Mct8-Deficient Mice Have Increased Energy Expenditure and Reduced Fat Mass That Is Abrogated by Normalization of Serum T_3 Levels

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Children with monocarboxylate transporter 8 (MCT8) deficiency lose weight, even when adequately nourished. Changes in serum markers of thyroid hormone (TH) action compatible with thyrotoxicosis suggested that this might be due to T_3 excess in peripheral tissues. Mct8-deficient mice (*Mct8KO*) replicate the human thyroid phenotype and are thus suitable for metabolic studies so far unavailable in humans. In the current work, compared with wild-type (*Wt*) mice, *Mct8KO* mice were leaner due to reduced fat mass. They tended to use more carbohydrates and fewer lipids during the dark phase. *Mct8KO* mice had increased total energy expenditure (TEE) and food and water intake, with normal total activity, indicating hypermetabolism. To determine whether this is due to the high serum T_3, we studied mice deficient in both Mct8 and deiodinase 1 (*Mct8D1KO*) with serum T_3 similar to *Wt* mice and *Wt* mice given L-T_3 to raise their serum T_3 to the level of *Mct8KO* mice. Contrary to *Mct8KO*, *Mct8D1KO* mice had similar fat mass, TEE, and food intake as their *D1KO* littermates, whereas T_3-treated *Wt* mice showed increased food intake and TEE, similar to *Mct8KO* mice. In skeletal muscle, *Mct8KO* mice had increased T_3 content and TH action and increased glucose metabolism, which improved in *Mct8D1KO* mice. These studies indicate that the high serum T_3 in MCT8 deficiency increases the TEE and fails to maintain weight despite adequate calorie intake. This is mediated by tissues that are not predominantly MCT8 dependent for TH transport, including skeletal muscle. Normalizing serum T_3 level by deleting deiodinase 1 corrects body composition and the metabolic alterations caused by the MCT8 deficiency. **(*Endocrinology* 154: 4885–4895, 2013)**

Mutations in the monocarboxylate transporter 8 (*MCT8*; *SLC16A2*) gene, coding for a specific thyroid hormone (TH) cell membrane transporter (1), have been recognized to cause a severe form of X-linked mental retardation and psychomotor impairment in more than 200 young males. The syndrome, known also as the Allan-Herndon-Dudley syndrome, comprises severe cognitive deficiency, truncal hypotonia, and poor head control, progressive spastic quadriplegia, diminished muscle mass with weakness, joint contractures, and dystonia (2, 3). However, most patients demonstrate a failure to thrive and an inability to gain weight. Their weight is below the third percentile, and often they require gastric tube feeding (4, 5). In addition to the psychomotor retardation, patients with MCT8 deficiency display a characteristic combination of thyroid function test abnormalities that include high serum concentration of T_3, low levels of T_4 and rT_3, and a normal or slightly elevated concentration of TSH (6, 7).

Although the neurological impairment causing difficulties with feeding could contribute to the inability to gain weight, the hypermetabolism caused by TH excess in some peripheral tissues may play an important role. This was suggested by the responses of several TH-responsive se-

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Abbreviations: AU, arbitrary unit; BAT, brown adipose tissue; BW, body weight; D1, deiodinase 1; D2, deiodinase 2; DEXA, dual-energy x-ray absorptiometry; KO, knockout; MCT8, monocarboxylate transporter 8; RER, respiratory exchange ratio; SM, skeletal muscle; TEE, total energy expenditure; TH, thyroid hormone; VCO_2, CO_2 production; VO_2, O_2 consumption; Wt, wild type.

rum markers, such as elevated serum SHBG, ammonium and lactic acid levels, and reduced cholesterol concentration. Of note, three patients showed weight gain closely correlated with the decline of T_3 while on treatment with diiodothyropropionic acid (8) or with combined L-T_4 and propylthiouracil treatment (5).

Initial studies in Mct8-deficient (*Mct8KO*) mice demonstrated TH deprivation in the brain resulting from severe impairment of T_3 transport reducing its access into neurons, even though the mice do not fully manifest the neuromuscular deficits observed in humans (9, 10). In contrast, the high serum T_3, observed in humans and mice, has been shown to increase its supply to mouse tissues, such as liver, which express other TH transporters, thus making them less dependent than brain on TH transport via Mct8. The high liver T_3 content in *Mct8KO*, accumulated through increased uptake or impaired efflux, produces an increase in deiodinase 1 (D1) and alterations in other markers of TH action (ie, increase in serum alkaline phosphatase and decrease in serum cholesterol and liver glutathione S transferase-α2 mRNA) (9, 10). This repetitive cycle of increased liver D1 expression stimulated by T_3 is in part responsible for the high serum T_3 level and contributes to the low serum T_4 concentration. By generating mice with combined Mct8 and D1 deficiencies (*Mct8D1KO*), we showed that absence of D1 corrected the serum T_3 and rT_3 abnormalities of Mct8 deficiency and increased the serum T_4 concentration, partially correcting the brain depletion of TH (11).

Given that the serum TH abnormalities observed in man have been reproduced in the *Mct8KO* mouse (9, 10), this mouse model continues to provide insights into the pathophysiology of MCT8 defects. A variable hormonal deficiency among tissues and cell types related to the redundancy of TH membrane transporters raises the possibility that the inability to gain weight observed in patients with MCT8 defects could be due to a TH excess in some peripheral tissues such as muscle. Because direct measurements of metabolism in Mct8-deficient mice and humans are still lacking, we used the *Mct8KO* mice to determine the whole-body energy homeostasis. To further investigate the metabolic effects of high serum T_3 levels in Mct8 deficient mice, we analyzed *Mct8D1KO* mice, which have normal serum T_3 levels, and T_3-treated *Wt* mice, with serum T_3 levels similar to *Mct8KO* mice (Table 1). Thus, we used two complementary experimental approaches to determine the role of excess serum T_3 in the hypermetabolism of Mct8 deficiency and the contribution of T_3 generated from T_4 in peripheral tissues.

Materials and Methods

Three separate experiments were performed.

Experiment 1

Body composition was measured by dual-energy x-ray absorptiometry (DEXA) in wild-type (*Wt*) and *Mct8KO* mice, five to eight mice for each group. They were subsequently placed in metabolic cages for determination of total energy expenditure (TEE), respiratory exchange ratio (RER), total activity, and food and water intake.

Experiment 2

To determine the role of the high serum T_3 levels on the hypermetabolism associated with Mct8 deficiency, studies were repeated in mice deficient in both Mct8 and D1 (*Mct8D1KO*), in which serum T_3 is not different from that of *Wt* mice and *D1KO* littermates, five to eight mice for each group. The results were compared with those obtained in experiment 1.

