Effect of weight loss on lactate transporter expression in skeletal muscle of obese subjects

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Metz L, Mercier J, Tremblay A, Alméras N, Joannis DR. Effect of weight loss on lactate transporter expression in skeletal muscle of obese subjects. J Appl Physiol 104: 633-638, 2008. First published December 13, 2007; doi:10.1152/japplphysiol.00681.2007.—The effects of weight loss on skeletal muscle lactate transporter [monocarboxylate transporter (MCT)] expression in obese subjects were investigated to better understand how lactate transporter metabolism is regulated in insulin-resistant states. Ten obese subjects underwent non-macronutrient-specific energy restriction for 15 wk. Anthropometric measurements and a needle biopsy of the vastus lateralis muscle before and after the weight loss program were performed. Enzymatic activity, fiber type distribution, and skeletal muscle MCT protein expression were measured. Muscle from nonobese control subjects was used for comparison of MCT levels. The program induced a weight loss of 9.2 ± 1.6 kg. Compared with controls, muscle from obese subjects showed a strong tendency (P = 0.06) for elevated MCT4 expression (+69%) before the weight loss program. MCT4 expression decreased (−7%) following weight loss to reach levels that were not statistically different from control levels. There were no differences in MCT1 expression between controls and obese subjects before and after weight loss. A highly predictive regression model (R² = 0.95), including waist circumference, citrate synthase activity, and percentage of type 1 fibers, was found to explain the highly variable MCT1 response to weight loss in the obese group. Therefore, in obesity, MCT1 expression appears linked both to changes in oxidative parameters and to changes in visceral adipose tissue content. The strong tendency for elevated expression of muscle MCT4 could reflect the need to release greater amounts of muscle lactate in the obese state, a situation that would be normalized with weight loss as indicated by decreased MCT4 levels.

monocarboxylate transporters; lactate transport; diet

The prevalence of obesity and associated comorbidities is increasing (30, 40), underscoring the importance of developing effective strategies for reducing obesity and the risk of metabolic disease linked to insulin resistance. Diet-induced weight loss can promote significant improvement in clinical and metabolic disease linked to insulin resistance (31). Moreover, lactate is now recognized as a substrate for ATP production in several tissues (skeletal muscles, heart, brain, and hepatocytes) (2, 28). Since skeletal muscle is the tissue responsible for the vasoactive component of peripheral glucose uptake in response to insulin action and plays a central role in lactate metabolism, an impairment in lactate exchange in this tissue could contribute to hyperlactatemia in insulin-resistant states.

Lactate flux across the plasma membrane occurs via facilitated diffusion (15). In the past few years a family of monocarboxylate transporters (MCTs) have been identified that can facilitate the flux of lactate as well as other monocarboxylates across the plasma membrane (15). In rat and human skeletal muscles, two isofoms (MCT1 and MCT4) are coexpressed with different kinetic properties and locations. MCT1 expression is highly regulated by endurance training (9) and is correlated with muscle oxidative capacity and slow fiber type proportion, whereas MCT4 expression is increased following intensive training and is also related to fast fiber type proportion and indexes of glycolytic metabolism (3). Indeed, it has been proposed that the expression of MCT1 is most closely associated with muscle characteristics favoring the uptake of lactate for oxidative disposal (26), whereas MCT4 expression is related to the need for lactate extrusion (39).

Numerous studies have shown that lactate transport and MCT expression in muscle could be regulated by physical activity and electrical stimulation in nonpathological states (3, 9). Recently it has been shown that caloric restriction could also affect lactate transport activity in rats (20). However, these authors investigated lactate metabolism in a nonpathological animal model where metabolic alterations are not as important as those found in obesity. Therefore, the present study was performed to investigate whether weight loss could affect MCT1 and MCT4 expression in skeletal muscle of obese subjects.

RESEARCH METHODS AND PROCEDURES

Subjects. Ten Caucasian obese individuals (5 men, 5 women) were recruited through the media and gave their written informed consent to participate in this study, which was approved by the Laval University Medical Ethics Committee. All individuals underwent a medical evaluation by a physician, including a medical history. Subjects with cardiovascular disease, diabetes mellitus, and endocrine disorders were excluded from the study. A detailed medical history was obtained to exclude the presence of any other condition that could interfere with the results of this study.

