Genetic ablation of myelin protein zero-like 3 in mice increases energy expenditure, improves glycemic control, and reduces hepatic lipid synthesis


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Czyzyk TA, Andrews JL, Coskun T, Wade MR, Hawkins ED, Lockwood JF, Varga G, Sahr AE, Chen Y, Brozinick JT, Kikly K, Statnick MA. Genetic ablation of myelin protein zero-like 3 in mice increases energy expenditure, improves glycemic control, and reduces hepatic lipid synthesis. Am J Physiol Endocrinol Metab 305:E282–E292, 2013. First published May 28, 2013; doi:10.1152/ajpendo.00228.2013.—Obesity continues to be a global health problem, and thus it is imperative that new pathways regulating energy balance be identified. Recently, it was reported: (Hayashi K, Cao T, Passmore H, Jourdan-Le Saux C, Fogelgren B, Khan S, Hornstra I, Kim Y, Hayashi M, Csiszár K. J Invest Dermatol 123: 864–871, 2004) that mice carrying a missense mutation in myelin protein zero-like 3 (Mpzl3) have reduced body weight. To determine how Mpzl3 controls energy balance in vivo, we generated mice deficient in myelin protein zero-like 3 (Mpzl3-KO). Interestingly, KO mice were hyperphagic yet had reduced body weight and fat mass. Moreover, KO mice were highly resistant to body weight and fat mass gain after exposure to a high-fat, energy-dense diet. These effects on body weight and adiposity were driven, in part, by a pronounced increase in whole body energy expenditure levels in KO mice. KO mice also had reduced blood glucose levels during an intraperitoneal glucose challenge and significant reductions in circulating insulin levels suggesting an increase in insulin sensitivity. In addition, there was an overall increase in oxidative capacity and contractile force in skeletal muscle isolated from KO mice. Hepatic triglyceride levels were reduced by 92% in livers of KO mice, in part due to a reduction in de novo lipid synthesis. Interestingly, Mpzl3 mRNA expression in liver was increased in diet-induced obese mice. Moreover, KO mice exhibited an increase in insulin-stimulated Akt signaling in the liver, further demonstrating that Mpzl3 can regulate insulin sensitivity in this tissue. We have determined that Mpzl3 has a novel physiological role in controlling body weight regulation, energy expenditure, glycemic control, and hepatic triglyceride synthesis in mice.

skeletal muscle; rough coat (rc/rc), Akt; immunoglobulin-like V-type domain; nonalcoholic fatty liver disease

Myelin protein zero-like 3 (Mpzl3) encodes a predicted type I transmembrane protein and was named after its closest homolog Mpzl2 (also referred to as EVA1). MPZL3 and EVA1 share roughly 60% similarity in their amino acid sequences (7). Similar to EVA1, MPZL3 has a predicted extracellular immunoglobulin (Ig)-like V-type (variable) structural domain and therefore has a putative role in cell adhesion. Although previous studies have documented a role for cell adhesion molecules of the Ig superfamily in mediating body weight (13, 18), members of this family differ vastly in their localization and in the number and type of Ig domains they contain (2). This has made it difficult to determine the role of individual Ig-containing subfamilies in energy balance. Moreover, few studies have focused on those subsets of molecules containing the Ig-like V-type domain and its relationship to obesity. Interestingly, adipocyte cell adhesion molecule, which contains both a V- and a C2-type domain, was found to be upregulated in obesity in white adipose tissue, thereby linking this molecule to adiposity (14).

The Mpzl3 locus is found on chromosome 9 in mice and chromosome 11 in humans and lies in a highly conserved chromosomal arrangement between Mpzl2 and Amica1, both encoding proteins with documented cell adhesion activity (19, 29). Interestingly, two genetic linkage studies have reported that the MPZL3 chromosomal location (11q23.3) is linked to body mass (20, 26) and energy expenditure (31) in Pima Indians, a population exhibiting a very high incidence of obesity and type 2 diabetes (23, 24). Two protein-coding transcripts for Mpzl3 of 96 and 237 AA in length are predicted in mouse, with the shorter transcript containing only a portion of the Ig-like domain and lacking the transmembrane-containing sequence (7). It is currently unknown whether the shorter, 96-AA transcript has functional relevance. The AA sequence of human MPZL3 is 86.8% identical to that of the mouse protein. Furthermore, orthologs of MPZL3 are present in lower vertebrate organisms including zebrafish, suggesting an evolutionarily conserved function for this protein across species (7) (34).

Although the function of MPZL3 remains unknown, Mpzl3 has recently been identified as being the site of a missense mutation in spontaneous rough coat mice (Mpzl3rc) (7). These mice have been characterized by their unkempt coat and progressive alopecia (12, 36). Additionally, male Mpzl3rc mice at 12 wk were reported to have a 10% reduction in body weight (21). In the present work, we utilized gene targeting in mice to delineate the in vivo metabolic function of MPZL3. Herein, we have identified a novel physiological role for Mpzl3 in the regulation of energy expenditure, glycemia, and hepatic lipid synthesis. Mpzl3-KO mice were found to be lean and resistant to weight gain, dislipidemia, and hyperglycemia associated
with adult-onset obesity and the consumption of a high-fat, high-energy diet. We also found that there was a reduction in the expression of hepatic lipogenic enzymes in liver tissue taken from KO mice. This resulted in a dramatic reduction of both circulating and hepatic triglyceride levels even in chow-fed KO mice. Last, we found that Mpzl3 expression was significantly increased in liver in diet-induced obese mice.

**METHODS**

**Generation of Mpzl3-KO mice.** All procedures were approved by the Institutional Animal Care and Use Committee of Eli Lilly and Co. and were in accordance with the National Institutes of Health (NIH) Guide for Care and Use of Laboratory Animals. Embryonic stem cells derived from the 129/OlaHsd mouse strain were used to generate male chimeric mice containing a 246-bp deletion of Mpzl3 (NM_001093749) (Deltagen, San Mateo, CA) (see Fig. 1A). Fl mice were generated by breeding a chimera with C57BL/6 females, and heterozygotes were back-crossed eight generations to C57BL/6 mice prior to obtaining homozygous mutants (Taconic Farms, Germantown, NY). Mice were maintained on a 12:12-h light-dark cycle (lights on 0600, lights off 1800) at 22°C with access to water and rodent chow ad libitum. All experiments were performed in male, littermate controls unless indicated otherwise.

