CALL FOR PAPERS | Integrative and Translational Physiology: Integrative Aspects of Energy Homeostasis and Metabolic Diseases

Effects of weight loss and leptin on skeletal muscle in human subjects

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Submitted 19 July 2011; accepted in final form 6 September 2011

Baldwin KM, Joanisse DR, Haddad F, Goldsmith RL, Gallagher D, Pavlovich KH, Shamoon EL, Leibel RL, Rosenbaum M. Effects of weight loss and leptin on skeletal muscle in human subjects. Am J Physiol Regul Integr Comp Physiol 301: R1259–R1266, 2011. First published September 14, 2011; doi:10.1152/ajpregu.00397.2011.—Maintenance of a 10% or greater reduced body weight results in decreases in the energy cost of low levels of physical activity beyond those attributable to the altered body weight. These changes in nonreverting energy expenditure are due mainly to increased skeletal muscle work efficiency following weight loss and are reversed by the administration of the adipocyte-derived hormone leptin. We have also shown previously that the maintenance of a reduced weight is accompanied by a decrease in the ratio of glycolytic (phosphofructokinase) to oxidative (cytochrome c oxidase) activity in vastus lateralis muscle that would suggest an increase in the relative expression of the myosin heavy chain I (MHC I) isoform. We performed analyses of vastus lateralis muscle needle biopsy samples to determine whether maintenance of an altered body weight was associated with changes in skeletal muscle metabolic properties as well as mRNA expression of different isoforms of the MHC and sarcoplasmic endoplasmic reticular Ca2+-dependent ATPase (SERCA) in subjects studied before weight loss and then again after losing 10% of their initial weight and receiving twice daily injections of either placebo or replacement leptin in a single blind crossover design. We found that the maintenance of a reduced body weight was associated with significant increases in the relative gene expression of MHC I mRNA that was reversed by the administration of leptin as well as an increase in the expression of SERCA2 that was not significantly affected by leptin. Leptin administration also resulted in a significant increase in the expression of the less MHC IIX isoform compared with subjects receiving placebo. These findings are consistent with the leptin-reversible increase in skeletal muscle chemomechanical work efficiency and decrease in the ratio of glycolytic/oxidative enzyme activities observed in subjects following dietary weight loss.

energy metabolism; exercise; obesity

* K. M. Baldwin and D. R. Joanisse contributed equally to the data presented in this manuscript.

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power generated per contraction (7, 30) provide a possible mechanism for the increased efficiency and utilization of free fatty acids as fuel following weight loss. Type I (MHC I isoform predominant, efficient, slow-twitch, mainly fatty acid oxidative) or type II (MHC II isoform predominant, less efficient but more powerful, fast-twitch, predominantly glycolytic) (5, 10) are coexpressed in muscle and are defined by the pH-responsive activation patterns of their myofibrillar ATPases. Type II MHC isoforms are subdivided into MHC IIA (more efficient and fatty acid oxidative) and MHC IIX (more powerful, less efficient, more glycolytic). The ATP consumption rate for MHC I predominant muscle fibers is ~40% of that of MHC IIX and 65% of that of MHC IIA (16, 22, 26, 33, 44, 54). The respiratory quotient during low work levels, and the inorganic phosphate content of skeletal muscle, are significantly correlated with the relative proportion skeletal muscle fiber types (62, 66). Shifts in relative content of MHC isoforms, i.e., ratios of MHC I/MHC II and MHC IIA/MHC IIX, could account for the decline in NREE and increase in muscle work efficiency observed in weight-reduced subjects (50). We have recently shown that a significant fraction of the increased efficiency, as well as reliance on free fatty acids as fuel, during low level exercise are reflected in a decline in the ratio of glycolytic [phosphofructokinase, (PFK)] to fatty acid oxidative [cytochrome c oxidase (COX)] in weight-reduced subjects (23) which would be consistent with a greater relative expression of the MHC I isoform.

The sarcoplasmic reticulum Ca\(^{2+}\)-ATPases (SERCAs) are a family of membrane-bound enzymes that drive free calcium ions from the cytosol into the sarcoplasmic reticulum by coupling ATP hydrolysis to the translocation of Ca\(^{2+}\) (41). SERCAs and MHCs contribute independently to muscle work efficiency (27). The SERCA2a isoform predominates in type I muscle fibers, while SERCA1 predominates in type II fibers and is uniquely able to uncouple ATP hydrolysis from Ca\(^{2+}\) transport, thereby releasing the enthalpy of ATP as heat (37, 41). We hypothesized that maintenance of a reduced body weight would induce leptin-reversible changes in activity of glycolytic and oxidative enzymes that were symptomatic of an overall increase in the relative expression of the more efficient MHC I and SERCA2 isoforms of skeletal muscle.