Experiment 3

Wt mice were treated with T_3 to increase the serum T_3 levels to those of *Mct8KO* mice. Five *Wt* mice received L-T_3 in the drinking water at a concentration of 220 ng/mL in 0.01% BSA solution. This treatment was given for 21 days, and for the last 6 days, from the 15th to the 21st day, the mice were placed in metabolic cages. The mice drank on average 11.58 mL/d per 100 g body weight (BW) of water, equating to approximately 2.5 μg per 100g BW of T_3 per day.

Experimental animals

Procedures performed in mice were approved by the University of Chicago Institutional Animal Care and Use Committee in which animals were housed and experiments were carried out. Animals were housed in temperature- (22 ± 2°C) and light (12

Table 1. Serum Thyroid Function Tests

Group	TSH, mU/L	T_4, μg/dL	T_3, ng/dL	rT_3, ng/dL
Wt	33.8 ± 6.5	3.7 ± 0.2	50.7 ± 2.9	22.4 ± 1.5
Mct8KO	74.6 ± 8.1[a]	1.1 ± 0.1[a]	90.6 ± 2.9[a]	3.2 ± 0.4[b]
D1KO	36.1 ± 7.7	5.2 ± 0.1[a]	40.1 ± 6.6	97.2 ± 6.8[a]
Mct8D1KO	49.5 ± 7.1[c,d]	5.1 ± 0.3[a,e]	40.5 ± 3.7[e]	17.4 ± 2.1[d]
Wt+T_3	<10[a,e]	<0.25[a,e]	98.6 ± 5.2[a]	<2.5[a]

Data expressed as mean ± SE. Eight to 10 mice were used per group. [a] $P < .001$, significant differences in comparison with the *Wt*. [b] $P < .01$, significant differences in comparison with the *Wt*. [c] $P < .05$, significant differences in comparison with the *Wt*. [d] $P < .05$, significant differences when comparing *Mct8D1KO* or *Wt*+T_3 with *Mct8KO* mice. [e] $P < .001$, significant differences when comparing *Mct8D1KO* or *Wt*+T_3 with *Mct8KO* mice.

h light, 12 h dark cycle; lights on at 6:00 AM)-controlled conditions and had free access to food and water. *Mct8KO*, *Mct8D1KO*, and *D1KO* mice were generated and genotyped as described (9, 11). Specifically, *Wt* and *Mct8KO* male mice were littermates as were *D1KO* and *Mct8D1KO* male mice. Because Mct8 is located on the X-chromosome, this was achieved by mating heterozygous $Mct8^{+/-}$ females with *Wt* males, whereas $Mct8^{+/-}D1^{-/-}$ females were crossed with $D1^{-/-}$ males. Each experiment was performed using different groups of 10- to 12-week-old male C57BL/6J mice. Tissues were obtained at the termination of all experiments.

Body composition

Body composition was measured by DEXA (Lunar PIXImus densitometer system; GE Healthcare) using PIXImus 2 software. The system was calibrated according to the manufacturer's instructions prior to the start of the experiment.

Indirect calorimetry

Animals were placed individually into an eight-cage combined, open circuit indirect calorimetry system (LabMaster system; TSE System), herein referred to as metabolic cages, that measures food and water intake and physical activity continuously as well as O_2 uptake and CO_2 production at 30-minute intervals (12). Mice were adapted to this environment for 48 hours before starting the recording periods. RER, TEE, and glucose and lipid oxidation were calculated from the O_2 consumption (VO_2) and CO_2 production (VCO_2) relative to body weight. In particular, glucose and lipid oxidations were calculated as described (13) with the following modifications: glucose oxidation = $4.55\ VCO_2 - 3.21\ VO_2$, and lipid oxidation = $1.67\ VO_2 - 1.67\ VCO_2$, where VCO_2 and VO_2 (not corrected for protein oxidation) are in liters per minute per kilogram body weight. Activity monitoring and detection of animal location were performed with infrared sensor pairs arranged in strips for horizontal and vertical activity, detecting every ambulatory movement. The sum of the spontaneous physical activity and the high-frequent activity (equivalent of breathing activity) represents the total activity. Use of infrared sensors for detection of movement allowed continuous recording in both light and dark phases.

Tissue T_3 content

Before tissue collection, mice were perfused, under anesthesia, with PBS containing heparin through a needle placed in the left ventricle. Tissues were collected rapidly on dry ice and stored at −80°C. T_3 was extracted from muscle and brown adipose tissue using a method described by Morreale de Escobar et al (14), and T_3 content was measured by RIA as previously detailed (see supplemental data in Reference 15). Recovery was monitored by the addition of labeled T_3 before tissue extraction.

Measurement of tissue mRNAs

Muscle (soleus and gastrocnemius), brown adipose tissue, and cerebrum were collected from 8–10 mice per group, *Wt*, *Mct8KO*, *D1KO*, *Mct8D1KO*, and T_3-treated *Wt*, to study gene expression. Methods for total RNA extraction from tissue, reverse transcription, and real-time quantitative PCR have been described previously (9). The oligonucleotide primers were designed to cross introns. Primer sequences used for the real-time quantitative PCR are provided in Supplemental Table 1, published on The Endocrine Society's Journals Online web site at http://endo.endojournals.org. Results are expressed relative to those in the *Wt* mice and normalized to RNA polymerase II (*RpII*) mRNA (16).

Determination of deiodinase 2 (D2) activity

D2 enzymatic activity was performed as described (15) with the following modifications: 100 μg of tissue homogenates (homogenized with Bullet Blender; Next Advance Inc) in 100 μL reaction mixture containing 0.1 M phosphate buffer (pH 7), 1 mM EDTA, 20 mM dithiothreitol, 1 mM propylthiouracil, 100 000 cpm [^{125}I]-T_4, and 2 nM unlabeled T_4 were incubated at 37°C for 1 hour. Saturating levels of unlabeled T_3 (1 μM) were added to the reaction mixture to inhibit the deiodinase 3 enzyme. The enzymatic activity was expressed in femtomoles per hour and milligrams of protein and was corrected for nonenzymatic deiodination observed in the tissue-free controls.

Measurement of serum thyroid function tests

Serum total T_4, T_3, rT_3, and TSH concentrations were measured by RIAs as detailed elsewhere (15).

Statistical analysis

All results are expressed as mean ± SEM. Statistical analysis was performed using a 2-tailed Student's *t* test for unpaired observations (comparison of values obtained in two groups) or 1-way ANOVA followed by a Newman-Keuls multiple comparison test (comparison among three or more groups). $P \geq .05$ was considered not to be significant (NS).

