Endurance Exercise Training Up-Regulates Lipolytic Proteins and Reduces Triglyceride Content in Skeletal Muscle of Obese Subjects

Katie Louche, Pierre-Marie Badin, Emilie Montastier, Claire Laurens, Virginie Bourlier, Isabelle de Glisezinski, Claire Thalamas, Nathalie Viguérie, Dominique Langin, and Cedric Moro


Context: Skeletal muscle lipase and intramyocellular triglyceride (IMTG) play a role in obesity-related metabolic disorders.

Objectives: The aim of the present study was to investigate the impact of 8 weeks of endurance exercise training on IMTG content and lipolytic proteins in obese male subjects.

Design and Volunteers: Ten obese subjects completed an 8-week supervised endurance exercise training intervention in which vastus lateralis muscle biopsy samples were collected before and after training.

Main Outcome Measures: Clinical characteristics and ex vivo substrate oxidation rates were measured pre- and posttraining. Skeletal muscle lipid content and lipolytic protein expression were also investigated.

Results: Our data show that exercise training reduced IMTG content by 42% ($P < .01$) and increased skeletal muscle oxidative capacity, whereas no change in total diacylglycerol content and glucose oxidation was found. Exercise training up-regulated adipose triglyceride lipase, perilipin (PLIN) 3 protein, and PLINS protein contents in skeletal muscle despite no change in mRNA levels. Training also increased hormone sensitive–lipase Ser660 phosphorylation. No significant changes in comparative gene identification 58, G0/G1 switch gene 2, and PLIN2 protein and mRNA levels were observed in response to training. Interestingly, we noted a strong relationship between skeletal muscle comparative gene identification 58 and mitochondrial respiratory chain complex I protein contents at baseline ($r = 0.87$, $P < .0001$).

Conclusions: Endurance exercise training coordinately up-regulates fat oxidative capacity and lipolytic protein expression in skeletal muscle of obese subjects. This physiological adaptation probably favors fat oxidation and may alleviate the lipotoxic lipid pressure in skeletal muscle. Enhancement of IMTG turnover may be required for the beneficial metabolic effects of exercise in obesity. (J Clin Endocrinol Metab 98: 4863–4871, 2013)

