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The metabolic phenotype of SCD1-deficient mice is independent of melanin-concentrating hormone

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Abstract

We propose that deletion of pro-melanin-concentrating hormone (pmCH) would increase energy expenditure and further improve glucose tolerance in mice lacking stearoyl-coA desaturase-1 (SCD1). To test our hypothesis, we bred and metabolically challenged *Pmch*^{−/−}; *Scd1*^{−/−} double-knockout mice, with comparison to *Pmch*^{−/−} mice; *Scd1*^{−/−} mice and C57Bl/6J controls. Deletion of both *Pmch* and *Scd1* increased both food intake and energy expenditure relative to control mice. *Pmch*^{−/−}; *Scd1*^{−/−} double-knockout mice had improved glucose tolerance relative to control mice. The majority of the metabolic effects were contributed by inactivation of the *Scd1* gene. We conclude that the increased food intake and increased energy expenditure of *Scd1*^{−/−} mice are independent of the neuropeptide melanin-concentrating hormone.

Keywords

Stearoyl-CoA desaturase-1; Energy metabolism; Melanin-concentrating hormone; Indirect calorimetry

1. Introduction

Leptin is an adipocyte-derived hormone that regulates energy intake, energy expenditure and substrate metabolism [23,25]. When circulating leptin levels decrease, the body acts to replenish energy stores by increasing food intake and decreasing energy expenditure. Leptin therapy reverses obesity in leptin-deficient mice and humans [5,7,14], but variable effects were seen among humans with common obesity [11]. Clinical interest in the leptin–melanocortin pathway remains high, and molecules downstream of leptin signaling have

become attractive candidates for drug development. Two molecules targeted by the leptin-responsive pathway are SCD-1 and pMCH.

Stearoyl-CoA desaturase-1 (SCD-1) is a key lipogenic enzyme involved in the biosynthesis of monounsaturated fatty acids [13]. *Scd1* is required for the full obese phenotype of *ob/ob* mice to manifest, and *Scd1*^{-/-} mice exhibit increased energy expenditure and hyperphagia [2]. Mice deficient in *Scd1* have decreased fat mass [2,13], reduced serum leptin levels [1] and improved insulin sensitivity [6]. *Scd1* is overexpressed in the livers of hyperphagic *ob/ob* mice, and *Scd1*-deficient *ob/ob* mice are even more hyperphagic than are *ob/ob* control mice [3,13]. *Scd1*^{-/-}; *ob/ob* mice also show increased lean mass, and a dramatic decrease in fat mass [3,13]. In fact, ablation of *Scd1* reduces obesity of *ob/ob* mice to a degree comparable with leptin replacement [2]. Removal of *Scd1* from *ob/ob* mice also normalizes hepatic lipid storage, a finding that proves liver *Scd1* is a target of leptin signaling [1]. However, *Scd1*^{-/-}; *ob/ob* mice have impaired insulin sensitivity relative to *ob/ob* mice, suggesting an uncoupling of the effects of SCD1 deficiency on body weight from those on glucose disposal in the *Scd1*^{-/-}; *ob/ob* model [12].

Prepro-melanin-concentrating hormone (pMCH) is a hypothalamic neuropeptide that, like *Scd1*, plays a crucial role in energy homeostasis [10]. The arcuate nucleus of the hypothalamus is a major center for coordination of the central nervous system (CNS) response to leptin [19]. Orexigenic neurons in the lateral hypothalamus that express pMCH project widely throughout the cortex [18]. Mice deficient in *Pmch* have hypophagia and/or increased energy expenditure, depending on the genetic background [10,21]. Investigation of the relationship between *Pmch* and leptin using double-knockout mice found that *Pmch* deficient *ob/ob* mice have attenuated weight gain (vs. *ob/ob* mice on a chow diet), but remain hyperphagic [20]. *Pmch*^{-/-}; *ob/ob* mice also have decreased fat mass and increased lean mass when compared with *ob/ob* mice [20]. *Pmch* deficient and *Pmch*^{-/-}; *ob/ob* mice are lean secondary to increased energy expenditure, a fact which confirms the role for *Pmch* downstream of leptin [20]. *ob/ob* mice overexpress *Pmch* in the hypothalamus [18] and also overexpress *Scd1* in the liver [2]. Furthermore, genetic ablation of *Pmch* attenuates *Scd1* overexpression in *ob/ob* mice [20]. We hypothesized that *Pmch* is a key link between leptin and *Scd1*. We further hypothesized that genetic removal of *Pmch* and *Scd1* *in vivo* should improve the body's metabolic profile over and above the improvements seen with knockout of the individual molecules. We therefore set out to examine energy balance phenotypes in *Pmch*^{-/-}; *Scd1*^{-/-} mice.

