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Pro-opiomelanocortin (POMC) modulates the thermogenic and physical activity responses to high fat feeding and markedly influences dietary fat preference

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Abstract

Complete 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. In order 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 a) changes in the components of energy balance in response to provision of a high fat diet and b) 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.

Keywords

Melanocortin; POMC; dietary fat; hypothalamus; diet-induced obesity

Introduction

Pro-opiomelanocortin (POMC) is a functionally inert polypeptide precursor molecule which is expressed in the hypothalamus, brainstem, anterior pituitary and skin. POMC undergoes extensive, tissue-specific post-translation modification to generate a range of smaller, biologically active peptides. These include ACTH and α-, β- and $\tilde{\gamma}$ melanocyte stimulating hormone (MSH), collectively known as the melanocortins. The biological effects of the melanocortins are diverse and largely mediated through one of the five melanocortin receptor isoforms, of which MC3 and MC4 receptor (MC3-R and MC4-R) have a functional role in appetite and body fat regulation.

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Data from human genetic and murine studies convincingly show that an intact central melanocortin signalling pathway is critical for normal energy homeostasis. Both humans (1) and mice (2) lacking all endogenous POMC peptides (*Pomc* $^{-/-}$) develop hyperphagia and extreme early-onset obesity.

Pomc haploinsufficiency can also result in disrupted energy homeostasis. Smart *et al.* have reported *Pomc*$^{+/-}$ mice to have a subtle obesity phenotype intermediate between wild type and *Pomc* null mice (3), while we have previously described obesity to develop in *Pomc*$^{+/-}$ mice with high fat feeding (2). Humans heterozygous for mutation in *POMC* have also been studied. The heterozygous parents of the probands reported in Krude *et al's* initial report were all found to have high normal or mildly elevated body weight, suggesting a dosage effect of *POMC* gene products on human weight regulation (1;4). This was borne out when a number of extended family members of a Turkish child with congenital POMC deficiency were also studies (5). Eleven of twelve subjects heterozygotes for the null mutation in *POMC* were either overweight or obese compared with only one of seven wild-type family members. The observation that even *POMC* haploinsufficiency confers a substantial obesity risk is in accord with the evidence implicating the region of human chromosome 2 containing the *POMC* gene as a susceptibility locus for common human obesity (6).

In addition to impacting upon total body weight, there are pharmacological and genetic data to suggest that disruption of melanocortin signalling can influence ingestive behaviour, in particular dietary fat consumption.

Agouti-related peptide (AgRP) is an antagonist at central nervous system melanocortin receptors and a potent orexigenic agent. When given centrally to rats, AgRP has been shown to preferentially increase intake of a high-fat over a low-fat diet (7). Peripheral administration of the non-selective melanocortin agonist melanotan II (MTII) can preferentially decreased fat consumption in a three choice diet paradigm, with no such effect seen in *Mc4-r* null mice (8). Agouti mice (A^y/a) develop obesity as a result of ectopic expression of the melanocortin antagonist agouti disrupting signalling at central MC-3 & 4 receptors. In a free-choice paradigm A^y/a mice consume a greater proportion of their daily intake from fat and less from carbohydrate compared to their wild-type littermates (9).

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*. suggested that human chromosome 2p22, a region containing *POMC*, might contribute to dietary macronutrient intakes and adiposity phenotypes in Mexican Americans (10).

We have previously shown that *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 have again used *Pomc* null mice to further investigate the interaction between melanocortin signaling and diet to determine if *Pomc* insufficiency not only alters the metabolic response to high fat feeding but also impacts upon ingestive behaviour to favour consumption of a high fat diet.

Method

Pomc$^{-/-}$ mice

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 maintained under controlled temperature (22°C) and light (1-2 h light from 7:00-19:00). All protocols were in accordance with the United Kingdom Home Office.

Diet composition

Standard chow (4.5% fat) was supplied by Special Diet Service. In addition, three other commercially available diets with different macronutrient composition were used; high-fat, medium-fat and low-fat (summarized in Table 1A and referred to as 60%F, 45%F and 10%F, respectively). 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 diet had the same source of fat, and the amounts of protein (20%) and mineral/fiber supplements were also identical. Each diet was a different colour to allow determination of intake. Studies of macronutrient selection used diet made fresh daily (Table 1B) in the laboratory 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 weeks of age. After 12 weeks 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 (CLAMS; Columbia Instruments, Columbus, OH). These consist of individual live-in cages instrumented for automated data collection.