Abbreviations: AMPK, 5′-AMP protein kinase; ATGL, adipose triglyceride lipase; CGI-58, comparative gene identification 58; DAG, diacylglycerol; HSL, hormone-sensitive lipase; IMTG, intramyocellular triglyceride; LD, lipid droplet; PUN, perilipin; TAG, triacylglycerol; T2DM, type 2 diabetes mellitus; $V_{O_2,max}$, maximal oxygen uptake.
Obesity and type 2 diabetes mellitus (T2DM) have been linked to ectopic fat deposition in nonadipose tissues such as skeletal muscle, which plays an important role in the etiology of insulin resistance (1). Ectopic lipids mainly accumulate as triacylglycerols (TAGs) stored within lipid droplets (LDs) in skeletal muscle. Intramyocellular triglyceride (IMTG) pools are now recognized as an important fuel source during exercise (2). IMTG content is increased in athletes and in lean healthy subjects in response to exercise training interventions (3, 4). However, IMTG content is paradoxically elevated in obese and T2DM individuals (5), and an inverse association between IMTG content and peripheral glucose disposal has been repeatedly reported in sedentary subjects (5, 6). It is now well established that IMTG is associated with elevated levels of lipotoxic intermediates such as diacylglycerols (DAGs) and ceramides disrupting insulin-stimulated glucose disposal (1). However, it is not clear so far how IMTG mediates lipotoxicity in obese sedentary subjects. LDs are dynamic organelles resulting from the balance between storage and breakdown of TAGs by lipases. We have recently shown that disturbances in lipase expression/activity in skeletal muscle may contribute to lipotoxicity and insulin resistance (7). Adipose triglyceride lipase (ATGL) expression is up-regulated, whereas hormone-sensitive lipase (HSL) expression is reduced in the muscle of obese subjects (8). A causal relationship between elevated ATGL and/or reduced HSL activity and insulin resistance was shown in vitro in human primary myotubes (7). ATGL is the main TAG hydrolase in mouse and human skeletal muscle (7, 9), whereas HSL exhibits a strong preference for DAG (10). ATGL activity is acutely regulated by comparative gene identification 58 (CGI-58) in human skeletal muscle (11). Recent data indicate that ATGL activity is inhibited by G0/G1 switch gene 2 (G0S2) (12), but nothing is known about its expression and potential role in human muscle. Both ATGL and HSL activity could also be regulated by LD-associated proteins of the perilipin (PLIN) family. At least 3 main isoforms are expressed in human skeletal muscle (PLIN2/3/5). Recent studies in transgenic mice with whole ablation or cardiac-specific modulations of PLIN5 have been insightful in determining the metabolic role of PLIN5 in skeletal and cardiac muscles (13, 14). PLIN5 seems to protect LDs from TAG hydrolysis and may channel fatty acids to mitochondrial \( \beta \)-oxidation. PLIN2 has also been associated with IMTG and insulin sensitivity in humans (15). Although PLIN3 is expressed in human muscle fibers (16), little is known so far about its function in skeletal muscle. Taken together, these results suggest that lipolytic proteins may play a role in the regulation of oxidative metabolism, lipotoxicity, and insulin sensitivity. Lifestyle interventions incorporating increased physical activity remain the primary preventive approach for metabolic diseases such as obesity and T2DM (17). Exercise training improves whole-body insulin sensitivity and metabolic flexibility in obese individuals and individuals with T2DM (18). One possible mechanism by which exercise training improves insulin sensitivity is by restoring lipase expression and reducing lipid content in skeletal muscle. Thus, enhancement of IMTG turnover may be required for the beneficial metabolic effects of exercise in obesity. The purpose of this study was to investigate the effect of 8 weeks of endurance exercise training on skeletal muscle neutral lipid content and lipolytic proteins in middle-aged obese male individuals.

### Materials and Methods

#### Subjects

Nineteen male obese subjects (mean age 36 ± 2 years; mean body mass index, 32.3 ± 0.7 kg/m\(^2\); range, 29.4–35.8 kg/m\(^2\)) were recruited at the Toulouse Clinical Investigation Centre. Subjects were included if they had a body mass index <40 kg/m\(^2\), blood pressure <140/90 mm Hg, and normal thyroid function. Participants were excluded if they had history of diabetes, cardiovascular diseases, or cancer or if they had any contraindication for local anesthetics and exercise. They were weight stable for the last 3 months and free of medications, chronic diseases, and known comorbidities. Ten subjects participated in an 8-week supervised endurance exercise training program in which paired biopsies were performed at rest before and after training. The participants were asked to refrain from vigorous physical activity for 48 hours before presenting to the clinical investigation center and ate a weight-maintaining diet consisting of 35% fat, 16% protein, and 49% carbohydrates 2 days before the experiment. Dietary intake was assessed by a dietician from a 3-day weighed food record, including 2 weekdays and 1 weekend day, the week before the first investigation day. Dietary records were assessed at baseline and during the last week of the program. Nutrient intake was calculated using PROFI software (version 6.7; Audit Conseil en Informatique Médicale) with the CIQUAL French food composition database for diet composition. Self-reported food intake did not change significantly during the training program (2567 ± 211 vs 2228 ± 158 kcal/d for pre- and posttraining, respectively).

