Daily exercise increases hepatic fatty acid oxidation and prevents steatosis in Otsuka Long-Evans Tokushima Fatty rats

R. Scott Rector,1# John P. Thyfault,1,2,3# R. Tyler Morris,4 Matthew J. Laye,4 Sarah J. Borengasser,3 Frank W. Booth,4,5,6 and Jamal A. Ibdah1,2,4

1Division of Gastroenterology and Hepatology, 2Harry S. Truman Memorial Veterans Medical Center, Departments of 3Nutritional Sciences, 4Medical Pharmacology and Physiology, 5Biomedical Sciences, and 6Dalton Cardiovascular Research Center, University of Missouri; Columbia, Missouri

Submitted 21 September 2007; accepted in final form 2 January 2008

Daily exercise increases hepatic fatty acid oxidation and prevents steatosis in Otsuka Long-Evans Tokushima Fatty rats. Am J Physiol Gastrointest Liver Physiol 294: G619–G626, 2008. First published January 3, 2008; doi:10.1152/ajpgi.00428.2007.—Exercise training is commonly prescribed for treatment of nonalcoholic fatty liver disease (NAFLD). We sought to determine whether exercise training prevents the development of NAFLD in Otsuka Long-Evans Tokushima Fatty (OLETF) rats and to elucidate the molecular mechanisms underlying the effects of exercise on hepatic steatosis.

Four-week-old OLETF rats were randomly assigned to either a sedentary control group (Sed) or a group given access to voluntary running wheels for 16 wk (Ex). No. rats were caged 2 days before euthanasia in the Ex animals, and both groups were euthanized at 20 wk old. Voluntary wheel running attenuated weight gain and reduced serum glucose, insulin, free fatty acids, and triglycerides in Ex animals compared with Sed (P < 0.001). Ex animals exhibited significantly reduced hepatic triglyceride levels and displayed fewer lipid droplets (Oil Red O staining) and reduced lipid droplet size compared with Sed. Wheel running increased % of palmitate oxidized completely to CO2 in the Ex animals but did not alter AMP-activated protein kinase-α (AMPKα) or AMPK phosphorylation status. However, fatty acid synthase and acetyl-coenzyme A carboxylase (ACC) content were significantly reduced (35 and 70%, respectively), and ACC phosphorylation and cytochrome c content were significantly elevated (~35 and ~30%, respectively) in the Ex animals. These results unequivocally demonstrate that daily physical activity attenuates hepatic steatosis and NAFLD in an obese rodent model and suggest that this effect is likely mediated, in part, through enhancement of hepatic fatty acid oxidation and reductions in key protein intermediates of fatty acid synthesis.

wheel running; nonalcoholic fatty liver disease; metabolic syndrome; physical activity; palmitate oxidation

WESTERNIZED SOCIETIES ARE EXPERIENCING A WEIGHT GAIN EPIDEMIC, AND RECENT EPIDEMIOLOGICAL STUDIES SUGGEST AN INCREASED RISK OF CORONARY HEART DISEASE, TYPE 2 DIABETES, AND NONALCOHOLIC FATTY LIVER DISEASE (NAFLD) IN OVERWEIGHT AND OBESE INDIVIDUALS. NAFLD REPRESENTS A MAJOR CAUSE OF CHRONIC LIVER DISEASE (8), AND IT IS ESTIMATED THAT UP TO 34% OF THE GENERAL ADULT POPULATION IN THE US HAS EXCESSIVE FAT ACCUMULATION IN LIVER (8), REACHING LEVELS AS HIGH AS 75–100% IN OBESE AND MORBIDLY OBESE INDIVIDUALS (6). NAFLD IS STRONGLY LINKED TO INSULIN RESISTANCE AND TYPE 2 DIABETES AND IS CONSIDERED THE HEPATIC REPRESENTATION OF THE METABOLIC SYNDROME (13, 34). IN ADDITION, CROSS-SECTIONAL STUDIES ESTIMATE THAT ~90% OF PATIENTS WITH NAFLD HAVE AT LEAST ONE CHARACTERISTIC OF METABOLIC SYNDROME AND ~33% HAVE THE COMPLETE DIAGNOSIS (23).

Lifestyle modifications targeted at increasing physical activity and reducing energy intake are recommended by health care providers for optimal health and are the most common prescribed therapy for individuals diagnosed with NAFLD (13). Recent cross-sectional studies in humans show that increased habitual physical activity (31) and cardiorespiratory fitness (10) are inversely associated with NAFLD. In addition, exercise training prevents the development of hepatic steatosis in rats fed a high-fat diet (18) and significantly reduces hepatic steatosis in obese patients with NAFLD (42). However, these prospective studies are limited and the underlying mechanisms of aerobic exercise training on the development and prevention of hepatic steatosis in humans or animal models remain largely unexamined.

