Sex difference in physical activity, energy expenditure and obesity driven by a subpopulation of hypothalamic POMC neurons

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ABSTRACT

Objective: Obesity is one of the primary healthcare challenges of the 21st century. Signals relaying information regarding energy needs are integrated within the brain to influence body weight. Central among these integration nodes are the brain pro-opiomelanocortin (POMC) peptides, perturbations of which disrupt energy balance and promote severe obesity. However, POMC neurons are neurochemically diverse and the crucial source of POMC peptides that regulate energy homeostasis and body weight remains to be fully clarified.

Methods: Given that a 5-hydroxytryptamine 2c receptor (5-HT2CR) agonist is a current obesity medication and 5-HT2CR agonist’s effects on appetite are primarily mediated via POMC neurons, we hypothesized that a critical source of POMC regulating food intake and body weight is specifically synthesized in cells containing 5-HT2CRs. To exclusively manipulate Pomc synthesis only within 5-HT2CR containing cells, we generated a novel 5-HT2CRfl mice line and intercrossed it with Cre recombinase-dependent and hypothalamic specific reactivatable Pomc mice to restrict Pomc synthesis to the subset of hypothalamic cells containing 5-HT2CRs. This provided a means to clarify the specific contribution of a defined subgroup of POMC peptides in energy balance and body weight.

Results: Here we transform genetically programed obese and hyperinsulinemic male mice lacking hypothalamic Pomc with increased appetite, reduced physical activity and compromised brown adipose tissue (BAT) into lean, healthy mice via targeted restoration of Pomc function only within 5-HT2CR expressing cells. Remarkably, the same metabolic transformation does not occur in females, who despite corrected feeding behavior and normalized insulin levels remain physically inactive, have lower energy expenditure, compromised BAT and develop obesity.

Conclusions: These data provide support for the functional heterogeneity of hypothalamic POMC neurons, revealing that Pomc expression within 5-HT2CR expressing neurons is sufficient to regulate energy intake and insulin sensitivity in male and female mice. However, an unexpected sex difference in the function of this subset of POMC neurons was identified with regard to energy expenditure. We reveal that a large sex difference in physical activity, energy expenditure and the development of obesity is driven by this subpopulation, which constitutes approximately 40% of all POMC neurons in the hypothalamic arcuate nucleus. This may have broad implications for strategies utilized to combat obesity, which at present largely ignore the sex of the obese individual.

Keywords: Pro-opiomelanocortin (Pomc); 5-HT2c receptor; Obesity; Energy expenditure; Brown adipose tissue; Hyperinsulinemia; Sexual dimorphism; Hypothalamus

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1. INTRODUCTION

When energy intake exceeds energetic demands, energy is stored primarily as fat. Excess adipose accumulation is the hallmark feature of obesity. Though men and women are exposed to the same environmental conditions, the World Health Organization (WHO) reports higher rates of obesity in women worldwide, reaching twice the prevalence of men in some regions of the world [1]. Obesity has a significant and widespread impact on human health, placing it at the forefront of healthcare priorities and challenges of this century. Obesity medications are in general prescribed without attention to the sex of the obese individual, implying the absence of sex-based differences in the molecular regulation of energy balance.

Insights from genetic research have led to the discovery of key regulators of energy balance [2], such as the melanocortin peptides encoded by the pro-opiomelanocortin gene (Pomc) [3]. Humans and animals unable to synthesize melanocortins or the receptor through which they primarily signal to influence energy balance, the melanocortin4 receptor (Mc4r/Mc4r), have dramatically increased food intake, reduced physical activity or energy expenditure and developed profound obesity [4,5]. Pomc/Pomc function also has broader application to common obesity; in both high fat diet-induced obesity and middle-age associated obesity, Pomc neuron activity within the arcuate nucleus of the hypothalamus (ARC) is diminished, which has been proposed to have a causal role in the increased acquisition of body weight and adiposity [6–8]. Treatment with a 5-hydroxytryptamine 2c receptor (5-HT2CR) agonist, such as the new obesity medication lorcanserin (Arena Pharmaceuticals), restores diminished Pomc neuron function and improves obesity [9–11]. Furthermore, inactivating 5-HT2CRs specifically on Pomc neurons in mice, a genetic strategy employed to manipulate 5-HT2CR expression, prevents the anorectic effect of 5-HT2CR agonists [12], thereby revealing that 5-HT2CR agonists modulate food intake via POMC neurons. Thus, Pomc peptides are an important driver of body weight and Pomc expressing neurons are amenable to pharmacological manipulation. Here, we sought to clarify the source of Pomc peptides that critically mediate body weight using a newly developed genetic approach.

