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*J Appl Physiol* 104:708-715, 2008. First published 13 December 2007;
doi: 10.1152/japplphysiol.01034.2007

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# Exercise-induced attenuation of obesity, hyperinsulinemia, and skeletal muscle lipid peroxidation in the OLETF rat

**R. Tyler Morris,[1] Matthew J. Laye,[1] Simon J. Lees,[5] R. Scott Rector,[3] John P. Thyfault,[2,3,4] and Frank W. Booth[1,5,6]**

[1]*Department of Medical Pharmacology and Physiology, School of Medicine;* [2]*Harry S. Truman Memorial Veterans Medical Center; Departments of* [3]*Internal Medicine and* [4]*Nutritional Sciences;* [5]*Department of Biomedical Sciences, College of Veterinary Medicine; and* [6]*Dalton Cardiovascular Research Center, University of Missouri, Columbia, Missouri*

Submitted 27 September 2007; accepted in final form 12 December 2007

**Morris RT, Laye MJ, Lees SJ, Rector RS, Thyfault JP, Booth FW.** Exercise-induced attenuation of obesity, hyperinsulinemia, and skeletal muscle lipid peroxidation in the OLETF rat. *J Appl Physiol* 104: 708–715, 2008. First published December 13, 2007; doi:10.1152/japplphysiol.01034.2007.—The Otsuka Long-Evans Tokushima fatty (OLETF) rat is a model of hyperphagic obesity in which the animals retain the desire to run voluntarily. Running wheels were provided for 4-wk-old OLETF rats for 16 wk before they were killed 5 h (WL5), 53 h (WL53), or 173 h (WL173) after the wheels were locked. Sedentary (SED) OLETF rats that were not given access to running wheels served as age-matched cohorts. Epididymal fat pad mass, adipocyte volume, and adipocyte number were 58%, 39%, and 47% less, respectively, in WL5 than SED rats. Contrary to cessation of daily running in Fischer 344 × Brown Norway rats, epididymal fat did not increase during the first 173 h of running cessation in the OLETF runners. Serum insulin and glucose levels were 77% and 29% less, respectively, in WL5 than SED rats. Oil red O staining for intramyocellular lipid accumulation was not statistically different among groups. However, lipid peroxidation levels, as determined by total *trans*-4-hydroxy-2-nonenal (4-HNE) and 4-HNE normalized to oil red O, was higher in epitrochlearis muscles of SED than WL5, WL53, and WL173 rats. mRNA levels of glutathione *S*-transferase-α type 4, an enzyme involved in cellular defense against electrophilic compounds such as 4-HNE, were higher in epitrochlearis muscle of WL53 than WL173 and SED rats. In contrast, 4-HNE levels in omental fat were unaltered. Epitrochlearis muscle palmitate oxidation and relative transcript levels for peroxisome proliferator-activated receptor-δ and peroxisome proliferator-activated receptor-γ coactivator type 1 were surprisingly not different between runners and SED rats. In summary, voluntary running was associated with lower levels of lipid peroxidation in skeletal muscle without significant changes in intramyocellular lipids or mitochondrial markers in OLETF rats at 20 wk of age. Therefore, even in a genetic animal model of extreme overeating, daily physical activity promotes improved health of skeletal muscle.

physical activity; oxidative stress; chronic disease

THE INCIDENCE OF OVERWEIGHT in children and adolescents has tripled to 17.1% in 2004 from the late 1960s in the United States (40). Obesity is caused by a positive energy balance, with excess energy intake and insufficient physical activity being major causal factors. An understanding of the biological basis for energy partitioning from fat to skeletal muscle is imperative for prevention of fat gain as young subjects grow to adults.

The Otsuka Long-Evans Tokushima fatty (OLETF) rat, which was selectively bred for null expression of the cholecystokinin (CCK)-1 receptor (27), is an animal model characterized by hyperphagia, obesity, decreased glucose infusion rate in a euglycemic clamp at 16 wk of age, and hyperinsulinemia at 24 wk of age in response to an intravenous glucose infusion and later development of Type 2 diabetes (26). We selected the OLETF rat as an obesity model, because it voluntarily runs long distances, in contrast to many other animal obesity models, which voluntarily run only short distances each day (6, 53). The result is a powerful model allowing the study of the effects of major increases in physical activity with concurrent hyperphagia and obesity. When introduced at 8 wk of age, voluntary running distance in the OLETF rat increased to a peak of ~10 km/day by 11 wk of age (6). Meal patterns, food intake, body weight, and leptin were subsequently maintained to levels of the nonobese littermates. Furthermore, long-term voluntary running for 13 wk reduced plasma glucose and immunoreactive insulin levels in response to a glucose load compared with a sedentary group (50).

The literature suggests that increased physical activity in normal, healthy rats would facilitate the following adaptations: *1*) lower levels of intra-abdominal fat (34) and of skeletal muscle lipid peroxidation via increases in SOD-1 (Cu/Zn SOD), SOD-2 (Mn SOD), and glutathione *S*-transferase (GSTα-4) (46, 48), inasmuch as a sedentary lifestyle in mice has been associated with enhanced vascular oxidative stress, which in turn was shown to be associated with vascular dysfunction (33), and *2*) higher values for skeletal muscle lipid oxidative capacity (24) and skeletal muscle mitochondrial markers [citrate synthase activity (24) and mRNAs for peroxisome proliferator-activated receptor (PPAR)-δ and PPAR-γ coactivator (PGC)-1α (5, 38)]. Since several studies suggest a link between lipotoxicity, oxidative stress, and insulin resistance (15, 25), lipid peroxidation was determined as an index of oxidative stress. Since 4-hydroxy-2-nonenal (4-HNE) is a biomarker of lipid peroxidation, oxidative stress, and subsequent pathophysiology (9, 12, 57), we hypothesized that increases in antioxidants, such as SOD, would lower 4-HNE. The rationale for determining SOD protein content is that it converts superoxide to $H_2O_2$, an outcome that would lower lipid peroxidation. The rationale for determining GSTα-4 mRNA is that it would be a marker for an enzyme that reduces electrophilic compounds, such as 4-HNE.