#### Ethics statement

This study was performed according to the latest version of the Declaration of Helsinki and the current International Conference on Harmonization guidelines. The application was approved by the Toulouse University Hospitals Ethics Committee, and all subjects gave written informed consent. The research was conducted at the Clinical Investigation Centre of Toulouse Hospitals under the supervision of trained staff and used standardized procedures.
Study design

Body composition, blood parameters, and maximal oxygen uptake (VO2max) were measured on one experimental day, and muscle biopsies and oral glucose tolerance tests (OGTT) were performed on a second experimental day 1 week apart. On each experimental day, subjects were investigated after a 10-hour overnight fast. VO2max was assessed using a graded exercise test conducted on an electromagnetically braked bicycle ergometer (Ergometrics 800; Ergoline) as described previously (19). The initial workload was 50 W, and it was increased by 30 W every 3 minutes until exhaustion. Heart rate was continuously monitored by telemetry using a heart rate monitor (Ergocard). We considered that the subjects achieved their VO2max when all of the following usual and accepted criteria were achieved: maximal heart rate measured at exhaustion was higher than 90% of the age-predicted maximal heart rate, respiratory quotient (i.e., VCO2/VO2) measured at exhaustion was higher than 1.1, and the subjects could not sustain a sufficient rate of cycling. Breath-by-breath measurements were taken at rest and throughout exercise to assess air flow and O2 and CO2 concentrations in expired gases by using a computerized ergospirometer (Ultima PFX; Medical Graphics). The VO2max exercise trial was performed in a ventilated room to ensure a constant room temperature and hygrometry from the calibration just before the trial. Muscle biopsy samples from vastus lateralis weighing 60 to 100 mg each were obtained using the Bergstrom technique (20). Pieces of muscle were collected in the respective buffers for ex vivo oxidation and glucose transport assays or blotted, cleaned, and snap-frozen in liquid nitrogen for gene expression and Western blot analyses. Ninety minutes after biopsy sampling, a 75-g oral glucose tolerance test (according to World Health Organization standards) was performed, and blood samples were collected at 0, 15, 30, 45, 60, 90, and 120 minutes. Body composition (considering a 3-compartment model) was determined before and after training using a total-body dual-energy x-ray absorptiometer (DPX software 3.6; Lunar Radiation Corp). Blood glucose was assayed using the glucose oxidase technique (bioMérieux), and plasma insulin was measured using a Bi-insulin IRMA kit (Bertin Pharma). Plasma free fatty acids were assayed with an enzymatic method (Wako kit; Unipath). Plasma cholesterol and triglycerides were determined using standard clinical biochemistry methods.

Exercise training

Endurance exercise was performed at the Centre de Ressources, d’Excellence et de Performance Sportives (CREPS) of Toulouse (Toulouse, France). Exercise sessions consisted mainly of cycling and running, 5 times per week for 8 weeks. Subjects exercised 3 times per week under supervision by an experienced physical coach during the first 4 weeks and 2 times per week during the last 4 weeks. They exercised on their own during other sessions. All daily sessions consisted of at least a 20-minute warm-up at 35% VO2max followed by progressively increasing exercise intensity (up to 85% VO2max) and duration (up to 1 hour) throughout the training program. The subjects exercised at a target heart rate corresponding to 35% to 85% of their VO2max. Heart rate was monitored with a Suunto T3 Cardiometer (MSE). Compliance with training was good, as checked by a training diary including day-to-day activities. The percentage of sessions completed was >85%.

Palmitate and glucose oxidation in muscle homogenates

This assay was performed as described previously (21, 22). In brief, 50 mg of muscle was minced and homogenized in a modified sucrose-EDTA medium (250 mM sucrose, 1 mM EDTA, and 10 mM Tris–HCl [pH 7.4]). Palmitate oxidation rates were determined by measuring production of [14C]-labeled acid-soluble metabolites (ASMs), a measure of tricarboxylic acid cycle intermediates and acetyl esters (incomplete oxidation), and [14C]CO2 (complete oxidation). The glucose oxidation rate was determined by measuring [14C]CO2. The radioactivities of CO2 and ASMs were determined by liquid scintillation counting. Data are expressed as nanomoles per hour and were normalized per gram of tissue weight.