**Determination of energy balance.** Mpzl3-KO and WT littermates were placed on a high-fat, energy-dense (HED) diet (Teklad 95217, 40% calories from fat, 4.3 kcal/g; Harlan Laboratories, Indianapolis, IN) at 8–9 wk of age. Chow-fed controls were maintained on a standard rodent diet (Teklad 2014, 13% calories from fat, 2.9 kcal/g, Harlan Laboratories). Mice were individually housed throughout the experiment with nestlets. Whole body composition was measured using NMR imaging (Whole Body Composition Analyzer; EchoMRI, Houston, TX). Indirect calorimetry measurements were taken when mice were 6 mo old and had been fed chow or the HED for 16 wk. A 16-cage custom calorimetry system (Oxymax, Columbus, OH), equipped with infrared beams surrounding the individual cages, was used to measure CO2 and O2 levels and total locomotor activity (combined horizontal ambulations and fine motor movements). The ambient temperature of the room that housed the calorimeters was set at 24°C. Twenty-four hour energy expenditure was calculated as caloric expenditure (kcal/h/kg body wt). Resting metabolic rate was estimated as the calculated caloric expenditure when total locomotor activity was measured to be near zero for a continuous 45-min-long period during the middle of the light phase. Respiratory quotient (RQ) was calculated as the ratio VCO2/O2. To determine nutrient absorption, feces were collected during indirect calorimetry and weighed after 48 h. The calorific contents of the fecal samples (calories per gram of feces) were measured using standard bomb calorimetry methods. The amount of calories excreted in the feces was subtracted from the number of calories consumed and the percent efficiency of absorption was compared for each genotype and diet. Mice were given 24 h to acclimate to the metabolic cages prior to calorimetry measurement and fecal collection. Food intake was measured for 3 consecutive days during indirect calorimetry measurements to determine the average food intake per day for each mouse.

**Histopathology.** Experimentally naïve, chow-fed Mpzl3-KO and WT littermates (4 mo old) were fasted for 4–6 h before being euthanized. Blood was collected, and major organs and tissues were dissected, weighed, and then placed in 10% formalin and paraffin wax embedded. sections (5 μm) were cut on a microtome and stained with hematoxylin and eosin (H&E). Sections were scored by a trained pathologist using a tiered grading scale: within normal limits, or minimal, mild, moderate, or marked lesion.

**Telemetric measurement of body temperature.** C57BL/6 WT and Mpzl3-KO (age-matched, 4-mo-old) male mice were anesthetized with isoflurane, and a TA-F20 transmitter (Data Sciences International, St. Paul, MN) was placed into the abdominal cavity of each animal. Animals were allowed to recover for 2 wk before body temperature measurements were taken. Home cages were placed on top of individual receiver pads in a sound-attenuated room, and temperature and activity measurements were taken at 5-min intervals for 48 h with ad libitum chow and water available. Chow was then removed for 24 h. After 24 h, food was then returned for additional measurements.

**In situ analysis of contractile force in muscle.** The contractile force generated by the gastrocnemius muscle was measured in response to sciatric nerve stimulation in age-matched C57BL/6 WT and Mpzl3-KO females at 14 mo. Mice were anesthetized and maintained under isoflurane throughout the procedure. The foot was held in a fixed position with a clamp attached to the physiology table. The knee was then placed in a rigid hold by first exposing the patella tendon, placing a Stevens tenotomy hook (AliMed/Arista; Dedham, MA) underneath, attached to a rack and pinion clamp (Harvard Apparatus; Holliston, MA), and putting it under tension by retracting the clamp in an anterior direction until the tibia was held in a fixed rigid position. The Achilles tendon was exposed and then cut and attached with surgical suture to an FT03 isometric force transducer (Grass Technologies, West Warwick, RI). The sciatric nerve was then exposed for indirect stimulation. A subminiature hook electrode (Harvard Apparatus) was placed around the sciatic nerve and gently fixed in place. The optimal resting tension of the gastrocnemius was determined as follows before force measurements were made. The muscle was subjected to increasing resting tension by adjusting the transducer by small discrete steps. The muscle was then stimulated directly with an S88 Dual Output Square Pulse Stimulator (Grass Technologies) at a voltage sufficient to elicit a contraction, and the force generated was recorded (10 V, 0.5 ms duration). This cycle of increasing resting tension followed by a single pulse stimulation was repeated until no further increase in force was observed. The muscle was then maintained at this resting tension, after which force measurements were obtained. Pulses of 0.1 ms duration were delivered from the Grass S88 stimulator to the sciatric nerve beginning at 10 V and increasing in 10-V steps 10 s apart until a further increase in voltage did not elicit an increase in force. Once the maximal twitch force was determined, a force frequency curve was generated. The nerve was stimulated for 2 s at 20 Hz using the stimulation settings required to elicit a supramaximal twitch response (0.1 ms duration, 2× supramaximal voltage). The frequency of stimulation was increased in 10-Hz steps 30 s apart up to 130 Hz. The force of contraction measured under maximal voltage and frequency was designated the maximal tetanic force of the muscle.

**Quantitative real-time PCR.** Tissues were collected from nonfasted age-matched (4–5 mo) male C57BL/6 WT and KO mice maintained on a standard chow diet. Total RNA was extracted from frozen tissue samples homogenized in TRIzol reagent (Invitrogen, Carlsbad, CA) and purified with the MagAttract RNA Universal Tissue M48 kit (Qiagen, Valencia, CA) with DNase treatment. cDNAs were synthesized and qPCR was performed as previously described (10). Taqman gene expression assays were obtained from Applied Biosystems (Madison, WI). Specific details are available upon request. All individual samples were measured in triplicate. Data were normalized to acidic ribosomal binding protein (ARBP) (10).