Otsuka Long-Evans Tokushima Fatty (OLETF) rats are a commonly studied model of obesity and Type 2 diabetes (20). This strain of rat is selectively bred for null expression of the cholecystokinin-1 receptor and, thus, exhibits a within-meal feedback defect for satiety, resulting in hyperphagia and obesity (26). OLETF rats spontaneously develop insulin resistance (26), Type 2 diabetes (30, 26), and multiple components of the metabolic syndrome (25). In addition, liver masses are elevated in OLETF rats compared with control animals (25), and OLETF rats develop hepatic steatosis and mild inflammation to levels comparable with NAFLD (44). These findings are limited and need to be further examined but suggest the applicability of the model for the study of NAFLD.

Importantly, OLETF rats exhibit an inherent ability to maintain daily physical activity levels using voluntary running wheels, a quality that is absent in most obese animal models (7, 38). Voluntary wheel exercise suppresses increases in body weight in OLETF rats (7, 25), enhances whole body insulin sensitivity (35), and prevents the development of Type 2 diabetes (35). Although it is well known that fatty acid accumulation in hepatocytes is related to dysregulation of both de novo lipogenesis and β-oxidation, the effects of exercise training on these pathways have not been thoroughly examined. We hypothesized that enhanced physical activity by OLETF rats would suppress liver fat accumulation by increasing fatty acid oxidation and reducing triglyceride (TG) formation. Therefore,

* R. S. Rector and J. P. Thyfault are co-first authors and contributed equally to this work.

Address for reprint requests and other correspondence: J. A. Ibdah, Division of Gastroenterology and Hepatology, M580 Health Science Center, Univ. of Missouri-Columbia, Columbia, MO 65212 (e-mail: ibdah@health.missouri.edu).

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.
the purpose of the present investigation was to test our hypothesis that exercise training would attenuate the development of NAFLD in OLETF rats through enhanced hepatic fatty acid oxidation and reductions in key protein intermediates of fatty acid synthesis.

METHODS

Animal protocol. The animal protocol was approved by the Institutional Animal Care and Use Committee at the University of Missouri-Columbia. OLETF male rats at 4 wk of age were kindly supplied by the Tokushima Research Institute, Otsuka Pharmaceutical (Tokushima, Japan). Animals were randomly separated into those with (Ex) or without (Sed) access to running wheels. The Ex group was immediately housed (at the age of 28 days) in cages equipped with voluntary running wheels outfitted with a Sigma Sport BC 800 bicycle computer (Cherry Creek Cyclery, Foster Falls, VA) for measuring daily running activity. Voluntary running was selected to approximate the more natural activity state of the animal. Cages were in temperature-controlled animal quarters (21°C) with a 0600–1800 light:1800–0600 dark cycle that was maintained throughout the experimental period. All animals were provided standard rodent chow (Formulab 5008, Purina Mills, St. Louis, MO) in new cages at the beginning of each week when cages were changed and body weights obtained between 0800 and 1000. Body mass and food intake were measured weekly throughout the investigation. Perhaps the best way to assess energy expenditure without direct or indirect calorimetry is by looking at food efficiency. Food efficiency was calculated by examining the amount of body weight gain divided by the amount of food intake (g gain/g intake). Running activity was observed every day of running between 0800 and 1000, and rats in the running group had access to wheels and food and water ad libitum until 20 wk of age, at which time (0600) wheels were locked. Rats were anesthetized [ketamine (80 mg/kg), xylazine (10 mg/kg), and acepromazine (4 mg/kg)] and killed by exsanguination by removal of the heart 2 days after locking of wheels; the sedentary rats (Sed) were euthanized at the same time. All animals had access to food until the day of euthanasia, when food was removed at 0600, resulting in a 5-h fast for each group of animals.

Tissue homogenization procedure. Livers were quickly excised from anesthetized rats and either flash frozen in liquid nitrogen, placed in 10% formalin, or placed in ice-cold isolation buffer (100 mM KCl, 10 mM Tris-base, 5 mM MgCl2·6H2O, 1 mM EDTA, and 1 mM ATP; pH 7.4). For fresh tissue hepatic fatty acid oxidation assays, ~50–100 mg of liver was thoroughly minced with scissors in 200 μl SET buffer (250 mM sucrose, 1 mM EDTA, 10 mM Tris·HCl, and 2 mM ATP; pH 7.4) and then the buffer volume was brought up to yield a 20-fold (wt/vol) diluted sample. This was transferred to a 3-ml Potter-Elvehjem glass homogenization vessel. Liver suspensions were homogenized on ice with a Teflon pestle at 10 passes over the course of 30 s at 1,200 rpm. Homogenates were kept on ice until oxidation experiments were performed.