MATERIALS AND METHODS

A total of 10 obese subjects (4 males and 6 females) were studied at their maximum lifetime weight that they had maintained within a 2-kg range for at least 6 mo prior to enrollment (35, 42). Recruitment procedures and exclusion criteria for these studies, and data regarding the effects of weight changes on energy expenditure in some of these subjects, have been previously reported (35, 46, 47). All studies were approved by the respective Institutional Review Boards and are consistent with guiding principles for research involving humans (2). Written informed consent was obtained from all subjects prior to enrollment. Subject characteristics are presented in Table 1.

The research protocol is schematized in Fig. 1. Subjects were inpatients in the Clinical Research Center at Columbia Presbyterian Medical Center throughout this study. They were weighed daily at 6 AM and were instructed to consume all meals before midnight. As described previously (35, 49), subjects were fed a liquid formula diet [40% of calories as fat (corn oil), 45% as carbohydrate (glucose polymer), and 15% as protein (casein hydrolysate)], plus vitamin and mineral supplements, in quantities sufficient to maintain a stable weight (defined as a mean daily weight variation of < 10 g/day for at least 2 wk). This weight plateau is designated as W\(_{\text{initial}}\).

Following completion of studies (described below), at W\(_{\text{initial}}\), subjects were provided 800 kcal of energy/day of the same liquid formula diet until they had lost ~10% of W\(_{\text{initial}}\). The duration of the weight loss phase ranged from 36 to 62 days. Once 10% weight loss had been achieved, intake was adjusted upward until subjects were again weight stable as described above. Subjects were then randomized to receive 5 wk of b.i.d. (8 AM and 8 PM) subcutaneous injections of saline (weight plateau is designated as W\(_{\text{10\%leptin}}\) or 5 wk of b.i.d. (8 AM and 8 PM) subcutaneous injections of recombinant human leptin (A-100, provided by Amgen, Thousand Oaks, CA and meteorelin provided by Amylin Pharmaceuticals, San Diego, CA) in doses that were calculated to achieve preinjection circulating leptin concentrations at 8 AM equal to those measured at W\(_{\text{initial}}\) (29, 48) (weight plateau is designated as W\(_{\text{10\%leptin}}\)). Initial leptin doses were: 0.08 mg/kg fat mass\(^{-1}\)dose\(^{-1}\) in males, and 0.14 mg/kg fat mass\(^{-1}\)dose\(^{-1}\) in females (48). Circulating leptin concentrations at 8 AM were measured weekly in subjects receiving leptin, and dosages were adjusted until circulating leptin concentrations were similar to those measured at 8 AM at W\(_{\text{initial}}\). Following completion of studies at W\(_{\text{10\%leptin}}\) or W\(_{\text{10\%placebo}}\), subjects underwent a 2-wk washout period during which they received no injections. They were then crossed over to receive either leptin or placebo injections for an additional 5 wk during which time the same measurements performed at W\(_{\text{initial}}\) and the previous weight loss study period (W\(_{\text{10\%placebo}}\) or W\(_{\text{10\%leptin}}\)) were repeated. The order of testing was preserved between study periods. Subjects were unaware of whether they were receiving leptin or placebo and remained on a diet isocaloric to that initially demonstrated necessary to maintain a 10% reduced body weight throughout the leptin or placebo arms of the study.

Studies

Subjects underwent the following studies at each study period.

Body composition. Fat-free mass and fat mass were determined by dual energy X-ray absorptiometry (43).

In vivo skeletal muscle fuel utilization and efficiency. In vivo skeletal muscle fuel utilization and efficiency were determined by graded cycle ergometry (52). After a 10-min period of accommodation, the subjects pedaled at 60 rpm against graded resistance to generate 10 W, 25 W, and 50 W of power in successive 4-min intervals using a Sensorsmedics 880S bicycle and ergometer with electrical braking (53). Oxygen uptake, carbon dioxide production, and the respiratory exchange ratio were measured continuously (21) using a Sensorsmedics VMAX 29 metabolic cart (53). Rates of oxygen consumption were converted to kilocalorie based on the respiratory

