Elevated intramyocellular lipid concentration in obese subjects is not reduced after diet and exercise training

PATRICK MALENFANT, ANGELO TREMBLAY, ÉRIC DOUCET, PASCAL IMBEAULT, JEAN-AIMÉ SIMONEAU, AND DENIS R. JOANISSE

Physical Activity Sciences Laboratory, Division of Kinesiology, Department of Social and Preventive Medicine, Faculty of Medicine, Laval University, Ste-Foy, Quebec, Canada G1K 7P4

Received 27 March 2000; accepted in final form 4 December 2000

Malenfant, Patrick, Angelo Tremblay, Éric Doucet, Pascal Imbeault, Jean-Aimé Simoneau, and Denis R. Joanisse. Elevated intramyocellular lipid concentration in obese subjects is not reduced after diet and exercise training. Am J Physiol Endocrinol Metab 280: E632–E639, 2001.—To determine the effects of weight loss on intramyocellular energy substrates, vastus lateralis muscle biopsies were taken from six obese subjects (body mass index 34 ± 5 kg/m²) before, after 15 wk of energy restriction (ER; −700 kcal/day), and after a further average 20.7 ± 1.6 wk of endurance training plus low-fat diet (ET-LFD). Body weight fell from 100 ± 6 to 89 ± 6 kg during ER and to 84 ± 4 kg after ET-LFD. Lipids and glycogen were histochemically measured in type I, IIA, and IIB fibers. Total muscle glycogen content (MGC; per 100 fibers) decreased after ER [from 72 ± 13 to 55 ± 8 arbitrary units (AU)]. A similar but not significant decrease was seen in total muscle lipid content (MLC; 14 ± 5 to 9 ± 1 AU). After ET-LFD, MGC returned to initial values (74 ± 8 AU), and MLC approached near-initial values (12 ± 3 AU). Individual fiber lipid concentration did not change throughout the protocol in all fiber types, whereas glycogen concentration increased after ET-LFD. The training effects of ET-LFD were measured as increasing activities of key mitochondrial enzymes. Although total muscle energy reserves can be reduced after weight loss, their concentration within individual myofibers remains elevated. Weight loss does not appear sufficient to correct the potential detrimental effects of high intracellular lipid concentrations.

obesity; weight reduction; triglyceride; lipid metabolism; Oil red O; glycogen

A REDUCTION IN ADIPOSE TISSUE MASS accounts for the major proportion of the weight loss in obese subjects after energy restriction (8, 33). However, the individual contribution of triglyceride stores located within peripheral tissues such as skeletal muscle to the overall fat mass losses in energy-restricted obese people is currently unknown. Results from computed tomography (CT) have shown that obese individuals are characterized by a higher amount of fat within muscle than lean controls (23), although this method cannot distinguish between intra- or extracellular lipids. Previous reports using Oil red O-stained muscle sections indicated that obese individuals have two to three times more intracellular fat in the main muscle fiber types than lean individuals (16, 28). This is in accord with the findings of other studies that either used quantification by histological scores of Oil red O-stained muscle (31) or involved triglyceride recovery from muscle homogenates of human skeletal muscle with varying degrees of obesity (30). Moreover, it appears that the proportion of lipid aggregates located in the central region of muscle fibers is also higher in obese subjects (28). These results suggest that fat deposits within the muscle of obese subjects could be less metabolizable, because the volume density of mitochondria is lower in the central region of the muscle cell than it is near the sarcolemma (19). Metabolic changes in muscle lipid metabolism observed in obesity (reviewed in Refs. 37, 39), such as reduced citrate synthase (CS), cytochrome c oxidase (COX), and carnitine palmitoyltransferase (CPT)-1 activities, with increased fatty acid binding protein (FABP) either at the plasma membrane or in the cytosol, could lead to the accumulation of their unused substrate, i.e., elevated muscle triglyceride deposits.

A growing number of studies suggest that the triglyceride content of muscle plays a significant role in the development of the insulin resistance syndrome in rats (40) and humans (25, 30, 36) and is related to a lower activation of glycogen synthase after a standardized meal (31). In addition, the accumulation of intracellular lipids has been shown to contribute to cell dysfunction, for example in pancreatic β-cells (44). An excessive accumulation of intramuscular triglycerides is thus a potentially harmful condition, in that it can enhance the metabolic perturbations seen in obesity through an impaired muscle insulin sensitivity. Although glucose disposal rate and insulin sensitivity are enhanced after a low-to-moderate weight loss in non-diabetic obese subjects (29), it is relevant to investigate the effects of energy restriction and exercise leading to important weight loss on the levels of intramyocellular lipid and glycogen. Although nuclear magnetic reso-

†Deceased 27 August, 1999

Address for reprint requests and other correspondence: D. R. Joanisse, Div. of Kinesiology, Dept. of Social and Preventive Medicine, Faculty of Medicine, Laval University, Ste-Foy, Quebec, Canada G1K 7P4 (E-mail: Joanisse@kin.msp.ulaval.ca).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
MUSCLE LIPID AFTER WEIGHT LOSS

1. Introduction

Phase (ER) and the endurance training program for women who had completed both the energy restriction and low-fat diet (ET-LFD) phases volunteered to undergo muscle biopsies. Muscle biopsies were obtained from the vastus lateralis muscle of 3 men and 3 women. Muscle samples were divided into two parts: one was frozen in liquid nitrogen for subsequent determination of muscle enzyme activities, whereas the other part was trimmed, mounted, and frozen in isopentane, cooled on liquid nitrogen, and stored at −80°C for histochemical analysis.

