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ORIGINAL CONTRIBUTION

K. Leigh Greathouse
Matthew Samuels
Nancy M. DiMarco
David S. Criswell

Effects of increased dietary fat and exercise on skeletal muscle lipid peroxidation and antioxidant capacity in male rats

Summary *Background* Elevated dietary fat increases oxidative metabolism and has been linked to increased oxidative stress, while exercise training may augment antioxidant capacity. Most studies examining oxidative stress in skeletal muscle employ extremely high levels of dietary fat and/or intense exercise training that may not adequately model human diet and activity patterns. *Aim* The purpose of this study was to examine the interaction between an elevated (40 % of calories) monounsaturated fat diet and a moderate-intensity exercise program similar to recommended human exercise prescriptions, on skeletal muscle oxidative stress and antioxidant defenses. *Methods* Twenty-four male Sprague-Dawley rats (~500 g) were randomly divided into 4 groups (n = 6/group): Standard Diet-Sedentary (SD-Sed), Standard Diet-Exercise (SD-Ex), Elevated Fat Diet-Sedentary (EFD-Sed), and Elevated Fat Diet-Exercise (EFD-Ex). The SD groups consumed 76 % of calories from CHO, 14 % from protein, and 10 % from fat, while the EFD groups received a diet of 46 % of calories from CHO, 14 % from protein, and 40 % from fat (high oleic sunflower oil). The exercise groups were progressively treadmill trained at 20 m/min, 4 days/week increasing from 15 min/day to 35 min/day by the end of 4 wks. *Results and conclusion* Antioxidant adaptations associated with exercise training or an elevated fat diet individually reduced basal lipid peroxidation levels in the plantaris muscle. However, the combination of exercise plus a monounsaturated fat diet increased lipid peroxidation levels above that with either treatment alone. This suggests an exhaustion of the antioxidant capacity in the plantaris muscle when both exercise and increased dietary fat diet are combined.

Key words oxidative stress – physical activity – monounsaturated fat – lipid hydroperoxides – plantaris muscle

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K. L. Greathouse · M. Samuels · N. M. DiMarco
Institute for Women's Health
Texas Woman's University
Denton, Texas

D. S. Criswell, Ph. D. (✉)
Center for Exercise Science
University of Florida
P. O. Box 118206
Gainesville (FL) 32611-8206, USA
Tel.: +1-352/392-9575, ext. 1399
Fax: +1-352/392-0316
E-Mail: dcriswell@hhp.ufl.edu

Introduction

The autoxidation of lipids, known as lipid peroxidation, may be linked with a wide variety of diseases such as arthrosclerosis, cancer, diabetes, and ischemia/reperfusion injury [1–4]. Therefore, mammalian cells have an elaborate defense system including antioxidant enzymes, like superoxide dismutase and glutathione peroxidase, membrane-bound antioxidants such as α-tocopherol (vitamin E), and soluble antioxidants like ascorbic acid (vitamin C) and glutathione. The imbalance between pro-oxidants and antioxidants in the favor of more pro-oxidants is referred to as oxidative stress. When the ratio of pro-oxidants to antioxidants is unbalanced in favor of pro-oxidants (or free radicals), such as in skeletal muscle during an acute bout of intense exercise, damage occurs to many cellular components in the form of lipid peroxidation, protein modifications, and DNA mutation [5, 6]. Mounting evidence suggests that intense exercise training negates the harmful effects of oxidative stress by up-regulating the activity of antioxi-

dant enzymes, such as superoxide dismutase and glutathione peroxidase [6–8]. This up-regulation has been postulated to be a result of the increased amounts of free radical ($R^{\bullet}$) formation that stimulate mitochondrial biosynthesis of antioxidant enzymes [9]. However, changes in skeletal muscle mitochondrial volume and oxidative capacity with exercise training do not always mirror changes in antioxidant capacity [10].