<table>
<thead>
<tr>
<th>Table 1. Subject clinical data</th>
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</thead>
<tbody>
<tr>
<td></td>
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<tr>
<td>Height, cm</td>
</tr>
<tr>
<td>Body weight, kg</td>
</tr>
<tr>
<td>Body mass index, kg/m(^2)</td>
</tr>
<tr>
<td>Fat-free mass, kg</td>
</tr>
<tr>
<td>Fat mass, kg</td>
</tr>
<tr>
<td>Plasma leptin, ng/ml</td>
</tr>
<tr>
<td>Respiratory exchange ratio at rest</td>
</tr>
<tr>
<td>Respiratory exchange ratio at 10 W power generated</td>
</tr>
<tr>
<td>Muscle efficiency at 10 W of power generated</td>
</tr>
</tbody>
</table>

Values are means (SD); 10 subjects, 6 males, 4 females. Subject population was ethnically diverse consisting of 3 African-American, 1 Hispanic-American, 1 Pacific Islander-American, and 1 South Asian-American female, 1 African-American, and 3 Caucasian-American males. *P < 0.05 compared with W\(_{\text{initial}}\), †P < 0.05 compared with W\(_{\text{initial}}\), and W\(_{\text{10\%leptin}}\).
were heated at 90°C for 5 min to stop the reaction and were then
protocol. At the end of the reverse transcription reaction, the tubes
of total RNA was reverse transcribed into cDNA for each sample
optical density at 260 nm (using the conversion factor of 40
RNA was precipitated from the aqueous phase with isopropanol, and,
biopsies (200–300 mg) were obtained under local anesthesia with
work performed above energy expenditure at rest (0 work performed)
to energy expended during exercise (21, 63). The lack of horizontal
in stationary bicycling minimizes the effects of weight loss on
work performed. Whatever decreased work there is from lifting the
weight-reduced leg up is theoretically matched by the decreased assist
from the weight of the lighter leg coming down. In fact, we have
previously shown that there are similar effects of weight loss on
skeletal muscle work efficiency (increased) and fuel utilization (de-
creased glucose utilization) in subjects studied following weight loss
with and without the addition of dead weight to the legs to replace lost
weight (50).

Biochemical analyses of skeletal muscle properties. Muscle needle
biopsies (~200–300 mg) were obtained under local anesthesia with 1%
xylcocaine from subjects in the postabsorptive state. Enzymatic
activity levels of creatine kinase, COX, hexokinase, glycogen phospho-
ylase, PFK, GAPDH, and 2-hydroxyacyl CoA dehydrogenase
(HADH), citrate synthase (CS; an indicator of mitochondrial mass),
and carnitine palmitoyl transferase-1 were determined spectrophot-
ometrically (14, 55, 56). The capacity of skeletal muscle to oxidize
fatty acids is reflected in the ratio of HADH/CS and the activity of the
mitochondrial respiratory chain is reflected in the ratio of COX/CS
(64). The ratios of PFK/COX and PFK/HADH activity in muscle
samples have been shown to provide estimates of the relative
glycolytic/fatty acid oxidative capacity of muscle in vivo (36, 64),
substantial difference being shown among different fiber types (31).

RNA analyses. Total RNA was extracted from preweighed frozen
muscle samples using the TRI Reagent according to the manufacturer’s
protocol (Molecular Research Center, Cincinnati, OH). Extracted RNA
was precipitated from the aqueous phase with isopropanol, and,
after washing with ethanol, the pellet was dried and suspended in
nuclease-free water. The RNA concentration was determined by
optical density at 260 nm (using the conversion factor of 40 μg/ml per
unit of optical density of 260 nm). The RNA samples were stored
frozen at −80°C for subsequent analyses for specific gene expression
using an end point RT-PCR approach. Prior to cDNA synthesis, RNA
integrity was checked by electrophoresis of 500 ng total RNA on 1%
agarose gel stained with Gelgreen stain (Biotium) and was found to be
high-quality RNA based on intact 28S and 18S bands. One microgram
of total RNA was reverse transcribed into cDNA for each sample
using the SuperScript II RT from Invitrogen and a mix of oligo(dT)
(100 ng/reaction) and random primers (200 ng/reaction) in a 20-μl
total reaction volume at 45°C for 50 min, according to the provided
protocol. At the end of the reverse transcription reaction, the tubes
were heated at 90°C for 5 min to stop the reaction and were then
stored at −80°C until used in the PCR reactions for specific mRNA
analyses.