Results

DEXA study

Body composition measurements indicated *Mct8KO* were leaner than *Wt* mice. Total body mass was 24.8 ± 0.4 and 27.0 ± 0.4 g in the *Mct8KO* and *Wt* mice, respectively (Figure 1A). Although these values were not significantly different, *Mct8KO* mice had reduced fat mass (15.0% ± 0.8% vs 18.6% ± 1.2%, $P < .05$) and increased lean mass (85.0% ± 0.8% vs 81.4% ± 1.2%, $P < .05$) as percentage of total body mass compared with *Wt* littermates (Figure 1B). In contrast, *Mct8D1KO* mice with combined Mct8 and D1 deficiency exhibited normal body composition compared with *D1KO* littermates, having similar total body mass (26.6 ± 0.8 vs 27.9 ± 0.6 g), fat (19.9% ± 1.0% vs 21.4% ± 0.7% total mass) and lean mass (80.1% ± 1.0% vs 78.6% ± 0.7% total mass). All these parameters did not differ from those of *Wt* mice (Figure 1, A and B).

Metabolic cage study

After placement in the metabolic cages, mice were allowed to acclimatize for 2 days with no recording. Subsequently, we gathered data for the following 5 days but used for analyses only data obtained from the last 3 consecutive days. For all considered parameters, results rep-

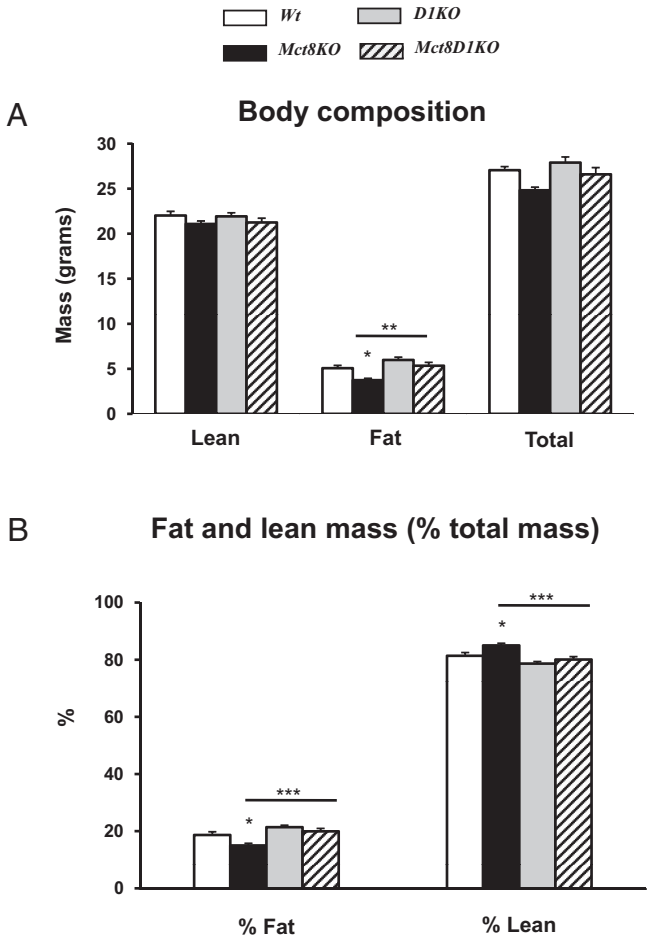

Figure 1. DEXA study of *Wt*, *Mct8KO*, *D1KO*, and *Mct8D1KO* mice. A, Body composition. B, Fat and lean mass expressed as percentage of total body mass. Genotypes are indicated. Asterisks represent *P* values by ANOVA and Newman-Keuls test. Significant differences compared with *Wt* animals are above the SEM bars. Comparisons of *Mct8KO* and *Mct8D1KO* are indicated by horizontal lines. *, $P < .05$; **, $P < .01$; ***, $P < .001$.

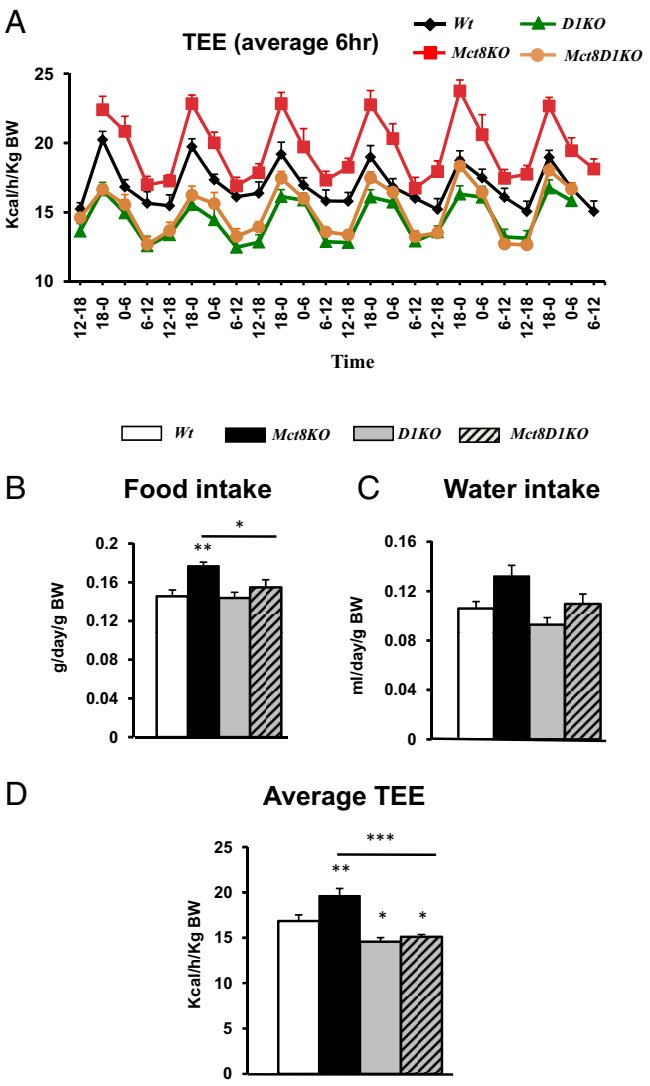

Figure 2. Metabolic cage study of *Wt*, *Mct8KO*, *D1KO*, and *Mct8D1KO* mice. A, An example of total TEE flow chart. B, Average 24-hour food intake. C, Average 24-hour water intake. D, Average TEE. Genotypes are indicated. Symbols indicating statistical significances are as indicated in the legend to Figure 1.

resent mean of the values obtained during the light phase (6:00 AM to 6:00 PM), dark phase (6:00 PM to 6:00 AM) and during the whole 24-hour period.

