Exercise training and calorie restriction increase SREBP-1 expression and intramuscular triglyceride in skeletal muscle


Intramuscular triglyceride (IMTG) deposition in skeletal muscle is associated with obesity and type 2 diabetes (T2DM) and is thought to be related to insulin resistance (IR). Curiously, despite enhanced skeletal muscle insulin sensitivity, highly trained athletes and calorie-restricted (CR) monkeys also have increased IMTG. Sterol regulatory element-binding proteins (SREBPs) are transcription factors that regulate the biosynthesis of cholesterol and fatty acids. SREBP-1 is increased by insulin in skeletal muscle in vitro and in skeletal muscle of IR subjects, but SREBP-1 expression has not been examined in exercise training or calorie restriction. We examined the relationship between IMTG and SREBP-1 expression in animal models of exercise and calorie restriction. Gastrocnemius and soleus muscle biopsies were obtained from 38 Sprague-Dawley rats (18 control and 20 exercise trained). Triglyceride content was higher in the gastrocnemius and soleus muscles of the trained rats. SREBP-1c mRNA, SREBP-1c precursor and mature proteins, and fatty acid synthase (FAS) protein were increased with exercise training. Monkeys (Macaca mulatta) were CR for a mean of 10.4 years, preventing weight gain and IR. Vastus lateralis muscle was obtained from 12 CR monkeys. Triglyceride content was higher in CR monkeys. This indicates that increased IMTG seen in skeletal muscle of highly conditioned athletes results via damaging effects of fatty acyl-CoA, ceramides, or diacylglycerols on the insulin-signaling cascade (13, 29, 40, 57, 72). Similarly, most interventions that improve insulin sensitivity decrease IMTG (23, 31, 54, 65–67, 71).

Curiously, it is also well supported that endurance-trained athletes have increased IMTG despite enhanced skeletal muscle insulin sensitivity (21, 27, 33, 43). The exercise training effect is not limited to elite athletes. Even moderate exercise training (1 h of cycle exercise at 45% of \( \text{VO}_2 \text{max} \)) in previously sedentary, elderly subjects increases IMTG (65). At the same time, exercise also improves generalized insulin sensitivity and normalizes insulin levels (6, 15, 16). Thus hyperinsulinemia is not the cause of increased IMTG with exercise. The paradox of increased IMTG with exercise training also suggests that the IMTGs themselves may not be directly causative of insulin resistance.

The mechanism controlling increased IMTG in insulin resistance and in exercise training has not been determined. One possibility is through upregulation of the lipid synthesis pathway in muscle. Sterol regulatory element-binding proteins (SREBPs) are a group of membrane-bound transcription factors that regulate expression of the genes involved in the production and uptake of cholesterol, fatty acids, triglycerides, phospholipids and the low-density lipoprotein (LDL) receptor (28). There are three isoforms of SREBP designated SREBP-1a, SREBP-1c, and SREBP-2. Whereas SREBP-1a can stimulate all SREBP-responsive genes, SREBP-1c preferentially activates fatty acid synthetic genes, and SREBP-2 activates mainly cholesterol synthesis genes (11). SREBPs are made as precursor proteins, requiring cleavage of the NH2-terminal domain, before the mature form can reach the nucleus and operate as a transcription factor (28).

Expression of SREBP-1 is enhanced by insulin in three major insulin target tissues: liver, fat, and skeletal muscle (20, 24, 34, 45, 59), and the levels of SREBP-1 are increased in the presence of hyperinsulinemia (7, 30, 60). Furthermore, we and others have previously shown that SREBP-1 is increased by insulin in skeletal muscle in vitro (45) and in skeletal muscle of insulin-resistant animals (46) via the MAP kinase signaling pathway (36, 45). In skeletal muscle, the MAP kinase pathway
remains intact in the setting of insulin resistance (8, 37), and hyperinsulinemia increases SREBP-1 despite defects in the muscle phosphatidylino-sitol 3-kinase pathway of insulin signaling (2, 8, 14, 19, 32, 35). Thus activation of SREBPs may be involved in the increased IMTG of insulin resistance, possibly through excess signaling by the hyperinsulinemia of this disease state. SREBP-1 expression has not been examined in the setting of exercise training.