Determination of neutral lipid content

Muscle tissue was homogenized in 1 mL of methanol-5 mM EGTA (2:1, v/v) with Fast-Prep (MP Biochemicals). Lipids corresponding to 20 mg of tissue were extracted according to Bligh and Dyer (23) in dichloromethane-methanol-water (2.5:2.5:2.1, v/v/v), in the presence of the internal standards (3 µg of stigmasterol, 3 µg of 1,3-dimyristine, 3 µg of cholesterol heptadecanoate, and 20 µg of glycerol trinonadecanoate) as described previously (21). The equivalent of 0.3 mg of tissue was evaporated under nitrogen, the dry pellets were dissolved overnight in 0.2 mL of NaOH (0.1 M), and proteins were measured with the Bio-Rad protein assay.

Reverse transcription and real-time quantitative PCR

Total RNA from muscle tissue was isolated with an RNaseasy mini kit according to the manufacturer’s instructions (QIAGEN GmbH). The quantity of RNA was determined on a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific). RT-PCR was performed on a GeneAmp PCR System 9700 using 1 µg

<table>
<thead>
<tr>
<th>Table 1. Clinical and Biochemical Variables in Obese Subjects at Baseline (Pretraining) and After 8 Weeks (Posttraining) of Endurance Exercise Training</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Body weight, kg</strong></td>
</tr>
<tr>
<td><strong>Body mass index, kg/m²</strong></td>
</tr>
<tr>
<td><strong>Fat mass, %</strong></td>
</tr>
<tr>
<td><strong>Lean body mass, kg</strong></td>
</tr>
<tr>
<td><strong>VO2max, L/min</strong></td>
</tr>
<tr>
<td><strong>VO2max, mL/min/kg</strong></td>
</tr>
<tr>
<td><strong>Fasting insulin, µU/mL</strong></td>
</tr>
<tr>
<td><strong>Fasting glucose, g/L</strong></td>
</tr>
<tr>
<td><strong>AUC0–120 glucose</strong></td>
</tr>
<tr>
<td><strong>AUC0–120 insulin</strong></td>
</tr>
<tr>
<td><strong>Plasma cholesterol, mM</strong></td>
</tr>
<tr>
<td><strong>Plasma triglycerides, mM</strong></td>
</tr>
<tr>
<td><strong>Plasma free fatty acids, µM</strong></td>
</tr>
</tbody>
</table>

Abbreviation: AUC, area under the curve; NS, not significant. Values are means ± SEM (n = 10).
of RNA and the MultiScribe reverse transcriptase method (Applied Biosystems). Real-time quantitative PCR was performed to determine cDNA content. Primers from Applied Biosystems were as follows: 18S (TaqMan assay identification number: Hs99999901_s1), PNPLA2 (patatin-like phospholipase domain-containing protein 2, also called ATGL, Hs00982040_g1), LIPE (hormone-sensitive lipase, Hs00943404_m1), CGI-58 (comparative gene identification 58, Hs00211205_m1), GOS2 (G5/G6, switch gene 2, Hs00274783_s1), PLIN2 (perilipin 2, Hs00605340_m1), PLIN3 (perilipin 3, Hs00998416_m1), and PLIN5 (perilipin 5, Hs00965990_m1). The amplification reaction was performed in duplicate on 10 ng of the cDNA samples in a final volume of 20 µL in 96-well reaction plates on a StepOne™ Plus real-time PCR system (Applied Biosystems). All reactions were performed under the same conditions: 50°C for 2 minutes, 95°C for 10 minutes, 40 cycles of 95°C for 15 seconds, and 60°C for 1 minute. All expression data were normalized by the 2−ΔCt method using 18S rRNA as an internal control.