2. Materials and methods

2.1. Mice and diets

Mice carrying the *Scd1*^{ab-2J} null allele [22] were backcrossed to C57BL/6J for 10 generations and then crossed to mice lacking the gene for prepro-MCH [21] to create double-heterozygous *Pmch*^{+/-}; *Scd1*^{+/-} mice. Double-heterozygotes were bred with each other, and the *Pmch*^{-/-}; *Scd1*^{+/-} offspring were intercrossed to produce *Pmch*^{-/-}; *Scd1*^{-/-} double-knockout (dKO) mice. *Pmch*^{+/-}; *Scd1*^{-/-} parents also produced *Pmch*^{+/+}; *Scd1*^{-/-} (*Scd1*^{-/-} or sKO) mice. *Pmch*^{+/-}; *Scd1*^{+/+} mice were also interbred to produce *Pmch*^{-/-};

Scd1^{+/+} (*Pmch*^{-/-} or pKO) mice and *Pmch*^{+/+}; *Scd1*^{+/+} (wild-type or WT) controls. Female mice were used for the following experiments. Animals were housed at the Child and Family Research Institute under an alternating 12:12-h light–dark cycle. Animals received a standard chow (LabDiet 5010 Autoclavable Rodent Diet; PMI Nutrition International, Richmond, IN). All experimental procedures were approved by the University of British Columbia's Animal Care Committee.

2.2. Genotyping

Genotypes were confirmed by PCR amplification of mouse tail genomic DNA. In *Pmch*^{-/-} mice, primers targeting the PGK-neo cassette [21] were used for PCR. The primers were: for *Pmch* 5'-GAATTTGGAAGATGACATAGTAT-3' (sense) and 5'-CAGCCCCGGTGAGTTACAAGATTCT-3' (antisense); for PGK-neo, 5'-CGGGTAGGGGAGGCGCT-3' (sense) and 5'-GCGCAAGGAACGCCCGTCGTG-3' (antisense) [20,21]; for the *Scd1* 5-ATCATACTGGTTCCCTCCTGCAAGC-3' (sense) and 5'-GAGCAAAGAGAAATCTGAGGCACTGG-3' (antisense).

2.3. Body weight and body composition measurements

Between 10 and 14 weeks of age, body weight was measured using a Denver Instrument XP-300 scale (Denver, CO). Body composition (fat and lean mass) was measured using quantitative magnetic resonance (QMR) (EchoMRI, Houston, TX).

2.4. Energy balance physiology measurements

Between 10 and 14 weeks of age, energy balance and home-cage activity were recorded using a combined indirect calorimetry system (LabMaster Cages, TSE-Systems, Bad Homburg, Germany). After 24–48 h of initial acclimatization, food intake, water intake, energy expenditure, resting energy expenditure, and respiratory exchange ratio were measured every 15 min for a total of 58 h. Resting energy expenditure was calculated by taking the average of the five lowest values of energy expenditure [4]. Along with total locomotor activity, stationary, ambulatory and rearing movements were recorded in the X, Y, and Z axes using infrared light beams [15].

2.5. Glucose tolerance test

Mice 4–6 months of age were fasted for 5 h. Glucose (2 g/kg of body weight) was administered through an intraperitoneal injection. Lateral saphenous blood was collected at 0, 15, 30, 60, 90 and 120 min. Blood glucose was measured using a glucometer (Breeze 2, Toronto, ONT).

2.6. Insulin tolerance test

Mice at 4–6 months of age were fasted for 5 h. Human insulin (Novo Nordisk, Mississauga, ONT) at a dose of 0.75 U/μ was administered through an intraperitoneal injection. Lateral saphenous blood was collected at 0, 15, 30, 60, 90 and 120 min.

2.7. Hormonal measurements

Mice 4–6 months of age were fasted for 5 h. Approximately 200 μ L of whole blood was collected via lateral saphenous vein into EDTA-coated Sarstedt, and non-coated Sarstedt, Microvette tube. Plasma insulin levels were measured using Enzyme Immuno Assay (EIA – Alpc Diagnostics, Salem, NH; Cat. No. 80-INSMSU-E01). Serum leptin levels were measured using Enzyme Immuno Assay (EIA – Alpc Diagnostics, Salem, NH; Cat. No. 22-LEPMS-E01).