Specific PCR primers to amplify target mRNA sequences were
designed using PrimerSelect software (LaserGene, DNASTar) and the
reference mRNA sequence from NCBI GenBank (see Table 2 for
primer information). The forward and reverse primers were designed
on different exons separated by large introns so that the GDNA
product will separate from the cDNA PCR product. Primers were
purchased from Operon Biotechnologies (Huntsville, AL). For each
target, The PCR reactions were carried out using (0.1 to 1 μl cDNA)
cDNA (corresponding to 5 to 50 ng of total RNA) in the presence of
2 mM MgCl2 by using standard PCR buffer (Bioline, Taunton, MA),
0.2 mM dNTP, 1 μM specific primer set, and 0.75 unit of DNA
polymerase (Bioline) in 25 μl total volume. For each primer set, PCR
conditions (cDNA dilution and PCR cycle number) were set to
optimal conditions so that the target mRNA product yields were in the
linear range of the semilog plot when the yield is expressed as a
function of the number of PCR cycles, and, for a given condition,
target mRNA PCR yields were tightly correlated to input cDNA (8).

Amplifications were carried out in a Stratagene Robocycler with an
initial denaturing step of 3 min at 96°C, followed by 24–27 cycles of
denaturing 1 min at 96°C, annealing 1 min at 59°C, and extending 1
min at 72°C, and a final extension step of 3 min at 72°C. PCR
products were separated on a 2.5% agarose gel by electrophoresis and
stained with ethidium bromide. The ultraviolet light-induced fluores-
cence of stained DNA bands was captured by a digital camera, and the
band intensities were quantified by densitometry with ImageQuant
software (GE Healthcare) on digitized images and were reported as
arbitrary scan units as reported previously (11). In this approach, each
specific mRNA signal is expressed in arbitrary units per ng of total
RNA.

Calculations and Statistical Analyses

Within-group comparisons (groups defined as the same subjects at
W_initial, W_initial–10%, or W_initial–5%) were made by repeated-measures
ANOVA. Before analyses were performed, normality of data distrib-
utions was confirmed by Wilk-Shapiro testing. Statistical signifi-
cance was prospectively defined as P < 0.05. Data were analyzed
using Statistica 6.0 software (60).

RESULTS

Subject Characteristics

Weight reduction was associated with a significant decrease in
body mass index, fat mass, and fat-free mass and in circulating
leptin concentrations (Table 1). We have previously
reported that leptin administration to weight-reduced subjects
resulted in a significant further loss of fat mass due to an increase
in 24-h energy expenditure (46). While we observed a tendency to
lose weight following leptin administration in the present study,
differences in weight and body composition between subjects at

Fig. 1. Schematic of protocol. Studies, including assessment of circulating leptin (lep) concentrations, energy expenditure, body composition, and skeletal muscle
biopsy, are identical at each testing period.
Wt\textsuperscript{initial} values following leptin repletion but differences in efficiency and fuel utilization were largely normalized to exercise by bicycle ergometry (Table 1, Fig. 2). Muscle catecholamine use of free fatty acids as fuel) at low levels of exercise and a significantly lower respiratory exchange ratio (indicating greater skeletal muscle chemomechanical work efficiency) and activity (whether expressed as the ratio of PFK to COX or PFK to HADH in muscle biopsy samples in subjects at Wt\textsuperscript{initial} and Wt\textsuperscript{10\%placebo} compared with both leptin replete states (Table 3).

Muscle Gene Expression

Subjects who were weight reduced without leptin repletion also demonstrated significantly increased expression of the more efficient MHC I isoform compared with the same subjects at Wt\textsuperscript{initial} or following weight loss but receiving leptin replacement (Wt\textsuperscript{10\%leptin}) (Fig. 3, Table 3). Expression of the more efficient SERCA 2 isoform was significantly increased following weight loss but did not appear to be affected by leptin administration to weight-reduced subjects. No significant effects of weight loss or of leptin replacement on MHC IIa or IIx expression were noted. The ratio of MHC IIa (more efficient/less efficient) was significantly greater at Wt\textsuperscript{10\%placebo} than Wt\textsuperscript{initial}. The expression of the more efficient SERCA 2 isoform was greater following weight loss and was not significantly affected by leptin repletion. Phentype alterations in slow Type I MHC and SERCA 2 mRNA expression occurred without any significant alterations in markers of muscle anabolic (IGF-I) or catabolic response (atrogin and myostatin) (Table 3). Furthermore, α-skeletal actin mRNA expression, which is fiber-type independent, remained unchanged in maintaining either a 10\% weight reduction or in response to leptin treatment.

When data were analyzed by ANCOVA in which gender, somatotype, and weight plateau were each entered as dichotomous variables, no significant effects of gender or somatotype were noted.