VO_2 was measured using, an eight-chamber open-circuit Oxymax syxtem that is a component of the CLAMS. Sample air was sequentially passed through oxygen and carbon dioxide sensors for determination of O_2 and CO_2 content. Prior to data acquisition, mice were acclimated to the CLAMS cages for 72 hours. Any loss of body weight during this period was taken as an indication of poor acclimatisation and mice so affected were excluded from subsequent analysis. All data acquisition started at the beginning of the light phase and continued for 3 consecutive 24-hours 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.

Physical activity was continuously measured by a dual array of infrared beams surrounding each cage. One array is situated 3.2cm above the floor of the cage and a second array at 7cm 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 7cm beam array. Beam breaks were monitored continuously and activity measurements were expressed in normalised units of beam/breaks per minute.

Feeding studies

1) 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. A week prior to the selection test, mice were acclimatised 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 (9-10am).

Once the 3 choice buffet (60% fat or 45% fat or 10% fat) was presented to the mice, they were given 48hours to acclimatize, with a measure of food intake and body weight taken daily for the 5 days following on from this acclimatization period.

In the corticosterone replaced study, an identical protocol was used but mice of all genotype had their drinking water replaced by corticosterone-supplemented drinking water for the duration of the study period.

2) Macronutrient Buffet

An identical apparatus 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 Table 1B) before data acquisition. All mice in the macronutrient study received corticosterone-supplemented drinking water.

Corticosterone supplementation

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

Statistical analysis

Data analysis was performed using Microsoft Excel and StatView (version 4.5; Abacus Concepts, San Francisco, CA). All values reported are the mean ± S.E.M. for each group. Statistical significance between groups was calculated by analysis of variance (ANOVA) or by unpaired 2-tail *t* test. The MedCalc software package was used for all analyses. Differences were considered to be significant if $P \leq 0.05$.

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 oxygen consumption than *Pomc*$^{+/-}$ and wild type littermates (4).

In this study, measurement of oxygen consumption (VO_2) over a 3-day period in the CLAM system also demonstrated that on standard chow *Pomc* null mice had a lower VO_2 than both *Pomc*$^{+/-}$ and wild-type mice. (Wt: 24.5± 0.4; *Pomc*$^{+/-}$: 23.6±0.4 and *Pomc*$^{-/-}$: 19.9±1.9ml/kg $^{0.75}$/min; Figure 1a, 1c)

A separate cohort of mice was fed a high fat diet (60% fat). After 12 weeks on this diet, VO_2 was measured in each genotype, again over a 3 day period in the CLAM system. Wild type mice increased VO_2 by 14% from 24.5±0.4 ml/kg$^{0.75}$/min to 27.8±0.6 ml/kg$^{0.75}$/min (P<0.01). Similarly, *Pomc*$^{+/-}$ mice significantly increased VO_2 by 12% from 23.6±0.4 ml/kg$^{0.75}$/min to 26.4±0.7 ml/kg$^{0.75}$/min (P<0.05). In contrast, there was no increase in oxygen consumption seen in *Pomc*$^{-/-}$ mice on high fat diet with corrected VO_2 unchanged from the value recorded with *Pomc*$^{-/-}$ mice on standard chow (Figure 1b, 1c).

In addition to measuring oxygen consumption, 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 60% fat diet, wild type mice significantly increased their activity levels in all 3 measured parameters. They increased total x-axis activity by 29%, ambulatory activity by 44% and rearing behaviour 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 60% fat, no measure of physical activity was significantly different from the level seen on standard chow (Figure 2a-c).

Pomc$^{-/-}$ and *Pomc*$^{+/-}$ mice preferentially consume diet with a higher fat content

To determine if Pomc insuffiency causes an increase preference for fat consumption we undertook a number of feeding studies in which mice had *ad libitum* access to a 3 -choice buffet of food pellets containing either 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 Kcal intake compared to the amount consumed when offered standard chow alone (standard vs. buffet, 17 ±0.3 vs 14.4±0.4 Kcal/day, P<0.001; Figure 3a). In contrast, *Pomc*$^{-/-}$ mice significantly increased their total Kcal intake when feeding from the "10/45/60 buffet" (standard vs. buffet, 17.7±0.6 vs 21.4±1.2 Kcal/day, P<0.05; Figure 3a). These data remained significant when corrected for total body mass (Figure 3b).