2. Methods

Subjects. Twenty Caucasian obese men (n = 12) and premenopausal obese women (n = 8) participated in this study (detailed in Ref. 43). All subjects were sedentary nonsmokers before the beginning of the study. Volunteers were recruited from the Quebec metropolitan area through newspaper advertisements. The subjects provided written informed consent according to the guidelines of the Medical Ethics Committee of Laval University. Individuals were excluded if blood glucose exceeded 7.8 mmol, blood pressure exceeded 160/90 mmHg, or if, based on medical history and physical examination, they had an underlying illness. Other exclusion criteria were participation in a low-calorie diet and/or an exercise training program as well as an unstable body weight. The maintenance of a stable weight during this period, albeit a crude index, was used to show a state of energy balance after intervention. Subjects were also asked to remain sedentary during this interval. This period allowed for the flushing out of medication after ER. All tests and muscle biopsies were performed at 0800 after an overnight fast (≥12 h), and subjects were instructed to avoid alcoholic beverages for 24 h and exercise for 48 h before testing. All tests were carried out within a 2-wk time frame at each time point.

Muscle biopsies. Muscle samples were obtained ≤3 wk before the start of the weight loss intervention, 4–6 wk after ER, and 4–6 wk after ET-LFD. Biopsies were taken from the middle region of the vastus lateralis muscle (15 cm above the patella) and 2 cm away from the fascia by use of the percutaneous needle biopsy technique described by Evans et al. (12) and regularly used in our laboratory (35). Muscle samples were divided into two parts: one was frozen in liquid nitrogen for subsequent determination of muscle enzyme activities, whereas the other part was trimmed, mounted, and frozen in isopentane, cooled on liquid nitrogen, and stored at −80°C for histochemical analysis.

Histochemical analysis. Cross sections (10 μm) of isopentane-frozen muscle were cut with a microtome at −20°C and stained for myosin ATPase (mATPase) (27, 35). The single-step staining procedure allowed the identification of three major fiber types (I, IIA, and IIB) from the same section. To measure the cross-sectional area of the different fiber types, sections were examined under a light microscope (Leitz Di- alux 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 with a Power Macintosh computer using the public domain NIH image analysis software developed by the US National Institutes of Health (available on the internet by anonymous FTP at zippy.nimh.nih.gov). 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.

Intracellular glycogen and triglycerides in muscle cross sections were measured using the periodic acid Schiff (PAS) reaction (3) and Oil red O staining, respectively. Glycogen content was quantified as the absorbance values within each session of endurance training (45–60 min/session) of moderate intensity (60–75% of maximal O2 uptake) in addition to a low-fat diet. Exercise intensity was progressively increased over a period of 1–2 mo, depending on the initial fitness level of the subjects, and was monitored with a heart rate monitor (Polar Vantage XL HRM, Stamford, CT). Unlike during the ER phase, the proportion of energy intake from macronutrients was 53, 30, and 17% for carbohydrates, lipids, and proteins, respectively. Underwater weighing was performed to assess changes in fat mass (FM) and fat-free mass (FFM) during both phases of the study protocol.

Resting metabolic rate, respiratory exchange ratio, fasting plasma variables, and oral glucose tolerance tests. Resting metabolic rate (RMR), respiratory exchange ratio (RER), fasting plasma variables [free fatty acids (FFA), triglyceride, insulin] and oral glucose tolerance tests (OGTT) were measured as described in Tremblay et al. (43). All tests and biopsies for the preintervention time point were taken ≤2 wk before ER. After the ER and ET-LFD phases, a period of ≥2 wk was allowed to pass before all tests and muscle biopsies, and all were performed within 24 h of the end of the intervention. The maintenance of a stable weight during this period, albeit a crude index, was used to show a state of energy balance after intervention. Subjects were also asked to remain sedentary during this interval. This period allowed for the flushing out of medication after ER (for the three subjects receiving fenfluramine), allowed us to time all tests during the follicular phase of the menses for female participants, and ensured that measures reflected the effects of weight loss and not an acute response to the weight loss protocol. All tests were performed at 0800 after an overnight fast (≥12 h), and subjects were instructed to avoid alcoholic beverages for 24 h and exercise for 48 h before testing. All tests were carried out within a 2-wk time frame at each time point.