Fasting and refeeding are also known to impact SREBP-1 expression. In muscle tissue, SREBP-1c mRNA is decreased in soleus, gastrocnemius, and white quadriceps muscle after short-term fasting (6–24 h) (5). In addition, after a 48-h fast, SREBP-1c mRNA is increased with refeeding in the same muscle groups (5), and after an 18-h fast, refeeding increases gastrocnemius SREBP-1c mRNA and SREBP-1 precursor and mature protein (12). However, long-term calorie restriction may have different effects on SREBP-1 expression than a short-term fast. Interestingly, Ortmeyer et al. (47) recently showed that monkeys subjected to long-term calorie restriction, like trained athletes, have increased IMTG. The relationship between SREBP-1 and IMTG with long-term calorie restriction, which, like exercise training, also increases insulin sensitivity, has not been examined.

We hypothesized that SREBP-1 expression would be enhanced in interventions that increase IMTG, even without hyperinsulinemia. To test this hypothesis, we examined models enhanced in interventions that increase IMTG, even without hyperinsulinemia. To test this hypothesis, we examined models enhanced in interventions that increase IMTG, even without hyperinsulinemia.

**Materials and Methods**

**Materials**

The spectrophotometer used was the DU 640 by Beckman (Fullerton, CA). The densitometer was the Flour-S MultiImager from Bio-Rad (Hercules, CA), with the Quantity One version 4.1.1 software being used. Also from Bio-Rad were the 12% Tris-HCl gel, the Kaleidoscope Prestained Standards, the polyvinylidene (PVFD) membranes, the iCycler system for quantitative real-time RT-PCR (qRT-PCR), the iScript cDNA Synthesis kit, the 1× SYBR Green RT-PCR supermix, 96-well PCR plates, and optical sealing tape. The MagicMark Western Standard was from Invitrogen (Carlsbad, CA). The DNase/RNase free water and phospho-buffered saline were from Gibco-BRL (Grand Island, NY). The tissue homogenizer (Tiszumizer), Wheaton Tenbroeck tissue grinders, shell vials, and 96-well tissue culture plates were from Fisher (Pittsburgh, PA), as were the chloroform, ethanol, isopropanol, formamide, and formaldehyde. The N-Evap 111 was from Organon (Berlin, MA). The Kinetic Microplate Reader was from Molecular Devices (Sunnyvale, CA), and the SPF 3.1.2 SOFTmax Pro software was used.

Reagents from Qiagen included the RNeasy minikit, DNase set (RNase free) and proteinase K. The amplification grade DNase I, and 0.24- to 9.5-kb RNA ladder were from Invitrogen (Carlsbad, CA). The SREBP-1 monoclonal IgG antibody was from Santa Cruz Biotechnology (Santa Cruz, CA), and the phospho-p44/42 MAP kinase (Thr202/Tyr204) polyclonal antibody was from Cell Signaling (Beverly, MA). The anti-α-actin monoclonal antibody was from Sigma (St. Louis, MO), and the purified mouse anti-fatty acid synthase (FAS) monoclonal antibody was from BD Transduction Laboratories (San Jose, CA). The enhanced chemiluminescence (ECL) Western Blotting Analysis System was from Amersham Biosciences (Piscataway, NJ). The mammalian lysis buffer was from Pierce (Rockford, IL). The Triglyceride Reagent, Free Glycerol Reagent, and all other reagents and chemicals were from Sigma.

Rat SREBP-1c and 36β4 (acidic ribosomal phosphoprotein P0) primers were provided by Dr. Victor Sorribus (University of Zaragoza, Zaragoza, Spain), who selected, designed, and tested all primers using Beacon Designer IDT technology (Corvalle, IA). Primer sequences were forward rat SREBP-1c forward primer (5’-GCAA-CACCTGCAAGATCTACGT-3’), rat SREBP-1c reverse primer (5’-TGGCAGGACACTTACGGA-3’), 36β4 forward primer (5’-CACCTTCCCACCTGCTGAA-3’), and rat 36β4 reverse primer (5’-TCCTCCGACTCTCTCTTG-3’). Primers were produced by Integrated DNA Technologies (Corvalle, IA).