**Glucose and insulin tolerance tests.** Blood glucose levels were measured with an Accucheck glucometer (Roche, Indianapolis, IN) in Mpzl3-KO and WT (littermates) male mice after injection of 2 mg/g body wt in-g glucose (Sigma, St. Louis, MO) for glucose tolerance test (GTT) or 0.75 U/kg insulin (Eli Lilly, Indianapolis, IN) for insulin tolerance test (ITT). For GTT, separate cohorts of both young (11 wk) and aged (24 wk) littermates were tested. For ITT, age-matched (28 wk) KO and C57BL/6 WT mice were used. All mice were fasted for at least 7 h prior to the taking of blood glucose measurements. Curves were analyzed with two-way repeated-measures ANOVA and post hoc analysis. Area under the curve (AUC) values were determined and analyzed with unpaired t-tests. Insulin levels were determined using
ELISA. Blood samples were collected at time of GTT. Homeostasis model assessment of insulin resistance (HOMA-IR) values were calculated using the following formula: [fasting blood glucose (mmol/l) × fasting insulin levels (mU/l)]/22.5 (1).

Triglyceride measurement. Frozen liver samples were weighed and homogenized in dH2O. Lipids were extracted by addition of chloroform. Following centrifugation, the chloroform layer was removed and dried under nitrogen. Dried lipids were reconstituted in isopropanol and assayed enzymatically using Triglyceride GPO reagent (Roche Diagnostics, Indianapolis, IN).

Western blot analysis. Western blot analysis was performed following standard procedures. UCP1 was detected with a rabbit polyclonal antibody (Calbiochem, San Diego, CA, no. 662045) at a dilution of 1:2,000. Calreticulin was detected with a rabbit polyclonal antibody (Abcam, Cambridge, MA, no. ab2907) at a dilution of 1:1,000 and served as an internal control for protein loading in each well. Phosphorylated Akt was detected with a rabbit polyclonal antibody (Cell Signaling Technology, Beverly, MA, 1:1,000 dilution), and total Akt was detected with a rabbit polyclonal antibody (no. 9272, Cell Signaling Technology, 1:1,000 dilution). Densitometry was performed with ImageJ 1.43u software (National Institutes of Health, Bethesda, MD).

In vivo lipogenesis assay. The rate of triglyceride synthesis was estimated using a [14C]acetate incorporation assay as previously described (10). Age-matched WT and Mpzl3-KO mice were 3 mo old at time of measurement and had been maintained on standard rodent chow.

Data analysis and statistics. Values are plotted as the mean ± SE for each genotype. Statistical analyses were conducted with GraphPad Prism 4 (GraphPad Software, San Diego, CA). Comparisons among genotype and diet were performed with two-way ANOVA and Bonferroni post hoc tests. Post hoc analysis results are indicated in the corresponding figure legends. Unpaired t-tests were also used to compare two groups as indicated. Growth curves and telemetry were analyzed with two-way repeated-measures ANOVA and Bonferroni post hoc tests.

RESULTS

Generation of Mpzl3-KO mice. To determine the function of MPZL3 in vivo, we generated Mpzl3-KO mice using standard gene-targeting methods (Fig. 1A). Deletion of Mpzl3 did not affect viability, as we obtained the expected Mendelian ratio of homozygous mutants from heterozygous breeding pairs (not shown). Gross inspection of offspring revealed that KO mice had evidence of localized alopecia at 8 wk. Detailed examination at 4 mo showed that the fur loss was primarily restricted to the ventral neck, interscapular, and flank regions. This pattern was maintained in all KO mice up to 24 mo (the oldest age examined). We also noted that all KO mice had areas of fur with reduced pigmentation on both the dorsal and ventral trunk regions at 4 mo of age. In addition, we examined 71 KO mice between the ages of 12 and 24 mo (42% male, 58% female), and all of them had complete fur loss in the area immediately surrounding the eyes. In 16 of these aged mice (22.5%), there was evidence of active or healed skin lesions in the ventral neck area but not on other areas of the body.

We performed a systematic histopathology analysis of major organs and tissues in male Mpzl3-KO and WT littermate controls (n = 5) that were fed a standard chow diet at 4 mo old. Whereas KO mice had a significant reduction in body weight (29.2 ± 0.8 WT vs. 26.6 ± 0.5, P = 0.03, unpaired t-test), organ weights were relatively consistent across genotypes (Table 1). A small increase in the average size of the kidney in KO mice was observed (P = 0.04), but this was in the absence of changes in serum blood urea nitrogen or protein levels (Table 2). All KO mice had a cutaneous phenotype characterized by enlarged and expanded sebaceous glands and a minimal increase in intradermal leukocytes near the necks of hair follicles in areas that had alopecia (Fig. 1B). In addition, KO mice had notable reductions in the size of cytoplasmic vacuoles in liver tissue, as described below. No significant histological differences were observed in other major tissues and organs examined from KO mice at 4 mo of age.

Mpzl3 is expressed in metabolically relevant tissues. To determine the expression pattern of Mpzl3 in metabolically active tissues, we performed qPCR analysis in eight distinct tissues from WT C57BL/6 mice. We found that the most significant expression of Mpzl3 was in liver and brown adipose tissue (BAT), followed by whole brain, adrenal glands, hypothalamus, and skeletal muscle (Fig. 1C). Detectable levels of Mpzl3 mRNA were also found in white adipose tissue (WAT) and the pituitary gland. Thus,
**Mpzl3** could potentially function directly in one or more of these tissues to control energy homeostasis. **Mpzl3**-KO mice have reduced body weight and adiposity and are protected against diet-induced obesity. An analysis of body weight and composition demonstrated that KO mice maintained on standard chow diet were significantly leaner than WT litterate controls. By 8 wk, male KO mice had small but significant reductions in body weight (WT 24.3 ± 1.0 vs. KO 22.3 ± 0.7 g, P = 0.0004, unpaired t-test, n = 6–8). At 4.5 mo, male KO mice continued to weigh less and had reduced adiposity compared with WTs (body wt WT 36.9 ± 1.0 vs. KO 25.2 ± 0.7 g; %fat WT 35.4 ± 1.1 vs. KO 15.8 ± 0.7%, P < 0.001, unpaired t-tests, n = 6–8; Fig. 2). Lean mass was also reduced but was significantly higher in KO mice compared with body weight (WT 63.6 ± 1.2 vs. KO 83.5 ± 0.7%, P < 0.001, unpaired t-test, n = 6–8). Small but significant reductions in body lengths were also noted in KOs at this age (nose to anus length WT 8.9 ± 0.05 vs. KO 8.5 ± 0.02 cm, P < 0.0001, unpaired t-test, n = 7).