Energy restriction program. Subjects followed a 15-wk treatment with a daily energy restriction (ER) of ~700 kcal below their estimated daily energy expenditure (detailed in Ref. 43). Three of the subjects received fenfluramine during the ER phase as described in Tremblay et al. (43). The proportion of energy intake from macronutrients was measured by a 3-day dietary record with a computerized version of The Canadian Nutrient File to ensure that the average 42, 39, and 19% of energy ingested from carbohydrates, lipids, and proteins, respectively, was maintained throughout the ER phase. Daily energy expenditure was estimated using values of measured resting metabolic rate (RMR) multiplied by 24 h and by 1.4, which corresponds to the level of energy expenditure for a sedentary individual (46).

ET-LFD. After a 4- to 6-wk interruption after the first phase of the program, those subjects maintaining a stable weight were asked to perform another weight loss phase (detailed in Ref. 43). Lasting on average 20.7 ± 1.6 wk (range 16–24 wk), the ET-LFD phase included three to five weekly sessions of endurance training (45–60 min/session) of moderate intensity (60–75% of maximal O2 uptake) in addition to a low-fat diet. Exercise intensity was progressively increased over a period of 1–2 mo, depending on the initial fitness level of the subjects, and was monitored with a heart rate monitor (Polar Vantage XL HRM, Stamford, CT). Unlike during the ER phase, the proportion of energy intake from macronutrients was 53, 30, and 17% for carbohydrates, lipids, and proteins, respectively. Underwater weighing was performed to assess changes in fat mass (FM) and fat-free mass (FFM) during both phases of the study protocol.

RESULTS

Resting metabolic rate, respiratory exchange ratio, fasting plasma variables, and oral glucose tolerance tests. Resting metabolic rate (RMR), respiratory exchange ratio (RER), fasting plasma variables [free fatty acids (FFA), triglyceride, insulin] and oral glucose tolerance tests (OGTT) were measured as described in Tremblay et al. (43). All tests and biopsies for the preintervention time point were taken ≤2 wk before ER. After the ER and ET-LFD phases, a period of ≥2 wk was allowed to pass before all tests and muscle biopsies, and all were performed within 24 h of the end of the intervention. The maintenance of a stable weight during this period, albeit a crude index, was used to show a state of energy balance after intervention. Subjects were also asked to remain sedentary during this interval. This period allowed for the flushing out of medication after ER (for the three subjects receiving fenfluramine), allowed us to time all tests during the follicular phase of the menses for female participants, and ensured that measures reflected the effects of weight loss and not an acute response to the weight loss protocol. All tests were performed at 0800 after an overnight fast (≥12 h), and subjects were instructed to avoid alcoholic beverages for 24 h and exercise for 48 h before testing. All tests were carried out within a 2-wk time frame at each time point.

Muscle biopsies. Muscle samples were obtained ≤3 wk before the start of the weight loss intervention, 4–6 wk after ER, and 4–6 wk after ET-LFD. Biopsies were taken from the middle region of the vastus lateralis muscle (15 cm above the patella) and 2 cm away from the fascia by use of the percutaneous needle biopsy technique described by Evans et al. (12) and regularly used in our laboratory (35). Muscle samples were divided into two parts: one was frozen in liquid nitrogen for subsequent determination of muscle enzyme activities, whereas the other part was trimmed, mounted, and frozen in isopentane, cooled on liquid nitrogen, and stored at −80°C for histochemical analysis.

Histochemical analysis. Cross sections (10 μm) of isopentane-frozen muscle were cut with a microtome at −20°C and stained for myosin ATPase (mATPase) (27, 35). The single-step staining procedure allowed the identification of three major fiber types (I, IIA, and IIB) 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 with a Power Macintosh computer using the public domain NIH image analysis software developed by the US National Institutes of Health (available on the internet by anonymous FTP at zippy.nimh.nih.gov). 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.

Intracellular glycogen and triglycerides in muscle cross sections were measured using the periodic acid Schiff (PAS) reaction (3) and Oil red O staining, respectively. Glycogen content was quantified as the absorbance values within each section of the muscle.
fiber, corrected for a background intensity previously standardized for all captured images. Oil red O, a Sudan-type stain that binds specifically to neutral lipids such as triglycerides, giving them an orange-red tint, has been used successfully in rat (9) and human skeletal muscle (31). A saturated isopropanol stock solution was made by dissolving 300 mg of Oil red O (Fisher Scientific, Fair Lawn, NJ) in 100 ml of isopropanol (99%). Muscle sections were immersed rapidly after sectioning (<15 min) for 10 min in a solution containing 12 ml of stock solution and 8 ml of distilled water previously filtered through a Whatman no. 42 filter paper. Thereafter, muscle samples were immersed twice in distilled water and rinsed with running water for another 10 min and then mounted with a drop of glycerol. Coverslips were sealed with an acetone-based nail polish.