Fatty acid oxidation. Fatty acid oxidation was measured with radiolabeled [1-14C]palmitate (American Radiochemicals) in fresh liver homogenate preparation using the methods from Noland et al. (29) and as modified by Dohm et al. (11). The oxidation rate of 14C]palmitate was measured by collecting and counting the 14CO2 and acid-soluble metabolites produced during incubation in a sealed trapping device. Palmitate (200 μM) and [1-14C]palmitate were bound to 0.5% BSA (final concentration) at 37°C and brought up in reaction buffer to yield the following final concentrations: 100 mM sucrose, 10 mM Tris·HCl, 10 mM KPO4, 100 mM KCl, 1 mM MgCl2·6H2O, 1 mM L-carnitine, 0.1 mM malate, 2 mM ATP, 0.05 mM CoA, and 1 mM DTT (pH 7.4). Reaction buffer (320 μl) was added to 80 μl of liver homogenate prepared in a sucrose-EDTA buffer in the well of the trapping device. A 0.65-ml centrifuge tube containing 400 μl of NaOH also was inserted in the well followed by sealing the well with a rubber gasket. After 60 min of incubation at 37°C on an orbital shaker, 200 μl of 70% perchloric acid was injected through the rubber gasket into the bottom of the well. 14CO2 driven out of the reaction by the perchloric acid during another 60-min incubation was trapped in the NaOH and then counted on a scintillation counter. The remaining homogenate was centrifuged at 18,000 rpm for 20 min and a sample of the resulting supernatant (acid soluble metabolites) also was counted on a scintillation counter. Specific activity was taken by counting a portion of the reaction buffer.

Fat pad collection and serum assays. Retroperitoneal and omental adipose tissue fat pads were removed from exsanguinated animals and weighed. Plasma glucose (Sigma), TG (Sigma), free fatty acids (FFA; Wako Chemicals, Richmond, VA), and insulin (Linco Research, St. Charles, MO) were measured with commercially available kits according to the manufacturer’s instructions. Serum alanine aminotransferase (ALT) concentrations were determined by the Clinical Pathology Laboratory in the College of Veterinary Medicine at the University of Missouri-Columbia utilizing an Olympus AU400e Chemistry Immuno Analyzer (Olympus America, Center Valley, PA).

Citrate synthase activity. Citrate synthase activity was determined by the methods of Sreer et al. (37). Briefly, liver homogenates were incubated in the presence of oxaloacetate, acetyl-CoA, and DTNB. Spectrophotometric detection of reduced DTNB at a wavelength of 412 nm served as an index of enzyme activity.

β-HAD activity. β-Hydroxyacyl-CoA dehydrogenase (β-HAD) activity was measured at 37°C in assay buffer containing 0.1 M triethanolamine-HCl, 5 mM EDTA, and 0.45 mM NADH (pH 7.0) (5). After an initial 2-min absorbance reading at 340 nm, the reaction was initiated by adding 0.1 mM acetoacetyl-CoA, and the rates of disappearance of NADH and the appearance of NAD were measured by change in absorbance every 10 s for 5 min. Enzyme activity was expressed as nanomoles per gram protein per minute.

Glycogen content. Approximately 15 mg of powdered liver was added to 0.5 ml of 1 N HCl secured in 2.0-ml screw-top tubes and then hydrolyzed for 2 h at 100°C (1). The tubes were cooled on ice and 1.5 ml of 0.67 N NaOH was added. Glycogen content was assessed in the samples by use of a glucose reagent kit (Thermo Electron, Louisiville, CO) and expressed as milligrams per gram wet weight.

Intrahepatic lipid content. Powdered liver (~30 mg) was added to 1 ml of lipid extraction solution composed of 1:2 vol/vol methanol-chloroform homogenized for 30 s and exposed to gentle agitation overnight at 4°C. One milliliter of 4 mM MgCl2 was added, vortexed, and centrifuged for 1 h at 1,000 g at 4°C. The organic phase was removed, evaporated, and reconstituted in butanol-Triton X-114 mix (3:2 vol/vol) and vortexed. Lipid content was measured from a commercially available kit (Sigma, F6428), and TG concentration was expressed as nanomoles per gram wet weight.