To determine whether KO mice were resistant to diet-induced obesity, we fed a separate cohort of KO and WT mice the HED beginning at 8 wk of age. WT mice gained significant body weight on the HED but KO mice did not (Fig. 2A). Both body weight and fat mass remained significantly reduced in KO mice after 10 wk of HED exposure (Fig. 2B and C). Similar results were found in female KO mice (data not shown). Unnormalized lean mass values as measured by qNMR were lower in KO mice (Fig. 2D) but were higher compared with body weights as they were in chow-fed KO mice. Serum leptin levels paralleled reductions in fat mass in KO mice (Fig. 2E). Interestingly, serum corticosterone levels increased in WT controls fed HED but not in KO mice (Fig. 2F). Body weight and adiposity measurements in heterozygotes were not different than in WTs, and thus they were not analyzed further (Fig. 2A). We also measured 24-h food intake over 3 consecutive days in **Mpzl3**-KO mice. KO mice were found to be significantly hyperphagic (Fig. 2, G and H). Interestingly, we did not find significant changes in the expression of hypothalamic NPY (not shown) in experimentally naïve male KOs. However, POMC levels were reduced in this region, and this is consistent with the observed hyperphagia (relative POMC mRNA WT 1.09 ± 0.04 vs. KO 0.86 ± 0.09, P = 0.05, unpaired t-test, n = 7). Fecal caloric contents were similar among the genotypes, suggesting that KO mice were not lean due to alterations in nutrient absorption (Fig. 2I). Collectively, these data demonstrate that **Mpzl3**-KO mice are lean and resistant to weight gain on HED and that this is driven by a reduction in accumulated fat mass but not increased serum corticosterone, reduced caloric intake, or altered nutrient absorption in the GI tract.

**Improved glucose tolerance in **Mpzl3**-KO mice.** Given the diet-independent reductions in adiposity in **Mpzl3**-KO mice, we measured serum glucose and insulin levels during an intraperitoneal GTT. KO mice had reduced blood glucose along with circulating insulin levels (Fig. 3A and B). Interestingly, no differences in blood glucose levels were observed after a systemic insulin challenge (Fig. 3C). However, we found that HOMA-IR values were significantly lower in KO mice (WT 3.2 ± 0.2 vs. KO 2.1 ± 0.3, P = 0.01, unpaired t-test, n = 8). To further investigate potential mechanisms for enhanced glycemic control under conditions of high glucose, we looked at how the levels of phosphorylated (p-)Akt would change in response to a bolus of insulin. Although basal p-Akt levels were essentially undetectable in both WT and KO mice, hepatic insulin-stimulated p-Akt levels were significantly elevated in KOs by 29% (Fig. 3D). A similar trend was found in skeletal muscle (gastroc bundle, WT 0.71 ± 0.13 vs. KO 0.87 ± 0.03, P = 0.13, unpaired t-test, n = 2–5). We also wanted to determine whether deletion of **Mpzl3** could prevent impairment of glycemic control, which occurs following prolonged exposure to HEDs in C57BL/6 mice. Indeed, KO mice fed a HED diet had significantly lower blood glucose levels during an ip GTT (Fig. 3E). Taken together, our findings suggest that loss of **Mpzl3** improves glycemic control, likely through an increase in insulin sensitivity in key tissues such as liver and muscle. Furthermore, lower blood glucose and insulin levels in KO mice were consistent with the observed reductions in adiposity.

### Table 1. *Organ weights in Mpz3-KO mice*

<table>
<thead>
<tr>
<th></th>
<th>WT 29.2 ± 0.8</th>
<th>KO 25.2 ± 0.5</th>
<th>P 0.05</th>
<th>NS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain, mg</td>
<td>417 ± 15</td>
<td>418 ± 6</td>
<td>0.04</td>
<td>NS</td>
</tr>
<tr>
<td>Heart, mg</td>
<td>156 ± 7</td>
<td>168 ± 7</td>
<td>0.04</td>
<td>NS</td>
</tr>
<tr>
<td>Kidney, mg</td>
<td>312 ± 7</td>
<td>342 ± 10</td>
<td>0.04</td>
<td>NS</td>
</tr>
<tr>
<td>Pancreas, mg</td>
<td>118 ± 7</td>
<td>114 ± 2</td>
<td>0.04</td>
<td>NS</td>
</tr>
<tr>
<td>Spleen, mg</td>
<td>74 ± 5</td>
<td>64 ± 5</td>
<td>0.04</td>
<td>NS</td>
</tr>
<tr>
<td>Thymus, mg</td>
<td>37 ± 4</td>
<td>31 ± 3</td>
<td>0.04</td>
<td>NS</td>
</tr>
<tr>
<td>Liver, g</td>
<td>1.44 ± 0.06</td>
<td>1.39 ± 0.06</td>
<td>0.04</td>
<td>NS</td>
</tr>
<tr>
<td>Gastroc, mg</td>
<td>124 ± 6</td>
<td>122 ± 3</td>
<td>0.04</td>
<td>NS</td>
</tr>
<tr>
<td>Biceps, mg</td>
<td>147 ± 10</td>
<td>137 ± 14</td>
<td>0.04</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are means ± SE. Shown are wet weights of organs from naïve male **Mpz3**-KO and WT littermate controls after approximately 5-h fast. Animals were 4 mo old at time of euthanasia and were maintained on a standard chow diet. Data were analyzed with unpaired t-tests (n = 5 per genotype). BW, body weight; Gastroc, gastrocnemius muscle; biceps, biceps femoris muscle.