**Image analysis.** Images of Oil red O-stained samples were captured at a total magnification of ×100 on a bright-field microscope (Olympus BX-50) with a CCD camera (Sony XC-77). mATPase and Oil red O-stained fibers were matched, and the Oil red O staining intensity of either type I, IIA, or IIB muscle fibers was quantified. A density threshold that represents the minimal absorbance values corresponding to lipid droplets was set manually for individual images in a blind procedure. Only pixel aggregates of >0.36 μm² and with higher absorbance values than the threshold values were quantified as fat aggregates. The variables retained for analyses were the mean number of lipid aggregates per muscle fiber, their size (μm²), the muscle fiber size (μm²), and the mean muscle fiber lipid content expressed relative to the percentage of area occupied by fat aggregates within the muscle fiber. To measure precisely the differences in the localization of intramuscular lipids after each treatment phase, aggregates were measured in each of nine successive bands of 2 μm in width, starting from the periphery to the central region of muscle fibers. For this approach, a total of 24 muscle fibers per subject was quantified (8 for each muscle fiber type).

**Substrate concentration and content determination.** Glycogen and lipid concentration and content were determined histochemically. At every time point and for each subject, at least 10 fibers of each type were measured at random for lipid determination, and at least 20 fibers of each type were measured at random for glycogen determination.

The intramyocellular concentration of glycogen and lipid, i.e., the relative amount of a given substrate within each muscle fiber, was measured. For glycogen, this was defined as the average staining intensity of the PAS staining of individual fibers of each type, expressed in arbitrary units (AU). For lipids, this was defined as the fraction of the total surface of a given fiber occupied by lipid aggregates detected with Oil red O staining and is expressed as the percentage of the surface occupied.

Muscle lipid and glycogen content, defined as the amount of each substrate contained within a given piece of muscle tissue, was calculated by taking into account fiber type proportion and fiber size to create an index representing the total amount of substrate located within 100 representative fibers. These values were calculated using the equations

**MLC =**

\[ \Sigma_{\text{LIA,IB}} \text{(lipid area} \cdot \text{fiber size} \cdot \text{fiber type proportion}) \cdot 10^{-8} \]

**MGC =**

\[ \Sigma_{\text{LIA,IB}} \text{(glycogen} \cdot \text{fiber size} \cdot \text{fiber type proportion}) \cdot 10^{-6} \]

where lipid area is the mean area occupied by lipid aggregates (μm²) in a specific fiber type; fiber size is the mean cross-sectional area of muscle fibers (μm²) of a given type; glycogen is the mean absorbance value (AU) of PAS-stained fibers of a given type; and fiber type proportion is the measured fiber type proportion (in %) of a given fiber type over the whole muscle section.

**Skeletal muscle enzyme activities.** Muscle samples were kept at −80°C until they were assayed for enzyme activities. Small pieces of the muscle sample (∼10 mg) were homogenized in a glass-glass homogenizer with 39 vol of ice-cold extracting medium (0.1 M Na-K-phosphate, 2 mM EDTA, pH 7.2), and enzyme activities were measured as previously described (38). The enzymes measured by this procedure were phosphofructokinase (PFK; EC 2.7.1.11), CS (EC 4.1.3.7), COX (EC 1.9.3.1), and 3-hydroxyacyl-CoA dehydrogenase (HADH; EC 1.1.1.35). Enzyme activities are expressed in units of micromoles of substrate consumed per minute per gram tissue (U/g). The intraindividual reproducibility for these measurements has been reported (15).

**Statistical analysis.** A two-way ANOVA with repeated measures over time (JMP v3.2.2 statistical software, SAS, Chicago, IL) was used to test the differences in the dependent variables after ER (diet effect) and after ET-LFD (diet-exercise effect) and their potential interactions with fiber types. The Student’s t-test was used for lean vs. obese comparisons. The Least Significant Difference test for repeated measures was used as a post hoc test to locate both fiber types and phase differences. Post hoc analyses were performed with Statview 5.0 (SAS Institute, Cary, NC). Data are presented as means ± SE.