Western blotting
Muscle tissue pieces were homogenized in a buffer containing 50 mM HEPES (pH 7.4), 2 mM EDTA, 150 mM NaCl, 30 mM NaPO4, 10 mM NaF, 1% Triton X-100, 10 µL/mL protease inhibitor (Sigma-Aldrich), 10 µL/mL phosphatase I inhibitor (Sigma-Aldrich), and 1.5 mg/mL benzamidine HCl. Tissue homogenates were centrifuged for 25 minutes at 15 000 g, and supernatants were stored at −80°C. Solubilized proteins (40 µg) were run on 4% to 12% SDS-PAGE (Bio-Rad), transferred onto a nitrocellulose membrane (Hybond ECL; Amersham Biosciences), and incubated with the primary antibodies: ATGL, HSL, pHSL Ser660, Akt, IRS1, AMPK (Cell Signaling Technology), OXPHOS (MitoSciences), CGI58 (Abnova Corp), and G0S2 (ProteinTech). For detection of PLINs, membranes were probed with PLIN2 and PLIN3 (Thermo Scientific), and PLIN5 (Progen) antibodies. Subsequently, immunoreactive proteins were visualized using a ChemiDoc MP Imaging System (Bio-Rad), and data were analyzed using Image Lab software (version 4.1; Bio-Rad). Glyceraldehyde-3-phosphate dehydrogenase (Cell Signaling Technology) served as an internal control.

Statistical analyses
Statistical analyses were performed using GraphPad Prism 5.0 for Windows (GraphPad Software Inc). Normal distribution and homogeneity of variance of the data were tested using the Shapiro-Wilk and F tests, respectively. Paired Student t tests were performed to determine the effect of training on anthropometric and clinical variables (Table 1). Pearson correlations were run to assess relationships between lipases and PLIN mRNA and proteins. Univariate linear regressions were applied to evaluate the relationship between ex vivo palmitate oxidation, complex I protein, and CGI-58 protein. All values in figures and the table are presented as means ± SEM. Statistical significance was set at a value of P < .05.
Results

Exercise training improves whole-body aerobic capacity and muscle glucose uptake

The 8-week training intervention tended to reduce body fat mass and to increase lean body mass, and it significantly increased VO$_2$max by about 10% (Table 1). The exercise intervention also tended to reduce the fasting plasma insulin level (−18%, $P = .07$) with no change in fasting glucose (Table 1). This result was associated with higher Akt Ser473 phosphorylation (3.4-fold), Akt total protein content (1.75-fold), and basal glucose uptake (2.65 fold) in skeletal muscle (Supplemental Figure 1 published on The Endocrine Society’s Journals Online web site at [http://jcem.endojournals.org](http://jcem.endojournals.org)). No change in 5’-AMP protein kinase (AMPK) total protein expression was observed in response to training (Supplemental Figure 1). The higher basal glucose uptake occurred in light of significantly elevated phosphorylation of the Akt substrate of 160 kDa (AS160) (3.3-fold), whereas no significant changes in skeletal muscle insulin receptor substrate-1 phosphorylation and total protein were observed (Supplemental Figure 2). No changes in the mRNA levels of the main PI3K subunits expressed in skeletal muscle were noted posttraining (data not shown). No significant effect of training on glucose tolerance and plasma lipids was observed (Table 1).

Exercise training increases muscle fat oxidative capacity and reduces lipid content

We next assessed the impact of the 8-week exercise training intervention on neutral lipid content in skeletal muscle. Exercise training strongly reduced resting IMTG content (−42%, $P = .008$) (Figure 1A), whereas no significant change in total DAG content was observed (Figure 1B). Interestingly, palmitate oxidation rates measured ex vivo in fresh muscle tissue tended to increase in response to training (71%, $P = .08$) (Figure 1C), whereas glucose oxidation remained unchanged (Figure 1D). Mitochondrial respiratory chain complex I (0.50 ± 0.08 vs 0.37 ± 0.04 arbitrary units, $P < .05$), complex III (0.45 ± 0.07 vs 0.27 ± 0.04 arbitrary units, $P < .01$), and complex V (1.59 ± 0.17 vs 1.28 ± 0.15 arbitrary units, $P < .05$) protein contents in skeletal muscle were also significantly up-regulated posttraining.