2. MATERIALS AND METHODS

2.1. Mice

5-HT2CR<sup>Cre</sup> line. 5.6 kb of genomic DNA containing portions of the final exon and the 3' UTR of the murine Htr2c gene was amplified by PCR from R1 ES cells [(129X1/SvJ x 129S1)F1 genetic background] and cloned into a plasmid for insertion of a FRT-NEO-FRT-IRES-CRE cassette between the STOP codon and the polyadenylation site, as previously described [17]. The targeting construct was linearized using NcoI and electroporated into R1 mouse embryonic stem cells at the University of Michigan Transgenic Animal Model Core. Neomycin-resistant clones were analyzed by quantitative real-time PCR [18] for copy number of the native Htr2c allele and further confirmed by Southern blotting using an external probe. Correctly targeted ES cells were injected into C57BL/6J blastocysts to generate chimeras. Male Southern blotting using an external probe. Correctly targeted ES cells Htr2c which they primarily signal to in

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2.2. Immunohistochemistry (IHC)

Tissue was processed for endogenous POMC and for 5-HT2CR<sup>Cre</sup> as previously described [9–11]. Briefly, under deep terminal anesthesia, mice were transcardially perfused with phosphate buffered saline (PBS) followed by 10% neutral buffered formalin (Sigma). Brains were extracted, post-fixed in 10% neutral buffered formalin at 4 °C, cryo-protected in 20% sucrose at 4 °C and then sectioned coronally on a freezing sliding microtome at 25 μm. Tissue was processed for POMC-immunoreactivity (IR) and 5-HT2CR<sup>YFP</sup> (GFP-IR) as previously described [14,15] using rabbit anti-POMC primary antibody (1:1000; H-029-30, Phoenix Pharmaceuticals, Burlingame, CA, USA), chicken anti-GFP (1:500; ab13970, AbCam, Cambridge, UK) and Alexa Fluor secondary antibodies (1:500 A-11012, Life Technologies, Paisley, UK), respectively. Single and dual-labeled POMC-IR and GFP-IR cells were counted in the ARC [16]. Analysis was carried out on 7 levels of ARC (−1.46 to −2.18 from Bregma) for each mouse (n = 4/sex).

2.3. Quantitative PCR

Total RNA was purified from whole hypothalamus, brainstem and interscapular brown adipose tissue (BAT) using RNA STAT 60 (AMS Biotechnology, Abington, UK) according to the manufacturer’s instructions and as previously described (9 months of age; n = 5–9/ genotype/sex) [13]. cDNA was obtained by reverse transcription of 500 ng hypothalamic RNA, 1000 ng brainstem RNA and 500 ng BAT RNA. Real-time PCR analysis of cDNA was performed in duplicate on an ABI Prism 7900 sequence detection system using Taqman or Sybr constructs and as previously described (9 months of age; n = 5–9/ genotype/sex) [13]. cDNA was obtained by reverse transcription of 500 ng hypothalamic RNA, 1000 ng brainstem RNA and 500 ng BAT RNA. Real-time PCR analysis of cDNA was performed in duplicate on an ABI Prism 7900 sequence detection system using Taqman or Sybr assays for POMC (ABI Tagman Gene expression assay Mm00435874_m1), elongation of very long fatty acids-like 3 (Elov3) and peroxisome proliferator-activated receptor gamma coactivator 1alpha (Pgc-1α). Data for levels of target gene mRNAs are expressed in arbitrary units corrected to the geometric average of four housekeeping genes: 18s, 36β, βactin and glyceraldehyde-3-phosphate dehydrogenase (Gapdh). Sequences of primers and probes used are listed in Supplementary Table 1.

