2 wk of high-fat intake, Pomc mRNA was also shown to be elevated (13). In addition, Hagan et al. (14) demonstrated that...
11 d of overfeeding rats via a gastric catheter can increase Pomp mRNA in the hypothalamic arcuate nucleus by 180% relative to levels in control animals.

The findings in this study that wild-type mice are able to increase activity levels and energy expenditure in the face of a high-fat diet has also been previously reported by a number of groups. Butler et al. (15) reported that a transition from standard chow to a higher-fat diet was associated with a significant increase in wheel-running activity in wild-type BL6 mice. Kokkotou et al. (16) also reported that wild-type SV129 mice increased their locomotor activity by 50% and their VO2 by 14% when fed a high-fat diet. Here we demonstrate that the diet-induced increase in locomotor activity was absent in both heterozygous and homozygous mutant mice, and it seems likely this relative hypoactivity contributes to the obesity seen in Pomp-insufficient mice. These findings are in keeping with previous data suggesting an intact central melanocortinergic system is required for appropriate metabolic responses to dietary changes as neither an increase in oxygen consumption nor an increase in physical activity were seen when Mc4-r null mice were challenged with an increase in dietary fat (15). Taken with our data, these findings indicate that important compensatory responses to a high-fat feeding are dependent on an intact melanocortin system. Interestingly, physical activity phenotypes in humans have also been associated with polymorphisms in the MC4-R, suggesting that variation at the MC4-R gene locus may contribute to the propensity to be sedentary (17).

The downstream pathways linking central melanocortin signaling with changes in physical activity remain to be fully elucidated. It may be that these changes are driven by modulation of sympathetic tone because there are previous data linking the leptin-melanocortin with activation of the sympathetic nervous system and subsequent diet-induced thermogenesis (18, 19). In addition, a recent report by Perel et al. (20) demonstrated that pro-TRH neurons can be stimulated by α-MSH, indicating that activation of the hypothalamic-pituitary-thyroid axis by melanocortins (21) may also be responsible, at least in part, for some of the diet-induced metabolic responses observed in the wild-type mice. Of note, a recent report has linked the neuropeptide melanin-concentrating hormone (MCH) with changes in activity levels when challenged with increased dietary fat (16). The authors demonstrated that on standard chow Mch-/- mice had only a minimal increase in activity levels and oxygen consumption, compared with wild-type mice. However, on a high-fat diet, the differences between WT and Mch-/- mice became far more pronounced, accounted for by a substantial increase in energy expenditure and activity levels in Mch null mice. This may explain why loss of Mch confers resistance to diet-induced obesity but also has relevance to our current study. In direct contrast to the knockout mice studied by Kokkotou et al. (16), Pomp null mice have an increased expression level of Mch in the lateral hypothalamus (2), which contribute to the failure of these mice to increase energy expenditure on a high-fat diet.

Genetic variability is known to influence macronutrient preference with patterns of fat consumption highly variable between inbred strains of mice. Smith et al. (22) reported the macronutrient diet selection in 13 different mouse strains. In this study, comparison of proportional fat intake across strains showed fat consumption ranged from 26 to up to 83% of total energy intake. For example, AKR/J, a strain highly sensitive to dietary obesity, self-selected around 27 kcal of fat per day, compared with only 7 kcal of fat selected by the carbohydrate-preferring CAST/Ei strain (22). Again, the underlying mechanisms leading to such marked differences in nutrient preferences remain elusive, but a number of pharmacological studies suggest that differences in activity within hypothalamic signaling pathways may offer a plausible explanation. For example, intracerebroventricular injection of galanin, an orexigenic 29-amino acid peptide found in the paraventricular nucleus, results in a selective enhancement of fat intake (23). Central administration of the endogenous opioid peptides, enkephalin and dynorphin, have also been reported to stimulate fat intake. Interestingly, neu-
ropeptide Y, the most potent hypothalamic orexigenic peptide, preferentially stimulates consumption of carbohydrate over fat or protein (24).

Our data demonstrate that loss of POMC peptides results in increased fat intake and is in accord with other murine data supporting a role for the leptin-melanocortin system in determining fat consumption. More than half a century ago Mayer et al. (25) evaluated nutrient selection in obese adult ob/ob mice and demonstrated a preference for fat consumption. More recently A^{3/4} mice have been reported to consume a greater proportion of their daily intake from fat than wild-type littermates (9). Central administration of the melanocortin antagonist AgRP also increases high-fat diet consumption (7), whereas administration of the melanocortin agonist melanotan II results in a preferential decrease in fat consumption (8).