The food choices made by each genotype were further analysed. Although 60 % fat made up the major part of the calorific intake of each genotype (WT *vs Pomc*$^{+/-}$ *vs Pomc*$^{-/-}$, 52% vs 54% vs 65%), *Pomc*$^{-/-}$ mice ate 83 % more of the 60% fat than wild type mice. Indeed, when corrected for total body weight, both *Pomc*$^{+/-}$ and *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 POMC deficiency is concomitant glucocorticoid deficiency and thus any interpretation of food preference data must be tempered by the fact that *Pomc*$^{-/-}$ have undetectable circulating corticosterone. We have previously demonstrated that restoration of plasma corticosterone to within the physiological range significantly worsens the hyperphagia seen in *Pomc* null mice. We therefore repeated the "10/45/60 buffet" test in a cohort of mice receiving corticosterone supplemented drinking water. This resulted in similar concentration of plasma corticosterone level in the three genotype groups (WT vs *Pomc*$^{+/-}$ vs *Pomc*$^{-/-}$; 26.1 ±11 *vs* 30.7 ±13 *vs* 51±13 ng/ml, p= n.s. vs WT). While 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 upon ingestive behaviour with both CORT-treated *Pomc*$^{+/-}$ and *Pomc*$^{-/-}$ increasing energy intake by 13% and 48%, respectively, on the "10/45/60 buffet" diet (Fig 4a &4b).

Upon analysing 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 day) but an 88% increase (from 13.9 to 26.2 kcal /day) in *Pomc* $^{-/-}$ mice. Once again, *Pomc* $^{+/-}$ showed the strongest preference for 45% fat chow (fig 4c &d).

Pomc insufficiency influences macronutrient selection

The data from the "10/45/60 buffet" study indicate that 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 1A). This reduction in carbohydrate content may have influenced pellet consumption patterns. Therefore, to further determine if fat is indeed the preferred macronutrient in 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 1b). All mice in the study were again given glucocorticoid supplemented water.

Given a free choice of three different macronutrients, total Kcal per mice was significantly increased in *Pomc* insufficient mice while *Pomc*$^{+/-}$ mice attained an energy intake intermediate to wild-type and *Pomc*$^{-/-}$ (Figure 5a). When this total energy intake was analysed 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 *Pomc*$^{+/-}$ and

Pomc$^{-/-}$ mice eating 45 % and 98 % more fat than wild type mice, respectively. When normalised for total body mass, *Pomc* $^{-/-}$ mice showed a significant preference for fat consumption at the expense of protein and carbohydrate (Figure 5b).

Discussion

In this study we extend our previous observations on the interaction between *Pomc* insufficiency and high fat feeding. We demonstrate that loss of only one copy of *Pomc* results in the inability to increase activity levels when challenged with a high fat diet. Further, 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.* studied neuropeptide expression in A/J mice, recognized as being resistant to diet-induced obesity. After 14 weeks on a high fat diet *Pomc* expression was increased whilst hypothalamic levels of NPY mRNA were decreased (11). In a separate study with only 2 weeks of high fat intake, POMC mRNA was also shown to be elevated (12). In addition, Hagan *et al.* demonstrated that 11 days of overfeeding rats via a gastric catheter can increased POMC mRNA in the hypothalamic arcuate nucleus by 180% relative to levels in control animals (13).

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.* have 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 (14). Kokkotou *et al.* also reported that wild type SV129 mice increased their locomotor activity by 50% and their VO_2 by 14% when fed a high fat diet (15).

Here, we demonstrate that the diet-induced increase in locomotor activity was absence in both heterozygous and homozygous mutant mice and it seems likely this relative hypoactivity contributes to the obesity seen in *Pomc* 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 *Mc4r*$^{-/-}$ mice were challenged with an increase in dietary fat (14). Taken with our data, these findings indicate that important compensatory responses to a high fat feeding are dependent upon 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 (16).

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 as there are previous data linking the leptin-melanocortin with activation of the sympathetic nervous system and subsequent diet-induced thermogenesis (17;18). Activation of the hypothalamic-pituitary-thyroid (HPT) axis by melanocortins (19) 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 (15). The authors demonstrated that on standard chow *Mch*$^{-/-}$ mice had only a minimal increase

in activity levels and oxygen consumption compared to wild-type mice. However, on a HFD, 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 and colleagues, *Pomc* null mice have an increased expression level of MCH in the lateral hypothalamus (2) which be contributing 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.* have reported the macronutrient diet selection in thirteen 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 to only 7 kcal of fat selected by the carbohydrate-preferring CAST/Ei strain (20). 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 signalling pathways may offer a plausible explanation. For example, intracerebroventricular (icv) injection of galanin, an orexigenic 29 amino acid peptide found in the PVN, results in a selective enhancement of fat intake. Central administration of the endogenous opioid peptides, enkephalin and dynorphin, have also been reported to stimulate fat intake. Interestingly, neuropeptide Y (NPY), the most potent hypothalamic orexigenic peptide, preferentially stimulates consumption of carbohydrate over fat or protein (22).