All measured parameters had a clear diurnal rhythm in all animal groups, with the highest values registered during the dark phase, corresponding to the period of major activity of the animals. As an example, a TEE flow chart is shown in Figure 2A.

Food and water intake

The *Mct8KO* mice maintained on regular chow were significantly hyperphagic relative to *Wt* littermates (Figure 2B). *Mct8KO* mice consumed 21% more regular chow per day than controls. The hyperphagia was associated with increased water intake, 25% more than *Wt* mice per 24 hours (Figure 2C). Increases in both food and water intake were observed only during the dark phase of the light-dark cycle (data not shown). Food and water intake in mice with *Mct8D1KO* did not differ from the intakes of the *D1KO* littermates or *Wt* mice (Figure 2, B and C).

Motor activity

There were no differences in the mean levels of total activity between *Mct8KO* and *Wt* mice in both light and dark phases [average per day: 38318 ± 3756 and 37943 ± 5674 arbitrary unit (AU), respectively]. The same held true for *Mct8D1KO* mice, which exhibited levels of activity (average per day: 34432 ± 2583 AU), similar to that of *Wt* mice and *D1KO* littermates (30531 ± 2701 AU).

Energy expenditure

Mct8KO mice had greater TEE than *Wt* mice in all periods of the day (Figure 2A), with daily mean values of

19.6 ± 0.8 vs 16.9 ± 0.7 kcal/h·kg BW of *Wt* ($P < .01$) (Figure 2D). The association of higher values of TEE with normal levels of motor activity suggests that the increase is not secondary to hyperactivity. TEE normalized in the *Mct8D1KO* mice during the dark phase and was not different than that in *D1KO* mice. However, TEE of both *Mct8D1KO* and *D1KO* mice was lower than that of *Wt* mice during the light phase (Figure 2A) with a consequent lower daily mean value (14.6 ± 0.5 and 11.1 ± 0.3 kcal/h·kg BW in the *D1KO* and *Mct8D1KO* mice, respectively, Figure 2D), a finding likely related to the absence of D1.

RER and substrate oxidation

To evaluate whether there were differences in substrate oxidation in mice lacking Mct8, we measured the RER, an indicator of metabolic fuel preference. The 24-hour average RER was similar in all four genotypes; thus, we further analyzed RER in the light and dark phases separately and found significant higher RER in *Mct8KO*, *D1KO*, and *Mct8D1KO* during the dark phase (Figure 3A).

Further analysis of glucose and lipid oxidation shows that during the dark phase, there is a significant increase ($P < .001$) in glucose oxidation and decreased ($P < .05$) lipid oxidation in *Mct8KO* vs *Wt* littermates (Figure 3, B and C). In the combined *Mct8D1KO*, the glucose oxidation normalized (Figure 3B), whereas lipid oxidation remains lower, although it did not reach significance (Figure 3C). The RER in *Mct8KO*, *D1KO*, and *Mct8D1KO* during the dark phase was close to 1, indicating that these mice are preferentially using carbohydrates as fuel during this interval.

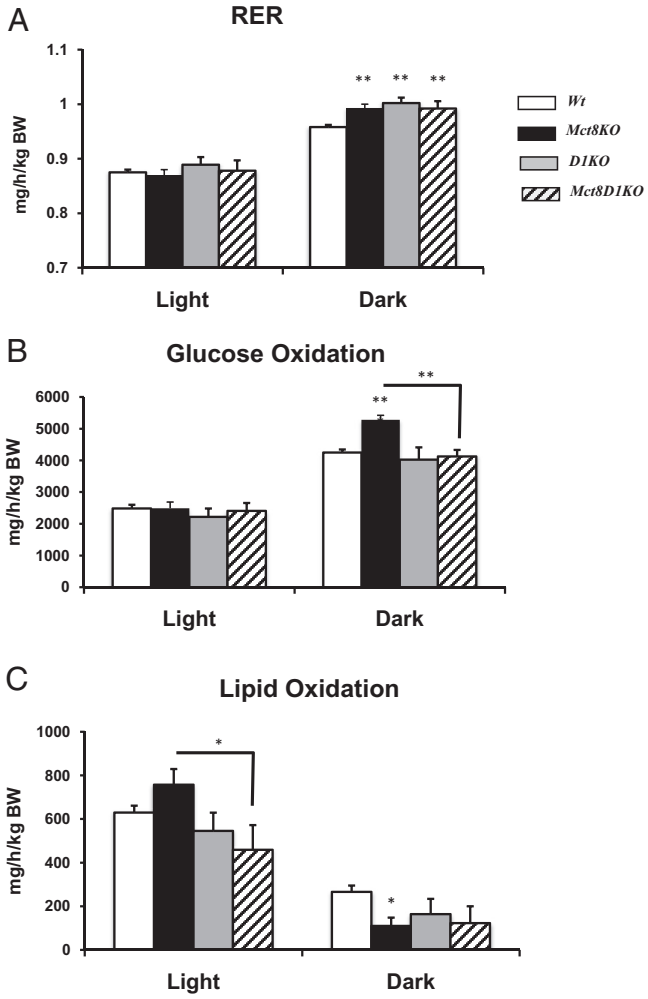

Figure 3. RER and substrate oxidation. A, RER, B, Glucose oxidation and C, Lipid oxidation in *Wt*, *Mct8KO*, *D1KO*, and *Mct8D1KO* mice. Genotypes are indicated. Symbols indicating statistical significances are as indicated in the legend to Figure 1.

TH status and glucose metabolism in skeletal muscle (SM), brown adipose tissue (BAT), and cerebrum

The finding that the normalization of serum T_3 levels in the *Mct8D1KO* mice ameliorated the phenotype of Mct8 deficiency indicates that the perturbation of TH status could be in part responsible for the observed hypermetabolism. It is known that TH plays an important role in energy expenditure and basal metabolic rate (17) both centrally and in periphery. Therefore, to investigate further, we assessed TH status and glucose metabolism in SM, BAT, and cerebrum.

Effect on SM

Data on muscle T_3 content are shown in Figure 4A. The SM of *Mct8KO* mice contained 30% more T_3 than *Wt* animals: 22.2 ± 2.1 vs 16.6 ± 1.6 pg/mg protein, respectively. Deletion of D1 reduced muscle T_3 content, making the values in *Mct8D1KO* mice intermediate between those in *Wt* and *Mct8KO* mice. The muscle T_3 content was 13.8 ± 0.9 and 18.1 ± 1.4 pg/mg of protein in the *D1KO* and *Mct8D1KO* mice, respectively. In general and for all genotypes, skeletal muscle T_3 content followed the concentrations observed in serum, indicating that TH uptake by skeletal muscle can occur independently of Mct8. In fact, we found that *Wt* mice have higher expression of *Mct10* than *Mct8* in muscle, both soleus (4.6 times) and gastrocnemius (40 times), and likely compensated for the absence of Mct8 in *Mct8KO* mice (Supplemental Figure 1).