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were excluded from the study. None had recently been on a diet or involved in a weight-reducing program, and their body weight had been stable during the last 2 mo before the study. Seven normal weight [body mass index (BMI) 22.8 ± 0.5 kg/m²] volunteers (4 men, 3 women) were included for the comparison of muscle MCT expression. They were slightly younger than subjects in the obese group (33.6 ± 2 vs. 40.6 ± 2.2 yr). All subjects were considered physically inactive on the basis that they engaged in no more than 1 h of leisure-related physical activity per week. Physical activity was controlled during the 15-wk weight loss protocol.

**Weight loss protocol.** Subjects in the weight loss protocol followed a 15-wk weight-reducing program involving a non-macronutrient-specific dietary restriction of −2,930 kJ/day combined with a drug therapy (fenfluramine 60 mg/day) or a placebo as previously described (7). Following the weight loss period, subjects were kept on a weight maintenance diet for 4–6 wk before muscle biopsy. This allowed us to avoid any potential acute effects of caloric restriction and active weight loss on MCT expression or other muscle changes.

**Anthropometric measurements.** Body weight was measured with a standard beam scale, and body density was determined by hydrodensitometry (1). The closed-circuit helium dilution method was used to assess residual lung volume (27). The Siri formula (36) was used to estimate body density, whereas fat mass and fat-free mass were estimated from the derived percentage of body fat and total body weight. Waist circumference was measured using standardized techniques (21).

**Oral glucose tolerance test.** A 3-h oral glucose tolerance test (OGTT) was performed in the fasting state according to procedures previously described in Tremblay et al. (38). These data were not available for the control subjects.

**Skeletal muscle biopsies.** Muscle samples were obtained 3 wk before the beginning of the weight-loss intervention and 4–6 wk after the end of the energy restriction. Both pre- (Pre-WL) and post-weight loss (Post-WL) muscle samples were obtained in a state of weight stability. Biopsies were taken from the middle region of the vastus lateralis muscle by use of the percutaneous needle biopsy technique as previously described (10). Muscle samples were divided in two parts: one was frozen in liquid nitrogen for subsequent determination of muscle enzyme activities and protein expression, whereas the other part was trimmed, mounted on corkboard, and frozen in isopentane cooled on liquid nitrogen and stored at −80°C for histochemical analysis.

**MCT1 and MCT4 expression.** Proteins were isolated from muscle as previously described by McCullagh et al. (26) and previously used in our laboratory (12, 34). All steps were carried out at 4°C in ice-cold buffers. Muscle protein concentrations were determined in duplicate by the bicinchoninic acid assay (Pierce, Interchim, Montluçon, France) with the use of BSA as a standard.

Affinity-purified polyclonal antibodies directed against the carboxy terminus of rat MCT1 were produced by immunization of New Zealand White rabbits with the synthetic peptide corresponding to amino acids 487–500 (KDTTEGPKKEEESPY) for MCT1 and amino acid 445–455 (GEVHTPETSV) of MCT4 (37). Twelve micrograms of protein for each sample and prestained molecular mass markers (Bio-Rad, Ivy-sur-seine, France) were separated on 12% SDS-polyacrylamide gels (200 V for 60 min) after the Novex system (Invitrogen, Groningen, The Netherlands). Proteins were transferred from the gels to polyvinylidene difluoride (PVDF) membranes (30 V, 60 min), and the membranes were incubated on a shaker for 1 h at room temperature in buffer D [20 mM Tris base, 137 mM NaCl, 0.1 M HCl adjusted to pH 7.5, 0.1% (vol/vol) Tween 20, and 5% (wt/vol) nonfat dried milk]. The membranes were then incubated with diluted MCT1 (1/60,000) or MCT4 antibody (1/90,000) in buffer D for 2 h at room temperature, followed by four washes in buffer E (buffer D without dried milk: 4 × 5-min washes) and then incubated for 50 min with goat anti-rabbit immunoglobulin G horseradish peroxidase-conjugated secondary antibody (1:1,500, Sigma France) in buffer E. Membranes were washed as above, and MCT1 or MCT4 expression was detected by ECL (Biomax MR films, Kodak, France). MCT1 and MCT4 protein band densities were determined by scanning the blots on a scanner (AGFA DuoScan T1200 NY) and analyzed with Scion Image software (Scion, Frederick, MD). Results are expressed in arbitrary OD units as used by Py et al. (34).