Oil Red O staining and liver histology. Frozen liver was sectioned serially at 5-μm thickness with a cryostat, placed on slides, and dried for 15 min at 37°C. Sections then were fixed for 10 min in neutral buffered 10% formalin. To detect neutral lipid accumulation, sections were stained with Oil Red O for 10 min, counterstained with hematoxylin for 2 min, and coverslipped with a water-based mounting medium. The area of positive staining for Oil Red O was calculated as a percentage of total section area, and an average lipid droplet size was calculated by utilizing ImagePro Plus (Media Cybernetics) from five to seven views per animal. To examine liver morphology, formalin-fixed paraffin embedded sections of liver were stained with hematoxylin and eosin (H&E).

Western blotting. AMP-activated protein kinase-α (AMPKα), AMPKβ Thr172 phosphorylation-specific, cytochrome c, acetyl-coenzyme A carboxylase (ACC), ACC Ser79 phosphorylation-specific, and fatty acid synthase (FAS) polyclonal antibodies were from Cell Signaling (Beverly, MA). Content of phospho-proteins (using phospho-specific antibodies) was calculated from the density of the band (total) by using the appropriate antibody.
Liver samples were homogenized using lysis buffer. Protein (20–40 μg) was loaded in SDS-PAGE gel and probed with primary antibodies. After washing, the membrane was incubated with horse-radish peroxidase-conjugated secondary antibodies. Protein bands were quantified via a laser densitometer (Molecular Dynamics). To control for equal protein loading and transfer, the membranes were then stained with 1% amido-black (Sigma). The total protein staining for each lane was quantified by laser densitometry and these values were used to correct for any differences in protein loading or transfer of all band densities.

**Statistics.** Each outcome measure was examined in six to eight animals. For each outcome measure, an independent-samples t-test was performed (SPSS/15.0, SPSS, Chicago, IL). Pearson correlations were used to examine associations between changes in physical characteristics and measures of lipid metabolism in both the Sed and Ex animals. Since the Sed animals were not allowed access to running wheels, animals were grouped into either no-exercise or exercise groups (dummy coded as 0 and 1) to assess the relative contribution of daily physical activity on the measured parameters. Values are reported as means ± SE, and a P value less than 0.05 denotes a statistically significant difference.

**RESULTS**

**Animal characteristics.** Sixteen weeks of daily activity suppressed (P < 0.001) weight gain in the Ex animals to ~70% of Sed controls (Table 1), a finding consistent with previous exercise studies using this animal model (7, 25). Body weights did not differ between groups at baseline, and weight gain during the 16-wk intervention in the Sed and the Ex animals ranged from 493.5 to 626.3 g and 286.9 to 412.9 g, respectively. Body weight was likely reduced by both increased daily energy expenditure due to voluntary wheel running (6.9 ± 0.3 km/day, range 5.9–9.3 km/day) in addition to decreases (~6%) in absolute food intake compared with the Sed animals (Table 1). Absolute food consumption ranged from 201.2 to 252.3 g/wk and 186.5 to 218.5 g/wk in the Sed and Ex animals, respectively. However, food consumption relative to body weight was significantly higher (P < 0.001) in Ex animals compared with Sed (0.46 ± 0.06 vs. 0.35 ± 0.05 g per wk/g body wt, respectively). Food efficiency also was significantly greater (P < 0.001) in the Sed compared with Ex animals (15.7 ± 0.2 vs. 11.1 ± 0.3%, respectively). These findings suggest that exercise was the primary contributor to reduced body weight.

The Ex group also had significantly reduced concentrations of serum glucose, insulin, TG, and FFA, whereas serum ALT levels did not differ between groups (Table 1). Fat pad masses (omental + retroperitoneal) were significantly reduced in Ex compared with Sed animals (Table 1). In addition, exercise significantly reduced insulin resistance as assessed by homeostasis model assessment (HOMA) (24) (data not shown). Higher heart weight to body weight ratios, another marker of training, were found in Ex animals compared with Sed (3.17 ± 0.22 vs. 2.65 ± 0.05 mg/g, P < 0.01). Liver glycogen content also was significantly higher in the Ex animals compared with the Sed (44.2 ± 3.9 vs. 32.8 ± 3.3 mg/g wet wt, respectively, P < 0.05).