We next measured the SM mRNA levels of well-documented markers of TH action and genes involved in glucose metabolism. These included the uncoupling protein-3 (*Ucp3*); the uncoupling protein-2 (*Ucp2*); the major isoforms of myosin heavy chain (*Mhc*); hairless (*Hr*); monocarboxylate transporter 1 (*Mct1*) and 4 (*Mct4*), which facilitate lactic acid and pyruvate transport; and pyruvate kinase muscle isoform (*Pkm*). Gene expression was studied separately in two different types of mouse SM: soleus,

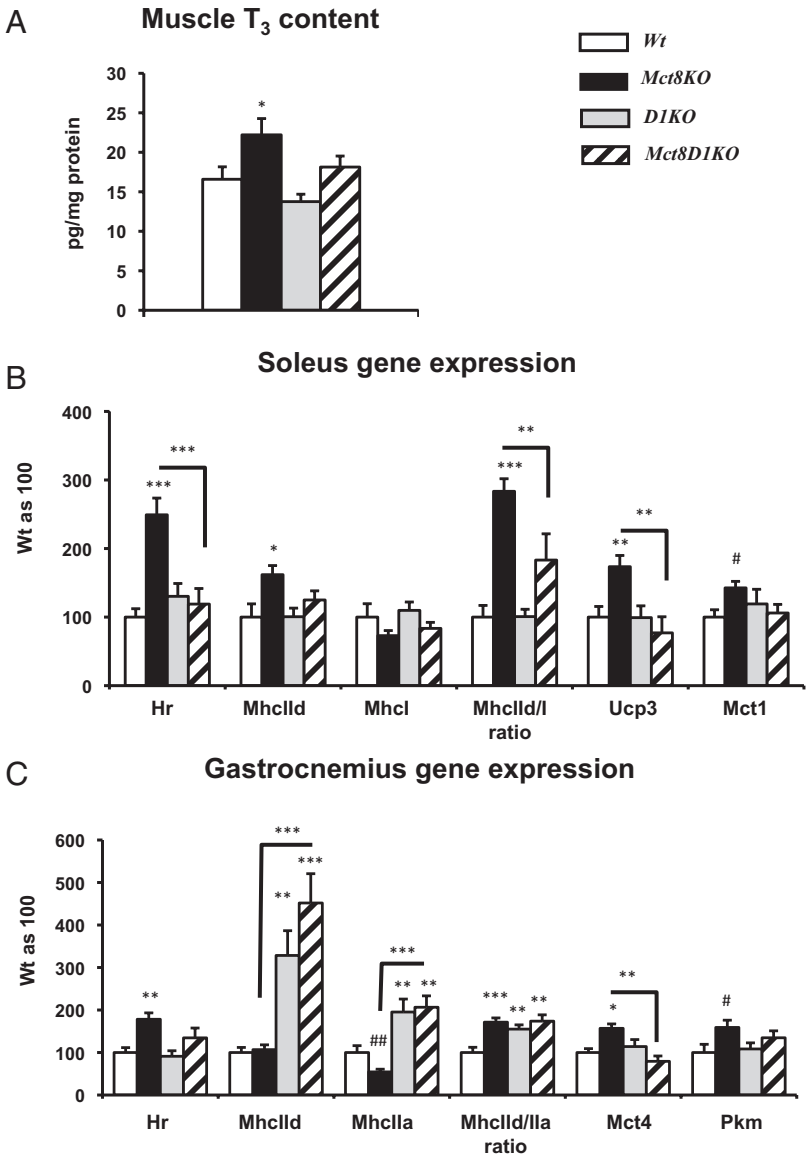

Figure 4. Measurements in skeletal muscle. A, T_3 content. B, Gene expression in soleus. C, Gene expression in gastrocnemius. Genotypes are indicated. Symbols indicating statistical significances are as in the legend to Figure 1. In addition, # and ##, $P < .05$ and $P < .01$, respectively, calculated by a 2-tailed Student's *t* test compared with *Wt*.

with predominantly slow-oxidative type I fibers, which control slow movement and posture, and gastrocnemius, with predominantly fast-glycolytic type IIB fibers, which control rapid movement (18). As expected on the basis of the tissue T_3 content, compared with *Wt* mice, *Mct8KO* mice had evidence of increased TH action (Figure 4, B and C) with increased *Hr* expression in both muscle types. *Mct8KO* mice exhibited in the soleus a reduction in *MhcI* with a significant increase in *MhcIId* mRNA levels, and in the gastrocnemius a reduction in *MhcIIa* with similar *MhcIId* as compared with the *Wt* mice (Figure 4C). These differences were more pronounced when the results were expressed as ratio between *IId/I* and *IId/IIa* isoforms of *Mhc* (Figure 4, B and C).

The results are in agreement with the higher muscle T_3 content producing a hyperthyroid state in muscle of Mct8-deficient mice because increasing levels of TH induce a consecutive shift from *MhcI* to *MhcIIa*, to *MhcIId*, and to *MhcIIb*, stimulating the next gene in the sequence and reducing or shutting down the expression of the former (18–20). We did not find any difference on *Ucp2*, *MhcIIb* mRNA levels between mice of two genotypes (data not shown). In soleus *Ucp3* mRNA was increased by 68% in *Mct8KO* mice as compared with the *Wt* littermates, whereas it was unchanged in gastrocnemius (data not shown). For lactate transporters *Mct1* expression was increased in soleus and *Mct4* expression was increased in gastrocnemius of *Mct8KO* mice compared with *Wt*, indicating increased shuffling of lactate between muscle fibers. Although Mct1 facilitates the uptake of lactate to be used as a respiratory fuel in soleus, which is primarily oxidative, Mct4 is the main transporter for efflux of lactic acid from a predominantly white fiber type muscle such as gastrocnemius, which is primarily glycolytic. *Mct4* overexpression in *Mct8KO* mice indicates relative increased lactic acid generation through glycolysis (21), as supported by the concomitant increased expression of *Pkm* in gastrocnemius in these mice (Figure 4C).

D1 deletion corrected the abnormalities related to Mct8 defects. *Mct8D1KO* mice showed in soleus normal *Hr*, *Ucp3*, and *Mct1* mRNA levels and normal *MhcIId* to *MhcI* mRNA ratio (Figure 4B). *Hr* and *Mct4* expression in gastrocnemius was no different from *Wt* in *Mct8D1KO* (Figure 4C). It is of interest to note that in the absence of D1, in gastrocnemius the mRNA levels of both *MhcIIa* and *MhcIId* were higher than in *Wt* and *Mct8KO* mice. The resulting ratio of *MhcIId* to *MhcIIa* is similar in these two genotypes and higher than the *Wt*. This is not well understood and needs further investigation.