2.4. Metabolic profile

Body weight was measured from weaning up to 1 year of age (n = 7– 17/genotype/sex), Home cage 24-h food intake was measured up to 6 months of age (n = 5–9/genotype/sex). At 9 months of age, a more detailed energy balance profile was performed, including light and dark cycle food intake, locomotor activity and energy expenditure assessment using indirect calorimetry in a Metabolic-Trace (Meta-Trace) system (Idea Studio, UK; n = 5–9/genotype/sex). Body composition was also analyzed at 7–9 months of age using dual-energy X-ray absorptiometry (DEXA) Lunar PIXImus2 mouse densitometer (General Electric Medical Systems, Fitchburg, WI, USA; n = 5–11/genotype/sex). Gonadal white adipose tissue (WAT) and interscapular BAT was dissected, fixed in 10% neutral buffered formalin, embedded in
paraffin, cut into 5 µm sections and stained with hematoxylin and eosin. WAT cell diameter (µm) and BAT lipid droplet size (% of total area) was measured on an inverted light microscope (Olympus BX41, Olympus UK Ltd, Southend-on-Sea, UK) using CellD Olympus Software (Shinjuku, Tokyo, Japan). Analysis was carried out on 5-9 sections for each mouse (9 months of age; n = 4–6/genotype/sex).

Blood samples were taken from the left ventricle in 6 h light cycle fasted terminal anesthetized mice (9 months of age; n = 5–9/genotype/sex) (phenobarbital sodium (Dolethal); Vétoquinol, UK). Insulin and leptin were assayed using a two-plex electrochemical luminescence microtiter plate immunoassay (MesoScale Discovery, Gaithersburg, MD, USA).

2.5. Insulin tolerance test
Mice (6–8 months of age; n = 5–9/genotype/sex) were fasted for 6 h before blood. Blood was sampled from tail vein immediately prior to insulin (1.1 U/kg IP, males; 0.8 U/kg IP, females) bolus, and 15, 30, 45, 60 and 90 min following bolus administration. Blood glucose was analyzed using AlphaTrak glucometer (Chicago, IL, USA).

2.6. Statistics
Data were analyzed with One-way, Two-way or Repeated Measures ANOVA or ANCOVA followed by Tukey post hoc tests. For all analyses, significance was assigned at P < 0.05. Data are presented as mean ± SEM.

3. RESULTS AND DISCUSSION

3.1. Generation of 5-HT2CRFlh line
To clarify the source of POMC peptides that underpin energy balance and body weight regulation, we utilized a Cre recombinase-dependent and ARC specific reactivatable PomcNeo line [13] to restore Pomc synthesis within the discrete subset of cells expressing 5-HT2CR. To achieve this, we first generated a 5-HT2CRFlh line. To confirm that 5-HT2CRFlh is contained in cells expressing endogenous 5-HT2CR, 5-HT2CRFlh mice were intercrossed with B6.129X1-Gt(RosA)26Sortm1(EYFP)Cos/J (Rosas2EYFP™) mice, which have a loxP-flanked STOP sequence followed by a YFP gene inserted into the 3′flanking region of the ROSA26sor locus. Interbreeding with 5-HT2CRFlh removes the STOP sequence and YFP is visualized in 5-HT2CRFlh expressing cells (Figure S1A).

Performing immunofluorescent staining in the ARC for YFP-immunoreactive (IR) and fluorescent in situ hybridization (FISH) to label endogenous 5-HT2CR mRNA revealed that the majority of Cre-containing cells expressed endogenous 5-HT2CR mRNA (Figure S1B).

3.2. Anatomical localization and genetic manipulation of subset of hypothalamic Pomc co-expressing 5-HT2CR
To determine the anatomical localization of the subset of ARC POMC neurons specifically co-expressing 5-HT2CR, dual-immunofluorescent analysis was performed for GFP-IR and Pomc-IR in the 5-HT2CRFlh line. This analysis revealed that approximately 40% of ARC POMC neurons co-express 5-HT2CR in male and female mice (Figure 1A–C).

This co-expression profile is similar to that previously observed in rats [9]. Further analysis of anatomical co-localization indicated that this 40% co-expression rate was consistent across the rostral-caudal extent of the ARC (Figure 1C).

To investigate the physiological importance of this specific source of POMC peptides in the regulation of energy balance and body weight, we intercrossed a Cre recombinase-dependent and ARC specific reactivatable PomcNeo line to restore Pomc expression only within cells expressing 5-HT2CR (Figure 1D). As expected, PomcNeo mice had no detectable hypothalamic Pomc mRNA, whereas Pomc was restored by 44% in male and 41% in female Pomc5-HT2CR mice (Figure 1E,F). This Pomc reactivation level is consistent with the anatomical co-localization determined above. No differences in Pomc expression among genotypes were detected in the brainstem, which includes the nucleus tractus solitarius (Figure S1C). Therefore, we created a genetic means to probe the specific function of a subset of ARC POMC neurons in the regulation of whole body energy balance and body weight.