**Liver morphology and Oil Red O staining.** Representative images of randomly selected sections of the liver stained for H&E in Sed and Ex are shown in Fig. 1 (A and B, respectively) at a ×40 magnification. There were notable histological changes in the Sed animals compared with Ex animals, including elevated numbers of vacuoles in the Sed images, most likely indicating lipid moieties. In addition, Ex animals had significantly reduced percent positive staining for neutral lipid and reduced lipid droplet size as indicated by Oil Red O images compared with Sed animals (Fig. 1, C and D). Percent positive staining (8.3 ± 0.9 vs. 0.3 ± 0.3%) and lipid droplet size (49.4 ± 3.3 vs. 19.7 ± 1.9 pixels) also were significantly different between Sed and Ex, respectively. Reduction in hepatic steatosis was further supported in the Ex animals by significant attenuation in hepatic TG accumulation compared with Sed (Fig. 1E).

**Fatty acid oxidation.** Measures of hepatic fatty acid oxidative capacity are shown in Fig. 2. Voluntary wheel running resulted in an approximately threefold increase in the percent of palmitate oxidized completely to CO2 in the Ex animals (Fig. 2A). However, total [incomplete (label incorporation into chain-shortened acid soluble metabolites, which provide an index of incomplete β-oxidation) + complete to CO2] palmitate oxidation was not significantly different between Sed and Ex groups (data not shown). Hepatic citrate synthase (49.5 ± 2.6 vs. 50.5 ± 0.7 nmol·min⁻¹·g⁻¹) and β-HAD activities (94.5 ± 5.2 vs. 93.8 ± 7.8 nmol·min⁻¹·g⁻¹) did not differ between Sed and Ex animals, respectively; however, liver cytochrome c protein content was significantly increased by ~30% in the Ex animals (Fig. 2B).

**AMPK, ACC, and FAS.** Voluntary wheel running did not alter AMPKα total protein or AMPKα Thr172 phosphorylation (representative blot shown in Fig. 3D). However, exercise training significantly reduced ACC total content by ~35% (P = 0.005) and increased ACC Ser79 phosphorylation by ~35% (P = 0.038) (Fig. 3, A and B). In addition, the Ex group exhibited a ~70% reduction in FAS protein concentration compared with Sed animals (Fig. 3C).

**Correlations.** We report that food efficiency (amount of weight gain per amount of food intake, an indirect assessment of energy expenditure) was positively associated with liver TG accumulation, fat pad mass, serum TG, and serum FFA concentrations and was negatively associated with total weight gain (Table 2). In addition, weight gain was positively correlated with liver TG accumulation, fat pad mass, food intake, and serum TG concentrations. Furthermore, neither circulating TGs nor FFAs were associated with hepatic TG content; however, serum FFAs were negatively associated with the complete oxidation of palmitate in liver (Table 2).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Sed</th>
<th>Ex</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>631.7±19.2</td>
<td>449.7±17.3*</td>
</tr>
<tr>
<td>Food consumption, g/wk</td>
<td>223.2±7.1</td>
<td>208.8±5.3*</td>
</tr>
<tr>
<td>Fat pad mass, g</td>
<td>30.2±1.9</td>
<td>8.3±0.7*</td>
</tr>
<tr>
<td>Serum glucose, mg/dl</td>
<td>71.3±25.7</td>
<td>58.4±21.8*</td>
</tr>
<tr>
<td>Serum insulin, mg/ml</td>
<td>1.98±0.71</td>
<td>0.48±0.04*</td>
</tr>
<tr>
<td>Serum TG, mg/dl</td>
<td>235.5±49.0</td>
<td>61.0±4.4*</td>
</tr>
<tr>
<td>Serum FFA, μM</td>
<td>199.5±17.2</td>
<td>147.9±16.8*</td>
</tr>
<tr>
<td>Serum ALT, U/l</td>
<td>64.4±8.0</td>
<td>51.4±2.7</td>
</tr>
</tbody>
</table>

Values are means ± SE (n = 6–8 rats). Sed, sedentary control group; Ex, voluntary wheel running group; TG, triglycerides; FFA, free fatty acids; ALT, alanine aminotransferase. *Significantly different than Sed (P < 0.01). Fat pad mass was the combination of omental and retroperitoneal fat pads.
Since the Sed animals were not allowed access to running wheels, animals were grouped into either no-exercise or exercise groups (dummy coded as 0 and 1) to assess the relative contribution of daily physical activity on the measured parameters. Daily physical activity was negatively associated with reduced liver TG content ($r = -0.567, P < 0.05$), fat pad mass ($r = -0.912, P < 0.001$), weight gain ($r = -0.909, P < 0.001$), serum TGs ($r = -0.676, P < 0.01$), and serum FFAs ($r = -0.499, P < 0.05$) and positively associated with the increased oxidation of palmitate to CO$_2$ in liver ($r = 0.528, P < 0.05$).

