Postprandial Oxidative Stress: Influence of Sex and Exercise Training Status

RICHARD J. BLOOMER[1], DAVID E. FEREBEE[1], KELSEY H. FISHER-WELLMAN[1], JOHN C. QUINDRY[2], and BRIAN K. SCHILLING[1]

[1]*Department of Health and Sport Sciences, University of Memphis, Memphis, TN; and* [2]*Department of Kinesiology, Auburn University, Auburn, AL*

ABSTRACT

BLOOMER, R. J., D. E. FEREBEE, K. H. FISHER-WELLMAN, J. C. QUINDRY, and B. K. SCHILLING. Postprandial Oxidative Stress: Influence of Sex and Exercise Training Status. *Med. Sci. Sports Exerc.*, Vol. 41, No. 12, pp. 2111–2119, 2009. An individual's sex and exercise training status may influence oxidative stress. No study has compared postprandial oxidative stress in exercise-trained and untrained men and women. **Purpose**: To compare oxidative stress biomarkers and triglycerides (TAG) in 16 trained and 16 untrained men and women after ingestion of a high-fat meal. **Methods**: Blood samples were collected before, and at 1, 2, 4, and 6 h after intake of a high-fat meal and analyzed for Trolox-equivalent antioxidant capacity (TEAC), malondialdehyde, hydrogen peroxide, xanthine oxidase activity, protein carbonyls (PC), and TAG. Area under the curve was calculated for each variable. **Results**: Sex main effects were noted for all variables ($P < 0.01$), except for PC and TEAC ($P > 0.05$), with higher values for men compared with women. A training status main effect was noted for TEAC ($P = 0.02$), with higher values for trained compared with untrained subjects. No interaction effects were noted ($P > 0.05$). Regression analysis indicated that TAG explained the greatest degree of variability for oxidative stress variables, and premeal TAG best predicted the TAG response to feeding ($R^2 = 0.50$). **Conclusions**: With the exception of TEAC, for which higher values were noted for trained compared with untrained subjects, our findings indicate that sex, not exercise training status, influences postprandial oxidative stress. Specifically, women experience a significantly lower oxidative stress response to feeding compared with men. This seems mediated in part by the TAG response to feeding. **Key Words:** TRIGLYCERIDES, REACTIVE OXYGEN SPECIES, SEX, NUTRITION

Oxidative stress is a condition in which an imbalance exists between oxidant and antioxidant levels in a way that oxidant production exceeds antioxidant defenses (3). Such a condition may lead to progressive oxidation of cellular components such as lipids, proteins, and nucleic acids and has been implicated in a wide variety of disease processes (9) as well as in aging (32). The generation of reactive oxygen and nitrogen species (RONS) occurs in part because of normal cellular metabolism (13); however, both physical and environmental stressors can further increase production of RONS.

A naturally occurring oxidative stress can vary in spectrum from a disease-causing process to a beneficial adaptive stimulus as in the cases of atherosclerotic plaque formation and acute physical exercise, respectively. Related to physical stress, acute exercise has been shown to result in oxidative stress as reviewed previously (3,11,26,36). This stimulus is thought to be necessary for the up-regulation of protective endogenous antioxidant defenses (17,18,24). In this way, the generation of RONS seems to be the "signal" needed to allow for such adaptations, which may function to protect cells from future elevations in RONS. Hence, individuals who are exercise-trained may exhibit attenuated oxidative stress after exposure to stressful conditions. This may be one additional positive adaptation associated with regular exercise training, highlighting the importance of regular exercise as a component of a healthy lifestyle.