In addition to exercise, high fat diets, especially high polyunsaturated fatty acid diets (PUFA), increase the production of free radical damage [11–13]. Unlike exercise training, however, many studies have suggested that chronic elevated-fat diets do not initiate the rise in antioxidant enzymes to counteract the rise in free radical production, and may actually depress antioxidant enzymes [11, 14, 15]. Whether the combined effects of dietary fat and exercise yield more antioxidant activity or free radical damage remains questionable. We hypothesized that monounsaturated fats in the diet would not hinder antioxidant adaptations in skeletal muscle. Therefore, the purpose of this study was to determine if an elevated-fat diet (40 % total kcalories), composed primarily of monounsaturated fat, would decrease basal lipid peroxidation in rat skeletal muscle, and if a moderate intensity exercise program of 4.5 weeks duration would enhance this effect.

Methods

Animals

Twenty-four male Sprague-Dawley rats (120 days old) were attained from Hilltop Lab Animals, Inc. (Scottsdale, PA) and housed individually in polycarbonate cages in the Texas Woman's University (TWU) Animal Facility. Animals were maintained on a 12 h light-dark cycle (light: 07:00–19:00 h) to maintain biological balance that mimics natural settings. The TWU Institutional Animal Care and Use Committee approved this study.

Dietary protocol

Prior to the beginning of the protocol, animals were randomly assigned to one of two dietary groups. Both groups consumed a standard AIN-M diet [16] consisting of 10 % total kcalories as fat, 76 % total kcalories as carbohydrate, and 14 % total kcalories as protein for the first week. Following this week of equilibration, Group 1 (elevated-fat diet, n = 12) was switched to a diet containing 40 % total kcalories as fat, 46 % total kcalories as carbohydrate, and 14 % total kcalories as protein (Table 1). Group 2 (standard diet, n = 12) consumed the standard AIN-M diet during the entire study. Both diets contained the AIN-M recommended vitamin and mineral supplements [16], including vitamin E (7500 IU/kg). All food was purchased through Dyets, Inc. (Bethlehem, PA).

Table 1 Composition of experimental diets

Ingredient (g/kg of diet)	SD	EFD
Casein	140	165
Cornstarch	465.7	326.8
Dextrose	155	110.5
Sucrose	100	88.1
Cellulose	50	80.7
Soybean oil	40	47.6
High oleic sunflower oil	0	132
AIN-M mineral mix	35	35
AIN-M vitamin mix	10	10
L-Cystine	1.8	1.8
Choline bitartrate	2.5	2.5

SD Standard diet; *EFD* Elevated-fat diet
High oleic sunflower oil contains 85 % monounsaturated fatty acids

Following dietary randomization, half of the animals in each group (n = 6) were again randomly assigned to either an exercising group or a sedentary group. This provided a total of four groups: (a) standard diet exercise (SD-Ex), n = 6; (b) standard diet sedentary (SD-Sed), n = 6; (c) elevated-fat diet exercise (EFD-Ex), n = 6; and (d) elevated-fat diet sedentary (EFD-Sed), n = 6. Body weight and food intake were recorded three times during the last 3 weeks of the study. Twenty-four hour food intake was calculated as the difference between the pre- and post-food weight measurements.

Exercise protocol

Exercise was performed once daily using a motor-driven rodent treadmill with ten separate running lanes. Treadmill speed was calibrated weekly using a stopwatch and a visual marker on the treadmill belt. All animals were introduced to the rodent treadmill during Week 1 by running at 20 m/min for 15 min/day. Subsequently, the duration was increased each day of running until all exercising animals were running at 20 m/min, 35 min/session, 4 days/week by the end of the training protocol (4.5 weeks). To control for the stress of handling and exposure to the treadmill, sedentary animals (SD-Sed and EFD-Sed groups) walked on the treadmill two times per week, 5 min per session at 15 m/min during the length of the study.