Because there was some interanimal variability in daily running distance in the Ex animals (range: 5.9–9.3 km/day), we sought to determine whether this variability was associated with responses in the measured parameters of the Ex group. When examining the Ex animals alone, only fasting insulin levels were negatively associated with higher daily running distance (km/day) ($r = -0.604, P < 0.05$), but not serum FFA, serum TG, liver TG, weight gain, or complete palmitate oxidation in liver (Supplemental Fig. S1). In addition, within the Ex animals, daily running distance was not significantly correlated with daily food intake ($r = 0.037, P = 0.914$). The present design prevents the ability to examine the titration of exercise on these peripheral and hepatic factors.

**DISCUSSION**

Although the present “gold standard” management of NAFLD and nonalcoholic steatohepatitis (NASH) includes increased physical activity (13), surprisingly, little is known about exercise’s impact on factors contributing to hepatic TG accumulation. To our knowledge, we are the first group to examine the effects of exercise training on hepatic fatty acid accumulation utilizing the OLETF rat, a well-characterized model of hyperphagia-induced obesity, insulin resistance, Type 2 diabetes, and the metabolic syndrome. Sedentary OLETF rats appear to develop significant hepatic steatosis by 20 wk of age without inflammation or fibrosis as confirmed by hepatic trichrome staining (data not shown). These findings indicate that OLETF rats are a model for early-stage development of NAFLD. When examined at 40 wk of age, Yeon et al. (44) found significant hepatic steatosis coupled with mild inflammation (NASH) in OLETF rats. Furthermore, it recently has been demonstrated that this model is more susceptible to...
The development of NASH with fibrosis when fed a methionine- and choline-deficient diet (30). This progression pattern is similar to that observed in the human disease, and hence the OLETF rat is a novel model to explore effects of lifestyle modifications on the prevention and progression of NAFLD and gain insights into the mechanisms underlying such effects.

We found that daily voluntary wheel running suppressed weight gain and significantly attenuated the development of hepatic steatosis in 20-wk-old OLETF rats fed a standard low-fat chow diet. In addition, we report for the first time that attenuation in hepatic steatosis in response to exercise training is associated with both increased hepatic fatty acid oxidation and likely reduced fatty acid synthesis, as indicated by significant reductions in key protein intermediates.

Voluntary wheel exercise prevents the development of Type 2 diabetes and obesity and increases glucose infusion rate and insulin sensitivity in OLETF rats (35). In addition, treadmill running (18) and swimming (27) suppress hepatic steatosis in Sprague-Dawley rats. Furthermore, exercise and weight loss in humans significantly reduce hepatic steatosis in obese patients with NAFLD (42) and Type 2 diabetes (40). The present finding of exercise-induced reduction in hepatic steatosis is in agreement with these observations. Furthermore, our study provides mechanistic insights into how exercise impacts hepatic function and hepatic steatosis in this novel animal model.

Some studies have shown that fatty acid oxidation is suppressed in intact hepatocytes (4) and perfused livers (3) from obese rodents whereas others have shown that obesity is associated with increased hepatic fatty acid oxidation in tissue homogenates (17, 33). Unfortunately, the complete degradation of fatty acids to CO2 is not always reported (33). In the present study, we measured both incomplete and complete fatty acid oxidation in liver homogenates of obese animals who underwent daily exercise or were sedentary. To our knowledge, this is the first report to determine whether exercise training could enhance either incomplete or complete hepatic fatty acid oxidation in an obese animal model. We found that the complete oxidation of palmitate to CO2 was increased approximately threefold in the wheel running animals compared with Sed, but there was no difference between Ex and Sed animals in total liver fatty acid oxidation (the combination of CO2 +

Fig. 2. Effects of voluntary running on liver fatty acid oxidation and cytochrome c protein content. Values are means ± SE (n = 6–8). Exercise significantly increased palmitate oxidation (A) and cytochrome c protein concentrations (B) (*P < 0.05).