Effect of endurance exercise training on skeletal muscle lipase expression

All biopsy samples analyzed were negative for PLIN1, excluding significant contamination by adipocytes (data not shown). Western blotting analyses on lysates of vastus lateralis muscle biopsy samples revealed a comparable increase in both ATGL protein (72%, $P = .04$) (Figure 2, A and B) and HSL Ser660 phosphorylation (78%, $P = .02$) (Figure 2, A and C). This occurred in the absence of a statistical change in total HSL protein (Figure 2, A and D). The higher HSL Ser660 phosphorylation observed after training remained significant after adjustment for total HSL protein (0.45 ± 0.08 vs 0.57 ± 0.09 arbitrary units, $P = .04$). Of note, no significant changes in ATGL (PNPLA2) and HSL (LIPE) gene expression were observed in response to training (Supplemental Table 1).

Effect of endurance exercise training on skeletal muscle colipase expression

Because ATGL activity is actively controlled by CGI-58 and G0S2, we next assessed the influence of exercise training on CGI-58 and G0S2 protein content in the skeletal muscle of obese subjects. We could show that exercise training did not significantly affect either CGI-58 (+20%, not significant) (Figure 3, A and B) or G0S2 (Figure 3, A and C) protein expression.

**Figure 3.** Effect of exercise training on skeletal muscle colipases. A, Representative blots of CGI-58, G0S2, and GAPDH. B–D, Quantitative bar graphs of CGI-58 protein (B), G0S2 protein (C) and the ratio of CGI-58 to G0S2 protein (D) in skeletal muscle of obese subjects before (Pre-) and after (Post-) exercise training. *, $P < .05$, comparing the effect of exercise training.
pression. No changes in CGI-58 and G0S2 mRNA levels were observed (Supplemental Table 1). However, exercise training increased the ratio of CGI-58 to G0S2 protein (48%, \( P < .04 \)) (Figure 3D).

Effect of endurance exercise training on skeletal muscle PLIN expression

PLINs are LD-associated proteins playing a role in the regulation of lipolysis. We observed a significant up-regulation of skeletal muscle PLIN3 (2.4-fold, \( P = .006 \)) (Figure 4, A and C) and PLIN5 (+26%, \( P < .05 \)) (Figure 4, A and D) proteins in response to training. No significant effect on muscle PLIN2 protein content was noted (Figure 4, A and B). As reported for lipases and colipases, the training intervention did not affect PLIN2/3/5 gene expression (Supplemental Table 1). No correlation between PLIN, lipase and colipase protein, and mRNA levels was observed at baseline. However, a significant positive relationship between respiratory chain complex I protein and palmitate oxidation (Figure 5A) and a robust positive correlation between respiratory chain complex I protein and CGI-58 protein were found at baseline in our obese cohort (Figure 5B).

Discussion

Exercise training is known to improve insulin sensitivity and muscle fat oxidative capacity. The major and novel findings of the present study are (1) that exercise training coordinately up-regulates fat oxidative capacity and lipolytic proteins (ATGL, HSL Ser660, PLIN3, and PLIN5) which together reduce resting IMTG content in obese subjects and (2) that G0S2 is significantly expressed in human skeletal muscle and not regulated by exercise training. This physiological adaptation may be seen as a favorable metabolic event (1) to alleviate the lipotoxic lipid pressure in skeletal muscle during obesity and (2) to enhance IMTG breakdown and utilization as fuel during exercise.