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 (23) evaluated nutrient selection in obese adult *ob/ob* mice and demonstrated a preference for fat consumption. More recently, *A*^{y}*/a* 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), while administration of the melanocortin agonist MTII 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 (24) and the mobilization of stored energy (i.e. glucose production; (25-28). 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 (SCAD). SCAD 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 (29).

Loss of POMC-derived peptides may also impact upon the rewarding and pleasurable aspects of appetitive behaviour. In addition to the melanocortins, POMC is also the precursor for the opioid β-endorphin (30). Pharmacological studies and data derived from genetically modified mice have established that the endogenous opioid system can modulate feeding behaviour. 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

and colleagues 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 (31). Rather unexpectedly, this study demonstrated that *β-end*-/- male mice had an increased body weight and increased food intake, suggesting that β-endorphin had an anorexic effect in energy homeostasis, more in keeping with POMC-derived melanocortins. Indeed, more recent data from Hayward *et al*. have suggested that the opioid enkephalin plays a more consistent role than β- endorphin in mediating the motivation for food reward (32) 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 upon the central rewarding system to favour fat consumption. Given the localization of POMC and MC4-R in brain reward regions (33-35) and functional interactions with psychostimulants (36;37), there is certainly evidence indicating melanocortins may have a role beyond that of helping to integrate a response to nutrient sensing. Genetically modified mice with selective loss of POMC fragments in specific brain regions may be useful to bring about a fuller clarification of such hypotheses. Finally, the orexin system may also play a role in the fat preference seen in Pomc null mice. We have recently demonstrated an elevation in orexin mRNA expression levels in the lateral hypothalamus of *Pomc* deficient mice (38). Orexin containing neurons project to reward-associated brain region (39) with ICV administration of orexin able to selectively stimulate consumption of a high fat diet (40). Interestingly, this orexigenic effect of orexin appears to be critically dependent upon activation of downstream opioid receptors.

As is evident from the discussion above, animal studies have 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 signalling 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 (16). 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 signalling, 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 signalling 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.

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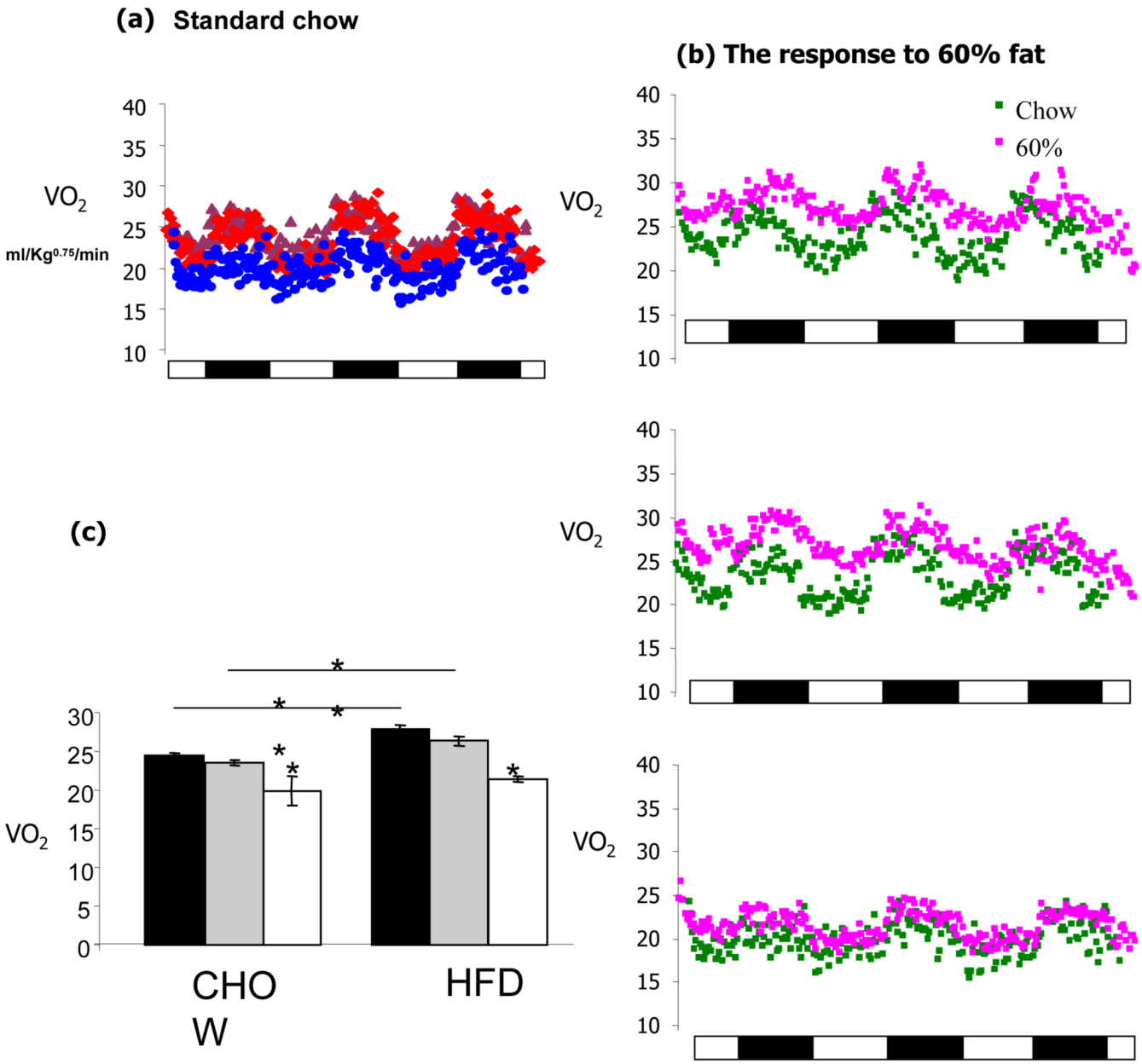