Fig. 3. Effects of voluntary running on acetyl-coenzyme A carboxylase (ACC) protein content and phosphorylation status and fatty acid synthase (FAS) protein content. Values are means ± SE (n = 6–8). ACC (A), ACC phosphorylation (pACC; B), and FAS protein content (C) were significantly different between groups (*P < 0.05). Representative blots from Ex and Sed animals are shown in D.
acid soluble metabolites produced). Koves et al. (21) have demonstrated that a high-fat-diet-induced elevation in β-oxidation is not matched with enhanced activity of the TCA cycle, leading to the incomplete oxidation of lipids, a possible indicator of metabolic dysregulation. Incomplete fat oxidation leads to an accumulation of acetyl CoA metabolites that can be exported as ketones (unlikely in the fed state) or converted to acetyl-carnitines by the enzyme carnitine acetyltransferase, which can then accumulate in the cell cytosol or leak into plasma. Increased acetyl carnitine levels in muscle and plasma have been found in obesity models or after chronic high-fat feedings (21, 41). Although not directly confirmed, previous reports indicate that the products of incomplete oxidation (acetyl CoA and acetyl-carnitine) may also be directed to fatty acid biosynthesis pathways (2). In findings similar to ours in liver, Koves et al. also found that exercise training increased the flux of both β-oxidation and TCA cycle improving the rate of complete fatty acid oxidation to CO2 in skeletal muscle, providing evidence that exercise training can enhance the complete oxidation of lipids in both liver and skeletal muscle. Although not studied herein, Koves, Muoio, and colleagues (21, 28) implicate that the accumulation of acetyl-carnitines resulting from incomplete fatty acid oxidation may impact tissue insulin sensitivity and mitochondrial oxidative stress factors that clearly play a role in hepatic function and thus deserve future attention.

In a further analysis of hepatic mitochondrial enzymes we witnessed no increase in liver β-HAD or citrate synthase activity indicating that exercise may not have increased specific enzymatic activity or increased mitochondrial content in liver. However, the electron transport chain protein cytochrome c was increased in the Ex group, demonstrating a possible enhancement of the final steps of oxidative phosphorylation. Also, the improved complete fatty acid oxidation appeared to be independent of changes in PPARα protein concentrations (R. S. Rector, preliminary observations). Thus our findings provide evidence that exercise training improves hepatic mitochondrial function, specifically, a tighter coupling of β-oxidation, TCA cycle, and oxidative phosphorylation that results in a more complete degradation of fatty acids in livers of obese rats.

Another possible explanation for the suppression of hepatic fatty acid accumulation relates to de novo fatty acid synthesis. Kinetic studies suggest that ~26% of hepatic TG accumulation can be accounted for by de novo lipogenesis (12). ACC is the committed step in fatty acid synthesis, catalyzing the carboxylation of acetyl-CoA to form malonyl-CoA. Phosphorylation inhibits ACC activity, reducing formation of malonyl-CoA and, hence, alleviating the allosteric inhibition of carnitine palmitoyltransferase I, which in turn increases the transfer of long-chain fatty acyl-CoA from the cytosol into mitochondria for oxidation. The Ex animals had both an increased phosphorylation of ACC and a reduced total protein content of ACC, indicating that there is likely a decrease in cytosolic malonyl-CoA levels. Reduction in malonyl-CoA concentrations would increase fatty acid oxidation and decrease available substrate for FAS, the enzyme responsible for the de novo synthesis of fatty acids. Another novel finding was that exercise training significantly reduced the protein content of FAS, which also likely decreased de novo fatty acid synthesis. Our findings support previous observations demonstrating the ability of exercise training to decrease hepatic FAS activity in response to a high-cornstarch diet (14) and streptozotocin treatment (15). In addition, treadmill running for 10 wk also downregulated liver FAS activity but not mRNA levels in obese Zucker rats fed a high-cornstarch diet (16).

ACC is a well-established downstream target of AMPK; although studies are limited, liver AMPK activity levels have been shown to be elevated 10–100 min after acute treadmill running (9). In addition, forced treadmill exercise training for 12 wk increased liver AMPK activity, AMPKα protein expression, and AMPK and ACC phosphorylation in healthy Wistar rats (39). However, the observed increases in ACC phosphorylation did not appear to be mediated by increased AMPKα content or AMPK phosphorylation in the present investigation. One possible explanation for the discrepancy with present findings could be related to the modality of exercise (forced treadmill vs. voluntary running), because the timing of the last bout of exercise was similar between investigations (48 h in Ref. 39 vs. 53 h in the present investigation). The lack of an exercise affect on AMPK also could have been related to the animal model (healthy Wistar vs. obese, insulin resistant OLETF rats) or the significant increases in liver glycogen content in the exercising animals, a factor known to attenuate AMPK activation (32). It should be noted that ACC phosphorylation observed following exercise could be due to protein kinase A activity (9). This possibility warrants future examination. Our novel findings of suppressed FAS and ACC protein content suggest that exercise training reduces hepatic lipogenesis and support the contention that exercise training beneficially attenuates hepatic lipid accumulation, in part by suppression of key intermediates in fatty acid synthesis.