Address for reprint requests and other correspondence: F. W. Booth, Univ. of Missouri, Dept. of Biomedical Sciences, 1600 E. Rollins, E102, Vet. Med. Bldg., Columbia, MO 65201 (e-mail: boothf@missouri.edu).

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Furthermore, our recent observations would suggest that these parameters would begin to return to preactivity levels upon a 7-day cessation of physical activity (31, 32, 34).

Taken together, we hypothesized that exercise-induced attenuation of obesity in young, rapidly growing OLETF rats would be associated with unchanged intramuscular lipid content; the hypothesis is based on reports that intramuscular triglycerides (IMTG) are high in sedentary obese and exercise-trained individuals, while insulin sensitivity is lower in the former than in the latter [denoted by Goodpaster et al. (19) as the "athlete paradox"]. We further hypothesized that factors underlying energy partitioning away from fat would be *1*) an enhanced capacity for skeletal muscle palmitate oxidation, as indicated by increases in PGC-1α and PPARδ mRNAs, and *2*) a reduction in hyperinsulinemia and hyperglycemia, suggesting increased glucose disposal to muscle. An overall purpose of the present study is to begin to link changes in skeletal muscle that cause energy to be partitioned to adipose tissue. Also, it was hypothesized that short-term physical inactivity for 53 or 173 h would result in a return to the maladaptations observed in the skeletal muscle of sedentary OLETF rats.

## MATERIALS AND METHODS

*Ethical approval.* All experimental protocols were approved by the Animal Care and Use Committee at the University of Missouri.

*Animals and housing.* Male OLETF rats ($n = 47$, 69–92 g body wt on the day of arrival) were received in two shipments (required for sufficient epitrochlearis muscle) from Tokushima Research Institute Otsuka Pharmaceutical in approximately the 4th wk of life. Numbers of rats in the first and second shipments, respectively, for the four experimental groups were as follows: $n = 5$ and 8 for the WL5 group (wheels locked to cease running for 5 h after 16 wk of voluntary running), $n = 5$ and 6 for the WL53 group (wheels locked for 53 h after 16 wk of running), $n = 6$ each for the WL173 group (wheels locked for 173 h after 16 wk of running), and $n = 6$ and 5 for the SED group (sedentary animals that never ran). Animals were housed one per cage with a 12:12-h light-dark cycle in a room maintained at 20–22°C and had free access to rat chow (17% of total calories from fat; Formulab 5008, Purina Mills, St. Louis, MO) and water. A running wheel outfitted with a Sigma Sport BC 800 bicycle computer (Cherry Creek Cyclery, Foster Falls, VA), as previously described (31), was immediately provided for all, except SED, animals.

*Experimental design.* On arrival, each rat was randomly designated a runner or a nonrunner (SED). The average initial body weights for runners and nonrunners were 79.0 ± 2.5 and 82.2 ± 4.2 g, respectively. OLETF runners were allowed free access to voluntary running wheels for 16 wk until 20 wk of age (see Fig. 1 in supplemental data for this article, available online at the *Journal of Applied Physiology* website) and showed a similar pattern of daily running distance as a function of age, as reported elsewhere (6, 50). We selected 16 wk of voluntary running, inasmuch as Ishida et al. (26) reported that OLETF rats develop hyperglycemia and hyperinsulinemia at 16 wk of age; our goal was to study the effects of running on a model that normally has developed to a diseased state.

At 20 wk of age, OLETF runners were divided into three additional experimental subgroups, so that the body weight means of each group were similar: wheel lock for 5 h (WL5), wheel lock for 53 h (WL53), and wheel lock for 173 h (WL173). Each wheel-lock group has been previously shown to be important for assessment of the effects of physical inactivity (31, 32). Age-matched, SED OLETF rats did not have access to running wheels throughout the duration of the study. Rat chow was removed 5 h before the animals were killed. Rats were deeply anesthetized by one intraperitoneal injection of ketamine (80 mg/kg), xylazine (10 mg/kg), and acepromazine (4 mg/kg), their tissues were removed, and they were killed by exsanguination.

*Fat pad mass and adipocyte size.* Epididymal and omental fat pads were removed and weighed, and a portion of epididymal adipose was fixed in 10% buffered formalin for 24 h and then embedded in paraffin for morphometric determinations of cell diameters, from which cell volume was calculated as follows: volume $= 4/3 \times \pi \times r^3$ (where $r$ is radius). Average adipocyte volume was then used to calculate average adipocyte mass, with the assumption of 0.95 mg/ml density for adipocytes. Cell number is calculated as follows: cell number = epididymal fat pad mass ÷ average epididymal adipocyte mass.

*Histological analysis.* Epitrochlearis muscle was carefully dissected, placed on cork with oxytetracycline (Fisher), and submerged in partially frozen (approximately −160°C) isopentane for ~1 min. Frozen muscle sections (10 μm) were cut at −20°C and stored at −80°C. Epitrochlearis muscle was used, because it is recruited during voluntary running and was studied in our previous wheel-lock experiment (31). The oil red O staining procedure was adapted from Koopman et al. (29). Oil red O in duplicate samples was observed under a Zeiss microscope at ×20 magnification on the day of staining, with 5–10 views per animal. The relative amount of intramuscular lipid accumulation was determined as arbitrary units by Image Pro-Plus software (MediaCybernetics, 2001).