Figure 1.

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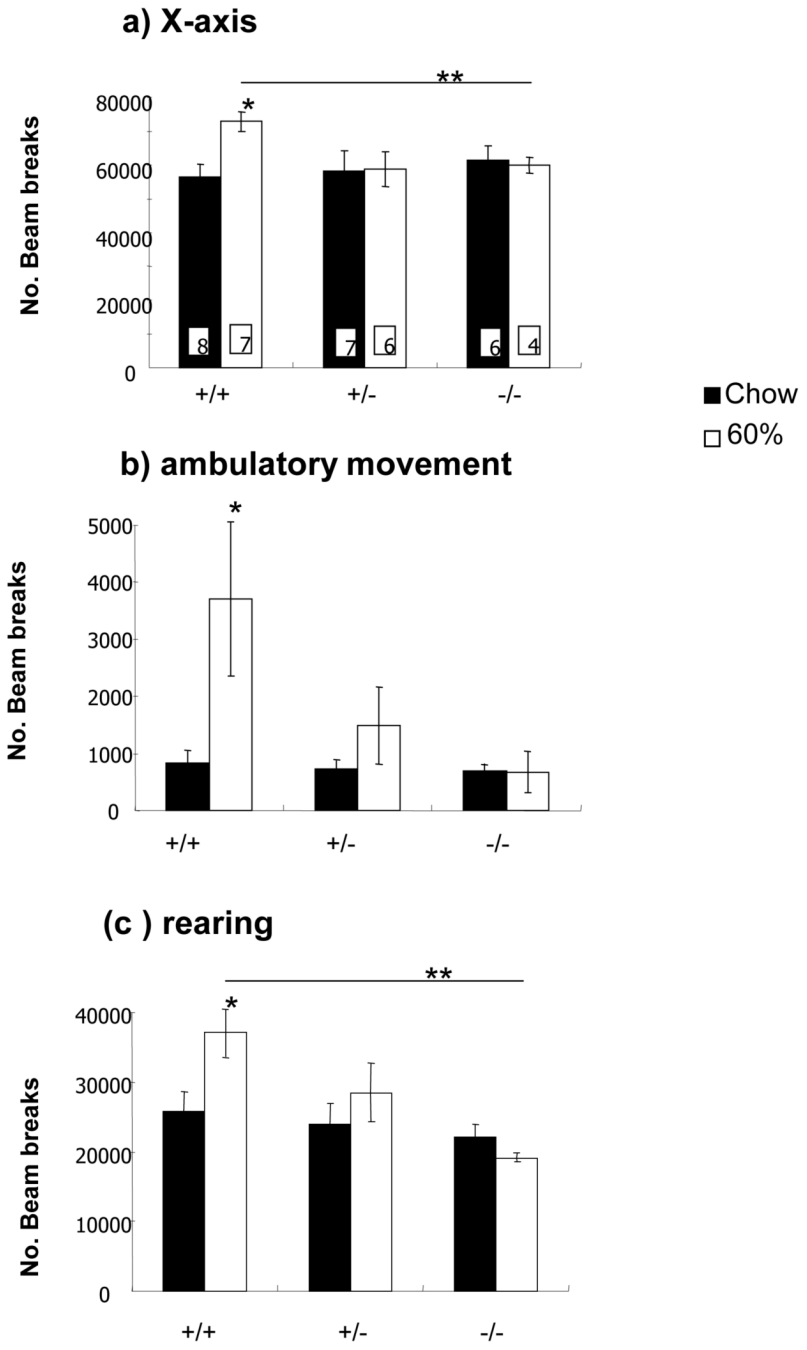