3.3. Hypothalamic Pomc expressed exclusively within 5-HT2CR containing cells modulates energy intake in male and female mice
Given that current obesity medication 5-HT2CR agonist lorcaserin improves obesity by influencing appetite, but not energy expenditure [19], and that 5-HT2CR agonists reduce food intake via increased activity of POMC neurons [9,20], we surmised that POMC peptides synthesized exclusively in neurons expressing 5-HT2CRs perform an essential role in the regulation of energy intake. To investigate this, we compared 24-hour food intake in PomcNeo, 5-HT2CR+/+, wild type and mice with restored Pomc expression only in cells expressing 5-HT2CRs (Pomc5-HT2CR). As expected, male and female PomcNeo mice exhibited significant hyperphagia (Figure 2A,B; Figure S2A–C, S2G–I), consistent with previous reports [13]. Restoration of hypothalamic Pomc expression only within 5-HT2CR cells normalized 24-h food intake in both male and female mice (Figure 2A,B). Additional circadian analysis revealed that PomcNeo energy intake was significantly higher during the dark cycle in male (Figure S2B,C) and female (Figure S2H,I) mice, and this was normalized by the restoration of Pomc expression in 5-HT2CR cells in both sexes. Next, we tracked 24-h food intake in male and female mice by genotype until 6 months of age. We observed that PomcNeo hyperphagia persisted with age, whereas Pomc5-HT2CR mice continued to consume food at a level consistent with control 5-HT2CR+/+ and wild type littermates (Figure S2A,G). Consequently, these data indicate that POMC peptides synthesized exclusively in neurons expressing 5-HT2CRs are sufficient to mediate POMC’s effects on food intake.

3.4. Unexpected sex difference in physical activity and energy expenditure modulated by hypothalamic Pomc exclusively expressed within 5-HT2CR containing cells
Analysis of energy homeostasis in Pomc5-HT2CR mice uncovered a substantial and unexpected sex difference in the molecular regulation of physical activity and energy expenditure. In both males and females, PomcNeo mice exhibited significantly reduced 24-h locomotor activity, which was accounted for by a reduction in activity within the dark, but not light cycle (Figure 2C,D; Figure S2E,F,K,L). However, restoration of Pomc5-HT2CR function fully normalized physical activity only in male mice (Figure 2C; Figure S2E,F). While a significant effect of body fat mass on total daily energy expenditure was detected in males (Figure 2E), this did not vary by genotype. Conversely, in female mice, restoration of Pomc5-HT2CR function had no impact on reduced physical activity (Figure 2D, Figure S2K,L) or total daily energy expenditure (Figure 2F). Thus, in females, a significant genotype effect of fat mass on total daily energy expenditure was observed. A general linear model using both fat and lean mass explained 80% of the variance in energy expenditure in pooled female wild type and 5-HT2CR+/+ mice. Both Pomc5-HT2CR and PomcNeo female mice displayed significantly reduced total daily energy expenditure compared to wild type and 5-HT2CR+/+ female siblings (Tukey’s post hoc P < 0.01) but did not differ from each other (P > 0.05). We next explored the impact of genotype and body composition on resting metabolic rate in female mice (Figure 2G).
A general linear model using both fat and lean mass explained 91.8% of the variance in energy expenditure in pooled female wild type and 5-HT2CRCRE mice. These results reveal that both PomcNEO and Pomc5-HT2CR female mice had significantly reduced resting energy expenditure compared to wild type and 5-HT2CRCRE siblings (Tukey’s post hoc, *P < 0.01) but did not differ from each other (*P > 0.05).

Brown adipocytes contain a large number of mitochondria for thermogenesis, and nutrient oxidation in BAT can account for up to 60% of the total energy expenditure of mice [21,22]. Melanocortinergic regulation of BAT thermogenesis and energy expenditure has been reported via Mc4rs expressed by cholinergic preganglionic sympathetic neurons within the intermediolateral nucleus of the thoracic spinal cord (IML) [23–25]. The IML is directly innervated by ARC POMC neurons [26,27] and postganglionic neurons innervating brown adipose tissue receive projections from the IML [28–30], suggesting a pathway through which POMC neurons may participate in the regulation of BAT thermogenesis and energy expenditure.