**RESULTS**

**Anthropometric measurements.** Results from anthropometric measurements are shown in Table 1. Obese subjects were weight stable before ER and experienced a significant weight loss after ER (100.1 ± 5.6 vs. 89.2 ± 4.0 kg for preweight loss and ER values, respectively, P < 0.01). Body weight was further decreased after ET-LFD (84.4 ± 3.6 kg) but not to a statistically significant extent. PM was reduced by 23% after ER (P < 0.01) and was further reduced by 23% after

| Table 1. Anthropometric, metabolic, and fasting plasma variables in obese subjects before and after ER and subsequent ET-LFD |
|-----------------|-----------------|-----------------|-----------------|-----------------|
|               | Pre             | ER              | ET-LFD          | Control         |
| Age, yr        | 42 ± 2          | 42 ± 2          | 42 ± 2          | 39 ± 3          |
| Weight, kg     | 100 ± 6         | 89 ± 4*         | 84 ± 4*         | 67 ± 3*         |
| BMI, kg/m²     | 34 ± 1          | 31 ± 1*         | 29 ± 1*         | 24 ± 1*         |
| Fat mass, kg   | 39 ± 2          | 30 ± 2          | 23 ± 1*         | 4 ± 1*          |
| Fat-free mass, kg | 61 ± 5     | 59 ± 5          | 62 ± 4          | 62 ± 4          |
| Energy expenditure, kcal/min | 1.6 ± 0.3 | 1.3 ± 0.1       | 1.1 ± 0.1       | 1.1 ± 0.1       |
| RER            | 0.78            | 0.77            | 0.81            |                 |
| FFA, mmol/l    | 0.5 ± 0.1       | 0.5 ± 0.1       | 0.5 ± 0.1       |                 |
| TG, mmol/l     | 1.6 ± 0.3       | 1.2 ± 0.3       | 0.9 ± 0.1*      |                 |
| Fasting insulin, pmol/l | 28 ± 2 | 67 ± 18         | 61 ± 19         | 33 ± 6*         |
| Insulin area, pmol·min⁻¹·m⁻² | 115 ± 27 | 82 ± 14         | 82 ± 18         |                 |

Values are means ± SE. Pre, before treatment; ER, energy restriction; ET-LFD, endurance trained + low-fat diet; BMI, body mass index; RER, respiratory exchange ratio; FFA, free fatty acid; TG, triglyceride. *Significantly different from Pre, P < 0.05; †significantly different from ER, P < 0.05; ‡significantly different from obese subjects either before or after ER and ET-LFD, P < 0.05.
ET-LFD ($P < 0.0001$) compared with initial values. FM after ET-LFD was also statistically lower than it was after ER ($P < 0.01$). FFM remained unchanged after either ER or ET-LFD phases. At all times, weight and body mass index (BMI) were higher in obese than in the lean controls. Fasting insulin levels and the insulin response after OGTT were not statistically different after ER or ET-LFD, despite being lowered by >30% compared with preweight loss data.

**Muscle morphology and intramyocellular lipid and glycogen.** Table 2 summarizes the morphological characteristics of muscle by fiber type. Fiber type distribution, fiber size, and capillary contact did not change significantly after ER or ET-LFD. The number of Oil red O-stained lipid aggregates was also unaffected by diet or by diet + exercise interventions in all three major muscle fiber types. The number of lipid droplets was statistically different between muscle fiber types ($P < 0.01$), type IIA and IIB fibers having 60 and 30% of the number of droplets found in type I fibers, respectively.

Intramyocellular concentrations of lipid (expressed as the percentage of the fiber occupied by lipids) and glycogen are shown in Fig. 1. The surface area of muscle fibers covered by fat aggregates was almost two times lower in type IIB fibers (2.1 ± 0.6%) compared with type I (5.4 ± 1.4%) or IIA (3.8 ± 1.2%) fibers. Lipid concentration did not change after the diet (ER) or ET-LFD phases in any fiber type. Intramyocellular glycogen concentration, measured as the average intensity of staining within each fiber, did not differ between fiber types either initially or after ER or ET-LFD phases. Muscle fiber glycogen concentration tended to be higher in type II fibers and increased in all fiber types after ET-LFD, although this reached statistical significance only for type IIB fibers.

Indexes of intramyocellular glycogen and lipids were used to measure total content in 100 representative fibers, taking into account changes in fiber type distribution and size. These indexes provide information on the total stores within muscle. Figure 2A shows muscle lipid content (MLC) before and after weight loss treatment. Although not statistically different, when expressed as the MLC index, the lipid content of 100 representative fibers was 35% lower after ER (from 14.4 ± 5.0 to 9.0 ± 0.8 AU) and remained decreased after ET-LFD (11.6 ± 2.7 AU). Retrospective sample size analysis estimates suggest that, given the high variability in measured MLC, at least 50 subjects would have been necessary to demonstrate statistical differences between preweight loss and ER results at 95% statistical power. Mean values of MLC remained well above those of lean controls (3.9 ± 1.3 AU) at all times of the protocol. Although derivatives of fenfluramine have been suggested to attenuate muscle triglyceride storage in rodent muscle (41), the three subjects