**Enzymatic activities.** Muscle (~10 mg) was homogenized in a glass-gram Diall homogenizer with 39 vol of ice-cold extracting medium (0.1 M Na-K-phosphate, 2 mM EDTA, pH 7.2), and maximal enzyme activities were measured spectrophotometrically as previously described (8). Enzymes chosen represent major energy-yielding pathways: phosphofructokinase (PFK), lactate dehydrogenase (LDH), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and hexokinase (HK) activities are representative of glucose metabolism and glycolysis; creatine kinase (CK) activity was used as indicator of the potential for anaerobic lactacid metabolism; and citrate synthase (CS), cytochrome-c oxidase (COX), and hydroxacyl-CoA dehydrogenase (HADH) are markers of aerobic (mitochondrial) and lipid metabolism potential. For LDH activity determination, muscle was homogenized in 20 vol of 210 mM sucrose, 2 mM EGTA, 40 mM NaCl, 30 mM HEPES, pH 7.4. The activity was determined in 50 mmol/l Tris, pH 7.5, 0.17 mmol/l NADH, and 2.5 mmol/l Na pyruvate. Enzyme activity was followed as changes in absorbance at 340 nm over 2.5 min at 25°C. All enzyme data are presented as units (micromoles of substrate consumed per minute) per gram of tissue (U/g).

**LDH isozyme distribution.** LDH isoenzymes present in muscle homogenates (1 μg of protein) were electrophoretically separated (90 V for 30 min) in agarose gels (1%) using a Bio-Rad SubCell system. An electrophoretic marker (LDH isostil, Sigma) containing LDH isozymes 1–5 was used as an aid to the identification of isozymes. LDH isozyme activities were visualized by nitroblue tetrazolium reduction to formazan (Sigma Procedure 705). The gels were fixed in 5% acetic acid. The different bands were scanned and quantified spectrophotometrically (AGFA Duo Scan T1200) using Scion Image software. LDH isozyme distribution was calculated by dividing the area × mean optical density product for each isozyme by the sum of area × mean optical density of the five isozymes. Results are expressed in percentage of all LDH isozymes.

**Histochemical analysis.** Cross sections (10 μm) of isopentane-frozen muscle were cut with a microtome at −20°C and stained for myosin ATPase (mATPase) (23). The single-step staining procedure allowed the identification of three major fiber types (I, IIA, and IIX) from the same section. To measure the cross-sectional area of the different fiber types, sections were examined under a light microscope (Leitz Dialux 20), which was connected to a charge-coupled device (CCD) camera (Sony C-350) with an analog-to-digital conversion system. Analysis of images of each fiber was performed on a Macintosh computer using the public domain NIH Image program (developed at the National Institutes of Health and available on the Internet at http://rsb.info.nih.gov/nih-image/). The mean cross-sectional area was determined by averaging the measurement of 30 randomly selected fibers of each type that had been obtained from the mATPase-stained sections.

**Statistical analysis.** Statistica (version 6.0, StatSoft) was used for all analyses. Effect of treatment (placebo/fenfluramine) or sex (male/female) was measured using paired Student’s t-test. No significant effects of treatment (fenfluramine vs. placebo) and sex were observed for any of the variables reported here. Differences before and after weight loss were tested using paired Student’s t-test. Enzyme activity and MCT protein level differences between groups were analyzed by two-way ANOVA. Simple regression and stepwise multiple regression analysis were performed to investigate predicting variables for MCT expression. Data are expressed as means ± SE, and effects were considered statistically significant at P values of less than 0.05.
RESULTS

Anthropometric data. The characteristics of the control subjects and of the obese subjects before and after weight loss are presented in Table 1. Caloric restriction in the obese subjects significantly reduced weight (−9.2 ± 1.6 kg), BMI (−3.1 ± 0.5 kg/m²), fat mass (−8.7 ± 1.5 kg), and waist circumference (−6 cm; all P < 0.05). No change was observed in fat-free mass.

OGTT. OGTT glucose values at 120 min did not decrease significantly with caloric restriction (6.98 ± 0.40 vs. 6.00 ± 0.53, P = 0.1). Glucose area under the curve did not change as a result of the intervention (Pre-WL, 1,263 ± 87; Post-WL, 1,146 ± 67 mmol·l⁻¹·min⁻¹), whereas for insulin area under the curve during the OGTT we observed a weak tendency to lower values (Pre-WL, 121,900 ± 16,058; Post-WL, 88,523 ± 12,913 μU·ml⁻¹·min⁻¹, P < 0.09).

Enzymatic activities and LDH isofrom distribution. Maximal enzyme activities are presented in Table 2. Except for slightly increased CK activity in obese individuals before weight loss, there were no differences between obese subjects and control subjects before or after weight loss.