2.8. Statistical analysis

Means $\pm$ SE are shown. Differences between the groups were calculated by one-way ANOVA or with repeated-measures ANOVA and with Bonferroni or Dunnett's post-tests.

3. Results

3.1. Body mass measurements

pKO mice weighed less than other strains, an effect which reached significance for sKO and dKO mice (Table 1, ANOVA $P = 0.0001$). aKO and dKO weighed more than dWT mice (Table 1, $P < 0.001$). pKO mice also had lower fat mass than any other strain (Table 1, ANOVA $P < 0.0025$). Lean mass was not different between pKO mice and controls, but both sKO and dKO mice had increased lean mass relative to pKO and dWT(B6) mice (Table 1, ANOVA $P < 0.0001$).

3.2. Hormone homeostasis

We found no differences in fasting glucose or insulin levels among the genotypes (Table 1); though plasma glucose in dKO mice was somewhat lower, the difference did not reach statistical significance. We did find improved glucose tolerance in all three knockout strains relative to dWT controls (Fig. 1A – repeated-measures ANOVA $P < 0.0001$, in agreement with AUC analysis Fig. 1B), though there were no significant differences in glucose tolerance between pKO, sKO and dKO mice. Insulin tolerance test in dKO and control mice found no difference between the strains (Fig. 1C and D). Fasting leptin levels did not differ among genotypes (Table 1).

3.3. Food intake

dKO and sKO mice show increased food intake during the light phases (Fig. 2A, ANOVA $P < 0.0353$) and during the dark phases (Fig. 2A, shaded, ANOVA $P < 0.0001$). This phenotype persisted when intake was normalized to body weight (Fig. 2A, left, middle, $P < 0.0001$) and lean mass (Fig. 2A, left, bottom, $P < 0.0005$).

3.4. Energy expenditure

dKO and sKO mice also show increased energy expenditure on a per mouse basis (data not shown) when compared with dWT control mice during the light ($P < 0.0001$) and dark phases ($P < 0.0001$). The effect of genotype on energy expenditure remains when normalized to body weight and when normalized to metabolically active lean tissue during the light and dark phases (Fig. 3A, all post hoc $P < 0.0001$). We observed a normal diurnal

fluctuation in food intake (Fig. 3A, left panels), and energy expenditure (Fig. 3A, right panels). Resting energy expenditure is increased in dKO mice when compared with dWT and pKO (Fig. 3B, left, $P < 0.0001$) mice. Resting energy expenditure also remains increased in these mice when normalized to metabolically active lean tissue (Fig. 3B, right, $P < 0.0001$). There was no significant difference in REE in dKO mice compared to sKO mice.

3.5. Physical activity

pKO mice show increased TLA relative to controls during the dark phase (Fig. 5, left, ANOVA $P = 0.0048$) but not during the light phase. We observed a normal diurnal fluctuation in total locomotor activity (Fig. 5, right).

3.6. Respiratory exchange ratio

The dKO mice show a significantly lower RER during the light phase than did controls ($P = 0.0291$, Fig. 4, left). The circadian-ultradian periodicity was preserved (Fig. 4, right).

4. Discussion

Leptin-deficient *ob/ob* mice overexpress *Pmch* in hypothalamus [18] and *Scd1* in liver [2], and genetic ablation of *Pmch* attenuates liver *Scd1* overexpression [20]. These previous findings suggested us that coordinate regulation of these two molecules by leptin might have physiological relevance. Information on energy balance in multiple tissues and organs is presented to the brain via humoral (endocrine) and neuronal afferent signals. When energy storage is excessive, the brain sends out efferent signals to enhance energy expenditure and lipolysis. In the rat brain, dense projections of MCH-immunoreactive axons from the dorsomedial and lateral hypothalamus arrive at the dorsal motor nucleus of the vagus, the nucleus tractus solitarius, and sympathetic premotor areas of the ventral medulla [26,9]. These caudal projections of MCH neurons to brainstem sites could plausibly regulate autonomic responses [16]. The liver is known to transmit autonomic signals to the brain via the afferent loop of the hepatic vagus nerve, which in turn modulates insulin sensitivity and energy expenditure [24]. It is possible that the efferent arm of the vagus receives input from the aforementioned MCH-ergic projections, and that this anatomical connection from the brainstem to the liver modulates hepatic *Scd1* activity.