Aside from exercise training status, it is possible that sex may influence the degree of oxidative stress. In particular, women may be less susceptible to oxidative stress because of the antioxidant properties of estrogen. It seems that estrogens bind estrogen receptors and activate both mitogen-activated protein kinase and nuclear factor κβ signaling pathways to induce an up-regulation in endogenous antioxidants (35). Mitochondria from female rats exhibit higher antioxidant enzyme gene expression compared with male rats, coupled with increased mitochondrial glutathione (4). We recently noted higher total and reduced blood glutathione, as well as vitamin E, in women compared with men (12). Lipid peroxidation (15), protein (20), and DNA oxidation (4) indices have also been reported to be lower in females compared with males. Taken together, these data indicate that women may be less susceptible to oxidative stress than

Address for correspondence: Richard J. Bloomer, Ph.D., Cardiorespiratory/Metabolic Laboratory, 161 Roane Field House, The University of Memphis, Memphis, TN 38152; E-mail: rbloomer@memphis.edu.
Submitted for publication January 2009.
Accepted for publication April 2009.

0195-9131/09/4112-2111/0
MEDICINE & SCIENCE IN SPORTS & EXERCISE®

DOI: 10.1249/MSS.0b013e3181a9e832

men, which may help to explain the increased longevity in women compared with men (35). However, most studies demonstrating lower oxidative stress in women have obtained measures during periods when estrogen levels are high (e.g., ovulation, estrogen replacement therapy). Therefore, it is uncertain whether women are less prone to oxidative stress compared with men during periods of low estrogen (e.g., early follicular phase).

Related to environmental considerations, ingestion of high-fat meals increases oxidative stress (for review, please see [29]). Individuals with frequent daily intake of high (saturated)-fat foods exist in a prolonged postprandial state, which may be a significant risk factor for atherogenesis (5,40). The elevations in blood triglyceride (TAG) levels peak between 2 and 4 h after feeding and typically mirror the increase in oxidative stress biomarkers (2,22,31). Postprandial hypertriglyceridemia is positively correlated with leukocyte superoxide production (1). The increased oxidative stress, coupled with hypertriglyceridemia, has a direct detrimental effect on endothelial function and plays a role in the development of atherosclerotic disease (1,10).

Together with a potentially heightened antioxidant capacity, trained individuals often have lower fasting TAG levels compared with untrained individuals and process TAG more efficiently after high-fat meals (6). This is mediated in part by a reduced chylomicron–TAG half-life and by an increased activity of lipoprotein lipase, the rate-limiting enzyme for serum TAG removal. However, caution should be taken when interpreting these findings because these may also simply be the result of an acute exercise bout (19). Women may have lower fasting TAG than men and may process TAG more efficiently than men (7), leading to lower postprandial hypertriglyceridemia. This finding may be related to serum estrogen (33,37). Because elevated blood TAG are associated with increased oxidative stress, exercise-trained individuals, in particular, women, may experience attenuation in postprandial oxidative stress. To date, no study has examined either the impact of exercise training status or sex on postprandial oxidative stress.

Therefore, the purpose of the present investigation was to compare blood oxidative stress biomarkers, antioxidant capacity, and TAG in exercise-trained and untrained men and women of similar age in response to a high-fat test meal. We hypothesized that because of the potential differences in endogenous antioxidant capacity and TAG processing, exercise-trained subjects would experience an attenuated oxidative stress response after feeding compared with untrained subjects. For these same reasons, we also hypothesized that women would experience an attenuated oxidative stress response compared with men.

METHODS

Subjects. Sixteen exercise-trained (8 men and 8 women) and 16 sedentary individuals (8 men and 8 women) between the ages of 18 and 40 yr participated in this study. Trained individuals were required to have participated in no less than 5 $h \cdot wk^{-1}$ of formal exercise (including aerobic and/or anaerobic) for no less than 1 yr immediately before enrollment. This was documented with each subject by completing a detailed exercise training history in conjunction with personal interviews. Sedentary individuals did not participate in any structured physical activity and did not have a physically demanding job. Persons with lactose intolerance were excluded from recruitment because of the test meal used (described later). Subjects completed a health history and underwent a physical examination before enrollment. Subjects were nonobese (body fat of $\leq$25% for men and $\leq$32% for women), normolipidemic (fasting TAG <150 $mg \cdot dL^{-1}$), and free of major signs and symptoms suggestive of cardiovascular, metabolic, or pulmonary disease as defined by the American College of Sports Medicine (ACSM) (38). Subjects did not use medications (e.g., anti-inflammatory or cardiovascular drugs) or nutritional supplements.