LDH activity decreased slightly (−10%) with weight loss, but this effect did not reach statistical significance. There was no difference in LDH isofrom distribution before and after weight loss, the proportions of LDH5 (43.9 ± 2.1 vs. 44.2 ± 2%, Pre-WL and Post-WL, respectively). LDH4 (24.7 ± 0.5 vs. 24.3 ± 0.6%), LDH3 (19.6 ± 0.7 vs. 19.4 ± 0.9%), LDH2 (8.7 ± 1.2 vs. 8.5 ± 1.1%), and LDH1 (2.9 ± 0.7 vs. 3.4 ± 0.6%) remaining unchanged.

Muscle fiber distribution. Muscle fiber type proportion did not change following weight loss (41.0 ± 3.4 vs. 44.8 ± 3.9%; 37.6 ± 2 vs. 39.5 ± 3.9%; and 17.2 ± 3.0 vs. 14.3 ± 2.7% for type I, IIA, and IIX, respectively).

Muscle MCT expression and relation to other variables. MCT1 expression in muscle homogenates did not show significant changes after weight loss (Fig. 1). Compared with controls, MCT4 expression tended to be higher (Fig. 2, P = 0.06) in obese subjects before weight loss and decreased slightly to become nonstatistically different from control values following weight loss.

Large interindividual variations were observed for changes to MCT1 expression following weight loss (Fig. 3). Such large interindividual differences following weight loss were not observed for other muscle phenotypes. Furthermore, no significant anthropometric or muscle phenotype differences were observed when comparing the groups of subjects who displayed increased or decreased MCT1 expression following weight loss (data not shown). In an attempt to explain the large variations in MCT1 expression, we used simple regression and multiple regression analyses. In simple regression analysis, changes to MCT1 levels were found to correlate with changes to fat-free mass (r = −0.734, P = 0.016) as well as changes in activities of enzymes of aerobic metabolism (ΔCS: r = −0.641, P = 0.046; ΔHADH: r = −0.741, P = 0.014). No correlations were found between changes to MCT1 expression and changes to the proportion of any muscle fiber type (r range 0.04–0.50, P range 0.12–0.91).

Stepwise multiple regression analyses were performed to investigate predicting variables for MCT expression. We investigated if one or more variables could explain the variation of MCT1 expression after weight loss. Since MCT1 expression has been linked to parameters relating to oxidative metabolism, we included muscle typology and enzymatic activities such as CS in the multiple regression matrix. Moreover, we hypothesis...
sized that variation of anthropometric data could be implicated in MCT1 expression. Despite the small number of subjects in our study, we identified a highly predictive model of the change in MCT1 expression ($\Delta$MCT1) based on changes in waist circumference, CS activity, and the proportion of type 1 fibers (Table 3; $R^2 = 0.93$, $P < 0.05$).

We also performed simple regression analysis and multiple regression modeling with MCT4 data to investigate if some metabolic and/or anthropometric variable could predict MCT4 levels or changes in its expression following weight loss. We found no such relationships in the analysis of the MCT4 data.

**DISCUSSION**

We investigated the effect of weight loss on lactate transporter protein expression in obese people. Our main findings are that 1) caloric restriction in obese humans did not change muscle MCT1 expression, 2) interindividual variation of MCT1 expression in skeletal muscle could nevertheless be explained by changes in oxidative parameters and in adipose tissue, and 3) MCT4 tended to be overexpressed in obese people before weight loss and slightly decreased after the energy restriction program and pharmacotherapy.

Insulin resistance is a primary disorder in obesity and Type 2 diabetes. In these states, an increase in blood lactate is present, its production mainly originating from skeletal muscle (4). Lactate exchange has also been shown to be impaired in obese Zucker fa/fa rats (34), but no data are available on muscular alteration of lactate metabolism in human obese subjects.

Activities and levels of lactate transporters (MCTs) in skeletal muscle have been shown to be regulated by physical activity (2) and caloric restriction (20). To our knowledge, this is the first study to report the effect of weight loss on MCT expression in obese subjects. The energy restriction program of the present study slightly affected muscle MCT expression, albeit with no significant change between before and after weight loss. Similar results have been reported in animal models (20). In fact, in a nonpathological rat model, food restriction was shown to increase sarcolemmal lactate transport without changes in MCT1 or MCT4 expression. Levels of other substrate transporters like GLUT4 can be increased by weight loss in humans (29, 32). It seems that weight loss can sometimes (6) but not always (13, 20) act on transport protein expression.