### Table 2. *Blood chemistry analysis in Mpz3-KO mice*

<table>
<thead>
<tr>
<th></th>
<th>WT 17.5 ± 0.9</th>
<th>KO 28.0 ± 1.5</th>
<th>P 0.07</th>
<th>0.02</th>
<th>0.004</th>
<th>0.008</th>
<th>0.01</th>
<th>0.007</th>
<th>NS</th>
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<tr>
<td>BUN, mg/dl</td>
<td>66 ± 8</td>
<td>44 ± 6</td>
<td>0.08</td>
<td>0.02</td>
<td>0.004</td>
<td>0.008</td>
<td>0.01</td>
<td>0.007</td>
<td>NS</td>
</tr>
<tr>
<td>ALT, IU/l</td>
<td>9.4 ± 0.5</td>
<td>9.7 ± 0.1</td>
<td>0.08</td>
<td>0.02</td>
<td>0.004</td>
<td>0.008</td>
<td>0.01</td>
<td>0.007</td>
<td>NS</td>
</tr>
<tr>
<td>Ca, mg/dl</td>
<td>88.8 ± 2.4</td>
<td>71.8 ± 4.4</td>
<td>0.08</td>
<td>0.02</td>
<td>0.004</td>
<td>0.008</td>
<td>0.01</td>
<td>0.007</td>
<td>NS</td>
</tr>
<tr>
<td>Chol, mg/dl</td>
<td>20.0 ± 0.7</td>
<td>18.3 ± 1.4</td>
<td>0.08</td>
<td>0.02</td>
<td>0.004</td>
<td>0.008</td>
<td>0.01</td>
<td>0.007</td>
<td>NS</td>
</tr>
<tr>
<td>Free Chol, mg/dl</td>
<td>79 ± 1</td>
<td>60 ± 3</td>
<td>0.08</td>
<td>0.02</td>
<td>0.004</td>
<td>0.008</td>
<td>0.01</td>
<td>0.007</td>
<td>NS</td>
</tr>
<tr>
<td>HDL Chol, mg/dl</td>
<td>6.0 ± 0.5</td>
<td>7.0 ± 1.0</td>
<td>0.08</td>
<td>0.02</td>
<td>0.004</td>
<td>0.008</td>
<td>0.01</td>
<td>0.007</td>
<td>NS</td>
</tr>
<tr>
<td>LDL Chol*, mg/dl</td>
<td>74 ± 7</td>
<td>46 ± 6</td>
<td>0.08</td>
<td>0.02</td>
<td>0.004</td>
<td>0.008</td>
<td>0.01</td>
<td>0.007</td>
<td>NS</td>
</tr>
<tr>
<td>Trig*, mg/dl</td>
<td>218 ± 1.0</td>
<td>172 ± 8.8</td>
<td>0.08</td>
<td>0.02</td>
<td>0.004</td>
<td>0.008</td>
<td>0.01</td>
<td>0.007</td>
<td>NS</td>
</tr>
<tr>
<td>Phos Lipid, mg/dl</td>
<td>8.6 ± 0.10</td>
<td>0.61 ± 0.05</td>
<td>0.08</td>
<td>0.02</td>
<td>0.004</td>
<td>0.008</td>
<td>0.01</td>
<td>0.007</td>
<td>NS</td>
</tr>
<tr>
<td>FFA, mmol/l</td>
<td>5.4 ± 0.2</td>
<td>5.3 ± 0.1</td>
<td>0.08</td>
<td>0.02</td>
<td>0.004</td>
<td>0.008</td>
<td>0.01</td>
<td>0.007</td>
<td>NS</td>
</tr>
<tr>
<td>TP, g/dl</td>
<td>20.4 ± 0.06</td>
<td>1.75 ± 0.10</td>
<td>0.08</td>
<td>0.02</td>
<td>0.004</td>
<td>0.008</td>
<td>0.01</td>
<td>0.007</td>
<td>NS</td>
</tr>
<tr>
<td>A/G*, g/dl</td>
<td>10.13 ± 3.3</td>
<td>10.13 ± 3.3</td>
<td>0.08</td>
<td>0.02</td>
<td>0.004</td>
<td>0.008</td>
<td>0.01</td>
<td>0.007</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are means ± SE. Serum was collected from naïve male **Mpz3**-KO and WT littermates after approximately 5-h fast. Animals were 4 mo at time of measurement and had been maintained on standard chow diet. Data were analyzed with unpaired t-tests. (n = 5 WT, n = 4 KO; *n = 3 WT, KO). BUN, blood urea nitrogen; ALT, alanine aminotransferase; CA, calcium; Chol, cholesterol; HDL Chol, high-density lipoprotein cholesterol; LDL Chol, low-density lipoprotein cholesterol; Trig, triglyceride; Phos Lipid, phospholipid; FFA, free-fatty acid; TP, total protein; A/G, albumin/globulin ratio.
Energy expenditure is increased in Mpzl3-KO mice. We next applied indirect calorimetry methods to determine whether metabolic rates were altered in Mpzl3-KO mice. Calculated energy expenditure values in KO mice were almost doubled compared with WTs (Fig. 4, A and B). These increases were not dependent on diet and were consistent over both the light and dark phases of the circadian cycle. Furthermore, these changes were independent of reductions in body weight, as 24-h VO$_2$ levels were also significantly increased in KO mice (Fig. 4, C and D). We also assessed spontaneous locomotor activity levels and found that total activity was significantly elevated in KO mice (Fig. 4, E and F). These changes in activity were more pronounced in KOs fed the HED (Fig. 4F) and were consistent with the observed reductions in body weight in KO mice compared with WTs on this diet. In addition, resting metabolic rates were also elevated, demonstrating that spontaneous activity was likely not solely driving the increased VO$_2$ in KO mice (Fig. 4G). We also found that the RQs in KO mice were elevated (Fig. 4H). This is consistent with the observed hyperphagia and suggests that KO mice might have a preference for carbohydrate utilization compared with WT mice.