**Methods**

**Exercise training.** A 20-wk involuntary exercise protocol in Sprague-Dawley rats was used as a model of long-term vigorous exercise, as previously described (9). Briefly, female Sprague-Dawley rats aged 3–4 mo were randomly assigned to either a sedentary (n = 18) or a running group (n = 20). All rats were housed in the same facility with a 12:12-h light-dark cycle and fed standard rat chow (Teklad 8640 rodent diet by Harlan) and water ad libitum. Rats in the trained group underwent 20 wk of forced treadmill running. During the first 8 wk, daily running speed was gradually increased from 20 to 35 m/min, and running time was gradually increased from 20 min at the end of week 1 to 1 h at the end of week 8. From 8 wk until time of death, the training protocol was 1 h/day, 5 days/wk of treadmill running up a 10% grade at 20 m/min for 15 min, 28 m/min for 30 min, and 35 m/min for 15 min. This training protocol is the standard used in our laboratory and results in a significant increase in skeletal muscle citrate synthase activity (25–40%) (61), a classic marker of exercise training (9, 10).

This study was conducted under guidelines accepted by the American Physiological Society and received prior approval from the Institutional Animal Care and Use Committee at the University of Colorado’s Boulder campus.

Rat characteristics after training are listed in Table 1. There were no significant differences between the sedentary or trained groups regarding weight or tibial length at the start or finish of the study. Average age at the time of death was similar in both groups (~8 mo). Rats were killed in the morning in a nonfasted state, 24 h after the last exercise bout. Gastrocnemius and soleus muscle samples were obtained and rapidly frozen in liquid nitrogen.

**Calorie restriction.** Male rhesus monkeys (Macaca mulatta) underwent caloric restriction as previously described (25). Briefly, the monkeys were individually housed and maintained in accordance with National Academy of Sciences guidelines for care and use of laboratory animals. After they reached mature adult stature, monkeys were placed on a protocol to stabilize body weight by weekly caloric adjustment. The calorie-restricted monkeys were given Purina monkey chow in restricted amounts to keep body weight at ~10–11 kg for a mean of 10.4 yr (8–13 yr). Thus weight gain was prevented by this caloric restriction, protecting against middle-age onset obesity, which otherwise occurs in this species (25). The daily caloric load required to maintain young adult weight was 40% less than that ingested by ad

| Body wt. g | 283 ± 4 | 275 ± 8 | 0.381 |
| Tibal length, cm | 38.0 ± 0.2 | 37.7 ± 0.5 | 0.571 |
| Age, days | 243.9 ± 9.1 | 233.4 ± 5.1 | NS |

Values are means ± SE. NS, not significant. Rat characteristics are listed after 20 wk of exercise training, as described in MATERIALS AND METHODS, compared with sedentary control rats of comparable age.
libitum-fed monkeys. Calorie-restricted monkeys remained normo-
glycemic (fasting plasma glucose 69 ± 3 mg/dl) and normoinsulin-
emic (fasting plasma insulin 51 ± 12 μU/ml). This is in sharp contrast
with the ad libitum-fed monkeys that gained weight, became hyper-
glycemic, and frequently became hyperinsulinemic. Complete char-
acteristics of all groups of monkeys have been published previously
(62).

Euglycemic hyperinsulinemic clamp studies were performed after
an overnight fast, as previously described in 12 monkeys (6 calorie
restricted and 6 controls) (62). Insulin-stimulated glucose disposal
rates were not significantly different in calorie-restricted vs. control
monkeys and were significantly higher than in obese or obese diabetic
monkeys (62). Biopsies of the vastus lateralis muscle were taken both
before and after insulin administration. Muscle samples were rapidly
frozen, lyophilized, and stored in liquid nitrogen.

Preparation of total cellular extracts. One-hundred milligrams of
muscle tissue were ground in a liquid nitrogen-cooled mortar and
pestle with mammalian lysis buffer. The slurry was then homogenized
on ice with a Tissuemiser at 30,000 rpm followed by centrifugation at
14,000 rpm for 10 min, and the supernatant was retained. Protein
concentration was quantified using a bichoninic acid standard
curve on a DU 640 spectrophotometer.