Figure 2. Physical activity when placed on 60% fat diet

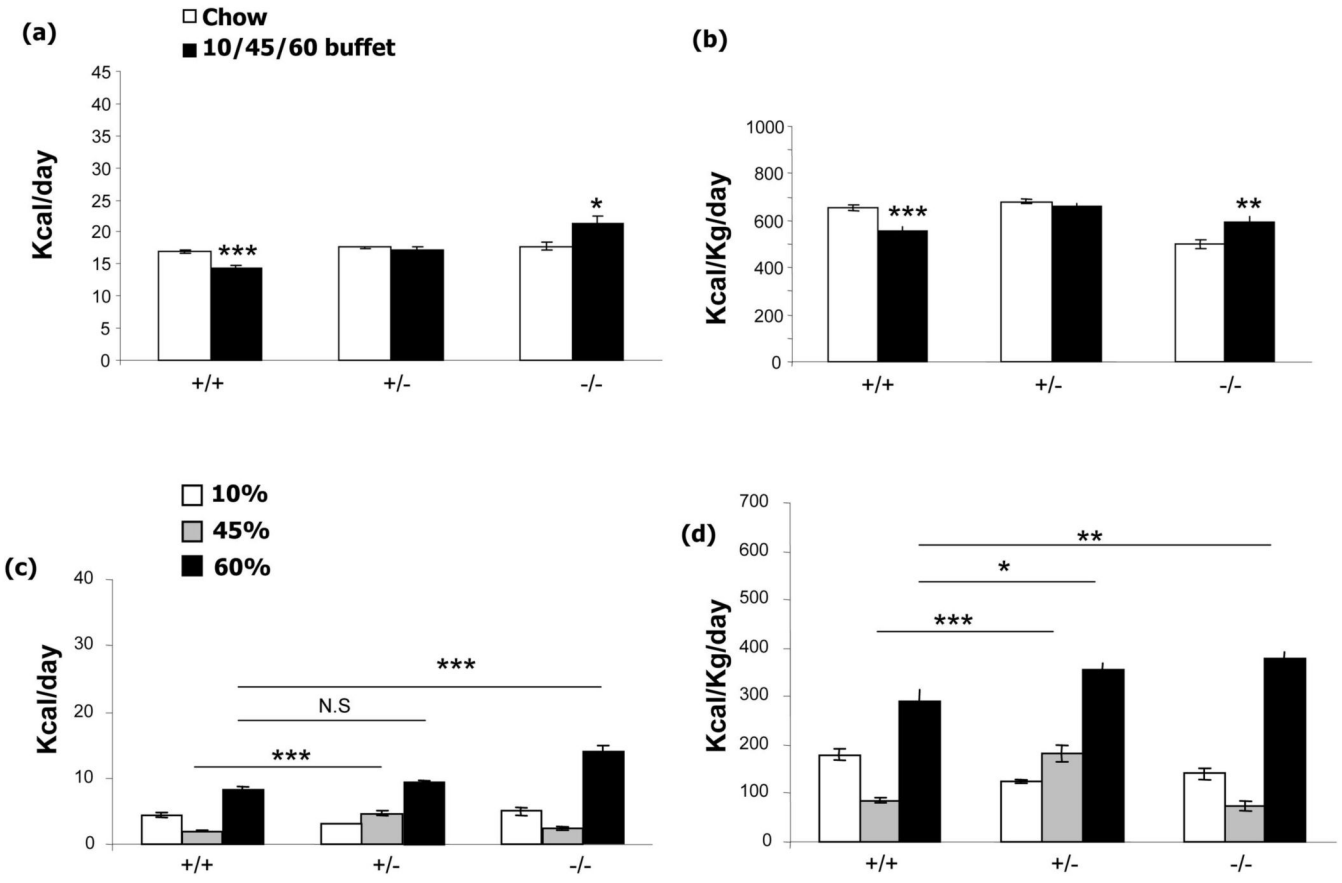

Figure 3.