Effects on BAT (Figure 5)

To evaluate the TH status and action in BAT, we measured T_3 content and expression of TH-regulated genes,

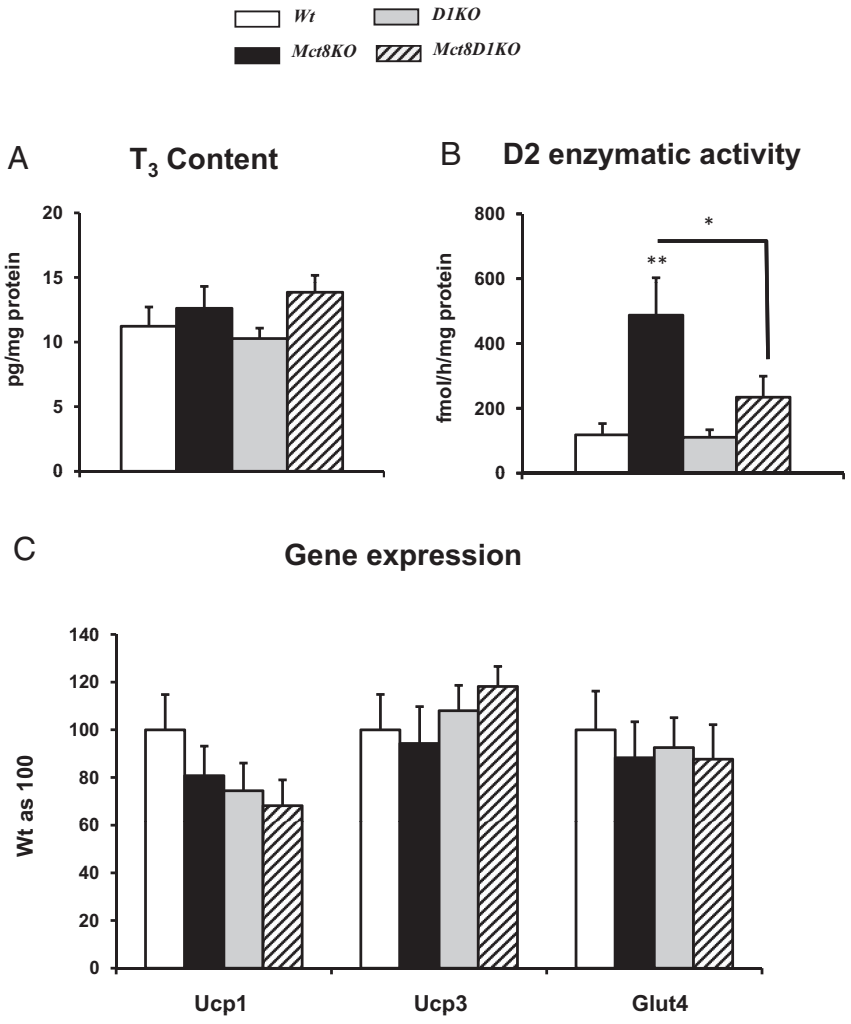

Figure 5. Measurements in BAT. A, T_3 content. B, D2 enzymatic activity. C, Gene expression. Genotypes are indicated. Symbols indicating statistical significances are as in the legend to Figure 1.

namely the uncoupling protein-1 (*Ucp1*), *Ucp3*, and *Glut4*. D2 enzymatic activity was also measured as an indicator of TH status. No differences were found in T_3 content and in the expression of all studied genes in all genotypes (Figure 5, A and C). Whereas D2 activity was increased in the *Mct8KO* mice as compared with the *Wt* controls (Figure 5B), the differences in D2 activity were abrogated in *Mct8D1KO* mice, probably as consequence of increased T_4 availability.

Effect on cerebrum (Supplemental Figure 2)

The TH status of the brain of *Mct8KO* mice has been previously reported (9–11), showing decreased T_3 content and expression of TH target genes. Considering the finding of overall increased glucose oxidation in *Mct8KO* mice (Figure 3B) and the fact that the brain is a major site for glucose consumption, we measured the expression of genes involved in glucose transport and metabolism: glucose transporter *Glut1*, *Mct1*, and *Mct2*, which shuffle lactic acid between astrocytes and neurons (21), and *Ucp2*, and *Pkm*, which is the predominant *Pk* isoform in brain (Supplemental Figure 2). *Glut1*, *Ucp2*, and *Mct1* show similar expression in the two genotypes, with *Mct2* and *Pkm* showing decreased expression in the *Mct8KO* mice compared with *Wt* littermates. This indicates similar or decreased glucose metabolism in the cerebrum consistent with the observed overall brain hypothyroidism.

T_3 treatment

To assess whether the observed differences in the metabolic profile of the *Mct8KO* mice correlate with peripheral hyperthyroidism, their baseline serum T_3 levels (90.6 ± 2.9 ng/dL) were replicated in *Wt* mice by treating them with T_3 (98.6 ± 5.2 ng/dL) (see Table 1). T_3-treated *Wt* mice displayed increased food and water intake, increased energy expenditure (Figure 6A), together with normal total activity (data not shown), similar to *Mct8KO* mice. However, in *Wt* T_3-treated mice, 24-hour average RER was decreased, glucose oxidation was normal, and lipid oxidation was increased compared with *Wt* untreated mice (Figure 6B). For *Mct8KO* mice, 24-hour average RER and lipid oxidation were normal, whereas glucose oxidation was increased. This indicates that some aspects of the hypermetabolism observed in the *Mct8KO* mice are replicated in the *Wt* T_3-treated mice, whereas there are some differences in substrate preference.