We first observed that exercise training intervention was effective in increasing maximal oxygen consumption and also tended to improve whole-body insulin sensitivity as reflected by lower fasting plasma insulin concentration posttraining, in line with several studies (18, 24). Although we did not directly assess skeletal muscle insulin sensitivity, we observed a significant up-regulation of basal glucose uptake and of Akt and its downstream target AS160 activation involved in GLUT4 trafficking and glucose transport (25, 26). Although AMPK is known to promote glucose transport in skeletal muscle (27), the higher basal glucose transport after training appeared to be independent of substantial changes in skeletal muscle AMPK protein content. These data are in agreement with other studies reporting a significant effect of strength and/or interval training on total Akt but no effect on total AMPK protein in skeletal muscle (28, 29). The improvement in whole-body aerobic capacity was paralleled by an up-regulation of skeletal muscle oxidative capacity. We also show that palmitate oxidation rates were closely associated with complex I protein expression in skeletal muscle, thus reflecting a surrogate marker of maximal oxidative capacity in this tissue. These data are in line with recent studies reporting a net increase in muscle ex vivo palmitate oxidation rates in obese subjects (30) and in subjects with T2DM (22) in response to several weeks of exercise training and are also consistent with an up-regulation of mitochondrial biogenesis and respiratory chain complex protein expression in response to training (22, 26).

An increase in muscle fat oxidative capacity may contribute to improving metabolic health in obese subjects. A
Strong inverse relationship between muscle lipid content and whole-body insulin sensitivity has been widely documented in sedentary subjects (5, 6). We report here a strong reduction in IMTG resting content in muscle of obese subjects in response to endurance exercise. Overall, this finding is in agreement with other studies (31, 32). However, it is worth mentioning at this point that several training studies in lean healthy subjects have reported a net increase in total IMTG content (4, 24, 33). IMTG content is also elevated in endurance-trained individuals compared with that in age-matched controls (3) and is positively associated with insulin sensitivity in fit individuals (6). This observation is at the basis of the so-called “athlete paradox” and the U-shaped relationship between IMTG content and insulin sensitivity (34). Thus, because IMTG content is increased several fold in obese and type 2 diabetic subjects (5, 6), the 42% decrease in total IMTG content observed in this study may not be sufficient to come back to the level observed in lean subjects. Of importance, the net decrease in IMTG may be due to a combination of increased fat oxidative capacity and lipase content. IMTG is a significant fuel source during exercise (2) and also potentially at rest because skeletal muscle largely relies on fat as a fuel during fasting (35). Consequently, IMTG lipolysis is a fine-tuning process to supply fatty acid fuels during periods of higher energy demand such as exercise.

Our data show a strong up-regulation of ATGL protein and HSL activation at Ser660 in skeletal muscle of obese subjects posttraining. This finding is in agreement with at least one other study in lean, healthy young men (31). HSL phosphorylation at Ser660 is mediated by cAMP-dependent protein kinase (36). HSL exhibits a strong preference for DAGs as substrates (10). However, the coordinated up-regulation of both ATGL and HSL did not significantly change muscle total DAG content in this study. This finding is in line with in vitro data showing that concomitant overexpression of ATGL and HSL does not significantly alter total DAG content (7). One study limitation here is that we did not directly measure membrane vs cytosolic DAG content, which may have been a better marker of bioactive DAG pools and insulin resistance (37). Muscle membrane DAG content is significantly reduced in athletes who are otherwise more insulin sensitive than matched control subjects. Thus, no significant changes in ATGL and HSL mRNA levels were observed in response to training. This result highlights the fact that lipases are actively regulated at the posttranscriptional level. ATGL is robustly activated by CGI-58 (38) and inhibited by G0S2 (12). We and others have shown that CGI-58 is highly expressed in human skeletal muscle and coactivates ATGL (11, 31). We also report for the first time significant expression of G0S2 at the mRNA and protein levels in human skeletal muscle. In this study, endurance training increased the ratio of CGI-58 to G0S2, which may result in an enhancement of ATGL activity.