### Table 2. PCR primers used in RT-PCR to amplify mRNA in skeletal muscle

<table>
<thead>
<tr>
<th>Target mRNA</th>
<th>PCR Primers, 5' → 3'</th>
<th>PCR Product Size, bp</th>
<th>GenBank Accession No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Actin</td>
<td>Fwd: GCGGCGATTCGAGAGACCA</td>
<td>214</td>
<td>NM_001100</td>
</tr>
<tr>
<td></td>
<td>Rev: CCAGCGATTCGAGAGACATT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atrogin</td>
<td>Fwd: GAGGCGGACCGAGCTTGGAGAGAGATG</td>
<td>198</td>
<td>NM_058229</td>
</tr>
<tr>
<td></td>
<td>Rev: TGAAGAACGGTGTATAGGCTTGGGGTTGAAA</td>
<td></td>
<td>NM_148177</td>
</tr>
<tr>
<td>Myostatin</td>
<td>Fwd: CTGCAAGGGAGAAGATCTTACCA</td>
<td>243</td>
<td>NM_005259</td>
</tr>
<tr>
<td></td>
<td>Rev: GTTGGAAGATCGAGATTGCGATAT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IGF-1</td>
<td>Fwd: AGGGCGTCTTTCTGTATTTTCTT</td>
<td>251</td>
<td>NM_000618</td>
</tr>
<tr>
<td></td>
<td>Rev: CAATACATCTCAGCGGCTCTTA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SERCA 1</td>
<td>Fwd: CCAGACGAGGGGAGAGATTT</td>
<td>213</td>
<td>NM_173201</td>
</tr>
<tr>
<td></td>
<td>Rev: CCAACAGACCAGGCTGAGATT</td>
<td></td>
<td>NM_003230</td>
</tr>
<tr>
<td>SERCA 2</td>
<td>Fwd: ATGTTATATTTTTTGGTGTGGTTGA</td>
<td>219</td>
<td>NM_170655</td>
</tr>
<tr>
<td></td>
<td>Rev: GCTTTAATCCGGCTGACACTCTTTC</td>
<td></td>
<td>NM_001681</td>
</tr>
<tr>
<td>MHC I</td>
<td>Fwd: GGTGGGCGGAGTGGAGAGATG</td>
<td>404</td>
<td>NM_000257</td>
</tr>
<tr>
<td></td>
<td>Rev: GGAGCTTCTGGGAGACCTC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MHC IIa</td>
<td>Fwd: GGTGACGCGAGCTGAGAGAGAGG</td>
<td>426</td>
<td>NM_017534</td>
</tr>
<tr>
<td></td>
<td>Rev: TTGAGGGAAATCGACAAATAGT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MHC IIx</td>
<td>Fwd: GAGGACACGCCGCGCCCATCT</td>
<td>524</td>
<td>NM_005963</td>
</tr>
<tr>
<td></td>
<td>Rev: TTTCTTCTGGACCTTGGAGACCTT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PGC1α</td>
<td>Fwd: CACCGAGCAAACACTGCTAAGTTAT</td>
<td>222</td>
<td>NM_013261</td>
</tr>
<tr>
<td></td>
<td>Rev: GGTGGAGGAGGCTACGTTGGTT</td>
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<td></td>
</tr>
<tr>
<td>PPARδ</td>
<td>Fwd: GCCAGTACTCGCGCTGAGA</td>
<td>221</td>
<td>NM_006238</td>
</tr>
<tr>
<td></td>
<td>Rev: GCGGGGCTTTTTTGGTGCAT</td>
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</tbody>
</table>

SERCA, sarcoplasmic reticulum Ca\textsuperscript{2+}-ATPase; MCH, myosin heavy chain; PPAR, peroxisome proliferator-activated receptor.
Table 3. In vitro analyses of muscle biopsies of vastus lateralis