Western blotting. The protein lysates were boiled for 10 min, and
25 μg of protein per lane dissolved in Laemmli buffer were resolved
by SDS-polyacrylamide gel electrophoresis (12% Tris/HCl gel).
MagicMark and Kaleidoscope standards were used to identify the
molecular weight. The protein was then electrotransferred to PVDF
membranes using standard Western blotting technique (100 V for
1–1.5 h for SREBP-1, ERK1/2, and α-actin, and 100 V for 4 h for
FAS). Membranes were prehybridized with nonfat powdered milk and
hybridized with mouse monoclonal anti-SREBP-1 antibody in a 1:500
dilution, anti-FAS antibody in a 1:250 dilution, anti-phospho-p44/42
MAP kinase antibody in a 1:1,000 dilution, or anti-total-p44/42 MAP
kinase antibody in a 1:1,000 dilution. The membranes were then
incubated with anti-mouse or anti-rabbit antibody conjugated with
horseradish peroxidase in a 1:1,000 dilution, and protein was detected
by chemiluminescence with the ECL Western Blotting Analysis
kit, following the manufacturer’s recommendations. Briefly, the reac-
tion of 240 nM total primers. Each qrt-RT-PCR reaction for
SREBP-1c was carried out in a 20-μl volume in 1× SYBR Green
supermix PCR reagents, 50 ng of reverse-transcribed total RNA, 120
nM of the forward rat SREBP-1c primer and 120 nM of rat SREBP-1c
reverse primers (240 nM total primers), and 10 μl of 2× SYBR Green
PCR Master Mix. Each qrt-RT-PCR reaction for the housekeeping
gene was carried out in a separate 20-μl volume in 1× SYBR
supermix PCR reagents, 50 ng of reverse-transcribed total RNA, 120
nM of the forward rat 36β4 primer and 120 nM of rat 36β4 reverse
primers (240 nM total primers), and 10 μl of 2× SYBR Green PCR
supermix.

qrt-RT-PCR was performed on a Bio-Rad iCycler iQ Real-Time
PCR detection system in 96-well plates. Triplicate amplifications
were performed for each control and sample. All 96-well PCR plates
were sealed with optical tape to prevent evaporation. The iCycler
protocol was as follows: 1 cycle of 95°C for 90 s, 40 cycles of 95°C for 30
s followed by 60°C for 60 s, and 1 cycle of 95°C for 60 s. A melt curve
of 80 cycles, starting at 55°C and increasing by 0.5°C every 10 s, was
done to determine primer specificity. Expression levels were calcu-
lated by the relative method by comparing the target amount of rat
36β4 mRNA, according to the manufacturer’s recommendations.

Muscle triglyceride analysis. Fifty milligrams of tissue per animal
were weighed and homogenized in a hand-held tissue homogenizer,
using a volume of distilled water equal to eight times the tissue weight
in milligrams. The protocol for triglyceride extraction was as follows:
5 M NaCl, methanol, and chloroform were added to the homogenate
and mixed, and the ternary phase was broken with water and chloro-
form after 5 min of incubation. The three-phase system was then
separated by centrifugation at top speed. The aqueous and protein
phases were reextracted with 9:1 chloroform-methanol wash solvent
and separated by centrifugation. The organic solvent was dried using
a stream of nitrogen gas with an N-Evap, and the triglyceride was
resuspended in isopropanol containing 2% Triton X-100.

Glycerol concentration was measured relative to a standard curve
made with glycerol and SDS. Standards and samples were loaded on
a 96-well plate, and Free Glycerol Reagent was added to each well.
The plate was incubated at 37°C and read on a Kinetic Microplate
Reader at 540 nm. Triglyceride Reagent was added to each well
and incubated at 37°C. The plate was read again on the Microplate
Reader at 540 nm, and the background glycerol was subtracted.

Data analysis. The results are expressed as means ± SE. An
unpaired *t*-test with a *P* value of <0.05 was used to determine
statistical significance.

RESULTS

Exercise Training

Exercise training increases skeletal muscle triglyceride content.
Initially, we compared the triglyceride content of control
(sedentary) and exercise-trained rats. Triglyceride content was sig-
ificantly higher in the gastrocnemius muscle of trained rats
than in sedentary controls (261 ± 3 vs. 185 ± 8 nmol/mg, 
P < 0.0001; Fig. 1). Similarly, there was a significant increase in
triglyceride in the soleus muscle of trained rats vs. sedentary
controls (372 ± 74 vs. 216 ± 37 nmol/mg, 
P < 0.001; Fig. 1).