### Table 2. Morphological changes of individual muscle fiber types of obese subjects before and after ER and ET-LFD

<table>
<thead>
<tr>
<th></th>
<th>Pre</th>
<th>ER</th>
<th>ET-LFD</th>
<th>Pre</th>
<th>ER</th>
<th>ET-LFD</th>
<th>Pre</th>
<th>ER</th>
<th>ET-LFD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proportion, %</td>
<td>35 ± 7</td>
<td>34 ± 6</td>
<td>39 ± 2</td>
<td>41 ± 3</td>
<td>43 ± 2</td>
<td>43 ± 3</td>
<td>24 ± 6</td>
<td>23 ± 7</td>
<td>18 ± 3</td>
</tr>
<tr>
<td>Capillary contact, n</td>
<td>4.7 ± 0.4</td>
<td>4.5 ± 0.3</td>
<td>5.1 ± 0.3</td>
<td>4.8 ± 0.3</td>
<td>4.1 ± 0.2</td>
<td>4.7 ± 0.3</td>
<td>3.5 ± 0.3</td>
<td>3.1 ± 0.1</td>
<td>3.6 ± 0.3</td>
</tr>
<tr>
<td>Lipid aggregate number, n</td>
<td>251 ± 58</td>
<td>253 ± 30</td>
<td>243 ± 37</td>
<td>165 ± 44</td>
<td>150 ± 14</td>
<td>143 ± 32</td>
<td>77 ± 18</td>
<td>82 ± 12</td>
<td>68 ± 14</td>
</tr>
<tr>
<td>Lipid aggregate size, μm²</td>
<td>1.05 ± 0.12</td>
<td>1.00 ± 0.07</td>
<td>0.97 ± 0.10</td>
<td>1.45 ± 0.36</td>
<td>1.23 ± 0.15</td>
<td>1.49 ± 0.15</td>
<td>1.22 ± 0.20</td>
<td>1.30 ± 0.18</td>
<td>1.42 ± 0.26</td>
</tr>
</tbody>
</table>

Values are means ± SE. *Significantly different from Type I Fibers, $P < 0.05$; †significantly different from Type I and IIA Fibers, $P < 0.05$.

---

**Fig. 1.** Intramyocellular lipid (A) and glycogen (B) concentration of human vastus lateralis before (Pre) and after energy restriction (ER) and endurance training plus low-fat diet (ET-LFD). AU, arbitrary units. Data are expressed as means ± SE. *Significantly different over time, $P < 0.05$; †significantly different from type I fiber values, $P < 0.05$. 

---

MUSCLE LIPID AFTER WEIGHT LOSS
who took fenfluramine during the ER phase had the lowest initial MLC and the smallest decrease in MLC after ER.

Total glycogen content in muscle fibers of obese subjects corrected for changes in their size and proportion [muscle glycogen content (MGC); Fig. 2B] was significantly diminished after ER (55.4 ± 7.9 AU, P < 0.05) but returned to initial values (72.4 ± 13.0 AU) after ET-LFD (74.2 ± 8.3 AU). Furthermore, MGC values were not different from those of lean subjects before and after ER or ET-LFD.

The relative behavior of lipid and glycogen stores after weight loss by ER is shown in Fig. 3. A significant linear relationship between MLC and MGC after ER is observed (r = 0.98; P < 0.05), with the greatest loss in MGC occurring in muscle from those subjects who also experienced the greatest loss in MLC. No relationship was observed between MLC and MGC after ET-LFD.

Intracellular localization of lipid stores. The distribution of lipids within muscle fibers is shown in Fig. 4. The proportion of lipid aggregates in 2-μm-wide bands decreased exponentially from ~48% immediately next to the periphery of muscle fibers to 6% at the onset of the central region. Treatment by diet alone or by diet and exercise had no effect on the distribution of lipids within muscle fibers overall (Fig. 3) or when each of the main muscle fiber types was considered separately (data not shown).

Enzymatic measurements. Results of the enzymatic assays are shown in Table 3. Treatment by energy restriction or endurance training did not result in statistically significant changes in any of the measured enzymes, although increases of 37 and 22% occurred for CS and COX maximal activities, respectively, after ET-LFD. The higher PFK-to-CS ratio of obese subjects before beginning the experiment was still above the values of lean controls after the ER phase of the protocol but was normalized after ET-LFD.

**DISCUSSION**

The effects of weight loss on intramuscular stores of metabolic fuels are largely unknown. The recently described increased levels of triglycerides in myofibers of obese individuals (17, 28) and the correlation of high muscle lipid content with insulin resistance (30) suggest that decreasing lipid levels after weight loss would be beneficial for the restoration of muscle insulin sensitivity. To our knowledge, the present study is the first...
to examine the effects of weight loss on the skeletal muscle intracellular content of energy substrates within each fiber type. As shown in this study, total skeletal MGC can be lowered by weight loss. Total MLC, however, did not decrease significantly (due to high variability) despite a 35% decrease in absolute values. Although the results of Goodpaster et al. (17) suggest a decrease in overall intracellular lipid concentration after an average weight loss of 15 kg by diet alone, these authors did not examine the specific contribution of different fiber types and their relative proportion. Our results show that the concentration of lipid as well as glycogen within all fiber types did not change with weight loss by diet alone. Thus changes to glycogen and lipid content in muscle appear to be attributable, at least in part, to changes in muscle fiber size. Diet with exercise also failed to modify the intracellular concentration of lipids, although glycogen concentration tended to increase, as is expected after exercise training. These results suggest that the events associated with increased lipid concentrations within muscle fibers are not corrected by weight loss, at least under the conditions of our experiment. This study does confirm that the differences in muscle fiber types with respect to their lipid concentration, previously described in nonobese individuals (11), are still present in obese subjects before or after substantial weight loss.