Women reported having normal (28–35 d) menstrual cycles and did not use oral contraceptives. After the screening procedure, subjects were scheduled for testing and given detailed instructions and data forms related to the recording of dietary and physical activity data during the 7 d before the test meal. All experimental procedures were performed in accordance with the ethical standards of the Helsinki Declaration and were approved by the university's human subjects review board. Subjects provided both verbal and written consent before participating. Participant characteristics are presented in Table 1.

Test meal. All subjects were tested in the morning after an overnight fast (minimum of 10 h). Women were tested on days 1–5 of their menstrual cycle to minimize any potential antioxidant effect of estrogen because circulating estrogen levels are lowest during this time. Serum estrogen levels were confirmed by biochemical analysis. A premeal blood sample was collected after a 10-min period of quiet rest. Subjects were then given 15 min to consume the test meal, which consisted of a milk shake made with a combination of whole milk, ice cream, and whipping cream. The milk shake consisted of 1.2 g of fat and carbohydrate and 0.25 g of protein per kilogram body mass, totaling approximately 71 kJ (17 kcal) per kilogram body mass. Subjects remained in the laboratory during the 6-h postprandial period and watched movies or studied. No additional meals or calorie-containing beverages were allowed, although water was allowed *ad libitum*.

Blood sampling and biochemistry. For each participant, approximately 20 mL of venous blood was taken from a forearm vein via needle and collection tube before meal (0 h) and at 1, 2, 4, and 6 h after meal. Blood collected in tubes containing ethylenediaminetetraacetic acid was processed immediately, and the plasma was stored in multiple aliquots at $-80°C$ until analysis. Blood collected in tubes with no additive was allowed to clot for 30 min at room temperature and was then processed. The serum was

TABLE 1. Descriptive characteristics of exercise-trained and untrained men and women.

Variable	Trained Men	Trained Women	Untrained Men	Untrained Women
Age (yr)	23 ± 1	23 ± 1	24 ± 2	25 ± 2
Height (cm)*	177 ± 1	165 ± 2	179 ± 3	162 ± 2
Weight (kg)*	83 ± 3	63 ± 4	83 ± 5	57 ± 3
BMI ($kg \cdot m^{-2}$)*	26 ± 1	23 ± 1	26 ± 2	22 ± 1
Body fat (%)*	12 ± 2	22 ± 1	16 ± 2	23 ± 2
Waist/hip*	0.81 ± 0.01	0.70 ± 0.01	0.78 ± 0.01	0.71 ± 0.01
Resting HR (bpm)†	64 ± 2	64 ± 3	66 ± 3	77 ± 4
Resting SBP (mm Hg)*	122 ± 2	110 ± 4	120 ± 5	116 ± 5
Resting DBP (mm Hg)	72 ± 2	70 ± 2	74 ± 4	74 ± 3
Total cholesterol ($mg \cdot dL^{-1}$)	156 ± 18	154 ± 10	170 ± 10	175 ± 13
HDL cholesterol ($mg \cdot dL^{-1}$)*	44 ± 3	56 ± 7	46 ± 4	58 ± 3
LDL cholesterol ($mg \cdot dL^{-1}$)	101 ± 19	89 ± 10	112 ± 11	110 ± 14
Total cholesterol/HDL	3.68 ± 0.56	2.98 ± 0.33	3.87 ± 0.38	3.08 ± 0.29
Glucose ($mg \cdot dL^{-1}$)	85 ± 3	78 ± 3	90 ± 2	87 ± 2
17β-Estradiol ($pg \cdot mL^{-1}$)	41 ± 11	64 ± 17	43 ± 9	57 ± 18

All blood-borne variables presented here represent fasting values.
Values are mean ± SEM.
* Sex main effect ($P < 0.05$).
† Training status main effect ($P < 0.05$). No other statistical differences were noted ($P > 0.05$).
BMI, body mass index; DBP, diastolic blood pressure; SBP, systolic blood pressure.

stored in multiple aliquots at −80°C until analysis. All assays were performed in duplicate and on first thaw.