Serial muscle sections were also stained for succinate dehydrogenase with use of a protocol modified by Pette and Tyler (43).

*Lipid peroxidation.* Protein was extracted from muscle and adipose tissue by glass-on-glass homogenization in Mueller buffer [50 mM HEPES (pH 7.4), 0.1% Triton X-100, 4 mM EGTA, 10 mM EDTA, 15 mM $Na_4P_2O_7 \cdot H_2O$, 100 mM β-glycerophosphate, 25 mM NaF, and double-distilled $H_2O$], protease inhibitor cocktail (Sigma), phosphatase inhibitor cocktail 1 and 2 (Sigma), β-mercaptoethanol (156 mM), and double-distilled $H_2O$. Samples were centrifuged at 13,000 *g* for 10 min, and the supernatant was stored at −80°C.

For measurement of *trans*-4-hydroxy-2-nonenal (4-HNE) levels, protein concentrations were determined by Bradford assay (Bio-Rad), and 10 μg of protein were loaded onto a slot-blot apparatus (Bio-Dot SF Microfiltration Apparatus, Bio-Rad). Immunoreactions were obtained with primary antibody for 4-HNE (AG Scientific; 1:2,500 dilution) followed by an anti-rabbit secondary antibody (Amersham Biosciences; 1:10,000 dilution) for 1 h at room temperature. Immunocomplexes were visualized using enhanced chemiluminescence (ECL; catalog no. 34076, Pierce), digitally captured with Kodak Image Station-4000R, and quantified by Molecular Imaging software.

*Western blot analysis.* Protein was isolated from muscle via a procedure similar to that described above for lipid peroxidation, except β-mercaptoethanol was not added during homogenization. Protein concentrations were determined by Bradford assay (Bio-Rad). Proteins were separated by a 12% acrylamide gel, transferred to nitrocellulose membrane, stained with Ponceau S for verification of equal loading, and then rinsed twice for 10 min each in TBS + Tween 20 (TBS-T). A 5% nonfat dry milk-TBS-T solution (pH 7.5) was used for blocking. Primary antibodies for SOD-1 (Stressgen; 1:5,000 dilution) and SOD-2 (Stressgen; 1:1,250 dilution) were incubated overnight with the membrane in 5% nonfat dry milk at 4°C. Membranes were then rinsed (TBS-T) four times for 10 min and incubated with an anti-rabbit secondary antibody (1:2,500 dilution for both) for 1 h at room temperature. Immunocomplexes were visualized using ECL (catalog no. 34076, Pierce), digitally captured with Kodak Image Station-4000R, and quantified by Molecular Imaging software.

*Palmitate oxidation.* Epitrochlearis muscle homogenate fatty acid oxidation was measured with radiolabeled palmitate ([1-$^{14}$C]palmitate, American Radiochemicals) in a fresh homogenate preparation according to the methods described by Noland et al. (39).

*RNA isolation and cDNA synthesis.* RNA was isolated from a section of epitrochlearis muscle according to a method adapted from Chomczynski and Sacchi (10). RNA in the homogenate was isolated and purified with RNeasy columns and DNase I (Qiagen). A 1%

agarose gel was run, and the integrity of the 28S and 18S bands was used to verify that no degradation of the RNA had occurred. cDNA was synthesized with 0.586 μg of purified total RNA with use of the Superscript III first-strand synthesis system according to the manufacturer's instructions (Invitrogen), 50 ng/μl of random hexamer primers, and a Mastercycler Gradient Thermocycler (Eppendorf).

*Quantitative real-time PCR.* Quantitative PCR was performed with an ABI 7000 sequence detection system using Taqman Gene Expression Chemistry (ABI). All PCR included 25 ng of cDNA, 90 nM primers (Integrated DNA Technologies), 250 nM fluorescent probe, and Taqman Universal PCR Master Mix (ABI). Each sample was assayed in a 25-μl reaction in duplicate. If the duplicate contained a cycle threshold ($C_T$) standard deviation of >0.5, it was reassayed. All experimental samples were analyzed relative to 18S RNA. The primer and probe sequences used for rat PPARδ and rat GSTα-4 were predesigned by ABI, and rat PGC-1α was generously provided by Dr. Tim Koves (Duke University). PCR product length was checked against a ladder for proper amplicon size using PCR Super Mix (Invitrogen) before the product was subjected to Taqman Gene Expression Chemistry.

Differences in gene expression were calculated relative to 18S RNA via the comparative $C_T$ method (User Bulletin No. 2, ABI PRISM 7700 Sequence Detection System). Comparison of the differences in raw $C_T$ values that did not differ ($P = 0.307$) among groups indicated that 18S RNA was an appropriate normalizer. Standard curves for each target were run to verify similar efficiency of the PCR.

*Citrate synthase assay.* Fifty micrograms of epitrochlearis muscle homogenate from samples were used for determination of citrate synthase activity (51).

*Blood measurements.* Postprandial blood was collected at the time of death (rats had access to food during the 12-h dark cycle and were denied food only during the first ~5 h of their sleep cycle), allowed to clot for 15 min, and centrifuged at 3,000 rpm for 10 min at 4°C, and serum was frozen at −80°C. Blood glucose and insulin in serum were measured with a glucose oxidase reagent kit (Sigma) and an ELISA kit (Linco), respectively. Samples from healthy humans were run to verify that the assay yielded expected values for this population.

*Statistical analysis.* A one-way ANOVA was used for all statistical comparisons (Sigma Stat 3.5); Student-Newman-Keuls post hoc analysis was used if $P < 0.05$.