We sought to investigate metabolic links between melanin-concentrating hormone and stearoyl coA desaturase. Since deletion of either melanin-concentrating hormone [21] or stearoyl coA desaturase [2,13] increases energy expenditure, we hypothesized that deletion of both genes would increase energy expenditure more significantly, and improve the metabolic phenotype of the B6 mouse strain. Removal of *Pmch* from an *ob/ob* background did not affect the food intake of hyperphagic *ob/ob* mice [20], so we hypothesized that deletion of *Pmch* from the hyperphagic *Scd1*^{-/-} background would leave food intake unchanged, and exert effects on energy balance primarily through effects on energy expenditure. Consistent with others, we observed an increased in TLA *Pmch*^{-/-} mice relative to controls along with the increased energy expenditure observed by others [8]. We observed a significant increase in both resting and total energy expenditure in double-

knockout mice relative to wild-type mice. The absence of SCD1 activity appeared to be the major quantitative contributor to the double-knockout phenotype, with a minor contribution from removal of *Pmch*. Consistent with earlier reports; deletion of the *Pmch* gene did not affect the food intake of B6 mice. Consistent with our hypothesis, the food intake of *Scd1*-deficient mice was not affected by deletion of the *Pmch* gene. This provides further weight to existing evidence that *Pmch* and its products exert their physiological effects primarily on the energy expenditure arm of the energy homeostasis equation.

Removal of *Pmch* from B6 mice had specific effects on fat mass, without affecting lean mass. Our QMR system does not measure bone mass, so the possibility that these mice also had some degree of reduction in bone mass remains unexplored. It is interesting to note that removal of *Pmch* from *Scd1*^{-/-} mice did not reduce fat mass. This suggests that the excess food intake that results from SCD1 deficiency outweighs the reduction in fat mass that would otherwise be associated with *Pmch* deficiency, even in the context of increased energy expenditure. Both sKO and dKO mice show reductions in RER during light phase dormancy, reflecting a relative shift away from carbohydrate and toward fat for energy requirements. Thus, the fact that fat mass does not differ between dWT, sKO and dKO genotypes cannot be explained through changes in macronutrient mobilization for energy needs. The quantitative effect of the reduced RER seen in the *Scd1*^{-/-} and *Pmch*^{-/-}; *Scd1*^{-/-} mice would be expected to reduce fat mass rather than increase it. We conclude that the hyperphagia caused by *Scd1* deficiency is quantitatively sufficient to increase fat mass despite the increase in energy expenditure mediated by deficiency of both molecules. Though deletion of the *Pmch* gene from *Scd1*^{-/-} mice may subtly increase the total and resting energy expenditure, result for REE only reached statistical significance relative to *Scd1*^{-/-} mice if a one-tailed post hoc *t*-test was used. The use of a one-tailed test was based on the prior evidence that removal of pMCH would only be expected to increase REE, not decrease it [10]. Nevertheless, a major effect of *Pmch* deficiency on the phenotype of the double-knockout mice was not detectable.

We did observe improvements in glucose tolerance in the dKO mice, but there did not appear to be any synergy of the individual gene knockouts on the phenotype, when compared to either of the parental genotypes. Further experimentation would be required to elucidate any such effects, perhaps via exposure of the mice to a high-fat and/or high-fat, high carbohydrate diet. We observed a small decrease in fasting insulin levels in *Pmch*^{-/-} mice, one that did not reach statistical significance when compared with wild-type mice. Since *Pmch* is a known trophic factor for islet beta cells, this is not surprising [17]. The fact that glucose tolerance was improved in all three single knockout strains relative to wild-type, attests to the utility of increasing energy expenditure as a protective factor against glucose intolerance, even in the context of a presumptive decrease in beta cell function.

The interplay between neuropeptides that regulate energy homeostasis and end-organs that process energy-containing nutrients is of interest from a clinical perspective. Insights into these processes may eventually lead to new treatments for excessive liver fat, as occurs in obesity and lipodystrophy syndromes. Though our data do not directly address whether elevated levels of MCH might increase liver *Scd1* activity via autonomic or other pathways,

they do show that the increased energy expenditure of *Scd1*-deficient mice is mediated largely by non-MCH-ergic pathways.

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Abbreviations

dKO	mice homozygous for inactivating alleles of both the <i>Pmch</i> and <i>Scd1</i> genes (<i>Pmch</i> $-/-$, <i>Scd1</i> $-/-$ genotype)
dWT	mice homozygous for normal alleles of both the <i>Pmch</i> and <i>Scd1</i> genes (<i>Pmch</i> $+/+$, <i>Scd1</i> $+/+$ genotype) (=B6)
pKO	“ <i>Pmch</i> knockout” mice, with a homozygous engineered deletion of the <i>Pmch</i> gene (<i>Pmch</i> $-/-$ genotype); pMCH, mouse pro-melanin-concentrating hormone protein
Pmch	mouse pro-melanin-concentrating hormone gene; SCD1, mouse stearyl-CoA desaturase-1 protein
Scd1	mouse stearyl-CoA desaturase-1 gene
B6	C57Bl/6J “wild-type” laboratory mice (=dWT); sKO, mice bearing homozygous <i>Scd1</i> ^{ab-2J} null alleles (<i>Scd1</i> $-/-$ genotype).