The study of gene expression in the *Wt* T_3-treated mice showed increase in *Hr* expression and *MhcIId* to *MhcIIa* ratio in gastrocnemius, confirming the hyperthyroid status obtained through T_3 treatment (Figure 7A). Considering the finding of increased lipid oxidation in *Wt* T_3-treated mice, the expression of genes involved in lipid metabolism (22) was studied in gastrocnemius (Figure 7B). Some of the genes studied include fatty acid translocase/Cd36 (*Cd36*), which facilitates fatty acid uptake into cells; lipoprotein lipase (*Lpl*); carnitin palmitoyltransferase 1b (*Cpt1b*), which is required to transport long-chain fatty acyl-CoAs from the cytoplasm into the mitochondria for β-oxidation; pyruvate dehydrogenase kinase

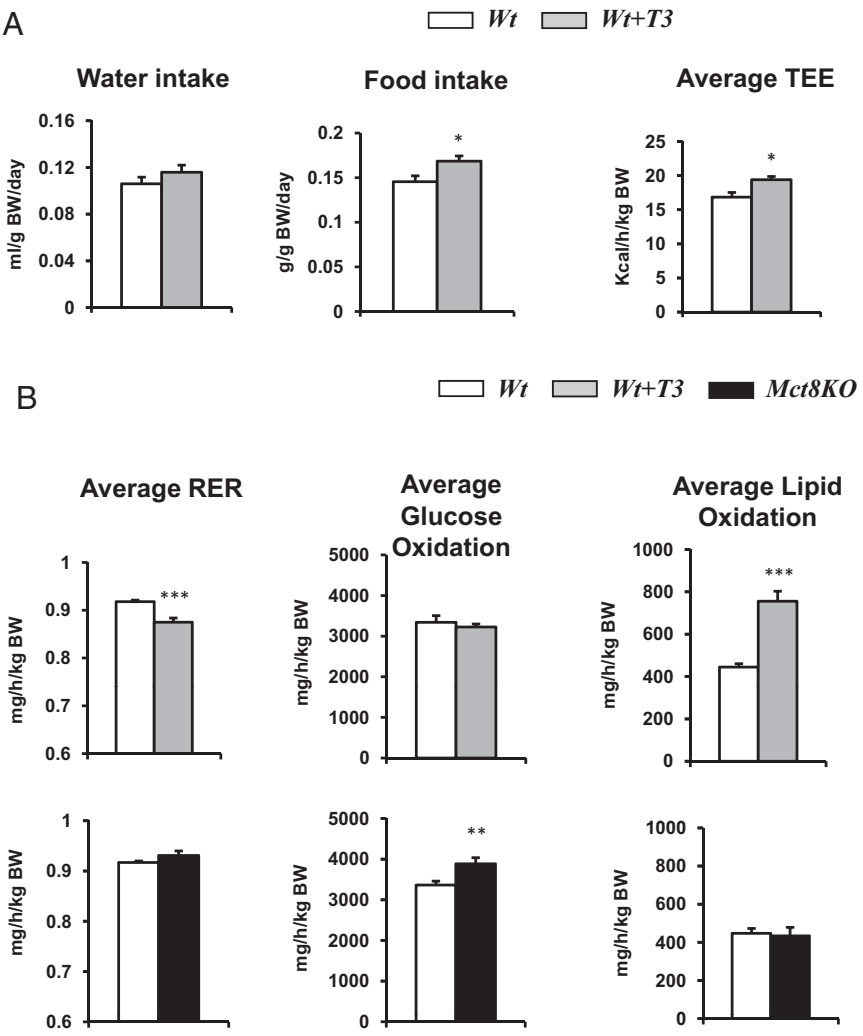

Figure 6. Metabolic cage study of *Wt* T_3-treated mice. A, Average 24-hour food intake, water intake, and TEE in *Wt* T_3-treated mice compared with *Wt*. B, Average 24-hour RER and glucose and lipid oxidation in *Wt* T_3-treated and *Mct8KO* mice compared with *Wt*. Genotypes are indicated. Statistical analyses were performed by a 2-tailed Student's *t* test for unpaired observations. *, $P < .05$; **, $P < .01$; ***, $P < .001$.

4 (*Pdk4*), which when increased determines the preference for lipid over glucose oxidation; and hormone-sensitive lipase (*Hsl*) (23, 24). When compared with *Wt* littermates, T_3-treated mice had increased expression of all genes studied, although not significant for *Hsl*, in agreement with the finding of increased lipid oxidation in T_3-treated mice. *Mct8KO* at baseline had normal expression of *Cd36*, *Lpl*, *Cpt1b*, and *Pdk4* but significantly low expression of *Hsl*, the latter one possibly reflecting, at least in part, the decreased lipid oxidation observed during the dark phase (Figure 3C).

Discussion

Several observations suggest that, in patients with inactivating mutations of *MCT8* gene, failure to gain weight may be the consequence of a hypermetabolic state resulting from elevated serum T_3 levels. A recent publication showed a close correlation between T_3 levels and body mass index in two twins with MCT8 deficiency, who showed an improvement in an SD score of weight with the decline of T_3 during treatment with the TH analog diiodothyropropionic acid (8). A similar observation was reported by Wemeau et al (5) in a child with MCT8 deficiency in whom T_3 generation was decreased during replacement therapy with levothyroxine by treatment with propylthiouracil, which reduces the conversion of T_4 to T_3 by D1 peripherally. The undernourished 16-year-old boy had previously difficulty to maintain his weight, even after placement of a gastric feeding tube. When T_3 levels normalized, there was a significant weight gain. The reduction of serum T_3 also resulted in a normalization of the low cholesterol and the high SHBG levels. These serum alterations, which are markers of TH action in peripheral tissues, have also been reported in other patients with *MCT8* gene mutations (4, 25). The increase of muscle metabolism in MCT8-deficient patients, as assessed by serum markers, is in agreement with the data found in mice.

Because detailed metabolic evaluation is lacking in individuals with MCT8 defects, we used the mouse model of Mct8 deficiency to study the whole-body energy homeostasis in this condition. Previous studies have showed that the *Mct8KO* mice have high serum T_3 levels (Table 1) and the other thyroid function test abnormalities described in humans with *MCT8* gene mutations (9, 10). In addition, they present a combination of tissue-selective hypothyroidism and hyperthyroidism (9, 10).

The present work shows that the *Mct8KO* mice are lean, have increased metabolism, and are hyperphagic, with the tendency to use as fuel more carbohydrates and less lipids during the dark phase and have higher RER during this time. The leanness in the presence of a normal activity is most likely a consequence of the increased en-