## RESULTS

*Physical characteristics.* Wet heart weight (mg) normalized to body weight (g) was 21% lower ($P < 0.05$) in SED than WL5 rats (Table 1). Left ventricular (LV) weight (not including septum) normalized to body weight was 23% and 9% lower ($P < 0.05$) in SED and WL173, respectively, than WL5 rats. In WL173 rats, LV weight fell 9% compared with WL5 rats, indicating ventricular atrophy. Also, access to voluntary running attenuated gain (58% less) in epididymal fat pad weight ($P < 0.05$) in WL5 compared with SED rats, with no changes from WL5 in the WL173 group. In WL5 rats, increases of epididymal adipocyte volume (pl), diameter (μm), and number ($10^6$) were lessened by 39%, 18%, and 47% ($P < 0.05$), respectively, compared with SED rats.

*Exercise prevented hyperglycemia and hyperinsulinemia.* Postprandial values were obtained to mimic the normal daily values. Food was removed from the cages at the end of the feeding cycle, when lights came on 5 h before the animals were killed. Voluntary wheel running significantly attenuated serum glucose and attenuated hyperinsulinemia in each of the wheel-lock groups compared with SED rats ($P < 0.05$; Table 2). Values did not return to SED levels 173 h after cessation of daily running physical activity.

*Voluntary running distances.* Initial OLETF running distance at 4 wk of age averaged 3–4 km/day. Peak distances of ~10 km/day were achieved between 8 and 11 wk and declined biphasically to ~5 km/day at 20 wk of age ($n = 16$; see supplemental Fig. 2 in the online version of this article).

*Food consumption.* Absolute weekly food consumption was significantly greater ($P < 0.05$) in OLETF SED rats in 7 of the 16 wk than in voluntary runners (Fig. 1*A*). However, relative to body weight (weekly food consumption/g of body wt), more food was consumed by the runners than by their sedentary counterparts in 14 of 16 wk (Fig. 1*C*). Food consumption during the 53- and 173-h wheel-lock periods did not significantly differ from daily average prelock values (data not shown).

*Prevention of weight gain with voluntary running and maintenance at WL173.* With the exception of 4–7 wk of age, body weights were significantly greater at all times in SED rats than in voluntary wheel runners ($P < 0.05$; Fig. 1*B*). Final body weights at 20 wk of age were 45% higher ($P < 0.05$) in SED than WL5 rats. Body weight of WL53 and WL173 rats did not significantly differ from body weight of WL5 rats (data not shown).

Food efficiency was 32% lower in runners (10.5 ± 0.2 g body wt gained/g food eaten) during the 16 wk of voluntary running ($P < 0.001$) than in SED rats (15.3 ± 0.2).

*Intramuscular lipid content is unaffected by running.* Although oil red O staining in epitrochlearis muscle was not significantly different ($P = 0.077$) among the groups, it tended to be more intense in WL5 and WL53 than SED rats (Fig. 2; see representative images at ×20 magnification in supplemental Fig. 3 in the online version of this article). Comparison of oil red O staining and succinate dehydrogenase enzyme activity in serial sections revealed that the predominant accumula-

Table 1. *Physical characteristics and serum insulin and glucose levels inWL5, WL53, WL173, and SED rats*

| | WL5 ($n = 5$) | WL53 ($n = 5$) | WL173 ($n = 6$) | SED ($n = 6$) |
|---|---|---|---|---|
| Heart wt/body wt, mg/g | 3.27±0.10* | 3.67±0.37* | 3.17±0.04* | 2.59±0.07 |
| Final body wt, g | 462±16* | 469±13* | 461±16* | 671±21 |
| Epididymal fat mass/body wt, g/g × 1,000 | 11.0±1.1* | 10.4±0.89* | 9.87±0.71* | 26.0±1.9 |
| Average epididymal adipocyte vol, pl | 1,760±150* | 1,690±100* | 1,630±210* | 2,900±130 |
| Average epididymal adipocyte diameter, μm | 137±4.2* | 136±2.9* | 133±6.0* | 168±2.9 |
| Epididymal adipocyte number, × $10^6$ | 35.5±5.4* | 30.4±3.8* | 34.8±5.5* | 66.8±6.8 |
| Serum glucose, mg/dl | 493±14* | 488±21* | 529±12* | 693±34 |
| Serum insulin, ng/ml | 0.46±0.01* | 0.51±0.04* | 0.46±0.01* | 1.97±0.73 |

Values are means ± SE. WL5, WL53, and WL173, 16 wk of voluntary running followed by 5, 53, and 173 h of no running, respectively; SED, sedentary. *$P < 0.05$ vs. SED.

Table 2. *Relative SOD-1 and SOD-2 protein expression levels in epitrochlearis muscle of WL5, WL53, WL173, and SED rats*

| | WL5 | WL53 | WL173 | SED |
|---|---|---|---|---|
| SOD-1 | 1.01±0.17 | 0.85±0.14 | 0.92±0.12 | 0.97±0.14 |
| SOD-2 | 1.30±0.20 | 1.19±0.14 | 1.23±0.09 | 1.15±0.08 |

Values are means ± SE, expressed in arbitrary density units from Western blot analysis ($n$ = 5–6 animals/group).

tion of intramuscular neutral lipids was in highly oxidative fibers (see supplemental Fig. 4 in the online version of this article), as has previously been demonstrated (37).

*Higher 4-HNE levels in SED rats.* 4-HNE, as index of lipid peroxidation, was significantly greater in epitrochlearis muscle of SED than WL5, WL53, and WL173 rats ($P < 0.05$; Fig. 3*A*). In contrast, in omental adipose tissue, 4-HNE was not different among WL5, WL53, WL173, and SED rats (Fig. 3*B*). 4-HNE, expressed as percent oil red O staining, in epitrochlearis muscle was 394%, 365%, and 199% ($P < 0.05$) greater in SED than WL5, WL53, and WL173 rats, respectively (Fig. 4). A significant positive association ($r = 0.614$, $P < 0.05$) was observed between lipid peroxidation products and serum glucose levels.