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Fig. 1.

Glucose homeostasis and plasma insulin levels. Using an intraperitoneal dose of 2 g/kg of body weight, a glucose tolerance test in 6 dWT, 6 pKO, 3 sKO, and 9 dKO mice (A), showed that dKO mice have improved glucose tolerance compared with dWT controls during the 120-min study period. Area under the curve values confirmed the results found in the glucose tolerance (B). Using an intraperitoneal dose of 0.75 U/μ of body weight, an insulin tolerance test in 7 dWT, and 5 dKO mice (C) showed no differences between the strains, results confirmed by area under the curve analysis (D). Values are means $\pm$ SEM. * P < 0.05 vs. WT.

Fig. 2.

Food intake. Ingestive behavior was calculated in 12 dWT, 8 pKO, 5 sKO, and 9 dKO mice given ad libitum access to standard chow diet. During a representative 58-h observation period (A, right) cumulative food intake during the dark phase (A, left, top) was increased in dKO mice, compared with dWT mice. This difference persisted when consumption was normalized to body weight (A, left, middle), and lean mass (A, left, bottom). Values are means $\pm$ SEM. * $P < 0.05$ vs. WT, and # $P < 0.05$ vs. pKO.

Fig. 3.

Energy expenditure, and resting energy expenditure. The average energy expenditure (A) and the basal metabolic rate (B) were measured in 12 dWT, 8 pKO, 5 sKO, and 9 dKO mice for a period of 58 h. dKO mice showed a significantly higher energy expenditure than dWT mice in both the dark and light phase (A, left, top). Normalization to metabolically active lean tissue persisted after the increase in energy expenditure (A, left, bottom). Basal metabolic rate was higher in dKO mice compared with dWT mice. Values are means $\pm$ SEM. * $P < 0.05$ vs. WT, and # $P < 0.05$ vs. pKO.

Fig. 4.

Respiratory exchange ratio. The average respiratory exchange ratio was measured in 12 dWT, 8 pKO, 5 sKO, and 9 dKO mice for a period of 58 h. dKO mice showed a significantly lower respiratory exchange ratio than dWT mice in the light phase (A, left). Values are means $\pm$ SEM. * $P < 0.05$ vs. WT.

Fig. 5.

Total locomotor activity. The total locomotor activity was measured in 12 dWT, 8 pKO, 5 aKO, and 9 dKO mice for a period of 58 h. pKO mice show a significantly increased total locomotor activity during the dark phases than other genotypes. Values are means $\pm$ SEM. * $P < 0.05$ vs. WT, and # $P < 0.05$ vs. pKO.

Table 1

Physical and metabolic characteristics (mean±SEM).

	dWT	pKO	sKO	dKO
Total body weight, g	18.6±0.5 (<i>n</i> = 12)	18.6±0.4 (<i>n</i> =8)	22.4±0.8 (<i>n</i> =5)	21.1±0.4 (<i>n</i> =9)
Fat mass, g	2.6±0.2 (<i>n</i> = 12)	1.8±0.1 (<i>n</i> =8)	2.6±0.1 (<i>n</i> =5)	2.4±0.1 (<i>n</i> =9)
Lean mass, g	14.8±0.4 (<i>n</i> = 12)	15.0±0.3 (<i>n</i> =8)	18.1±0.2 (<i>n</i> =5)	18.0±0.4 (<i>n</i> =9)
Baseline glucose, mmol/L	9.8±0.4 (<i>n</i> = 15)	9.4±0.8 (<i>n</i> =6)	10.0±0.7 (<i>n</i> =8)	8.5±0.4 (<i>n</i> =9)
Insulin, ng/mL	0.50±0.20 (<i>n</i> = 14)	0.10±0.03 (<i>n</i> =3)	0.40±0.09 (<i>n</i> =7)	0.30±0.04 (<i>n</i> = 11)
Leptin, ng/mL	0.4±0.3 (<i>n</i> =4)	0.3±0.1 (<i>n</i> =3)	0.4±0.2 (<i>n</i> =2)	0.3±0.1 (<i>n</i> =8)