Exercise training increases SREBP-1c mRNA. We then
examined muscle SREBP-1 expression in exercise-trained and
sedentary rats. As SREBP-1c is the isoform most related to
triglyceride production in vivo, we assessed the effect of exercise
on SREBP-1c mRNA levels using qrt-RT-PCR.
SREBP-1c mRNA was significantly higher in gastrocnemius
tissue from trained rats than sedentary controls (8.92 ± 1.64
vs. 3.69 ± 0.56, 
P < 0.002; Fig. 2). Similarly, soleus tissue
from trained rats expressed significantly more SREBP-1c than
sedentary controls (1.63 ± 0.23 vs. 1.04 ± 0.04, 
P < 0.03; Fig.
2). There were no significant differences in 36β4 mRNA from gastrocnemius of soleus tissue of trained or control rats.

**Exercise training increases SREBP-1 precursor and mature protein.** To determine whether the increased SREBP-1c mRNA expression translated to increased SREBP-1 protein, we used immunoblotting. Western blotting studies from gastrocnemius of trained rats revealed significantly higher SREBP-1 precursor protein levels than sedentary controls (0.38 ± 0.02 vs. 0.26 ± 0.02, P = 0.001; Fig. 3). Similarly, SREBP-1c precursor expression was significantly higher in soleus muscle of trained rats than in sedentary controls (0.39 ± 0.03 vs. 0.29 ± 0.02, P = 0.03; Fig. 3).

Activation of SREBP-1 requires its cleavage and the entry of the mature protein into the nucleus. Therefore, we also assessed the amounts of mature (or active) SREBP-1. Expression of the mature SREBP-1 protein was significantly higher in the gastrocnemius and soleus muscles of trained rats than in sedentary controls (0.56 ± 0.07 vs. 0.37 ± 0.07, P = 0.03 and 0.39 ± 0.03 vs. 0.29 ± 0.02, P = 0.01, respectively; Fig. 4).

**Exercise training increases FAS protein.** To evaluate the physiological relevance of increased SREBP-1, we assessed expression of FAS protein, an enzyme stimulated by SREBP-1 (24). Expression of FAS protein was significantly higher in the gastrocnemius muscle of trained rats than in sedentary controls (0.32 ± 0.03 vs. 0.56, P = 0.001; Fig. 5). Similarly, there was a trend toward an increase in FAS in the soleus muscle of exercise-trained rats compared with sedentary controls [0.45 ± 0.07 vs. 0.36 ± 0.07, P = not significant (NS); Fig. 5].
Calorie Restriction

Chronic calorie restriction increases skeletal muscle triglyceride content. To assess the effect of calorie restriction on muscle triglyceride, vastus lateralis muscle biopsies were obtained from four calorie-restricted yet normally insulin-sensitive male rhesus monkeys (10 ± 0.1 kg) and three normal control monkeys (8 ± 0.8 kg) (47). Skeletal muscle triglyceride was significantly lower in the control (4 ± 2 nmol/mg dry wt) than in the calorie-restricted (43 ± 11 nmol/mg dry wt) monkeys (P < 0.05).

Chronic calorie restriction increases SREBP-1 precursor and mature protein. To determine whether SREBP-1 is increased in calorie-restricted monkeys with high muscle triglyceride, we examined muscle SREBP-1 expression in both groups. Western blotting studies revealed that SREBP-1 precursor protein was significantly higher in vastus lateralis muscle of calorie-restricted monkeys than controls (0.66 ± 0.10 vs. 0.34 ± 0.07, P = 0.019; Fig. 6). Similarly, vastus lateralis SREBP-1 mature protein was significantly higher in calorie-restricted than in control monkeys (0.70 ± 0.09 vs. 0.43 ± 0.08, P = 0.038; Fig. 7).