<table>
<thead>
<tr>
<th>Enzyme activity in 8 obese subjects (4 males, 4 females)</th>
<th>Wtinitial</th>
<th>Wt−10%placebo</th>
<th>Wt−10%leptin</th>
</tr>
</thead>
<tbody>
<tr>
<td>PFK, μM·min⁻¹·g⁻¹</td>
<td>47.9 (5.6)</td>
<td>40.4 (5.0)*</td>
<td>54.0 (4.9)</td>
</tr>
<tr>
<td>CK, μM·min⁻¹·g⁻¹</td>
<td>445.3 (28.5)</td>
<td>434.8 (41.6)</td>
<td>445.1 (29.2)</td>
</tr>
<tr>
<td>GAPDH, μM·min⁻¹·g⁻¹</td>
<td>455.8 (30.3)</td>
<td>427.1 (49.6)</td>
<td>465.6 (28.1)</td>
</tr>
<tr>
<td>CS, μM·min⁻¹·g⁻¹</td>
<td>7.7 (0.4)</td>
<td>8.0 (0.6)</td>
<td>6.9 (0.4)</td>
</tr>
<tr>
<td>HADH, μM·min⁻¹·g⁻¹</td>
<td>12.6 (1.2)</td>
<td>12.8 (0.9)</td>
<td>11.8 (0.4)</td>
</tr>
<tr>
<td>COX, μM·min⁻¹·g⁻¹</td>
<td>4.1 (0.4)</td>
<td>4.5 (0.3)</td>
<td>3.5 (0.5)†</td>
</tr>
<tr>
<td>Glycogen phosphorylase, μM·min⁻¹·g⁻¹</td>
<td>33.4 (3.1)</td>
<td>34.9 (3.2)</td>
<td>32.4 (2.2)</td>
</tr>
<tr>
<td>COX/CS</td>
<td>0.5 (0.1)</td>
<td>0.6 (0.1)</td>
<td>0.5 (0.1)</td>
</tr>
<tr>
<td>HADH/CS</td>
<td>1.6 (0.1)</td>
<td>1.6 (0.1)</td>
<td>1.7 (0.1)</td>
</tr>
<tr>
<td>PFK/COX</td>
<td>13.2 (2.8)</td>
<td>9.5 (1.5)*</td>
<td>17.3 (2.3)</td>
</tr>
<tr>
<td>SERCA1/SERCA2</td>
<td>5.1 (1)</td>
<td>3.3 (0.4)*</td>
<td>4.6 (0.4)</td>
</tr>
</tbody>
</table>

mRNA expression in 10 obese subjects (6 males, 4 females) in arbitrary units (AU/μg RNA)

<table>
<thead>
<tr>
<th>Gene</th>
<th>Wtinitial</th>
<th>Wt−10%placebo</th>
<th>Wt−10%leptin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Actin</td>
<td>83.1 (4.8)</td>
<td>84.0 (9.9)</td>
<td>81.7 (9.8)</td>
</tr>
<tr>
<td>Atrogin</td>
<td>25.6 (3.0)</td>
<td>21.7 (3.3)</td>
<td>19.3 (2.1)</td>
</tr>
<tr>
<td>Myostatin</td>
<td>72.2 (13.5)</td>
<td>61.1 (13.1)</td>
<td>70.3 (13.1)</td>
</tr>
<tr>
<td>PGC1-α</td>
<td>111.9 (8.4)</td>
<td>104.5 (11.1)</td>
<td>101.3 (12.0)</td>
</tr>
<tr>
<td>PPAR-δ</td>
<td>20.4 (3.3)</td>
<td>21.4 (3.3)</td>
<td>20.9 (3.6)</td>
</tr>
<tr>
<td>IGF-1</td>
<td>48.1 (5.5)</td>
<td>46.4 (6.8)</td>
<td>51.5 (5.3)</td>
</tr>
<tr>
<td>MHC I</td>
<td>68.9 (7.4)</td>
<td>86.0 (12.1)*</td>
<td>75.4 (12.9)</td>
</tr>
<tr>
<td>MHC IIA</td>
<td>90.5 (12.1)</td>
<td>90.9 (11.3)</td>
<td>99.8 (10.6)</td>
</tr>
<tr>
<td>MHC IIX</td>
<td>78.7 (12.8)</td>
<td>76.4 (11.9)</td>
<td>89.4 (9.5)†</td>
</tr>
<tr>
<td>SERCA1</td>
<td>168.1 (19.1)</td>
<td>157.6 (21.2)</td>
<td>165.3 (19.6)</td>
</tr>
<tr>
<td>SERCA2</td>
<td>99.6 (18.3)</td>
<td>131.8 (17.9)*</td>
<td>115.6 (17.9)</td>
</tr>
<tr>
<td>MHC I/MHC IIA</td>
<td>0.99 (0.22)</td>
<td>1.00 (0.21)</td>
<td>0.85 (0.18)‡</td>
</tr>
<tr>
<td>MHC I/MHC IIX</td>
<td>0.94 (0.17)</td>
<td>1.13 (0.21)*</td>
<td>0.98 (0.26)</td>
</tr>
<tr>
<td>MHC IIA/MHC IIX</td>
<td>1.59 (0.38)</td>
<td>1.36 (0.21)</td>
<td>0.66 (0.08)‡‡</td>
</tr>
<tr>
<td>SERCA1/SERCA2</td>
<td>2.43 (0.53)</td>
<td>1.44 (0.22)</td>
<td>1.91 (0.40)</td>
</tr>
</tbody>
</table>