We therefore next examined whether Pomc exclusively synthesized within 5-HT2CR expressing neurons influences BAT function. In male and female mice, genetic inactivation of ARC Pomc was associated with increased lipid accumulation in BAT (Figure 2H–J) and reduced expression of Pgc-1a and Elovl3, mitochondrial genes important for BAT thermogenesis (Figure 2K–L). In male mice, restoration of Pomc5-HT2CR function normalized BAT lipid accumulation (Figure 2H) and Pgc-1a and Elovl3 expression (Figure 2K). In contrast, Pomc5-HT2CR female mice displayed an increase in BAT lipid content (Figure 2H,J) and a downregulation in thermogenic gene expression (Figure 2L) compared to control littermates. These data suggest that impaired BAT thermogenesis in female PomcNEO and Pomc5-HT2CR mice contributes to the observed reduction in whole body energy expenditure.

Taken together, these data signify that ARC POMC regulates both physical activity related energy demands and resting metabolism and that restoration of POMC function within the subset of 5-HT2CR expressing neurons is not sufficient to appropriately regulate energy expenditure or BAT function in female mice. No differences in respiratory exchange ratio (RER) were detected by genotype in either sex, and, in the combined data set, there was no sex effect on the RER (Figure S2D,J). However, we uncovered an unexpected sex difference in the molecular underpinnings driving physical activity and determined that Pomc5-HT2CR have a broader, sex-specific, function in the regulation of energy usage.
3.5. Unexpected sex difference in body weight regulation modulated by hypothalamic Pomc exclusively expressed within 5-HT2CR containing cells

We next investigated the ramifications of this discrete source of ARC POMC peptides on body weight and adiposity. Consistent with the metabolic consequence of restored energy balance in male Pomc5-HT2CR mice, a prevention of obesity in male Pomc5-HT2CR mice was observed compared to PomcNEO littermates (Figure 3A). Furthermore, male Pomc5-HT2CR mice displayed levels of lean mass (Figure 3C,D), fat mass (Figure 3F) and leptin levels (Figure S3A) that were comparable to control 5-HT2CRone and wild type littermates. Likewise, male
HT2CRs was not sufficient to regulate adiposity in female mice. In contrast, ARC Pomc synthesized within neurons expressing 5-HT2CRs was geneti
cally predisposed to develop obesity compared to wild type and 5-HT2CRCRE female siblings (Figure 3B), with increased fat mass (Figure 3C,G) and displayed significantly larger white adipocytes (Figure 3H,J). This elevation in body weight and fat mass was less severe than that produced by full ARC Pomc nulls, though both PomcNEO and Pomc5-HT2CR mice showed comparable increases in white adipocyte size. However, only PomcNEO mice exhibited statistically increased lean mass (Figure 3E) and leptin levels (Figure S3B) and this was corrected in Pomc5-HT2CR mice. These data reveal that the subpopulation of ARC POMC expressed within 5-HT2CR containing neurons is sufficient to regulate whole body energy balance, body weight and adiposity in male, but not female, mice.

3.6. Hypothalamic Pomc expressed exclusively within 5-HT2CR containing cells modulates insulin sensitivity in male and female mice

Recent reports revealed that though PomcNEO mice exhibit insulin resistance, they display normal glucose levels and improved glucose tolerance, primarily by increasing glycosuria [31,32]. We next considered whether Pomc synthesized exclusively within 5-HT2CR expressing cells is sufficient to normalize Pomc hyperinsulinemia and impaired insulin tolerance. As expected, severely hyperphagic and obese male and female PomcNEO mice exhibited pronounced hyperinsulinemia (Figure 4A,B) with normal fasting blood glucose (Figure 4C,D), a pattern indicating insulin resistance compensated by increased insulin secretion from pancreatic beta cells. Consistent with the metabolic consequence of normalized energy balance and adiposity in male Pomc5-HT2CR mice, hyperinsulinemia was corrected in male Pomc5-HT2CR mice compared to PomcNEO littersmates (Figure 4A). Despite disrupted energy balance and pronounced obesity, female Pomc5-HT2CR mice also displayed insulin levels comparable to wild type and 5-HT2CRCRE siblings (Figure 4B). Examining insulin sensitivity further, we found that both male (Figure 4E,F) and female (Figure 4G,H) PomcNEO mice exhibited impaired responses in an insulin tolerance test compared with control littersmates, and this was normalized by restoring Pomc in 5-HT2CR expressing cells. Taken together, these results indicate that despite a sex difference in energy balance and obesity, Pomc synthesized in 5-HT2CR expressing cells is sufficient to mediate POMC’s effects on insulin sensitivity in both male and female mice.