Serum antioxidant capacity was measured using the Trolox-equivalent antioxidant capacity (TEAC) assay (Sigma Chemical, St. Louis, MO; coefficient of variation (CV) = 5.5%), as previously described (27). Plasma malondialdehyde (MDA) was measured using the method described by Jentzsch et al. (16) (CV = 4.9%). Plasma hydrogen peroxide (H_2O_2), xanthine oxidase (XO) activity, and catalase (CAT) activity were measured using the Amplex Red reagent method (Molecular Probes, Invitrogen Detection Technologies, Eugene, OR). The CV for H_2O_2, CAT, and XO was 4.4%, 4.5%, and 4.1%, respectively. Serum superoxide dismutase (SOD) activity was measured using enzymatic procedures (Cayman Chemical, Ann Arbor, MI; CV = 5.3%). Plasma glutathione peroxidase (GPx) activity was measured using enzymatic procedures (Cayman Chemical), where values for GPx were calculated using the nicotinamide adenine dinucleotide phosphate (NADPH) extinction coefficient and are presented in nanomoles per minute per milliliter, where one unit is defined as the amount of enzyme needed to oxidize 1.0 nmol of NADPH to $NADP^+$ (CV = 6.8%).

Plasma protein carbonyls (PC) were assessed using a dot blot technique per Oxyblot oxidized protein detection kit specifications (Chemicon International, Germany). In brief, plasma protein concentrations were determined from diluted samples using the spectrophotometric Bradford method. Plasma samples were then normalized to 4 μg protein$\cdot \mu L^{-1}$ and derivatized with 2,4-dinitrophenylhydrazine. Twenty microliters of total protein was then loaded onto Bio-Rad nitrocellulose membranes (Bio-Rad Labs, Hercules, CA) using a Topac dot blotter (Topac, Cohasset, MA). Membranes were then sequentially incubated with a blocking solution of 5% nonfat milk, 1° antirabbit anti-DNP (2,4 dinitrophenol) antibody, and 2° antigoat antirabbit immunoglobulin G conjugated to horseradish peroxidase. Blots were then exposed using a Western blotting detection reagent (ECL Plus; GE Healthcare, UK) and exposed using a Kodak Gel Logic 2200 imaging system (Eastman Kodak, Rochester, NY). Sample data were analyzed using Kodak digital software under blinded conditions concluding with a net intensity value proportional to the PC content present. Data are expressed as a percent of premeal baseline values (100%).

Assays for TAG, glucose, total cholesterol, and HDL cholesterol were performed using serum following standard enzymatic procedures as described by the reagent provider (Thermo Electron Clinical Chemistry Waltham, MA). The CV for these assays was <5%. LDL cholesterol was calculated using the Friedwald equation. Serum estradiol (17β-estradiol) was measured using a competitive enzyme immunoassay following procedures provided by the reagent provider (Cayman Chemical; CV = 7.2%). Related to all above assays, only TEAC, MDA, H_2O_2, XO, PC, and TAG were measured before meal and at all times after meal. The other assays were only performed before meal, with values being used for further descriptive characteristics of subjects.

Dietary and physical activity records. All subjects were instructed to maintain their normal diet and physical activity levels and to record these variables during the 7-d period before the test meal day. Each subject was instructed on

TABLE 2. Dietary variables of exercise-trained and untrained men and women.