Values are means ± SE. Weight loss and leptin effects on skeletal muscle. PFK, phosphofructokinase; CK, creatine kinase; CS, citrate synthase; HADH, hydroxacyl CoA dehydrogenase; COX, cytochrome c oxidase. *P < 0.05 compared with Wtinitial; †P < 0.05 compared with Wt−10%placebo; ‡P < 0.01 compared with Wt−10%placebo.

DISCUSSION

Maintenance of a reduced or elevated body weight is associated with respective decreases and increases in 24-h energy expenditure and NREE (35, 49). These effects are predominantly due to changes in skeletal muscle work efficiency (1, 32) and are largely reversed by leptin repletion following weight loss (46). The major findings of the present study are: 1) maintenance of a reduced body weight is associated with a decreased activity of the glycolytic enzyme PFK, which is typical of more efficient skeletal muscle (23) and that is reversed by leptin repletion; and 2) maintenance of a reduced body weight is associated with changes in skeletal muscle gene expression, which are also consistent with greater skeletal muscle efficiency and some of which are reversed by leptin repletion. More specifically, the weight loss-associated increased expression of the more efficient MHC I isoform is reversed by leptin repletion, while the increased expression of the more efficient SERCA2 isoform does not appear to be affected by leptin. The observed effects of weight loss and of leptin repletion on skeletal muscle biochemistry and gene expression in vitro are all consistent with the changes that are observed in vivo ergometry studies (23, 46, 50). We are not aware of any other studies that have reported these effects of either weight loss or leptin on skeletal muscle in humans. These novel findings are interesting with respect to muscle plasticity in the maintenance of a lower body weight and its implications to whole body physiology and energy metabolism.

As discussed in MATERIALS AND METHODS, caloric intake at Wtinitial and following a 10% weight loss, was titrated to achieve a degree of weight stability that would be extremely difficult to sustain outside of a Clinical Research Center. This weight stability following weight loss is further demonstrated by the persistence of the resting respiratory exchange ratio at ~0.85, which is the same value as the formula quotient of the liquid formula. The observation that leptin administration to weight-reduced subjects, which promotes a negative energy balance and further weight loss via increased energy expenditure on an isocaloric diet (46), does not result in a decrease in the respiratory exchange ratio due to increased oxidation of fatty acids (35) (Table 1) suggests that leptin administration affects muscle in a manner that blunts the increase in fatty acid oxidation that would normally occur during weight loss.

We (23) and others (32) have detected no changes in skeletal muscle fiber type following weight change when fiber types are qualitatively identified by histochemical staining for the predominant form of myofibrillar ATPase. This lack of change in skeletal muscle fiber type is not inconsistent with the results of this study. There is substantial evidence that changes in the exercise or hormonal milieu of muscle produce fiber-type specific changes in gene expression (4–6, 54, 58) without...
necessarily affecting the relative preponderance of the fiber types themselves. Direct assessment of changes in MHC isoform expression derived by high fidelity mRNA analyses is a more sensitive predictor of alterations in muscle function than assessment of changes in muscle fiber type. Since muscle fibers are classified by the predominant MHC isoform which is expressed, assessment of weight loss and leptin replacement effects on fiber type would be biased toward detecting changes in those fibers that are most mixed (closest to a 1:1 ratio of MHC I to II). Furthermore, the specificity of effects of weight loss and leptin replacement on MHC and SERCA is supported by the absence of an effect of these manipulations on levels of actin expression.

The leptin-responsive decline in the activity of PFK and the resultant decline in the ratio of glycolytic/oxidative enzyme ratios following weight loss are entirely consistent with the leptin-responsive increase in MHC I expression and the ratios of MHC I/MHC II and MHC IIa/ MHC IIX expression observed in weight-reduced subjects. A lower potential to oxidize glucose relative to fatty acids (as reflected in the lower PFK/COX and PFK/HADH ratios following weight loss) is characteristic of a great relative expression of the more efficient MHC I isoform (relative to MHC IIa or IIX) and MHC IIa (relative to MHC IIX) isoforms (5, 10). The increase in expression of the more efficient SERCA 2 isoform, which was not affected by leptin administration, would also contribute to the increased skeletal muscle work efficiency noted following weight loss.