After the last exercise session, animals were allowed a 72-h recovery period before being sacrificed by decapitation. This was for the purpose of measuring the dependent variables in a rested state. Immediately after decapitation, the plantaris muscles were removed, placed on dry ice, frozen, and stored at –80 °C until analysis.

Biochemical analyses

The following physiological parameters were measured in the plantaris muscle as previously described: concentration of lipid hydroperoxides [17], thiol content [18], glutathione peroxidase [19], superoxide dismutase [20], and citrate synthase [21] activities.

Statistical analysis

Data were analyzed by a factorial 2 x 2 (dietary fat x exercise) ANOVA performed using SPSS for Windows, version 10.0. If a significant difference was present, a pairwise multiple comparison was performed using the Student-Newman-Keuls method. Alpha was set at $p \leq 0.05$ for all tests.

Results

Body weights were significantly higher in the EFD-Sed (542 ± 18 g) and EFD-Ex (572 ± 13 g) groups compared to the SD groups (SD-Sed = 499 ± 17 g, SD-Ex = 504 ± 15 g). Additionally, kcalorie intake (kcal/wk) was significantly increased in the EFD-Sed and EFD-Ex groups compared to SD-Sed. However, no differences were found between groups for total food intake (g/wk).

The lipid hydroperoxide levels in the plantaris were significantly reduced in the SD-Ex group compared to the control group (SD-Sed) (Fig. 1). The groups fed the moderate-fat diet had opposite results with the lipid hydroperoxide levels in the EFD-Sed decreased significantly from the SD-Sed group, and the EFD-Ex group increased compared to the EFD-Sed group. Overall, the lipid hydroperoxide levels demonstrated a significant interaction between diet and exercise treatments.

There was a significant exercise main effect for total thiols, with exercise groups being higher than the sedentary groups. There was, however, no significant main effect for the diet treatments (Fig. 2). Specifically, total thiol level was significantly increased in the SD-Ex group above the SD-Sed group and in the EFD-Ex group compared to EFD-Sed (i. e. exercise effect). However, total thiols in the EFD-Ex group were significantly lower than in the SD-Ex group indicating a blunted response to the exercise in the EFD groups.

Antioxidant enzymes

Mn-SOD activity was higher in the EFD groups compared to the SD groups. However, there was no significant difference between sedentary and exercise treatment groups and no significant interaction between diet and exercise treatments with regard to Mn-SOD activity

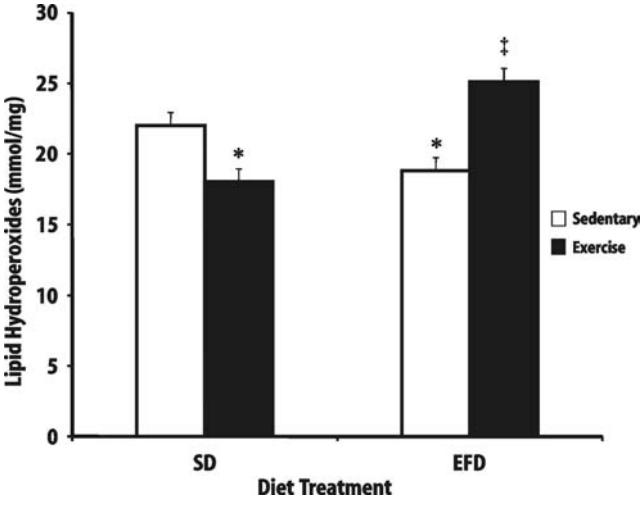

Fig. 1 Concentration of lipid hydroperoxides in plantaris muscle. * = Significantly different from the SD-Sed group, $p < 0.05$. ‡ = Significantly different from EFD-Sed and SD-Ex groups, $p < 0.05$