*Antioxidants.* SOD-1 ($P = 0.887$) or SOD-2 ($P = 0.864$) protein expression in epitrochlearis muscle after 16 wk of voluntary running or short-term physical inactivity was not significantly different among the groups (Table 2). GSTα-4 mRNA was lower ($P < 0.05$) in WL173 and SED than WL53 rats (Fig. 5). GSTα-4 mRNA between WL5 and SED rats did not achieve statistical significance but had a nonsignificant trend ($P = 0.053$).

*Mitochondria.* Neither PPARδ nor PGC-1α mRNA levels expressed relative to 18S RNA in epitrochlearis muscle were significantly different among groups (Fig. 5; $P = 0.285$ for PPAR-δ and $P = 0.363$ for PGC-1α). Citrate synthase activity was greater in WL53 rats than in all other groups (Fig. 6; $P < 0.05$).

*Palmitate oxidation.* Total palmitate oxidation in epitrochlearis muscle was not significantly different among groups: 3,022 ± 412, 2,891 ± 568, 2,381 ± 229, and 2,512 ± 426 nmol $CO_2 \cdot g^{-1} \cdot h^{-1}$ for WL5, WL53, WL173, and SED, respectively ($n$ = 5–8 animals/group).

## DISCUSSION

One aim of the present study was to determine some of the biological bases for better health associated with physical activity during the increase of body fat in an extreme, genetic model of obesity. Whereas body mass was 44.3% higher in OLETF SED than OLETF runners, cumulative food intake was not different between these groups until the final 4 wk of the 16 wk of running; at 16 wk, OLETF SED rats had consumed 4.7% more food than OLETF runners during the entire 16 wk of running. Together, these findings suggest an important role for energy expenditure in reducing the rate of increase in body mass and intra-abdominal fat. As expected from an earlier report (6), 16 wk of access to a wheel for voluntary running in the present study attenuated the gain in epididymal (58% less) and omental (69% less) fat pad weights compared with their sedentary cohorts.

A novel observation in the present study was lower 4-HNE, a marker of lipid peroxidation, in epitrochlearis muscle of the OLETF runners. Surprisingly, lower 4-HNE was not accompanied by a change in mitochondrial markers (PGC-1α mRNA and palmitate oxidation) in epitrochlearis muscle. A number of

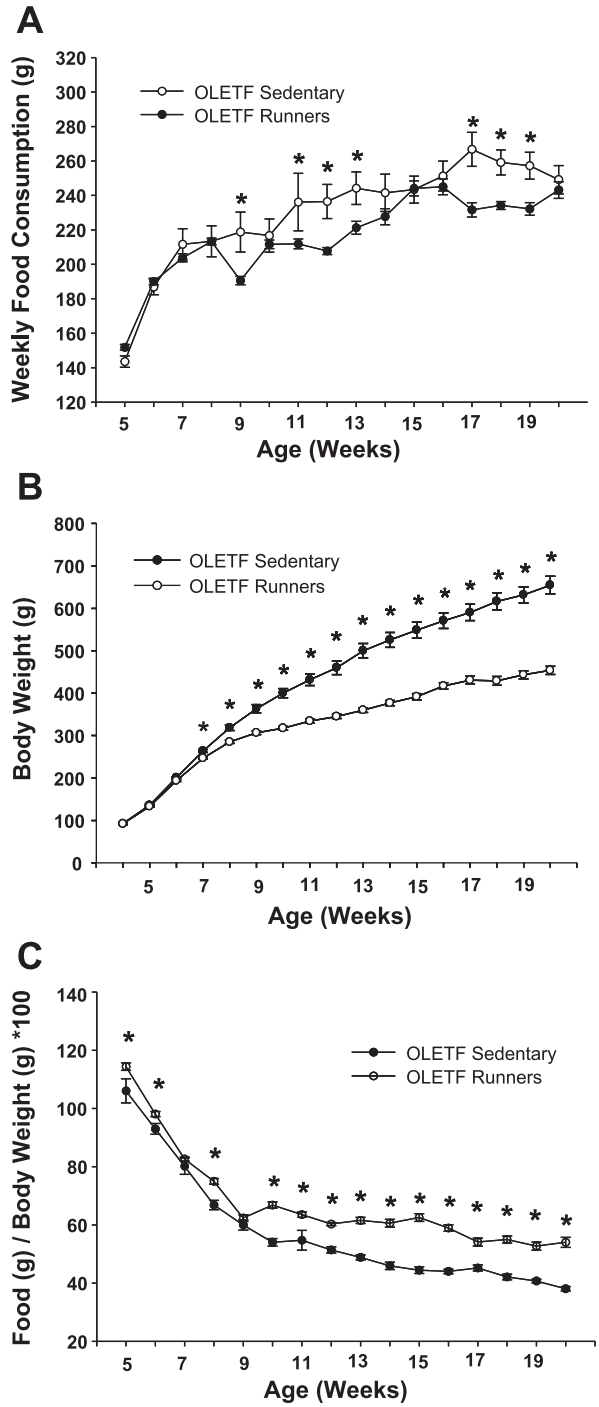


Fig. 1. *A*: weekly food consumption during 16 wk of voluntary running as a function of age in Otsuka Long-Evans Tokushima fatty (OLETF) rats. *B*: effect of voluntary running on body weights in OLETF rats during running at 4–20 wk of age. *C*: food-to-body weight ratio in OLETF rats during running at 4–20 wk of age. Values are means ± SE ($n$ = 6 sedentary and 16 runners). *$P <$ 0.05, runners vs. sedentary in a given week (2-way repeated-measures ANOVA).