Energy expenditure is increased in Mpzl3-KO mice in the absence of fur loss. Since fur loss was noted in KO mice, one might expect to see an increase in energy expenditure to maintain core body temperature in this strain. We therefore repeated analysis of energy expenditure in a separate cohort of KO mice at 6 wk of age, before there were any visible signs of fur loss. Energy expenditure was again found to be significantly increased in KO mice (Fig. 5, A–E) in the absence of changes in body weight and adiposity or an increase in locomotor activity (Fig. 5, F–I).

A significant portion of the energy expended by an animal is to maintain core body temperature. We used telemetry to determine that core body temperatures in KO mice were similar to those in WT controls and had normal diurnal variations (Fig. 6A). However, body temperature dropped significantly lower in KO mice compared with controls during fasting (Fig. 6A). Leptin is a known regulator of the maintenance of body temperature during fasting (17); thus, these results are consistent with their reduced serum leptin levels and overall reduction in fat stores (Fig. 2). UCP1 mediates the uncoupling of oxidative phosphorylation in BAT to dissipate energy in the form of heat, and its activity is a significant contributor to overall metabolic rate in rodents. Thus, UCP1 levels are commonly assessed as a surrogate marker for BAT activity. In Mpzl3-KO mice, UCP1 mRNA levels were slightly elevated compared with WTs (Fig. 6B), but protein levels were not different among the genotypes at least at ambient temperature (Fig. 6C). Collectively, these data support that fur loss, increased locomotor activity levels, and UCP1 activity in BAT...
Skeletal muscle function is enhanced in Mpzl3-KO mice. We wanted to directly assess the functional capacity of skeletal muscle in KO mice, since 1) skeletal muscle is an important determinant of whole body resting metabolic rate (47), 2) the embryonic loss of Mpzl3 might have impaired the development of skeletal muscle, and 3) the observed reductions in body weight could be due to cachexia. To do this, we measured the contractile force generated by the gastrocnemius upon stimulation of the sciatic nerve in situ using a naïve cohort of aged female KO and WT mice. Interestingly, more force per unit muscle mass was generated in KO mice than in controls (Fig. 7A). After analysis, we dissected out the gastrocnemius bundle and measured wet weights of the intact muscle tissue (gastrocnemius, soleus, and plantaris muscles) from each mouse. These data were used to normalize the force data, and we determined that the amount of muscle relative to body weight was increased by 37% in KO mice (WT 3.6 ± 0.1 vs. KO 5.7 ± 0.1 g body wt, *P < 0.0001, unpaired t-test, n = 8–9).

We also performed qPCR analysis in muscle to determine the expression patterns of common molecular markers used to assess mitochondrial oxidative capacity in skeletal muscle (4). Interestingly, we found that Ucp3 mRNA levels were increased in muscle of KO mice. This is consistent with the protective role of UCP3 overexpression in the development of diet-induced obesity (9). The levels of peroxisome proliferator-activated receptor-γ coactivator 1α (PGC-1α) and PGC-1ß mRNA were unchanged (Fig. 7B). PGC-1 is a major regulator of mitochondrial biogenesis in skeletal muscle (46). Collectively, these data demonstrated that an increase in the mitochondrial capacity, but not increased mitochondrial density, are likely not the sole contributors to the increased energy expenditure observed in Mpzl3-KO mice.

**Skeletal muscle function is enhanced in Mpzl3-KO mice**. We wanted to directly assess the functional capacity of skeletal muscle in KO mice, since 1) skeletal muscle is an important determinant of whole body resting metabolic rate (47), 2) the embryonic loss of Mpzl3 might have impaired the development of skeletal muscle, and 3) the observed reductions in body weight could be due to cachexia. To do this, we measured the contractile force generated by the gastrocnemius upon stimulation of the sciatic nerve in situ using a naïve cohort of aged female KO and WT mice. Interestingly, more force per unit muscle mass was generated in KO mice than in controls (Fig. 7A). After analysis, we dissected out the gastrocnemius bundle and measured wet weights of the intact muscle tissue (gastrocnemius, soleus, and plantaris muscles) from each mouse. These data were used to normalize the force data, and we determined that the amount of muscle relative to body weight was increased by 37% in KO mice (WT 3.6 ± 0.1 vs. KO 5.7 ± 0.1 g body wt, *P < 0.0001, unpaired t-test, n = 8–9).

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was likely contributing to the observed enhancements in contractile force in KO mice. Furthermore, these data demonstrated that *Mpzl3*--KO mice were not cachexic.

*Mpzl3--KO mice have reduced hepatic triglyceride synthesis and are protected from hepatic steatosis.* Consistent with a reduction in hepatic cytoplasmic vacuoles in *Mpzl3*--KO mice (Fig. 8A), total liver triglyceride content was reduced by 92% in KOs compared with WT controls (Fig. 8B). To determine whether there were changes in hepatic lipogenic enzymes that could account for this dramatic reduction in triglycerides, we performed gene expression analysis of liver tissue from KO and WT mice. *Acc1*, *Scd1*, FAS, and *Ucp2* mRNA levels were significantly lower in hepatic tissue isolated from KOs (Fig. 8C). SREBP1, a transcription factor that controls triglyceride synthesis by positively regulating *Acc1*, *Scd1* and FAS (33), was also downregulated in KO mice in liver tissue (Fig. 8C). We also assessed the rate of de novo triglyceride synthesis in vivo and found that there was a 56% reduction in [14C]acetate-incorporated triglycerides in hepatic tissue taken from KO mice (Fig. 8D). Consistent with reduced hepatic lipid synthesis, levels of serum cholesterol, phospholipids, triglycerides ($P = 0.08$), and free-fatty acids ($P = 0.07$) were also reduced (Table 2). As expected, WT mice fed the HED had accumulated significant hepatic lipids, as evidenced by the increased size of cytoplasmic vacuoles in this tissue, but *Mpzl3*--KO mice were completely protected from diet-induced steatosis (Fig. 8A).