Variable	Trained Men	Trained Women	Untrained Men	Untrained Women
kcal*	3239 ± 342	2173 ± 219	2102 ± 227	2196 ± 220
kJ*	13,526 ± 1428	9074 ± 915	8778 ± 948	9170 ± 919
Protein (g)*	178 ± 15	98 ± 8	73 ± 6	78 ± 8
Carbohydrate (g)*	416 ± 59	235 ± 38	258 ± 35	263 ± 24
Fat (g)	89 ± 11	77 ± 9	76 ± 6	82 ± 11
Vitamin C (mg)*	251 ± 80	89 ± 14	72 ± 19	71 ± 9
Vitamin E (mg)	12 ± 3	11 ± 3	7 ± 2	11 ± 4
Vitamin A (IU)†	9235 ± 1295	8014 ± 2257	2361 ± 489	4141 ± 1057
Selenium (μg)†	103 ± 21	68 ± 29	34 ± 4	42 ± 8

Values are mean ± SEM.
* Training status × sex interactions were noted for kilocalories/kilojoules, protein, carbohydrate, and vitamin C, with trained men higher than all other groups ($P < 0.05$).
† Training status main effect ($P < 0.05$). No other statistical differences were noted ($P > 0.05$).

TABLE 3. Antioxidant enzyme activity of exercise-trained and untrained men and women.

Variable	Trained Men	Trained Women	Untrained Men	Untrained Women
CAT ($U \cdot mL^{-1}$)	113.5 ± 3.6	114.6 ± 2.3	110.2 ± 2.6	111.2 ± 3.3
SOD ($U \cdot mL^{-1}$)	0.145 ± 0.008	0.149 ± 0.015	0.123 ± 0.009	0.141 ± 0.012
GPx ($nmol \cdot min^{-1} \cdot mL^{-1}$)	149.3 ± 1.2	156.3 ± 2.8	168.7 ± 3.9	154.7 ± 3.1

Values are mean ± SEM. No statistical differences were noted between groups for any above variable ($P > 0.05$).

proper recording including portion sizes. Diet records were analyzed for total calories, macronutrients, and a variety of antioxidant micronutrients (Food Processor SQL, version 9.9; ESHA Research, Salem, OR). Physical activity records were analyzed to determine the amount of activity completed during the week before the test day. Activity records (classified as light, moderate, or hard) were reviewed with each participant upon return to enhance accuracy of reporting and entry. Light activity (e.g., walking the dog) required some effort but maintained normal breathing. Moderate activity (e.g., housework or gardening) was somewhat difficult with an increase in breathing and heart rate. Hard activity (e.g., strenuous manual labor) involved labored breathing and difficulty holding a conversation during the activity. Subjects were given specific instructions to avoid physically stressful tasks (including exercise) during the 24-h period preceding the test meal. This was important to control for any acute effects of physical activity on postprandial oxidative stress as recently demonstrated (22).

Statistical analysis. Area under the curve (AUC) was calculated for each using the trapezoidal method (AUC_G) as described in detail by Pruessner et al. (25). All variables were then analyzed using a 2 (training status) × 2 (sex) ANOVA. Tukey's *post hoc* tests were performed where appropriate. Effect size calculations were performed using Cohen's *D*. Pairwise correlations were performed for all outcome variables. Multiple stepwise forward regression analysis was performed to determine the contributions of the following predictor variables on our oxidative stress biomarkers: TAG, sex, and premeal (fasting) estradiol. Nonsignificant *F* ratios were noted for the potential predictor variables of exercise training status, LDL cholesterol, CAT, SOD, GPx, vitamin C, vitamin E, vitamin A, and selenium using the probabilities to enter and exit of 0.25 and 0.10, respectively. Therefore, these were not considered in the model. An additional regression analysis was performed to determine the contributions of the following predictor variables on the TAG response to feeding: premeal (fasting) TAG, sex, and TEAC. Nonsignificant *F* ratios were noted for the potential predictor variables of exercise training status, estradiol, HDL and LDL cholesterol, CAT, SOD, GPx, vitamin C, vitamin E, vitamin A, and selenium using the probabilities to enter and exit of 0.25 and 0.10, respectively. Therefore, these were not considered in the model. Variables were checked for colinearity before being included in the model. Dietary, physical activity, and descriptive data were also analyzed using a 2 (training status) × 2 (sex) ANOVA. The data are presented as mean ± standard error of the mean. All analyses were performed using JMP statistical software (version 4.0.3; SAS Institute, Cary, NC). Statistical significance was set at $P \leq 0.05$.