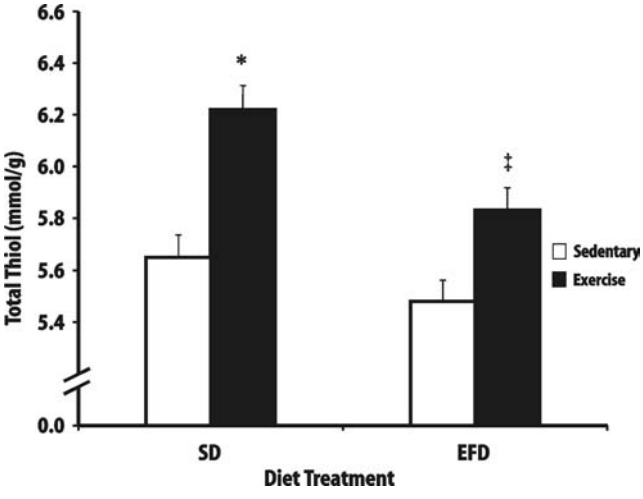

Fig. 2 Total thiol level in the plantaris muscle. * = Significantly different from the SD-Sed group, $p < 0.05$. ‡ = Significantly different from EFD-Sed and SD-Ex groups, $p < 0.05$

(Table 2). Total SOD activity and activity of the Cu,Zn isoform of SOD did not differ between any groups (data not shown). There was also a significant main effect of diet on plantaris GPX activity. However, no significant exercise main effect or interaction between activity and diet was observed (Table 2).

Citrate synthase

Citrate synthase activity tended to be higher in all treatment groups as compared to the control group (SD-Sed) (Table 2). Statistically, there was a significant main effect observed for the diet treatment; however, no significant

Table 2 Antioxidant and oxidative enzyme activities in plantaris muscle

Groups	Mn-SOD (mmol/g)	GPX (mmol/mg)	CS (mmol/mg)
SD-Sed	1.76±0.04	53.4±2.2	206.4±5.3
SD-Ex	1.84±0.07	56.3±2.4	218.6±7.2
EFD-Sed	1.97±0.04*	66.3±2.6*	246.4±4.4*
EFD-Ex	2.05±0.05*	66.2±2.1*	244.4±5.5*

Values are Mean ± SEM. *Mn-SOD* Manganese superoxide dismutase; *GPX* glutathione peroxidase; *CS* citrate synthase. *SD* standard diet; *EFD* elevated fat diet; *Sed* sedentary; *Ex* treadmill exercised
* Significantly different from SD-Sed, $p < 0.05$

exercise main effect or interaction between the two treatment groups was observed.

Discussion

Oxygen-centered radicals, such as superoxide, and related compounds, such as hydrogen peroxide, are produced normally as a by-product of oxidative metabolism [22]. These compounds, often referred to as reactive oxygen species (ROS), have the potential to damage cellular components including lipid membranes, proteins, and DNA [5, 6]. Therefore, metabolically active tissues are equipped with antioxidant defenses. These include enzymatic systems (e. g. superoxide dismutase and glutathione peroxidase) and scavenger systems such as glutathione, ascorbic acid, α-tocopherol, etc. Chronic elevation of dietary fat intake increases reliance on fatty acid oxidation and decreases glucose disposal. Therefore, dietary fat increases the overall production of ROS and may contribute to oxidative stress in skeletal muscle and other tissues [15].

Skeletal muscle is unique in that its metabolic activity varies tremendously between rest and intense exercise [5]. Therefore, if the antioxidant systems are not adapted to regular physical activity, skeletal muscle may be susceptible to oxidative damage during acute bouts of exercise. Although physical exercise increases the production of oxy-radicals, most studies agree that chronic exercise training increases the antioxidant capacity of skeletal muscle [6]. Nevertheless, the precise intensity and duration of training necessary to elicit increases in skeletal muscle antioxidant capacity remain elusive. Therefore, we sought to examine the interaction between a moderate (40 % of calories) monounsaturated fat diet and a moderate-intensity exercise training program to determine if exercise further exacerbates oxidative stress or if a balance could be achieved.