We also investigated whether there were alterations in lipolytic mechanisms in WAT that could account for the greater than 50% reduction in fat mass in KO mice (Fig. 2C). Interestingly, we found no difference in the rate of de novo triglyceride synthesis in epididymal WAT in KO mice (Fig. 8E). However, mRNA levels of adipose triglyceride lipase (ATGL) were significantly downregulated in this tissue (Fig. 8F). ATGL is the rate-limiting enzyme in the breakdown of WAT triglyceride stores (45). Thus, increased lipolysis in WAT was likely not a major contributor to the reduced adiposity observed in *Mpzl3*--KO mice. In summary, our data demonstrate that both de novo and dietary-induced pathways promoting hepatic triglyceride production were downregulated in KO mice. Last, we measured *Mpzl3* mRNA expression in liver in response to HED exposure. Hepatic (but not hypothalamic) expression of *Mpzl3* was increased in mice fed the HED for 8 wk (Fig. 8G), suggesting that *Mpzl3* is regulated in liver in response to dietary fat.

**DISCUSSION**

The present study has identified MPZL3 as an essential regulator of metabolic homeostasis in mice. Genetic ablation of
Mpzl3 in mice led to profound changes in metabolic physiology. Mpzl3-KO mice had decreased body weight and adiposity, increased overall energy expenditure, improved glycemic control, and reduced both circulating and liver triglyceride levels. Furthermore, KO mice were protected from weight gain, hyperglycemia, and hepatic steatosis from both adult-onset and diet-induced obesity that are characteristic of mice on the C57BL/6 background strain (25). These effects occurred in the absence of 1) reductions in food intake, 2) global increases in fat oxidative pathways (since RQs were elevated) 3) significant increases in BAT activation, and 4) increases in lipolysis in WAT. However, our evidence suggests that several of the metabolic improvements were likely driven by reductions in hepatic lipogenesis, which is consistent with its relatively high expression in liver. Since we found very low expression levels of Mpzl3 in WAT, we speculate that a reduction in lipolytic activity in this tissue could be a compensatory mechanism in an effort to reduce overall lipolysis and retain fat to defend body weight and energy stores. We were further able to demonstrate that the overall leanness in these mice was not due to cachexia, as skeletal muscle function remained intact in KO mice. Last, we found that Mpzl3 was increased by feeding a high-fat diet and would increase food intake in response to metabolic demand (15). Total caloric energy expenditure is controlled by several distinct mechanisms, including increases in lean skeletal muscle mass and oxidative capacity, non-exercise-induced thermogenesis such as increased locomotor activity due to grooming or foraging for food, and activation of BAT as measured by increased UCP1 expression. We found evidence that all of the above parameters were modestly enhanced in KO mice, and thus all likely contribute (possibly additively) to the observed increase in whole body energy expenditure. It is interesting to note that EVA1 (the closest known homolog of MPZL3) has been identified as being highly enriched in met-

KO mice utilized much more glucose to support the massive increases in energy expenditure and muscle contractility. Glucose uptake in muscle will need to be directly compared between KO and WT mice. Overall, our data suggest that whole body insulin sensitivity might be increased in KO mice, and this is supported by significantly lower HOMA-IR values in KO mice. In addition, we saw an increase in insulin-stimulated expression of p-Akt in livers in KO mice. Hyperinsulinemic-euglycemic clamp studies will need to be performed to directly measure whole body insulin sensitivity and hepatic glucose production (3).

Mpzl3-KO mice had one of the more dramatic diet-independent increases in energy expenditure that has been reported in a KO mouse strain to date. Elevated energy expenditure along with increased glucose and fat utilization levels in KO mice could certainly drive reductions in body weight and adiposity and would increase food intake in response to metabolic demand (15). Total caloric energy expenditure is controlled by several distinct mechanisms, including increases in lean skeletal muscle mass and oxidative capacity, non-exercise-induced thermogenesis such as increased locomotor activity due to grooming or foraging for food, and activation of BAT as measured by increased UCP1 expression. We found evidence that all of the above parameters were modestly enhanced in KO mice, and thus all likely contribute (possibly additively) to the observed increase in whole body energy expenditure. It is interesting to note that EVA1 (the closest known homolog of MPZL3) has been identified as being highly enriched in met-

**Fig. 6.** Analysis of thermogenesis in Mpzl3-KO mice. A: core body temperatures were measured by telemetry before and during fasting in chow-fed WT and Mpzl3-KO mice. Gray bars, dark phase of circadian cycle. Fasting was initiated 1 h before lights-off, indicated by horizontal line. Data were analyzed with two-way repeated-measures ANOVA and posttests (*P < 0.01; n = 8 WT, n = 7 KO). B: UCP1 mRNA levels in BAT of Mpzl3-KO mice. C: Western blot analysis of UCP1 protein at room temperature. Shown is relative density of UCP1 protein levels normalized to calreticulin in BAT. *P = 0.02, unpaired t-test; n = 6-7.

**Fig. 7.** Contractile force of skeletal muscle in Mpzl3-KO mice is increased. A: in situ analysis of contractile force generated by gastrocnemius muscle upon stimulation of the sciatic nerve. Data were normalized to mg of muscle for each individual mouse. Mice were age-matched WT and KO female mice at 14 mo (paired t-test, *P = 0.0009; n = 8 WT, n = 7 KO). B: qPCR analysis of mRNA expression in skeletal muscle. Data were normalized to ARBP. Total RNA was prepared from the gastrocnemius bundle from naïve males at 4–5 mo of age (*P = 0.003, unpaired t-test; n = 7). All mice were maintained on standard chow.
abnormally active BAT compared with white or beige adipocytes (39, 44). Consistent with this, we found that \( \text{Mpzl3} \) expression was about four times higher in BAT than in epididymal WAT. Thus, although UCP1 levels in KO mice were modestly increased in KO mice, lipid synthesis in the sebaceous glands of SCD-1 or DGAT1-KO mice leads to gland atrophy, which in turn causes the hair follicle to die, resulting in widespread alopecia (8, 28, 37). Taken together, increased energy expenditure in \( \text{Mpzl3} \)-KO mice is likely not a direct consequence of fur loss and subsequent dissipation of heat with a loss of insulation. However, a water immersion test is needed to quantitatively compare the insulating properties of skin in KO and WT mice (8).