RESULTS

Sex differences in anthropometric characteristics were as anticipated ($P < 0.05$; Table 1). It is important to note that serum estradiol concentrations were not statistically different between groups ($P > 0.05$). Regarding dietary intake, several differences were noted ($P < 0.05$; Table 2). In particular, trained men had higher total energy, protein, carbohydrate, and vitamin C intake compared with all other groups ($P < 0.05$). Trained subjects had higher vitamin A and selenium intake compared with untrained subjects ($P < 0.05$). No other group differences were noted for dietary intake ($P > 0.05$). As expected, the total amount of habitual physical exercise performed by trained men (aerobic: 3.6 ± 1.9 yr; 1.1 ± 0.5 $h \cdot wk^{-1}$; anaerobic 7.2 ± 0.6 yr; 6.7 ± 1.3 $h \cdot wk^{-1}$) and trained women (aerobic: 5.7 ± 1.5 yr; 4.9 ± 0.6 $h \cdot wk^{-1}$; anaerobic 5.4 ± 1.5 yr; 1.8 ± 0.3 $h \cdot wk^{-1}$) was greater than for untrained men and women ($P < 0.05$) who reported little to no structured physical activity. The amount of self-reported physical activity during the week before the test meal mimicked that of subjects' habitual exercise regimen.

No statistically detected differences were noted for CAT, SOD, or GPx activity ($P > 0.05$; Table 3). A training status main effect was noted for TEAC, with greater values for trained compared with untrained subjects ($P = 0.02$). No other training status effects were noted ($P > 0.05$). No interactions were noted either ($P > 0.05$). However, sex main effects were noted for MDA, H_2O_2, XO, and TAG ($P < 0.01$) but not for PC ($P > 0.05$). Specifically, AUC

TABLE 4. Postprandial AUC data of exercise-trained and untrained men and women.

Variable	Trained Men	Trained Women	Untrained Men	Untrained Women
TEAC ($mmol \cdot L^{-1} \cdot 6\ h^{-1}$)	4.49 ± 0.23†	4.41 ± 0.27†	3.91 ± 0.26	4.12 ± 0.21
MDA ($\mu mol \cdot L^{-1} \cdot 6\ h^{-1}$)	7.62 ± 1.15	6.26 ± 1.11*	8.75 ± 0.87	6.10 ± 0.85*
H_2O_2 ($\mu mol \cdot L^{-1} \cdot 6\ h^{-1}$)	101.35 ± 17.52	59.45 ± 11.03*	100.75 ± 13.18	71.90 ± 13.60*
XO ($mU \cdot mL^{-1} \cdot 6\ h^{-1}$)	76.62 ± 11.09	52.15 ± 8.61*	78.10 ± 10.50	49.90 ± 7.65*
PC (% of premeal $\cdot 6\ h^{-1}$)	867.08 ± 140.06	783.16 ± 136.43	1085.86 ± 93.48	761.86 ± 191.60
TAG ($mg \cdot dL^{-1} \cdot 6\ h^{-1}$)	567.15 ± 67.00	398.68 ± 43.50*	593.23 ± 88.50	361.50 ± 70.00*

Data are mean ± SEM.

* Denotes sex main effect ($P < 0.01$); women < men for each indicated variable.

† Denotes training status main effect ($P = 0.02$); trained > untrained. No training status × sex interactions noted ($P > 0.05$).