Why skeletal muscle from obese individuals has a higher lipid concentration and why it is not normalized after weight loss are still unclear. Numerous studies suggest defective lipid oxidation in obese subjects, and others further indicate that this disturbance is worsened after weight loss and may even contribute to the potential for weight regain (42). Severe energy restriction protocols cause either no change (6) or a drastic reduction in whole body lipid oxidation (7, 14). Furthermore, reduced lipid oxidation occurs in postobese subjects after ingestion of a high-fat diet compared with weight-matched never-obese individuals (4), and Buscemi et al. (7) reported similar findings after gastrointestinal bypass in morbidly obese subjects. The underlying mechanisms contributing to changes in lipid oxidation with weight change are unclear. Flatt (13) has suggested that an overabundance of carbohydrate could, in fact, promote carbohydrate oxidation over lipid oxidation, as well as stimulating lipogenesis.

Regardless of the mechanism responsible for decreased lipid oxidation after weight loss, this change could account for the maintained high intramyocellular lipid concentrations that we observed. Our results could also be explained, in part, by the well known decrease in RMR of obese subjects after long-term moderate energy restriction (1, 45). Formerly obese women have been shown to have lower energy expenditure as well as a twofold reduction in fat oxidation (32). More specifically addressing skeletal muscle metabolism with the leg balance technique, Kelley et al. (22) reported a 25% decrease in skeletal muscle FFA uptake after weight loss. This reduced lipid uptake observed after weight loss could be explained, in part, by reduced muscle lipoprotein lipase activity (10), circulating triglycerides thus likely shunted away for storage in adipose tissue. Because a close link exists between FFA uptake and oxidation in skeletal muscle, the reduction of FFA taken up after weight loss could reflect a proportionate decrease in lipid oxidation during fasting (22). Increased oxidation of lipids is also unlikely after diet in our study, given the unchanged whole body RER and mitochondrial enzyme activities (HADH, CS, and COX). An absence of effect of diet on muscle enzyme activities and muscle morphology is not novel (6, 24). Simoney et al. (39) have further shown that the CPT-to-FABP plasma membrane ratio was unaltered by weight loss and remained far below that of lean controls, thus predisposing the muscle cell to lipid storage. Taken together, these changes could help maintain high muscle lipid concentrations after significant weight loss.

As measured for lipids, we do not observe any decrease in the concentration of glycogen within individual muscle fibers after ER, indicating that muscle carbohydrate reserves are not specifically targeted for consumption after weight loss. It is not surprising that MGC does not decrease, given the well known improved insulin sensitivity (6, 14, 26) and insulin-stimulated glucose transport after weight loss (29). When combined, the well documented increase in nonoxidative glucose metabolism (glycogen synthesis) (14) and elevated insulin-stimulated leg respiratory quotient (22) after weight reduction maintain the glycogen equilibrium so that levels remain essentially identical to those found before weight loss. Indeed, improved glucose utilization has been shown in subjects following the same protocol as that used in this study (43).

The addition of exercise to the weight loss program also failed to normalize lipid concentrations within muscle fibers. The physiologically relevant (but statistically not significant) 30% increase in activities of oxidative enzymes (i.e., CS and COX) coupled to the 8% increase in HADH activity after diet and exercise is indicative of the beneficial effects of adding exercise to the weight loss program, but clearly these changes are not sufficient (at least in the time frame of our study) to remove excess lipid from the cell. Coupled with the normalization of the glycolytic/oxidative potential (measured as PFK/CS) after ET-LFD, however, this improved oxidative machinery

Table 3. Skeletal muscle enzymatic activities of obese subjects before and after ER and ET-LFD