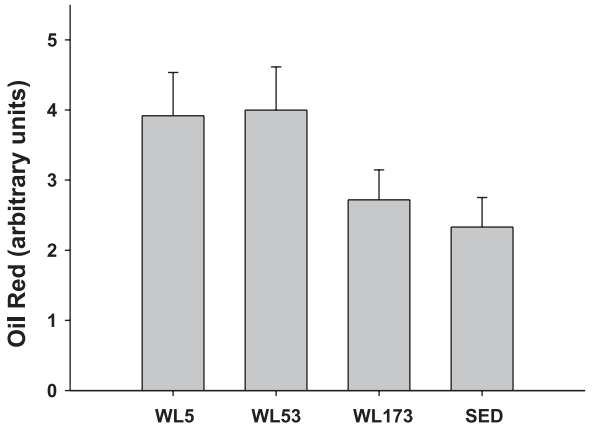


Fig. 2. Effect of 16 wk of voluntary running followed by 5 (WL5), 53 (WL53), or 173 (WL173) h of wheel lock or no running (SED) on intramuscular lipid accumulation (percent stained with oil red O) in epitrochlearis muscle of OLETF rats. Values are means ± SE ($n$ = 5–6 animals per group). $P$ = 0.77.

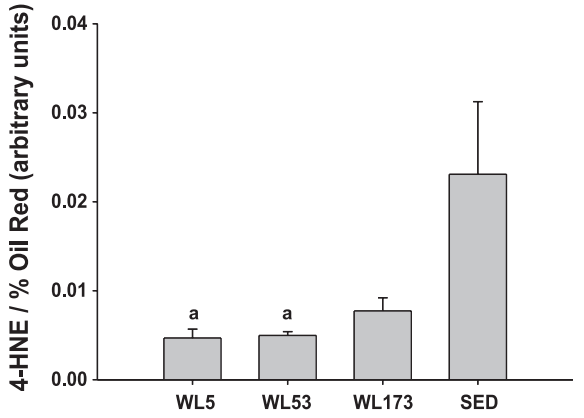


Fig. 4. Accumulation of 4-HNE/intramuscular lipid in epitrochlearis muscles of WL5, WL53, WL173, and SED rats. Values are means ± SE ($n$ = 5–6 animals per group). [a]$P < 0.05$ vs. SED.

other novel observations (lower adipocyte numbers, postprandial attenuation of hyperinsulinemia and hyperglycemia, and higher muscle GSTα-4 mRNA in the OLETF runners) were outcomes of our study. An additional unique observation was the failure of epididymal fat mass growth to catch up during the first 173 h of cessation of daily voluntary running. Possible explanations for the differences between the present findings and our previous report are differences in rat strain and voluntary running durations. In Fischer 344 × Brown Norway rats, epididymal fat increased 1 wk after cessation of 3 or 6 wk of voluntary running (32, 34); in OLETF rats, however, there was no change in epididymal fat 1 wk after cessation of 16 wk of voluntary running. Furthermore, 4-HNE in the epitrochlearis muscle was unchanged for the first 173 h after cessation of running. Whether the maintenance of the new lower levels of epididymal fat mass and muscle 4-HNE is due to differences in strain or the absence of CCK-1 receptor requires additional investigation.

4-HNE is an electrophile that reacts with amino acid side chains of proteins (11, 16, 54), thereby altering protein function (2, 20, 45, 56). 4-HNE, an end product of lipid peroxidation, is widely recognized as a specific marker of oxidative stress (41); increased reactive oxygen species (ROS) levels are an important trigger for insulin resistance in numerous settings (25). 4-HNE is known to produce nitrosylation of Akt, causing reduced insulin signaling transduction in insulin-resistant muscle (35). The potential clinical significance of lowered ROS levels in skeletal muscle by voluntary physical activity could be its amelioration of skeletal muscle insulin resistance. Reaven (47) emphasizes that the combination of insulin resistance and hyperinsulinemia (known as syndrome X, the insulin resistance syndrome, or cardiometabolic syndrome) greatly increases the likelihood of developing a cluster of closely related abnormalities, such as cardiovascular disease and Type 2 diabetes.

The present findings also provide new evidence that physical activity induces preferential increases of GSTα-4 mRNA levels at one of three time points in epitrochlearis muscle of