Rough coat mice (\( \text{Mpzl3}^{rc} \)) have a missense mutation (Arg\( ^{100} \) to Gln substitution), which presumably results in a loss of MPZL3 function (7). Thus, \( \text{Mpzl3}^{rc} \) mice might be expected to have a similar phenotype as \( \text{Mpzl3} \)-KO. In our KO model, almost the entire exon 3 of \( \text{Mpzl3} \) was deleted, including the Gln substitution), which presumably results in a loss of MPZL3 function (7). Thus, \( \text{Mpzl3}^{rc} \) mice might be expected to have a similar phenotype as \( \text{Mpzl3} \)-KO. In our KO model, almost the entire exon 3 of \( \text{Mpzl3} \) was deleted, including the

Measuring energy expenditure under conditions of thermoneutrality would have been ideal to rule out a significant contribution of nonshivering thermogenesis to increased energy expenditure (37), although its necessity has been much debated (27, 32, 40, 43). Such experiments require maintaining the mice in alternative housing conditions that were not permitted to be achieved in our laboratory. Importantly, we were able to demonstrate that younger \( \text{Mpzl3} \)-KO mice have increased energy expenditure levels before visible fur loss (Fig. 5). It is also worth noting that, although subcutaneous fat deposits in \( \text{Mpzl3} \)-KO mice are likely smaller as part of a general reduction in percent body fat, we found no evidence for a reduction in the number or size of the lipid-producing sebaceous glands in \( \text{Mpzl3} \)-KO mice. Moreover, the sebaceous glands of KO mice appeared hypertrophic compared with WT controls (Fig. 1B) similar to \( \text{Mpzl3}^{rc} \) mice (7). This is opposite to what has been found in mice lacking the genes encoding the lipogenic enzymes stearoyl-CoA desaturase-1 (SCD-1) and diaclylglycerol acyltransferase-1 (DGAT1). The reduction of lipid synthesis in the sebaceous glands of SCD-1 or DGAT1-KO mice leads to gland atrophy, which in turn causes the hair follicle to die, resulting in widespread alopecia (8, 28, 37). Taken together, increased energy expenditure in \( \text{Mpzl3} \)-KO mice is likely not a direct consequence of fur loss and subsequent dissipation of heat with a loss of insulation. However, a water immersion test is needed to quantitatively compare the insulating properties of skin in KO and WT mice (8).

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Several other phenotypic changes were noted in \( \text{Mpzl3}^{rc} \) mice, including elevated blood calcium levels and myocardial fiber degeneration (21). Our studies failed to find these differences
(Table 2 and T. A. Czyzyk, unpublished observations). However, most of our studies were done in relatively young mice (4–6 mo) compared with the 12- to 20-mo-old mice used for the Mpzl3 

and Mpzl3 KO strains needs to be performed. It should be noted that Tang et al. (42) have recently reported the generation of both Mpzl2- and Mpzl3-KO mice as part of a large-scale effort to knock out secreted and membrane-bound proteins in mice. Similar to our findings, they reported reductions in body weight and fat mass and evidence of alopecia in Mpzl3-KO mice. Interestingly, such pronounced phenotypes were not observed in Mpzl2-KOs suggesting that Mpzl2 and Mpzl3 have distinct roles in metabolism even though they are structurally homologous (19, 22).

Many questions remain, including how MPZL3 is posttranslationally processed and how it might control hepatic lipogenesis. Overexpression of Mpzl3 in WT mice should help to elucidate some of its direct effects, particularly in liver. Furthermore, MPZL3 interactions with cellular proteins and intracellular signaling pathways have yet to be determined in a mammalian system (5, 35). Immunoglobulin domain-containing cell adhesion molecules are known mediators of immune cell recruitment during inflammation, and their expression in plasma is increased in obesity (41) and after consumption of a single high-fat meal in humans (30). Thus, MPZL3 might potentially function in the inflammatory response to dietary fat intake (6). Further studies to determine whether changes in the inflammatory pathways are involved in the lean phenotype of Mpzl3-KO mice are necessary.

The significance of our findings to human obesity remains to be determined, since human models of MPZL3 deficiency have yet to be reported in the literature. However, two genetic linkage studies have reported that the MPZL3 chromosomal location (11q23.3) is linked to body mass (20, 26) and energy expenditure (31) in Pima Indians, a population exhibiting a very high incidence of obesity and type 2 diabetes (23) (24). Specific analysis of MPZL3 in candidate gene association studies may reveal that MPZL3 mutations can modify metabolism in Pima Indian, diabetic, and/or dyslipidemic populations. Furthermore, the potential role of MPZL3 in human skin conditions is intriguing (34). In humans, the prominent feature of both acne and male pattern baldness (androgenic alopecia) is sebaceous gland hypertrophy. Conversion of weak androgens into more potent androgens such as 5α-dihydrotestosterone (DHT) is suspected to increase the size and metabolic rate of the sebaceous gland in these conditions (11, 38). Further biochemical studies including lipid profiling of skin and analysis of androgen sensitivity in Mpzl3-KO mice are needed to understand their possible contributions to the lean phenotype of these mice.

MPZL3 remains a protein of unknown function, but elucidation of its mechanism of action will surely lead to a better understanding of pathways that control energy and glucose homeostasis. Therapeutics that target MPZL3 or related pathways might someday prove to be beneficial in the treatment of obesity.

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GRANTS

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DISCLOSURES

All authors were employees of Eli Lilly and Company at the time the research was conducted.

AUTHOR CONTRIBUTIONS


REFERENCES