<table>
<thead>
<tr>
<th></th>
<th>Pre</th>
<th>ER</th>
<th>ET-LFD</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>PFK</td>
<td>64 ± 4*</td>
<td>65 ± 4*</td>
<td>59 ± 4*</td>
<td>46 ± 4</td>
</tr>
<tr>
<td>CS</td>
<td>9.7 ± 1.1</td>
<td>10.0 ± 0.7</td>
<td>13.3 ± 1.6</td>
<td>10.0 ± 0.7</td>
</tr>
<tr>
<td>PFK/CS</td>
<td>7.1 ± 1.0*</td>
<td>6.8 ± 0.8*</td>
<td>4.6 ± 0.4</td>
<td>4.7 ± 0.4</td>
</tr>
<tr>
<td>COX</td>
<td>6.9 ± 0.5</td>
<td>6.4 ± 1.0</td>
<td>8.4 ± 0.9</td>
<td>8.1 ± 0.8</td>
</tr>
<tr>
<td>HADH</td>
<td>15.0 ± 1.6</td>
<td>15.0 ± 1.4</td>
<td>16.2 ± 0.7</td>
<td>14.2 ± 0.5</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SE. Units are all U/g of tissue. PFK, phosphofructokinase; CS, citrate synthase; COX, cytochrome c oxidase; HADH, 3-hydroxyacyl-CoA dehydrogenase. *Significantly different from nonobese controls, P < 0.05.
should not be discounted, because it hints at an improved capacity for lipid utilization that may result in long-term decreases in muscle lipid concentration. As expected, endurance training led to increases in the intramyocellular concentration of glycogen. Thus the level of exercise training in the current study specifically augmented the energy density of muscle by increasing intramyocellular glycogen concentration but not that of lipid. Whether this would also be true for sedentary nonobese individuals undergoing the same training routine is not clear. Reasons for the lack of intramyocellular lipid concentration augmentation with exercise in the obese could include the activation of a protective mechanism to prevent further metabolic dysfunction from lipid overabundance. It is also conceivable that the energy density that can be stored as lipids within the myofiber has already reached its maximum.

Despite the lack of effect of weight loss by diet on the intracellular concentrations of glycogen, the muscle content of this substrate (the relative quantity stored within 100 representative fibers) was lowered. The observed changes appear to be largely the result of decreased fiber size after weight loss by diet. When regular exercise was added to caloric restriction to promote a greater weight reduction in the ET-LFD phase, whole muscle stores that had previously been reduced after diet alone returned to initial values, in large part due to increasing muscle fiber size. Muscle lipid content followed the same pattern as glycogen content, but high interindividual variability precluded the demonstration of statistically significant changes. Increased muscle glycogen storage after diet and exercise is in accord with previous reports (e.g., Ref. 18). In addition to exercise, changes in the carbohydrate content of the diet could also have played a significant role in our study, because high-carbohydrate diets are generally associated with higher muscle glycogen content (2). The measured 11% increase in macronutrients ingested as carbohydrate between ER and ET-LFD could thus have contributed to increased muscle glycogen stores. However, the reciprocal decrease in lipid intake (a drop of 9% between ER and ET-LFD phases) did not yield a decrease in muscle lipid stores. This also occurred despite the generally reported increase in lipid utilization after long-term endurance training (21) and the report of Schrauwen et al. (34) that showed that obese subjects oxidized 40% more fat after weight loss by diet and exercise than those after a low-fat diet alone.

The distribution of lipid droplets within a myofiber, unchanged after weight loss by diet or diet and exercise (Fig. 4), remains different from that of lean individuals. Obese subjects were shown to have a higher proportion of lipids in the central area of muscle fibers than lean controls (28), clearly indicating perturbed muscle lipid deposition patterns that remain after weight loss. Because mitochondria are located primarily near the sarcolemma (19, 20), lipids located in the central region of the fibers may be more difficult to mobilize for oxidation, favoring their storage. However, the unchanged lipid distribution within myofibers after weight loss, coupled with an overall decrease in muscle lipid content, suggests, in fact, that lipids throughout the cell are mobilized.

The physiological significance of supranormal myocellular triglyceride concentrations is still unclear, although indications that they might contribute to muscle insulin resistance and studies showing cellular dysfunction in the presence of lipid accumulation suggest a detrimental effect. Our data show that an apparently improved (though not statistically significant) insulin sensitivity after weight loss, measured as a decrease in fasting insulin and a decrease in insulin secretion during OGTT (Table 1), is not associated with changes to the intramyocellular lipid concentration of muscle cells. However, it is important to point out that the postweight loss levels of these markers of insulin sensitivity are still much above values normally measured in sedentary controls (e.g., Ref. 43). Also, lipid accumulation is certainly not the only factor contributing to insulin resistance. Thus our data do not preclude a role for intracellular lipid levels in insulin resistance.

Our data clearly show that weight loss affects muscle energy reserves. However, weight loss by diet or diet and exercise does not influence the concentration of these reserves within muscle fibers, at least within the time frame of our study and/or the intervention protocol used. Our data suggest that the normalization of the intramyocellular environment with respect to lipid concentration is, in fact, quite difficult and may require longer-term intervention, most probably coupled with a relatively high level of physical activity, to improve lipid oxidation.

We give special thanks to Dr. Serge Rivest, who graciously provided microscope access and made the completion of this project possible. We also thank Yves Gélinas and Sophie Raymond for technical assistance. We gratefully acknowledge the contribution of the late Dr. Jean-Aimé Simoneau to this paper.

REFERENCES