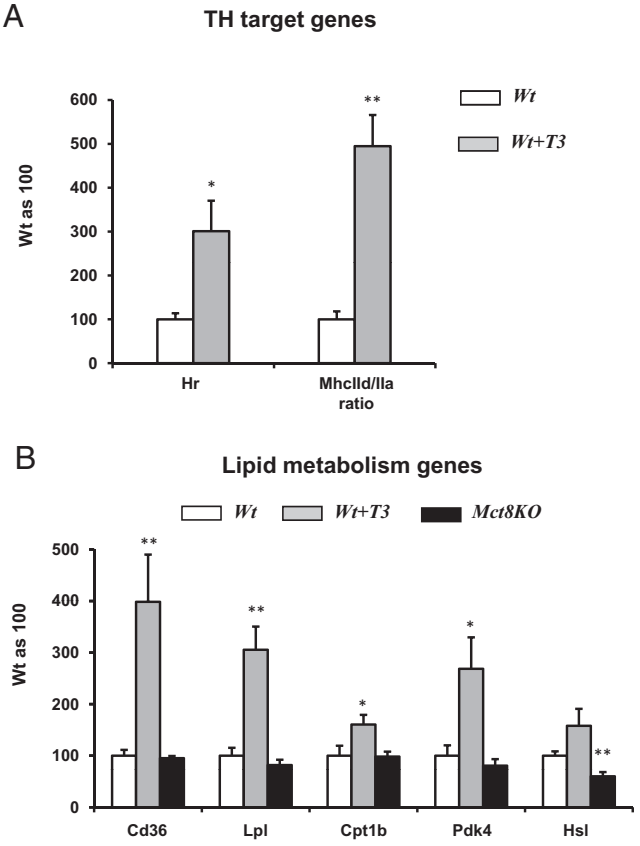

Figure 7. Gene expression in gastrocnemius of *Wt* T_3-treated mice. A, TH target genes in *Wt* T_3-treated mice compared with *Wt*. B, Lipid metabolism genes in *Wt* T_3-treated and *Mct8KO* mice compared with *Wt*. Statistical analyses were performed by a 2-tailed Student's *t* test for unpaired observations. *, $P < .05$; **, $P < .01$.

ergy expenditure, whereas the food intake is increased for substrate replenishment.

Of note, *D1KO* mice had normal food intake but decreased TEE compared with *Wt*; however, they have normal body composition paradoxically without an increase in fat mass, as one would predict. The reason for this finding is unclear. D1 was shown to have a functional role in white adipose tissue, being involved in the metabolism and/or accumulation of adipose tissue, with a stimulatory effect of leptin on D1 activity (26). Therefore, the relative lack of increased fat mass in *D1KO* mice could be due to this role of D1 in white adipose tissue.

The study of the *Wt* mice treated with L-T_3 to have serum T_3 levels similar to those of untreated *Mct8KO* mice showed that some of the metabolic parameters, such as increased food and water intake, increased energy expenditure in a setting of normal level of total activity, were similar in these groups of mice. However, other parameters were different between the two groups, such as the 24-hour average RER, and glucose and lipid oxidation. In agreement with the finding of increased lipid oxidation in *Wt* T_3-treated mice, the expression of *Cd36*, *Lpl*, *Cpt1b*, and *Pdk4*, genes involved in lipid metabolism, was increased. The decreased *Hsl* expression observed in *Mct8KO* mice possibly reflects the decreased lipid oxidation observed during the dark phase. Some of the observed differences between the *Wt* T_3-treated and *Mct8KO* mice could be in part due to different thyroid function tests because T_3-treated *Wt* mice have undetectable TSH and T_4 compared with the *Mct8KO* mice (Table 1). Also, we cannot exclude the possibility that the unique tissue-specific TH availability characteristic of the MCT8 defect has intricate consequences on the metabolic homeostasis, compared with global hyperthyroidism. This requires further studies.

The involvement of TH in the regulation of energy expenditure and the metabolic rate is well known (17), being in part dependent on central control from the brain as well as on peripheral tissues. As previously shown (27), the TH status of the brain can modulate the metabolic status in peripheral tissues through sympathetic and parasympathetic pathways. Considering the central hypothyroidism in *Mct8KO* mice (9, 10), compared with a relative state of hyperthyroidism in *Wt* T_3-treated mice based on undetectable TSH, the autonomic outflow of the brain to metabolic organs is expected to be different. In fact, our data on gene expression involving glucose transport and metabolism in cerebrum (*Glut1*, *Mct1* and *Mct2*, *Ucp2*, and *Pkm*) indicate similar or decreased glucose metabolism in the cerebrum of *Mct8KO* mice, consistent with the previously reported brain hypothyroidism. Although it is likely that brain metabolism contributes to the global metabolism measured in these mice, it seems that peripheral tissues such as muscle have a bigger contribution because the overall TEE is actually increased in *Mct8KO* mice.

Muscle and BAT are peripheral tissues playing important roles on the metabolism and energy expenditure. The higher T_3 content in muscle of *Mct8KO* mice compared with *Wt* mice was associated with markers of increased TH action including increased *Hr* gene expression and the switch in the expression of the myosin isoforms. Thus, it seems that as for liver and kidney, Mct8 is not critical for TH transport into muscle, and other transporters can compensate for its lack. This is supported by a recent study showing that in addition to MCT8, other transporters are expressed in human skeletal muscle, including MCT10 (28). In fact, we find that mice have higher expression of *Mct10* than *Mct8* in muscle and likely compensates for the absence of Mct8 in *Mct8KO* mice.

The study of genes involved in glucose metabolism showed increased expression of *Pkm* and *Mct4* in the gastrocnemius of *Mct8KO* mice, indicating increased glycolysis, and efflux of lactic acid, whereas the increased expression of *Ucp3* and *Mct1* in soleus indicates increased

lactate uptake and overall increased mitochondrial oxidative capacity (21). Overall, muscle manifests a hyperthyroid status with increased glucose metabolism and energy expenditure.

Contrary to muscle, BAT seems to respond to the low serum T_4 levels of Mct8 deficiency because *Mct8KO* mice show a significant increase in BAT D2 enzymatic activity. These results are in agreement with the estimated important contribution of D2 to T_3 levels in BAT, even at room temperature (29). The finding of similar T_3 levels and mRNA level of genes regulated by TH in *Mct8KO* and *Wt* mice is likely due to this compensatory increase in D2.

When we performed the same studies in mice deficient in both Mct8 and D1, (*Mct8D1KO*), which have normal serum T_3 levels (Table 1) and normal cerebrum T_3 content (11), the body composition and food intake normalized. In the combined *Mct8D1KO*, the glucose oxidation normalized, whereas lipid oxidation remained lower. The muscle of *Mct8D1KO* mice showed a euthyroid state in terms of *Hr* expression and the metabolic genes studied, in agreement with the metabolic cage data and DEXA study. These data support the hypothesis that normalization of serum T_3 concentration and that of brain T_3 levels can ameliorate the metabolic phenotype of Mct8 deficiency.

In conclusion, this study demonstrates that in MCT8 defects, failure to maintain normal weight despite increased caloric intake is in part due to increased energy expenditure associated with high serum T_3 levels. This is true for tissues that are not predominantly MCT8 dependent for TH transport. Skeletal muscle is among these tissues, manifesting thyrotoxic increase in energy expenditure. Also, the tissue-specific thyroid hormone availability characteristic of MCT8 defect plays a role in the observed metabolic homeostasis.

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