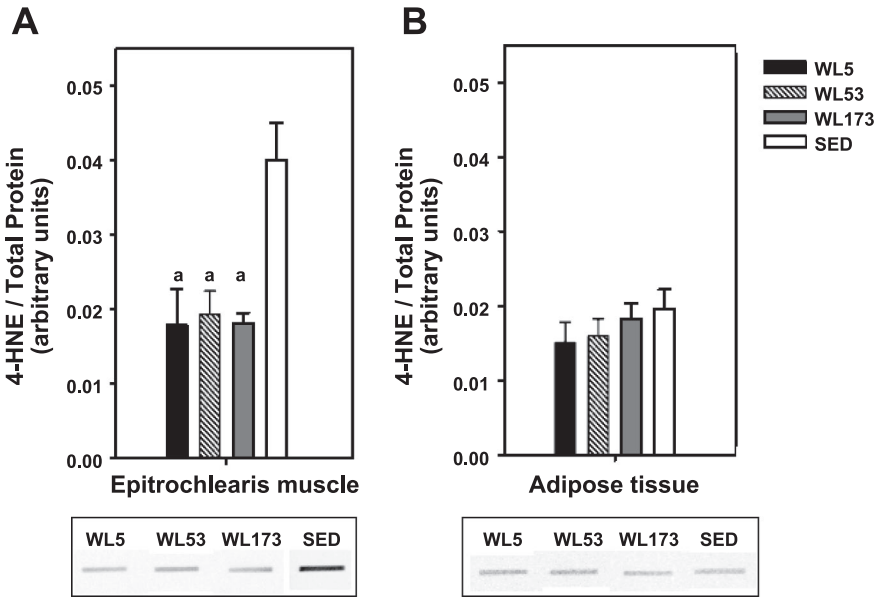


Fig. 3. Production of 4-hydroxy-2-nonenal (4-HNE) in epitrochlearis muscle (*A*) and omental adipose tissue (*B*) of WL5, WL53, WL173, and SED rats. Data are measured in arbitrary units and interpreted as relative changes within each tissue. All samples for a given tissue were analyzed on the same membrane in a slot-blot apparatus, and representative samples are shown. Values are means ± SE ($n$ = 5–6 animals per group). [a]$P < 0.05$ vs. SED.

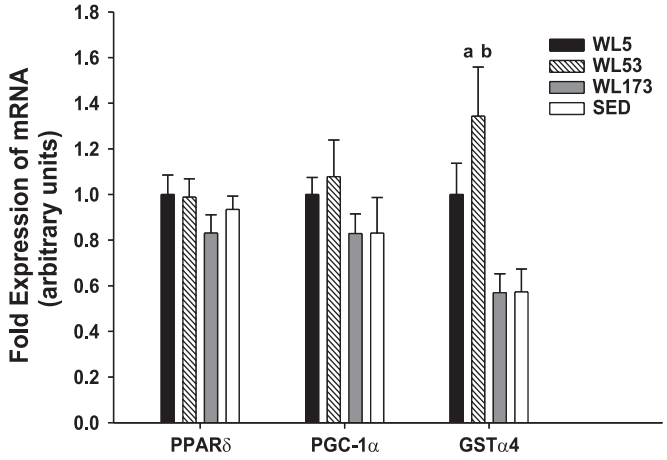


Fig. 5. mRNA levels of peroxisome proliferator-activated receptor (PPAR)-δ, PPARγ coactivator (PGC-1α), and glutathione *S*-transferase (GSTα-4) in epitrochlearis muscles of WL5, WL53, WL173, and SED rats. Values were normalized to WL5 group levels. Values are means ± SE ($n$ = 5–8 animals per group). [a]$P < 0.05$ vs. SED. [b]$P < 0.05$ vs. WL173.

OLETF rats. Glutathione transferases inactivate endogenous α,β-unsaturated aldehydes, quinones, epoxides, and hydroperoxides formed as secondary metabolites during oxidative stress (23). GSTα-4 mRNA levels significantly increased ~135% in WL53 vs. both WL173 and SED groups, with a nonsignificant trend for higher levels in WL5 than SED rats. The GST family of enzymes catalyzes the conjugation of reduced glutathione with electrophilic compounds, such as 4-HNE, via Michael addition (21, 22). This enzymatic reaction serves to reduce 4-HNE levels via GSTα-4. GSTα-4-knockout (GSTα-$4^{-/-}$) mice exhibit reduced ability to conjugate 4-HNE (17). Consequently, steady-state levels of 4-HNE and malondialdehyde (an end product of lipid peroxidation by ROS) in the liver were significantly higher in GSTα-$4^{-/-}$ than wild-type mice at 4 mo of age. Also, the survival time of GSTα-$4^{-/-}$ mice was significantly reduced after an intraperitoneal injection of paraquot, a generator of superoxide radicals (17). Although changes in mRNA levels may not reflect functional protein activity, measurements of GSTα-4 mRNA levels suggest enhanced gene transcription and/or improved mRNA stability for GSTα-4 in response to exercise in this model. Taken together, the above-mentioned findings suggest that if the exercise-induced increase in GSTα-4 mRNA would increase GSTα-4 protein and activity, 4-HNE levels in vivo could be reduced and, thus, protect against oxidative stress. Lower 4-HNE levels could also result from less lipid peroxidation, which was not determined in the present study.

The lack of change in SOD-1 and SOD-2 proteins in skeletal muscle of physically active OLETF rats is surprising. SODs are enzymes that catalyze the conversion of superoxide to $H_2O_2$ and oxygen. Exercise training is a powerful stimulus for increased antioxidant capacity via improvements of ROS scavenging (14, 49, 55). We speculate that, in OLETF runners, either the physical activity stimulus is insufficient or some inhibitory factor related to the loss of the CCK receptor in the OLETF rat is increased. A limitation of our study is that other antioxidant factors were not determined.

The expected increases in mRNA levels for PPARδ and PGC-1α, the capacity to oxidize palmitate, or citrate synthase activities were not found in epitrochlearis muscle of physically active OLETF (WL5) rats. Several studies have shown that exercise training increases skeletal muscle PGC-1α and PPARδ mRNAs in nonobese (1, 3, 4, 44) and PGC-1α in obese (42, 52) subjects. Activation of PPARδ (38) and overexpression of PGC-1α (36) upregulate fatty acid oxidation. Endurance-type training has also been shown to increase fatty acid oxidation in the vastus lateralis muscle of obese women (13) and in red and white gastrocnemius muscle of rats run on a treadmill for 10 wk (30). Additional studies demonstrate that the capacity to oxidize palmitate in skeletal muscle is significantly reduced with human obesity and negatively associated with body mass index (28). Although citrate synthase activity was higher in WL53 rats, it was not different in WL5 rats, an observation that we are unable to explain.