Of note, we found a strong positive relationship between CGI-58 protein and mitochondrial respiratory chain complex I protein expression at baseline in our obese cohort. This finding suggests a tight coupling of lipolytic and mitochondrial fat oxidative capacity in skeletal muscle. We also observed a significant up-regulation of PLIN5, which is a known peroxisome proliferator–activated receptor-target gene, in muscle after training (39). PLIN5 is predominantly expressed in oxidative tissues such as cardiac and skeletal muscles and may actively control lipid storage and lipolysis in those tissues (13, 14). Our data are in agreement with another recent study showing increased muscle PLIN5 protein content in response to 6 weeks of endurance or sprint interval training in lean healthy volunteers (33). We also report a robust up-regulation of muscle PLIN3 and no change in PLIN2 protein expression after training. PLIN2 and PLIN3 are abundant PLIN proteins in human skeletal muscle (16, 33). PLIN2 is a constitutive LD-associated protein coating large LDs. On the other hand, PLIN3 is an exchangeable LD-associated protein that preferentially coats small LDs in response to lipolytic or lipogenic stimuli (40). Collectively, the up-regulation of PLIN proteins (PLIN3 and PLIN5)
may reduce access of the main lipases (ATGL and HSL) to LDs in the basal state (rest) and favor IMTG-derived fatty acid channeling to mitochondria in the stimulated state (exercise).

We acknowledge that our study may have been underpowered to capture significant changes in some secondary outcomes presented here. Potential contamination of muscle biopsy samples by infiltrated adipocytes was excluded by immunoblotting PLIN1, which is not present at the protein level in skeletal muscle (31). Skeletal muscle insulin sensitivity and signaling were not specifically assessed by a hyperinsulinemic-euglycemic clamp. The strength of the current study is the longitudinal intervention with detailed metabolic phenotyping of skeletal muscle ex vivo.

In summary, our study shows that aerobic exercise training enhances basal ATGL expression and HSL phosphorylation in human skeletal muscle. This physiological adaptation probably contributes to a reduction in resting IMTG content in obese subjects. Thus, the concomitant up-regulation of muscle fat oxidative capacity and lipolytic proteins in response to training, as well as the strong baseline relationship between these 2 parameters, highlights the fact that IMTG lipolysis is an important metabolic function of skeletal muscle. An enhancement in IMTG turnover may be required for the beneficial metabolic effects of exercise in obesity.

Acknowledgments

We thank Marie-Adeline Marques (Institut National de la Santé et de la Recherche Médicale [INSERM] Unité Mixte de Recherche 1048, Toulouse, France) for outstanding technical assistance. We thank J. Bertrand-Michel and V. Roques (Lipidomic Core Facility INSERM 1048, part of the Toulouse Metatoul Platform) for lipidomic analysis, advice, and technical assistance. We are also grateful to the study participants and to Dr. François Crampes for critical reading of the manuscript and helpful discussions.

Address all correspondence and requests for reprints to: Cédric Moro, PhD, Institut National de la Santé et de la Recherche Médicale, Unité Mixte de Recherche 1048, Institut des Maladies Métaboliques et Cardiovasculaires, Centre Hospitalier Universitaire Rangueil, BP 84225, 1 Avenue Jean Poulhès, 31432 Toulouse Cedex 4, France. E-mail: cedric.moro@inserm.fr.

This study was supported by the National Research Agency (grant ANR-12-JSV1-0010-01), the European Federation for the Study of Diabetes/Novo Nordisk and Société Francophone du Diabète (C.M.), the Fondation pour la Recherche Médicale, GlaxoSmithKline, the National Research Agency National Research Agency LIPOB, INSERM Direction de l’Hospitalisation et de l’Organisation des Soins Recherche Translationnelle, and AOF Hôpitaux de Toulouse (D.L.).

This study was registered with clinical trial registration number NCT01083329.

Disclosure Summary: The authors have nothing to disclose.

References

20. Bergstrom J. Percutaneous needle biopsy of skeletal muscle in phys-