These data reveal a discrete and specific sex difference in the regulation of body weight, fat accumulation and adipocyte size driven by the subpopulation of hypothalamic POMC peptides exclusively synthesized in neurons expressing 5-HT2CRs. Interestingly, POMC within the subpopulation of ARC leptin receptor expressing neurons was sufficient to normalize energy balance and body weight in both male and female PomcNEO mice [32]. Thus, these data reveal a specific and functionally distinct role of POMC within the subset of cells examined here, providing further support for the functional heterogeneity of ARC derived POMC peptides.

Figure 3: Subpopulation of Pomc differentially modulates body weight and adiposity in male and female mice. (A,B) Body weight (male, F3,19 = 60.80, P < 0.001); female F3,19 = 9.43, P < 0.001; female F3,19 = 42.04, P < 0.001). (C, F, G) Fat mass (male F3,20 = 13.98, P < 0.001; female F3,13 = 11.87, P < 0.001) were increased in ARC Pomc null (PomcNEO) mice and normalized by restoration of Pomc in 5-HT2CR neurons (Pomc5-HT2CR) in male but not female mice. However, female Pomc5-HT2CR mice still exhibited significantly greater body weight and fat mass, but not adipocyte size, compared with Pomc5-HT2CR mice. (D, E) Both male and female PomcNEO mice exhibited significantly greater lean mass compared to Pomc5-HT2CR mice and controls. Scale bar, 2000 μm.

Figure 4: Arnt1 is sufficient to restore energy balance, body weight and adiposity in male and female mice. (A) Body weight (male, F3,19 = 60.80, P < 0.001); female F3,19 = 9.43, P < 0.001; female F3,19 = 42.04, P < 0.001). (C, F, G) Fat mass (male F3,20 = 13.98, P < 0.001; female F3,13 = 11.87, P < 0.001) were increased in ARC Pomc null (PomcNEO) mice and normalized by restoration of Pomc in 5-HT2CR neurons (Pomc5-HT2CR) in male but not female mice. However, female Pomc5-HT2CR mice still exhibited significantly greater body weight and fat mass, but not adipocyte size, compared with Pomc5-HT2CR mice.
The sex difference in energy storage is consistent with significantly reduced energy usage in female Pomc5-HT2CR mice. Given that food intake is normalized in female Pomc5-HT2CR mice, this genetic model reflects a new example of the cumulative impact of reduced physical activity and energy expenditure on body weight and adiposity with time. It may be noted that in the PomcNEO mice, the impact on energy expenditure was much greater in the females compared to males (compare Figure 2E,F) and the corresponding fat mass also much greater (compare Figure 3F–G). Therefore, the localized synthesis of Pomc peptides within a subset of neurons in the discrete brain region the ARC produces a substantial difference in the magnitude of adiposity accumulation in male and female mice. This phenotype cannot simply be explained by impairing Pomc activity by estrogens, because the inactivation of estrogen receptor specifically within ARC Pomc neurons does not impact fat mass or reduce energy expenditure [33].

4. CONCLUSIONS

These findings support the functional heterogeneity of ARC Pomc, revealing that the source synthesized within 5-HT2CR expressing neurons is sufficient to regulate energy intake and insulin sensitivity in male and female mice. Moreover, these data provide evidence for a specific neurochemical basis for levels of reduced physical activity and reveal that the molecular underpinnings of the impetus to engage in physical activity are differentially modulated in males and females. However, physical activity comprises only one means of utilizing energy and our data further show that the same neuronal population plays a key role, modulated by sex, in the regulation of resting rates of energy expenditure. Consequently, these data uncovered an unexpected sex difference, mediated by Pomc, in total energy expenditure, thermogenic activity of BAT and adiposity. These findings provide evidence that males and females are hardwired differently in their regulation of energy balance. Given the reported reduction of Pomc neuron activity in middle age in mice [7], these data may have translational relevance by providing a potential molecular explanation for the global sex differences in obesity prevalence. Finally, these data may have broad implications for future sex-specific strategies in treating overweight and obesity.