In the present investigation, running wheels were locked and daily running ceased 2 days before the observed measurements. This timing was used to mirror what is often seen in human exercise training studies, to examine the chronic effects

Table 2. Correlations among individual animal characteristics and lipid parameters

<table>
<thead>
<tr>
<th></th>
<th>Food Intake, g/wk</th>
<th>Food Efficiency, g/g</th>
<th>Weight Gain, g</th>
<th>Serum TG, mg/dl</th>
<th>Serum FFAs, μM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver TG, nmol/g</td>
<td>0.316</td>
<td>0.576*</td>
<td>0.551*</td>
<td>0.310</td>
<td>0.475</td>
</tr>
<tr>
<td>Fat pad mass, g</td>
<td>0.567†</td>
<td>0.880‡</td>
<td>0.973‡</td>
<td>0.857‡</td>
<td>0.386</td>
</tr>
<tr>
<td>Palmitate oxidation, % oxidized to CO₂</td>
<td>−0.291</td>
<td>−0.301</td>
<td>−0.400</td>
<td>−0.191</td>
<td>0.638*</td>
</tr>
<tr>
<td>Food intake, g/wk</td>
<td>0.300</td>
<td>0.617†</td>
<td>0.725†</td>
<td>0.197</td>
<td></td>
</tr>
<tr>
<td>Food efficiency, g/g</td>
<td>−0.938‡</td>
<td>0.643†</td>
<td>0.446*</td>
<td>0.405</td>
<td>0.287</td>
</tr>
<tr>
<td>Weight gain, g</td>
<td>0.798‡</td>
<td>0.700‡</td>
<td>0.191</td>
<td>0.446*</td>
<td></td>
</tr>
<tr>
<td>Serum TG, mg/dl</td>
<td>0.938‡</td>
<td>0.643†</td>
<td>0.446*</td>
<td>0.405</td>
<td>0.287</td>
</tr>
</tbody>
</table>

Significant association (*P < 0.05; †P = 0.01; ‡P < 0.001). Liver palmitate oxidation (% oxidized to CO₂); Food efficiency was calculated as gram of weight gain per gram of food intake.
of exercise training vs. the acute effects of the last exercise bout. Indeed, it is well documented that the timing of the last bout of voluntary wheel running influences physiological measurements (19), and it is quite possible that if we had taken our measurements within a few hours of the last training bout the responses may have been more magnified. This possibility warrants future investigation.

It is likely that the exhibited increases in complete hepatic fatty acid oxidation and the attenuated protein expression of de novo lipogenesis markers do not entirely explain the grossly blunted lipid accumulation found in the wheel-running animals, suggesting the potential role of other contributing factors. It is well known that adipose tissue lipolysis significantly contributes to circulating fatty acids and the development of hepatic steatosis. In the present investigation, we found significant reductions in body weight and fat pad mass in the exercising animals in coordination with reduced serum TG and FFA concentrations. Furthermore, body weight and fat pad mass were significantly associated with liver and serum TG concentrations, and circulating FFAs were negatively correlated with hepatic palmitate oxidation. Exercise also improved insulin sensitivity, as indicated by improvements in insulin, glucose, and the surrogate marker HOMA. These findings would collectively result in less hepatocyte exposure to lipids and hyperinsulinemia, potent regulators of hepatic lipogenesis and inhibitors of VLDL secretion (22, 36, 43). Taken together, it is likely that these exercise-induced changes (decreased adiposity, lipid, and insulin) significantly contributed to the observed attenuation in hepatic TG in the Ex animals. Future mechanistic studies are needed to separate exercise-mediated peripheral changes known to influence liver metabolism from the direct effects of exercise on liver metabolism.

In conclusion, findings from the present investigation suggest that exercise training attenuates the progression of hepatic steatosis in OLETF rats. These effects appear to be at least partially mediated by increasing complete hepatic fatty acid oxidation and by reducing key protein intermediates linked to de novo hepatic fatty acid synthesis. Collectively, these findings demonstrate the powerful contributions of physical activity in altering metabolic health within this animal model. The OLETF rat represents a novel model for exploring the effects of lifestyle modifications on development of fatty liver disease, which is a major public health problem in Westernized societies.

ACKNOWLEDGMENTS

The authors thank Dr. James Turk, Dr. Javad Habibi, Jennifer Casati, Suzie Clark, and Grace Upptergrove for excellent technical assistance to this work. The authors also thank the Veterinary Medicine Diagnostics Laboratory at the University of Missouri for help with the histological sections and serum ALT measurements. The OLETF rats were a generous gift of the Tokushima Research Institute, Otsuka Pharmaceutical (Tokushima, Japan).

GRANTS

Partial funding was obtained from the University of Missouri Research Board and the College of Veterinary Medicine.

REFERENCES