The results of our study suggest that oxidative stress in the plantaris muscle is reduced with exercise training or elevated monounsaturated-fat diet consumption, individually. This was evidenced by the lower lipid hydroperoxide levels in the SD-Ex and EFD-Sed groups as compared to the SD-Sed group. Also, total thiol levels were elevated in the SD-Ex group compared to the SD-Sed group (Figs. 1 and 2). However, when the exercise program is combined with the EFD (EFD-Ex), total thiols are statistically unchanged compared to the SD-Sed group and lipid hydroperoxides are higher than the EFD-Sed group. This suggests that the elevated fat diet limited the ability of the plantaris to up-regulate antioxidant capacity. However, in response to the exercise stimulus alone (i. e. standard diet), overall muscle antioxidant capacity was likely increased.

CS activity

Citrate synthase activity, which is indicative of mitochondrial volume, was measured to evaluate training effect and oxidative capacity of the plantaris. The activity of CS increased significantly in the EFD groups (Table 2), which correlates with other studies [15, 23, 24]. Specifically, in the study by Pereira et al., CS activity was most dependent upon the amount and type of dietary fat (PUFA vs. SFA) rather than physical activity levels. There was no exercise main effect on CS activity indicating that our training intensity was not sufficient to cause significant increases in muscle mitochondrial volume during the relatively short 4.5-week duration of the study. Nevertheless, significant exercise effects were found for lipid hydroperoxides and total thiols, suggesting that antioxidant adaptations to exercise occur prior to, and/or have a lower threshold for induction than adaptations in skeletal muscle oxidative potential.

Mn-SOD activity

In our study, no significant differences in Mn-SOD activity were found in the exercise groups, although the EFD increased Mn-SOD activity above the standard diet groups (Table 2). While studies by Laughlin et al. [25] and Alessio et al. [7] agree with our study, the majority report an increase in Mn-SOD activity with exercise training [10, 15, 23, 24, 26–30]. This discrepancy may be attributed to the low level of training used in this study, which may not have allowed for a true exercise-response adaptation to occur. With respect to dietary fat effects, the majority of the literature indicates either no change or a decrease [11, 15, 31] in Mn-SOD activity. Consistent with the findings of Pereira et al., however, our study found increased Mn-SOD with incorporation of fat in the diet. Although our data are not conclusive, an up-regulation of Mn-SOD most likely contributes to the lower resting lipid peroxidation levels in the plantaris following exercise training or elevated dietary fat.

GPX activity

Glutathione peroxidase has a high specificity for glutathione (GSH) and a low specificity for hydroperoxide; therefore, it plays a major role in inhibition of lipid peroxidation [8]. In addition, this enzyme has consistently been reported to increase its activity with adaptation to exercise training [8, 10, 23, 24, 28, 32, 33] (Table 2). In this study, elevated fat in the rat diet causes an up-regulation in GPX activity. Although exercise training tended to increase GPX activity in the SD groups, the effect was not additive with the diet effect, since GPX activity in the EFD-Ex group did not differ from the EFD-Sed group. It is possible that a more intensive and/or prolonged exercise regimen would have produced a significant exercise effect on GPX activity.