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CONFLICTS OF INTEREST

The authors declare that no conflict of interest exists.

APPENDIX A. SUPPLEMENTARY DATA

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.molmet.2016.01.005.
REFERENCES

[1] World Health Organisation, 2014. Global Health Observatory (GHO) obesity situation and trends.
[2] Williams, K.W., Elmquist, J.K., 2012 Oct. From neuroanatomy to behavior: central integration of peripheral signals regulating feeding behavior. Nature Neuroscience 15(10):1350–1355.
[3] Ellacott, K.L., Cone, R.D., 2006 Jul. The role of the central melanocortin system in the regulation of food intake and energy homeostasis: lessons from mouse models. Philosophical Transactions of the Royal Society B: Biological Sciences 361(1471):1265–1274.
[4] Yeo, G.S., Heisler, L.K., 2012 Oct. Unraveling the brain regulation of appetite: lessons from genetics. Nature Neuroscience 15(10):1343–1349.
[5] Ramachandrappa, S., Farooqi, I.S., 2011 Jun. Genetic approaches to understanding human obesity. Journal of Clinical Investigation 121(6):2080–2086.
[6] Mori, H., Inoki, K., Münzberg, H., Opland, D., Faouzi, M., Villanueva, E.C., et al., 2009 Apr. Critical role for hypothalamic mTOR activity in energy balance. Cell Metabolism 9(4):362–374.
[7] Yang, S.B., Tien, A.C., Boddupalli, G., Xu, A.W., Jan, Y.N., Jan, L.Y., 2012 Aug. Rapamycin ameliorates age-dependent obesity associated with increased mTOR signaling in hypothalamic POMC neurons. Neuron 75(3):425–436.
[8] Newton, A.J., Hess, S., Paeger, L., Vogt, M.C., Fleming Lascano, J., Nillni, E.A., et al., 2013 Jan. AgRP innervation onto POMC neurons increases with age and is accelerated with chronic high-fat feeding in male mice. Endocrinology 154(1):172–183.
[9] Heisler, L.K., Cowley, M.A., Tecott, L.H., Fan, W., Low, M.J., Smart, J.L., et al., 2002 Jul. Activation of central melanocortin pathways by fenfluramine. Science 297(5581):609–611.
[10] Lam, D.D., Przydzial, M.J., Ridley, S.H., Yeo, G.S., Rochford, J.J., Opland, D., Louis, G.W., Garfield, A.S., Patterson, C., Skora, S., Gribble, F.M., Reimann, F., Evans, M.L., et al., 2012 Nov. Serotonin 5-HT2C receptor agonist promotes hypophagia via downstream activation of melanocortin 4 receptors. Endocrinology 149(3):1232–1238.
[11] Burke, L.K., Deslikova, B., D’Agostino, G., Garfield, A.S., Farooq, G., Balthasar, N., Olson, D., Scott, M., Berglund, E.D., Lee, C.E., et al., 2013 Jan. Melanocortin 4 receptors reciprocally regulate sympathetic and parasympathetic preganglionic neurons. Cell Metabolism 17(3):591–605.
[12] Balmes, J., 2012 Oct. From neuroanatomy to behavior: central integration of peripheral signals regulating feeding behavior. Nature Neuroscience 15(10):1350–1355.
[13] Soliman, G.A., Ishida-Takahashi, R., Gong, Y., Jones, J.C., Leshan, R.L., Saunders, T.L., et al., 2007 Oct. A simple qPCR-based method to detect correct insertion of homologous targeting vectors in murine ES cells. Transgenic Research 16(5):665–670.
[14] Martin, C.K., Redman, L.M., Zhang, J., Sanchez, M., Anderson, C.M., Smith, S.R., et al., 2011 Mar. Lorcaserin, a 5-HT(2C) receptor agonist, reduces body weight by decreasing energy intake without influencing energy expenditure. Journal of Clinical Endocrinology and Metabolism 96(3):837–845.
[15] Deslikova, B., Garfield, A.S., Shaw, J., Evans, M.L., Burdakov, D., Billups, B., et al., 2013 Jun. 5-HT2C receptor agonist anorectic efficacy potentiated by 5-HT1B receptor agonist coapplication: an effect mediated via increased proportion of pro-opiomelanocortin neurons activated. Journal of Neuroscience 33(23):9800–9804.