Interindividual variation in MCT1 levels has previously been reported following endurance training (9), but our data are the first to show this variation following weight loss. Interestingly, we found a large variation in MCT1 expression following weight loss, such that it increased in five subjects and decreased in five others. To understand this large interindividual variability, and despite the small number of subjects that certainly limits our interpretation of the results, we used stepwise multiple regression analysis. Our results show that...
changes in the expression of MCT1 could be strongly explained by a regression model, including waist circumference and two parameters of oxidative metabolism (CS activity and type I fiber proportion). These results could explain why MCT1 expression is sometimes (9) but not always (11) correlated with indexes of muscle oxidative capacity. It is possible that substantial changes in muscle oxidative capacity or a combination of modest changes of different parameters are required to induce changes in the levels of this lactate transporter isoform.

The association between changes to type I fiber proportion and changes to MCT1 expression is not surprising since MCT1 is known to be expressed in oxidative muscle and to be localized preferentially in type I fibers (26). However, in our regression model, changes to fiber type proportion only weakly contributed to the large variation in MCT1 expression. The association between changes in muscle morphology (type I fiber proportion) and variations in MCT1 levels should therefore be considered in a context of interaction with changes in muscle metabolic potential (CS and HADH activities) and body composition (waist circumference).

The relationship between changes in MCT1 expression and changes in waist circumference is interesting. Waist circumference is used as an indicator of the visceral accumulation of adipose tissue, recognized as a site particularly important in the development or aggravation of insulin resistance. Moreover, this relation seems logical given the role of adipose tissue in the alteration of lactate metabolism in insulin-resistant states. Several studies have shown that adipocytes, especially from old obese or insulin-resistant animals, are able to release lactate produced from glucose metabolism (5). Recent data (16) on the lactate-sensitive transcription factor network in cultured L6 rat myocytes suggest mechanisms by which chronic exposure to elevated lactate levels could explain our data relating to MCT1 (Table 3). Indeed, among the adaptive responses observed by these authors following exposure to high lactate levels were increased RNA and protein expression of MCT1 and numerous mitochondrial proteins. Thus we could hypothesize that decreases in visceral adiposity following weight loss could significantly modify lactate release by adipose tissue, and as a consequence of changes to circulating lactate levels, muscle MCT and mitochondrial marker expression would be modified. However, considering that the effects of lactate in L6 cells were measured at concentrations more closely related to those measured following intense exercise (10 and 20 mM), it remains to be seen if chronic small changes in resting lactate (∼1–2 mM in the obese) following interventions such as weight loss are sufficient to impact on gene expression at all.

Unfortunately, we did not have measures of lactate fluxes (or of other substrates of interest, such as glucose, leucine, and free fatty acids) under basal conditions or during the OGTT to compare with MCT levels. Nevertheless, changes in lactate levels are not a prerequisite for changes to MCT levels, as it has been shown that lactate levels and MCT expression can vary independently (18, 20). Our results indicate that the metabolic potential and obesity status should be added to the list of parameters that could influence MCT expression.

MCT4 expression tended to be higher in muscle of obese subjects before weight loss compared with controls. This result contrasts with a previous study on animals that showed that obese rats had lower levels of MCT4 expression than controls rats (34). The MCT4 isoform is characterized by a low affinity for lactate but a high capacity for its transport, suggesting a role in lactate extrusion from the cell (25). In the obese state an increase in muscle lactate levels has been reported (34) possibly resulting from the observed shift toward a greater dependence on glycolytic metabolism (35). The tendency toward increased MCT4 expression found in the present study could reflect the need to extrude these increased levels of lactate from skeletal muscle in obese individuals. Although our data contrast with what has been described in rodents (3), the absence of a correlation between MCT4 protein levels and type II fiber proportion in our data agrees with the work of Pilegaard et al. (33) in human muscle.

Whatever changes might occur to muscle lactate levels following weight loss (i.e., decreased production or increased clearance or oxidation), our results show that changes to total LDH activity or isozyme distribution do not play a role. This result is consistent with data from others showing that weight loss only has a small (24) or no (17) effect on LDH enzymatic activity.

In conclusion, our results show that weight loss is accompanied by important interindividual variation in expression of MCT1. Although weight loss per se did not lead to changes in MCT1 expression, levels of this transporter were modified in concert with changes to markers of oxidative metabolism of skeletal muscle as well as changes to visceral adiposity. The strong tendency toward MCT4 overexpression in muscle of obese people could reflect the need to release a greater amount of muscle lactate. Caloric restriction induced a slight decrease in MCT4 expression to levels approaching control values, suggesting a normalization of lactate metabolism with weight loss.

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