Lipid hydroperoxide levels

Perhaps the most interesting finding in this study is the dramatic interaction between the two treatments, diet and training, $p < 0.01$, such that exercise training lowered hydroperoxide levels in the standard diet groups, but elevated this variable in the elevated fat groups. Two studies, which evaluated an increased fat diet and training effects, also found a predominate effect on lipid hydroperoxide levels from increased fat in the diet [15, 34]. Pereira et al. reported that when training was added to the increased fat diet, lipid peroxide levels decreased from the exercise and fat diet groups. This contradicts our study. However, Mataix et al. [34] found that rats consuming a fat diet and exercising continued to have elevated lipid peroxide levels (above exercise or fat diet alone), as was seen in our study. To our knowledge, these are the only two studies that have compared exercise training and a fat supplemented diet in rats. One possibility for these conflicting results is the type of fat used for supplementation. Mataix et al. [34] used monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) and Pereira et al. [15] used PUFA and saturated fatty acids (SFA). PUFA are known to be more susceptible to lipid peroxidation than are MUFA or SFA. This effect is clearly evident in the study by Mataix et al. [15], which demonstrated only a small increase in lipid peroxidation levels in a group ingesting a MUFA supplemented diet as compared to the significant increase of the PUFA group. Our study, showing a decrease in lipid hydroperoxides with a moderate MUFA-supplemented fat diet, is consistent with Mataix et al. [15] and supports the MUFA-supplemented diet effects reported by Lopez-Bote et al. [35] who fed rats a diet high in MUFA and found low levels of lipid peroxidation. Although the mechanism for the protective effect of our EFD on lipid peroxidation cannot be determined from this study, at least two possibilities exist. 1) The EFD contained more casein than the SD. Rival et al. [36] have shown that caseins and casein-derived peptides have antioxidant properties. This could have reduced lipid peroxidation in the EFD-Sed group. 2) Also, diets containing MUFA result in greater incorporation of vitamin E into lipid membranes [37]. This would seem to be the most likely explanation of our observed EFD effect.

The significant decrease in lipid peroxidation levels observed in the SD-Ex group ($p < 0.02$) vs. SD-Sed suggests that antioxidant capacity of the plantaris muscle was enhanced by exercise, thereby protecting against lipid peroxidation. However, data failed to show significant increases in Mn-SOD and GPX activities in the trained rats receiving the standard diet (Table 2). Total thiol levels, on the other hand, were significantly elevated (Fig. 2), indicating an increased buffer against oxidative radical-mediated lipid damage.

The elevated fat diet resulted in an increase in the oxidative capacity of the plantaris muscle as determined by citrate synthase activity. This presumably would have allowed for a greater metabolic production of ROS. However, the moderate fat diet also caused an up-regulation in GPX and Mn-SOD activities in the plantaris. This would seem to explain, at least in part, the decreased lipid hydroperoxides in the EFD-Sed group. Conversely, the EFD-Ex group exhibited no change in lipid hydroperoxides compared to the SD-Sed, despite the increases in antioxidant enzyme activities. This is logical, given that the effects of exercise training and dietary fat on antioxidant enzyme activities were not additive. The combined elevated fat and exercise group (EFD-Ex) exhibited GPX and Mn-SOD activities that were not different from the EFD-Sed group. Therefore, the rats receiving the moderate fat diet were unable to adapt to the added stress of the exercise program.

Conclusion

In summary, the incorporation of an increased level of MUFAs into the diet of rats decreased markers of lipid peroxides in the plantaris muscle. Moderate exercise training also reduced indicators of lipid peroxidation. These main effects of monounsaturated dietary fat and exercise training are likely due to independent actions; 1) up-regulation of glutathione and the activities of the antioxidant enzymes GPX and Mn-SOD, and 2) the protective effects of dietary monounsaturated fatty acids. The effects of dietary fat and exercise training on antioxidant enzymes were not additive, as evidenced by the failure of the exercise protocol to increase GPX and Mn-SOD activities above the levels caused by the moderate fat diet alone. This lack of antioxidant up-regulation may explain why lipid hydroperoxides were decreased in the SD-Ex group and in the EFD-Sed group, yet were elevated in the EFD-Ex group. Overall, these

data suggest that short-term, moderate intensity exercise, performed while consuming a moderate fat diet may increase oxidative stress in skeletal muscle. Since it is not uncommon for humans in Western society to consume 40 % of calories from fat, moderate-intensity physical activity may be a significant source of oxidative stress. Further study is required to determine if more intense and/or longer duration exercise training will allow for sufficient antioxidant adaptation to prevent this effect.

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