[16] Harms, M., Seale, P., 2013 Oct. Brown and beige fat: development, function and therapeutic potential. Nature Medicine 19(10):1252–1263.
[17] Virtue, S., Vidal-Puig, A., 2013. Assessment of brown adipose tissue function. Frontiers in Physiology 4:128.
[18] Swanson, L.W., Kuppers, H.G., 1980 May. A direct projection from the ventromedial nucleus and retrochiasmatic area of the hypothalamus to the medulla and spinal cord of the rat. Neuroscience Letters 15(1):134–135.
[19] Harms, M., Seale, P., 2013 Oct. Brown and beige fat: development, function and therapeutic potential. Nature Medicine 19(10):1252–1263.
[20] Harms, M., Luthold, U., 2013. Assessment of brown adipose tissue function. Frontiers in Physiology 4:128.
[21] Rossi, J., Balthasar, N., Olson, D., Scott, M., Berglund, E., Lee, C.E., et al., 2011 Feb. Melanocortin-4 receptors expressed by cholinergic neurons regulate energy balance and glucose homeostasis. Cell Metabolism 13(2):195–204.
[22] Berglund, E.D., Liu, T., Kong, X., Sohn, J.W., Vong, L., Deng, Z., et al., 2014 Jul. Melanocortin 4 receptors in autonomic neurons regulate thermogenesis and glycemia. Nature Neuroscience 17(7):911–913.
[23] Sohn, J.W., Harris, L.E., Berglund, E.D., Liu, T., Vong, L., Lowell, B.B., et al., 2013 Jan. Melanocortin 4 receptors reciprocally regulate sympathetic and parasympathetic preganglionic neurons. Cell 152(3):612–619.
[24] Elias, C.F., Lee, C., Kelly, J., Aschkenasi, C., Ahima, R.S., Caccamo, P.R., et al., 1998 Dec. Leptin activates hypothalamic CART neurons projecting to the spinal cord. Neuron 21(6):1375–1385.
[25] Swanson, L.W., Kuppers, H.G., 1980 May. A direct projection from the ventromedial nucleus and retrochiasmatic area of the hypothalamus to the medulla and spinal cord of the rat. Neuroscience Letters 17(3):307–312.
[26] Banesh, M., Song, C.K., Bartness, T.J., 1999 Jun. CNS origins of the sympathetic nervous system outflow to brown adipose tissue. American Journal of Physiology 276(6 Pt 2):R1569–R1578.
[27] Rothwell, N.J., Stock, M.J., 1987. Effect of diet and fenfluramine on energy balance and glucose homeostasis. Journal of Physiology 276(6 Pt 2):R1569–R1578.
[28] Vanes, L.W., Flier, J.S., 1997. Brown adipose tissue, beta 3-adrenergic receptors, and obesity. Annual Review of Medicine 48:307–316.
[29] Chhabra, K.H., Adams, J.M., Fagel, B., Lam, D.D., Qi, N., Rubinstein, M., et al., 2014 Jun. Leptin activates hypothalamic CART neurons projecting to the spinal cord. Neuron 21(6):1375–1385.
[30] Lowell, B.B., Flier, J.S., 1997. Brown adipose tissue, beta 3-adrenergic receptors, and obesity. Annual Review of Medicine 48:307–316.
[31] Chhabra, K.H., Adams, J.M., Fagel, B., Lam, D.D., Qi, N., Rubinstein, M., et al., 2015 Oct. Hypothalamic POMC-deficiency improves glucose tolerance despite insulin resistance by increasing glycosuria. Diabetes.
[32] Lam, D.D., Attard, C.A., Mercer, A.J., Myers, M.G., Rubinstein, M., Low, M.J., 2015 Apr. Conditional expression of Pomp in the Lepr-positive subpopulation of POMC neurons is sufficient for normal energy homeostasis and metabolism. Endocrinology 156(4):1292–1302.
[33] Xu, Y., Nedungadi, T.P., Zhu, L., Sobhani, N., Irani, B.G., Davis, K.E., et al., 2011 Oct. Distinct hypothalamic neurons mediate estrogenic effects on energy homeostasis and reproduction. Cell Metabolism 14(4):453–465.