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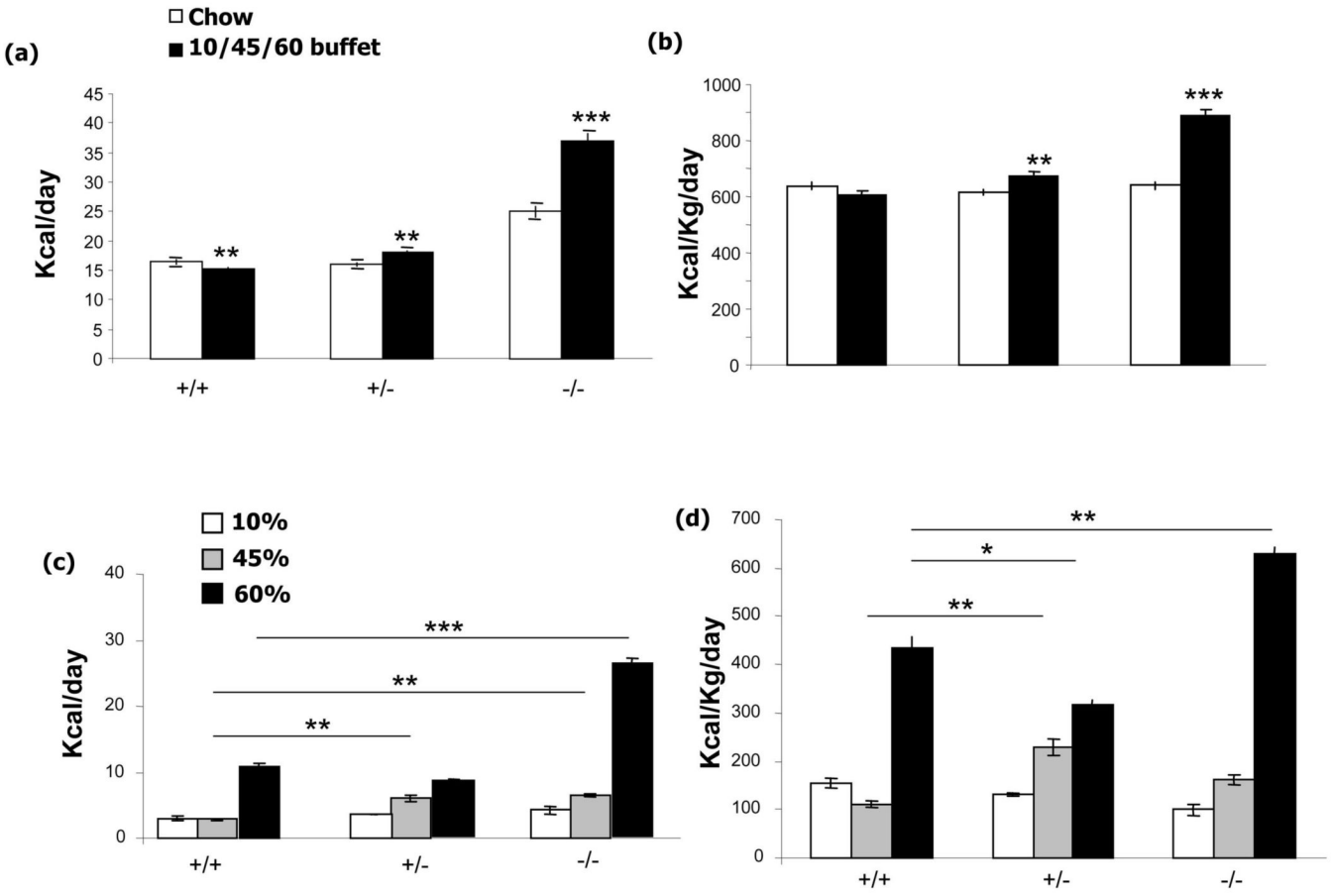

Figure 4.