There are several potential mechanisms linking a loss of activity of POMC-derived peptides with a preference for fat. Although not directly addressed in this study, reduced melanocortin tone may affect hypothalamic nutrient sensing. A number of studies have demonstrated that hypothalamic metabolism of fatty acids functions as a biochemical sensor for nutrient availability that in turn modulate nutrient intake (26) and the mobilization of stored energy [i.e. glucose production (27–30)]. Interestingly, a study using BALB/cByJ mice has linked fatty acid metabolism with food preference. These mice bear a spontaneous mutation in Acads, the gene that encodes for the short-chain acyl-CoA dehydrogenase, which is a mitochondrial enzyme with a role in the β-oxidation of C_{4–C_{6}} fatty acids. BALB/cByJ mice select a low percentage of fat intake in a free-choice paradigm and in particular avoid dietary fat when the source is predominantly long-chain fatty acid (31).

Loss of POMC-derived peptides may also impact on the rewarding and pleasurable aspects of appetitive behavior. In addition to the melanocortins, POMC is also the precursor for the opioid β-endorphin (32). Pharmacological studies and data derived from genetically modified mice have established that the endogenous opioid system can modulate feeding behavior. These roles have historically been defined as being involved in the hedonics of feeding rather than the regulation of energy homeostasis. However, the endogenous opioid system is complex with a number of peptide ligands and a range of widely expressed opioid receptors. Appleyard et al. (33) published a study whereby they genetically removed β-endorphin to differentiate the effects of this POMC-derived opioid from that of the other endogenous opioid peptides like enkephalin and dynorphin. Rather unexpectedly, this study demonstrated that β-end null male mice had an increased body weight and increased food intake, suggesting that β-endorphin had an anorectic effect in energy homeostasis, more in keeping with POMC-derived melanocortins. Indeed, more recent data from Hayward and Low (34) suggested that the opioid enkephalin plays a more consistent role than β-endorphin in mediating the motivation for food reward, indicating that a lack of POMC derived β-endorphin may not be linked to the preferences seen in the current study.

However, it may be that loss of melanocortins in Pomc^{−/−} mice are exerting an effect on the central rewarding system to favor fat consumption. Given the localization of POMC and MC4-R in brain reward regions (35–37) and functional interactions with psychostimulants (38, 39), there is certainly evidence indicating melanocortins may have a role beyond that of helping to integrate a response to nutrient sensing. One additional caveat to the interpretation of the data we present is that the mice studied lack POMC peptide in both the arcuate nucleus and the nucleus of the solitary tract. Both regions of the brain have important roles in the control of energy homeostasis, so studying the responses to increased dietary fat in mice lacking POMC in only one of these discrete anatomical lesions would help further finesse our understanding of the mechanisms determining energy expenditure and food preference.

Finally, the orexin system may also play a role in the fat preference seen in Pomc null mice. We recently demonstrated an elevation in orexin mRNA expression levels in the lateral hypothalamus of Pomc-deficient mice (40). Orexin-containing neurons project to reward-associated brain region (41) with intracerebroventricular administration of orexin able to selectively stimulate consumption of a high-fat diet (42). Interestingly, this orexigenic effect of orexin appears to be critically dependent on activation of downstream opioid receptors.

As is evident from the discussion above, animal studies contributed significantly to our knowledge of the genetic determinants of macronutrient intake. In humans, although the heritability of body mass is well validated, our understanding of the genetic mechanisms underlying nutrient selection is less clear. In particular, to date, there are no published human data demonstrating that a loss of POMC peptides or a disruption in MC4-R signaling causes a preference for fat consumption. However, in a genome-wide scan based on data derived from food questionnaires evidence of linkage with saturated fat intake was found on chromosome 2, a region containing the POMC gene (10). In addition, an ethnic-specific AGRP polymorphism has been demonstrated to show significant associations with lowered dietary fat intake (43). Yet questions remain as to the extent to which POMC-derived peptides mediate an individual’s response when exposed to a high-fat diet. In particular, it would be intriguing to determine whether differences in central melanocortinergic tone account for resistance or susceptibility to weight gain on a high-fat diet. Given the marked phenotypic similarities in humans and rodents with disrupted melanocortin signaling, it seems reasonable to speculate this may indeed be the case.

In conclusion, this study shows that loss of POMC results in increased preference for fat and lends further support to the notion that an individual’s appetite for palatable, energy-dense food is genetically determined. Moreover, these results indicate that impaired melanocortin signaling disrupts adaptive metabolic changes normally seen when challenged with an increase in dietary fat content. The cumulative effect of these perturbations results in substantial weight gain when a high-fat diet is available, highlighting an important gene-environment interaction and further supporting the hypothesis that genetic variation around the POMC locus confers a risk for developing obesity.
Acknowledgments

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