In contrast to our previous study in lean Fischer 344 × Brown Norway rats (31), we did not observe an increase in markers of mitochondrial oxidative capacity in OLETF rats 5 h after the cessation of voluntary running (WL5). There are several potential explanations for our present observations. *1*) Wheel running may be lower intensity exercise than typical rodent treadmill training sessions, but when performed over many hours the total energy expenditure may equal or exceed that of treadmill running. However, some of the muscle adaptations may require development of the higher-intensity activity. We previously showed that, for equal 2-h durations of running, the increase in mitochondrial markers by daily voluntary running (33) is only one-quarter of exercise training produced by forced treadmill running (19). No significant increase in four mitochondrial markers was found in Sprague-Dawley rats that ran one 10-min daily bout on a treadmill 5 days/wk for 12 wk (18). Furthermore, the average running speed of OLETF runners is less than that of Fischer 344 × Brown Norway rats: 2.2 vs. 2.5 km/h (31). Together, these findings lead us to the hypothesis that threshold duration for intermittent bouts or threshold speed is required for some biochemical adaptations to exercise, explaining in part why intermittent bouts <10 min result in smaller increases in mitochondrial concentration. *2*) The daily running distance for OLETF rats fell ~50% to ~5 km/day at 16 wk of running from a peak of ~10 km/day during 8–11 wk; potential causes for decreased distances are increases in body mass and/or onset of prediabetes. In contrast, citrate synthase activity in epitrochlearis

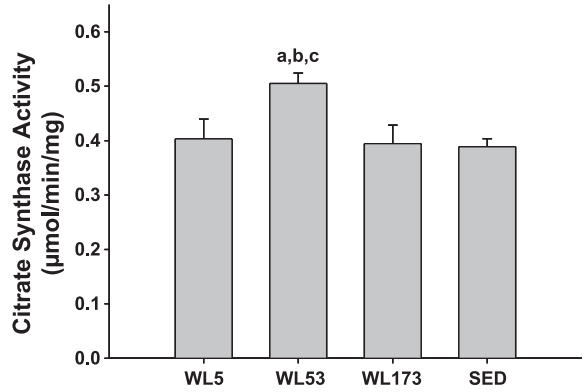


Fig. 6. Citrate synthase enzyme activities in epitrochlearis muscles of WL5, WL53, WL173, and SED rats. Values are means ± SE ($n$ = 5–6 animals per group). [a]$P < 0.05$ vs. SED. [b]$P < 0.05$ vs. WL173. [c]$P < 0.05$ vs. WL5.

muscle increased by 26% in Fischer × Brown Norway rats that were voluntarily running a distance [5.7 km/day (33)] similar to that run by the OLETF rats in their last week of voluntary running. *3*) Some of the variation could be due to differences in rat strains or a difference in gait between the muscles tested. A limitation of the present study is that we used only epitrochlearis muscle, and other muscles could respond differently. Another limitation is that the OLETF model of obesity differs from human obesity, in that OLETF rats would have more voluntary activity and they were fed a diet consisting of 17% of total calories from fat.

The number and diameter of adipocytes in each epididymal fat pad were decreased by 47% and 18%, respectively, in runners compared with sedentary rats in the OLETF model. These values imply that physical activity in this genetic-obese model hindered adipogenesis via decreased hyperplasia as the animals grew from 4 to 20 wk of age. Further research is required for extrapolation of the amount and intensity of exercise performed by the OLETF rats to translate to a similar adipogenenic response in rapidly growing children.

Postprandial hyperinsulinemia and hyperglycemia were 77% and 29% less, respectively, in OLETF rats 5 h after cessation of daily running than in sedentary OLETF rats. A potential clinical significance of a delay in the magnitude of the rise in postprandial hyperinsulinemia and hyperglycemia would be delay in the onset of Type 2 diabetes (8). It is known that hyperglycemia induces an overproduction of superoxide by the mitochondrial electron transport chain (7). Interestingly, a significant positive association ($r = 0.64$) was revealed between serum glucose levels and 4-HNE in the epitrochlearis muscle of OLETF rats. When expressed as group averages, the total content of 4-HNE in the epitrochlearis muscle, when expressed relative to protein or intramyocellular lipid, in OLETF rats that ran for 16 wk was 25% of that in cohorts that never ran (Fig. 3*A*). Similarly, Russell et al. (48) found 5.4- and 4.6-fold higher 4-HNE levels and 4-HNE-to-IMTG ratios, respectively, in skeletal muscle of obese than endurance-trained subjects. They concluded that the protective effect of endurance training on IMTG peroxidation, rather than just the amount of IMTG, which is elevated in endurance athletes, may provide a link between the lipid peroxidation-to-IMTG ratio and insulin resistance (48).

Taken together, the present study provides evidence that levels of 4-HNE (a marker of lipid peroxidation and oxidative stress) in the epitrochlearis muscle are lower when OLETF rats voluntary run during a period of growth, and this adaptation was maintained after the cessation of exercise for up to 1 wk. Our study also indicates that protection against lipid peroxidation in skeletal muscle may be related, in part, to increases in GSTα-4. Furthermore, daily voluntary running did attenuate adipocyte hyperplasia, epitrochlearis lipid peroxidation, hyperinsulinemia, and hyperglycemia. In summary, natural daily running in a hyperphagic animal model with obesity resulted in adaptive lowering of lipid peroxidation in skeletal muscle without notable changes in skeletal muscle mitochondria, palmitate oxidation, or intramyocellular lipid, suggesting that these events may not be connected. Therefore, even in a genetic animal model of extreme overeating, daily physical activity promotes improved health of skeletal muscle.

## ACKNOWLEDGMENTS

We thank Sarah Borengasser and Scott Naples for help with the data collection.

## GRANTS

This research was supported by the University of Missouri Research Board (F. W. Booth), an anonymous gift (F. W. Booth), and the University of Missouri Life Sciences Predoctoral Fellowship (M. J. Laye).

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