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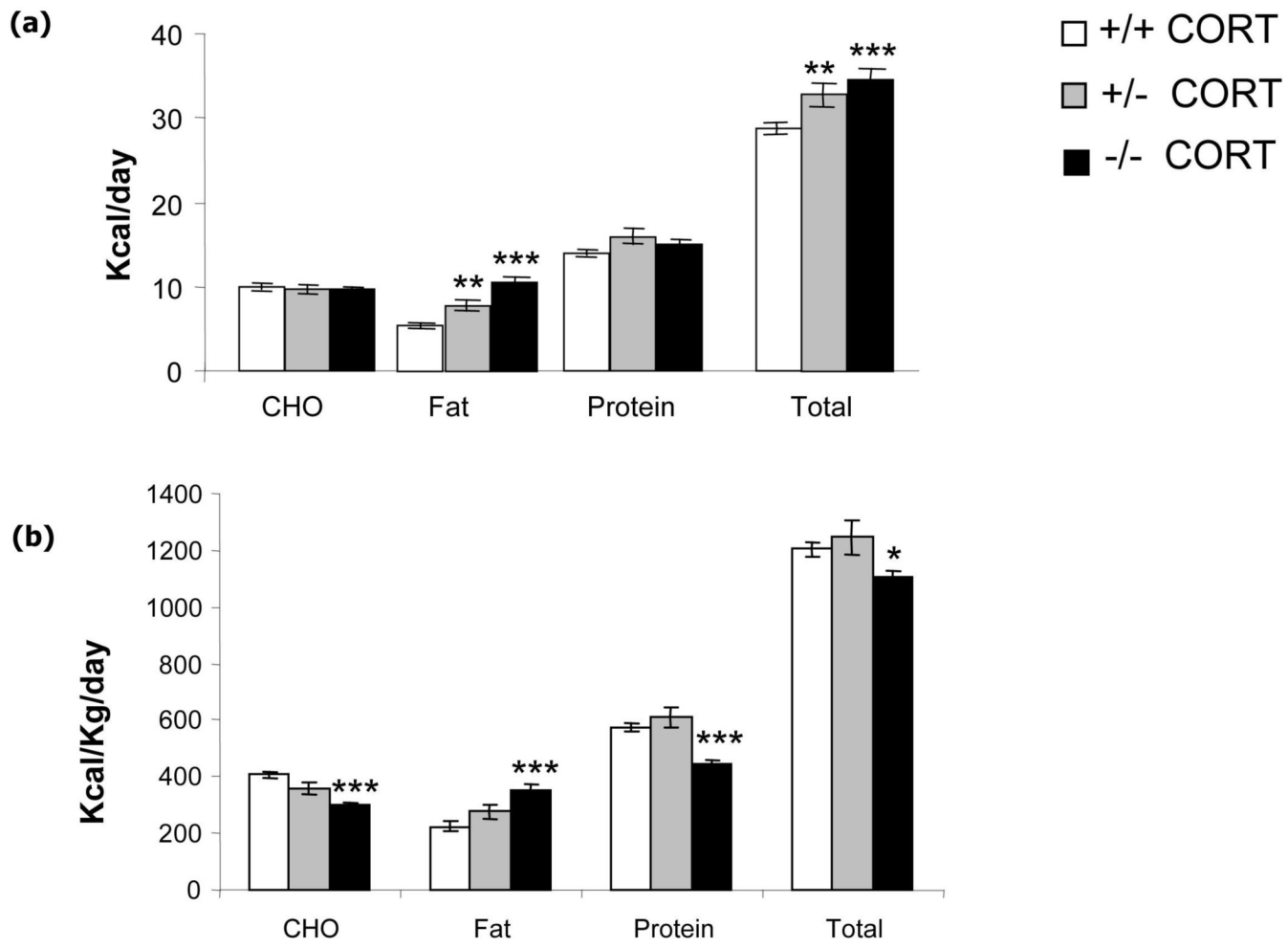

Figure 5.

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Table 1

(a) self-selected-diet test

Diet	10%fat	45%Fat	60% fat	Sweetened	Chow
Protein (Kcal %)	20	20	20	17	27
Carbohydrate (Kcal %)	70	35	20	44	61
Fat (Kcal %)	**10**	**45**	**60**	38	12
Total (Kcal/gm)	3.85	4.73	5.24	4.16	3.61

Table 1

(b) Macronutrient dietary components

Fat component (7.85Kcal/g)
93.6% Lard
6.2% Safflower Oil
0.2% Vitamin mix
Protein component (3.76Kcal/g)
99.8% Casein
(with gelatine)
0.2% Vitamin mix
Carbohydrate component (3.3Kcal/g)
59.8% Flour
28.6% Dextrin
10.4% Sucrose
1% Solka-flock
0.2% Vitamin mix