Insulin infusion increases SREBP-1 protein in controls, but not with calorie restriction. To determine the effect of insulin administration on the skeletal muscle SREBP-1 expression, SREBP-1 protein was examined in a subset of both groups before and after a hyperinsulinemic euglycemic clamp (46). Interestingly, although insulin infusion significantly increased SREBP-1 protein in control monkeys (0.17 ± 0.02 vs. 0.26 ± 0.04, P = 0.04; Fig. 8), calorie-restricted monkeys showed no increase in SREBP-1 after exposure to insulin (0.54 ± 0.06 vs. 0.49 ± 0.05, P = NS; Fig. 8). Of note, this lack of SREBP-1 response to insulin does not indicate generalized insulin resistance, because the calorie-restricted animals were otherwise very sensitive to insulin (62). Instead, it likely indicates that maximal SREBP-1 expression has already occurred at baseline.

Chronic calorie restriction increases FAS protein. To assess signaling downstream of SREBP-1, expression of FAS protein was measured by Western blotting. FAS protein levels were significantly higher in vastus lateralis muscle of calorie-restricted monkeys than controls (2.83 ± 0.39 vs. 1.23 ± 0.33, P = 0.026; Fig. 9).

Chronic calorie restriction activates the ERK1/ERK2 pathway. Finally, we looked for a mechanism to increase SREBP-1 in calorie restriction. We have previously shown that SREBP-1 is increased via ERK1/ERK2 in hyperinsulinemia (45). On the basis of these data, we examined the activity of the ERK1/ERK2 pathway in calorie restriction. Despite equal amounts of total ERK1/ERK2 (Fig. 10A), ERK1 phosphorylation increases with calorie restriction compared with controls (0.24 ± 0.05 vs. 0.08 ± 0.03, P = 0.02; Fig. 10B), as does ERK2 phosphorylation (0.65 ± 0.11 vs. 0.15 ± 0.05, P = 0.003; Fig. 10C).
DISCUSSION

Even though increased levels of IMTG are commonly found in obesity and T2DM, which are two well-defined insulin-resistant states (1, 2, 5–9, 11), unexpectedly, increased IMTG is also observed in two models of enhanced insulin sensitivity: calorie restriction and exercise. Both the physiological significance and the biochemical mechanism underlying these paradoxical observations remain largely enigmatic.

Increased IMTG with exercise has been reported previously by several groups (21, 27, 32, 43, 65). Exercise training increases both intramuscular lipid and oxidative capacity of vastus lateralis muscle (53). Increased IMTG with calorie restriction, however, was only recently reported (47). The present study is the first to begin to explain the recent observation that increased IMTG is associated with long-term calorie restriction and to suggest a mechanism that might explain the increased IMTG in exercise training as well. We found that, as is the case with T2DM and the metabolic syndrome (46), this increased IMTG in skeletal muscle of trained and calorie-restricted monkeys is associated with increased expression of SREBP-1c and FAS. The two yet-unanswered questions are whether the mechanism explaining increased IMTG in the setting of exercise or calorie restriction is different from the cause of increased IMTG seen with insulin resistance and whether the consequences of increased IMTG in these settings are the same.

It is not yet clear what is responsible for regulating SREBP-1. Because the compensatory hyperinsulinemia of insulin resistance increases SREBP-1 in multiple tissues (20, 24, 34, 45, 59), insulin is a likely culprit. In contrast, as the present study demonstrates, insulin levels are not elevated with calorie restriction or exercise, and yet the levels of skeletal muscle SREBP-1 are also increased. One potential mechanism is that exercise and chronic calorie restriction increase SREBP-1 expression in skeletal muscle through the MAP kinase signaling pathway. Our previous observations and the results from the literature indicate that in skeletal muscle, insulin increases SREBP-1 via the MAP kinase pathway (24, 45). Therefore, it is conceivable that MAP kinase activation also increases SREBP-1 in exercise and calorie restriction.

MAP kinase activation is a feasible mechanism to link both exercise and calorie restriction with SREBP-1 expression.
skeletal muscle, as the MAP kinase pathway has been shown to be activated in both settings. For example, stimulation of the ERK1/2 kinase pathway is increased in human skeletal muscle after acute cycling exercise (68, 74) and marathon running (73). This appears to be a local, not systemic, effect of muscle contraction, because it occurs only in the exercising limb (68). This observation has also been confirmed in vitro with isolated contracting rat skeletal muscle (56, 69, 70). Thus skeletal muscle contraction might increase SREBP-1 directly via the MAP kinase pathway. Similarly, calorie restriction can activate the MAP kinase pathway. Mice subjected to a 30% reduction in calorie intake compared with control littersmates showed increased MAP kinase pathway activation (1). Our data confirm that MAP kinase activation may be the trigger increasing SREBP-1 in calorie restriction.