Candidate signaling molecules that could account for these leptin-reversible changes in skeletal muscle physiology and biochemistry following weight loss are necessarily limited to leptin itself and molecules whose circulating concentrations are leptin-sensitive. Decreased circulating leptin concentrations following or during weight loss (34) may directly affect skeletal muscle, which expresses both the long and short leptin receptor isoforms (61). Direct effects of leptin on muscle could account for both the leptin-responsive decline in energy expenditure and utilization of glucose as fuel, since leptin directly stimulates thermogenesis (18), futile substrate cycling between lipogenesis and lipolysis (59), and glucose flux through the Krebs cycle in muscle (12, 13). However, central leptin administration also increases muscle thermogenesis (28), suggesting indirect effects of leptin on skeletal muscle.

Maintenance of a reduced body weight is associated with leptin-reversible declines in sympathetic nervous system tone and circulating concentrations of triiodothyronine (3, 46, 47), either or both of which may contribute to the observed leptin-mediated changes in muscle following weight loss. Declines in circulating concentrations of T3, working synergistically with decreased sympathetic nervous system tone, may constitute a mechanism by which the relative proportion of slow type I MHC versus fast type II MHC isoforms in muscle is increased following weight loss and reversed by leptin. Catecholamine administration induces increased expression of MHC II and decreased expression of MHC I in muscle (38, 39), while chemical sympathectomy in rats attenuates leptin-induced thermogenesis (17). There is a putative thyroid response element in the MHC I gene promoter, a mutation of which results in a loss of response of MHC I to T3 (65), and declines in circulating concentrations of bioactive thyroid hormones promote increased expression and protein content of MHC I and MHC IIA (10, 44), whereas MHC I gene expression is inhibited by T3 (19). Thyroid hormone may also affect and play a role in weight-loss induced changes in SERCA expression, which were not reversed by leptin-administration. The SERCA1 promoter gene contains multiple T3 response elements and the transcription rate of SERCA1 is increased fourfold in the presence of T3 in vitro (57). Hyperthyroidism induces an increase in SERCA1 expression accompanied by a decrease in SERCA 2, whereas hypothyroidism decreases both SERCA1 and SERCA2 expression and protein content (41, 57).

The meticulous maintenance of diet composition, fitness, and exercise patterns as well as the within-subjects design permitting each subject to serve as their own control significantly increase the sensitivity of this study to detect significant changes in skeletal muscle. However, there are a number of limitations to this study. First, the subject population is restricted to obese adults. While these results cannot be conclusively extrapolated to leaner populations, it should be noted that we have not detected significant differences in the metabolic responses to leptin administration or weight loss in previous studies of mixed populations of lean and obese subjects (35, 46, 47). Second, sample sizes were generally insufficient for assessment of protein content in addition to gene expression studies. In this regard, previous studies have reported that mRNA expression and protein content of different MHC and SERCA isoforms (1, 30) as well as PGC1α (6, 33) and peroxisome proliferator-activated receptor-δ (33, 45) vary similarly in response to exercise, weightlessness, hypothyroidism, and pulmonary disease. It is therefore likely that the maintenance of a reduced body weight is in fact associated with a leptin-sensitive increase in skeletal muscle MHC 1 protein content as well as in increase in SERCA 2 protein content that is not leptin sensitive. While most studies report concordance between the change in protein and mRNA levels; it has been reported that sometimes there is a discordance between some MHC mRNA and protein levels when determined at a single time point (4). This can be attributed to a dynamic state of transition whereby the mRNA change occurs faster than that of the protein. Clearly, further studies are needed which examine both lean and obese subjects and in which gene expression and protein content of muscle are both analyzed under different conditions.

**Perspectives and Significance**

These studies show that maintenance of an altered body weight is associated with in vivo and in vitro changes in skeletal muscle fuel utilization that are only evident at low levels of muscle work. Concordant changes are seen in vivo and in vitro and suggest possible mechanisms to account for the specific changes in skeletal muscle work efficiency that are present in individuals maintaining an altered body weight (50). They also suggest that interventions to decrease skeletal muscle work efficiency, either by exercise or pharmacotherapy, during and/or following weight reduction may circumvent some of the metabolic opposition to dynamic and sustained weight loss.

**ACKNOWLEDGMENTS**

We thank our research subjects and members of the nursing and nutrition staffs of the Irving Center for Clinical Research at Columbia Presbyterian Medical Center for dedicated help with their care. We also thank Anqi X. Qin...
REFERENCES


EFFECTS OF WEIGHT LOSS AND LEPTIN ON MUSCLE