Another potential mechanism controlling SREBP-1 expression is dietary cholesterol intake. The Schoenheimer effect describes the process whereby a low dietary cholesterol intake increases cholesterol synthesis and high dietary cholesterol intake decreases cholesterol synthesis (58). Recent evidence shows that SREBP-1 regulation also may control this phenomenon. Insign proteins are required for the sterol-mediated processing of SREBP to its nuclear form (11). Cholesterol feeding reduces nuclear SREBP-1 and downstream lipogenic enzymes; this effect is blocked in Insig 1 and Insig 2 double knockouts (17). Similarly, lower cholesterol intake leads to increased nuclear SREBP-1. Therefore, a calorie-restricted diet, leading to absolute decreases in cholesterol intake, could lead to increased nuclear SREBP-1 expression.

If the pathophysiological consequences of increased skeletal muscle IMTG in insulin-resistant states include the pathogenesis of insulin resistance itself, their role in exercise and chronic caloric restriction is much less apparent. Although this issue is beyond the scope of the present investigation, it is likely that increased IMTG in exercised muscle may represent an important fuel depot that is ready for mitochondrial oxidation.

Exercise training increases the oxidative capacity of skeletal muscle in rats (3, 26, 42), guinea pigs (4), and humans (46, 53). Specifically, in rats undergoing treadmill running, the ability to oxidize fat and carbohydrate is doubled (3, 26, 42). When a muscle cell is geared for oxidation, as with exercise (21), the fat will be oxidized by mitochondria to supply the energy needs of exercise. Increasing fatty acid oxidation in L6 muscle cells can also increase their insulin sensitivity without decreasing their lipid content (49). In contrast, cells in subjects with T2DM are not poised to use lipids as fuel, and turnover of lipids is slow because their mitochondria have defects limiting oxidation (41, 55). Similarly, offspring of patients with T2DM who do not yet have diabetes also have impaired mitochondrial activity, implying that the mitochondrial defect occurs early on rather than as a consequence of the diabetes (51).

Increased IMTG may also be an important defense mechanism in the face of limited glycogen stores during chronic caloric restriction. Thus the metabolic milieu may be quite different in cells from insulin-sensitive vs. insulin-resistant subjects once fat is deposited. Because our study was not designed to examine lipid oxidation in exercise or caloric restriction, future studies are necessary to address this issue.

Even though our study is the first to demonstrate that caloric restriction increases skeletal muscle SREBP-1, increased precursor and mature SREBP-1 proteins have been found in adipose tissue from male Wistar rats upon refeeding after both short- and long-term calorie restriction (64). These investigators concluded that calorie restriction increases the capacity for fatty acid biosynthesis. Unfortunately, neither muscle triglyceride, SREBP-1, nor FAS were assessed in the aforementioned study. However, taken together with our present observation, these results support the notion that chronic calorie restriction increases SREBP-1, which could lead to increased IMTG deposition.

In summary, we found increased expression of SREBP-1 mRNA and protein, FAS protein, and increased IMTG in skeletal muscle of exercise-trained rats and chronically calorie-deprived monkeys. With better understanding of the mechanism leading to increased IMTG, the next logical step would be to evaluate why and how accumulation of IMTG in obesity and T2DM is associated with insulin resistance, whereas in exercise and chronic caloric restriction it is associated with normal insulin sensitivity.

ACKNOWLEDGMENTS

We express gratitude to Dr. Marc Goalstone for his expert advice and Dr. Victor Sorribas for providing primers for qRT-PCR.

GRANTS

This work was supported in part by the Veterans Affairs Research Service (B. Draizin), National Institutes of Health/Division of Research Resources Grant no. 1K23 RR-020038-01 and National Institutes of Health/National Institute of Diabetes and Digestive and Kidney Diseases Grant no. 5P30-DK-48520-10 (J. Nadeau), National Institutes of Health Grants R01-DIC-06741 and P01-56481 (J. E. B. Reusch), and National Heart, Lung, and Blood Institute Grant no. PHS-ROI-HL-72790 (R. L. Moore).

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