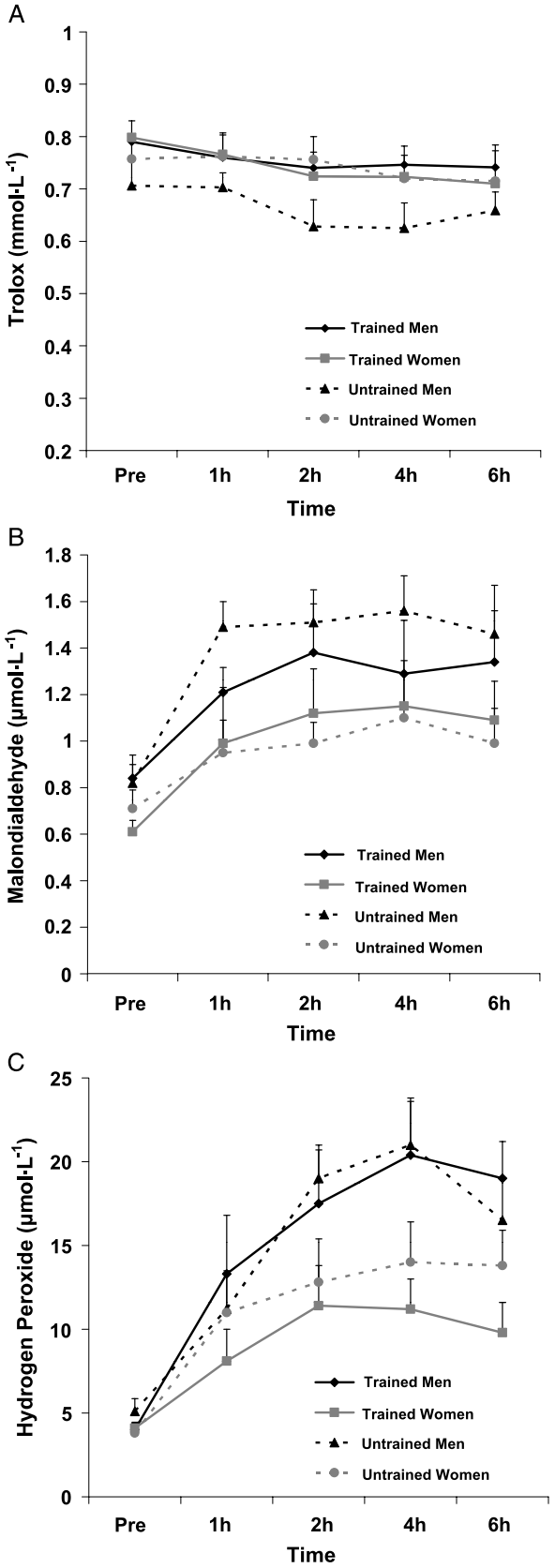

FIGURE 1—Serum TEAC (A), plasma MDA (B), and plasma H_2O_2 (C) before and after intake of a high-fat meal in exercise-trained and untrained men and women.

values were higher for men compared with women. Data are shown in Table 4 and in Figures 1 and 2. A representative sample of the PC dot blots is presented in Figure 3. Effect size calculations between trained and untrained subjects for all variables were small (≤0.18), providing support for our lack of statistical differences in the main model. Regarding comparisons between men and women, effect size calculations for all variables with the exception of TEAC and PC were considered moderate, ranging from 0.43 to 0.68.

Malondialdehyde, H_2O_2, XO, and TAG were all positively correlated with one another (correlation range: 0.45–0.77; $P < 0.00001$ for all). Significant correlations ($P < 0.00001$) were noted between TAG and MDA ($r = 0.55$), H_2O_2 ($r = 0.67$), XO ($r = 0.62$), and PC ($r = 0.18$; $P = 0.02$). Conversely, all of these variables were negatively correlated with TEAC, although the correlations were weak (correlation range: 0.11–0.33; $P < 0.03$ for all).

Regression analysis indicated that TAG contributed to the greatest degree of variability in the response for MDA ($R^2 = 0.30$), H_2O_2 ($R^2 = 0.44$), XO ($R^2 = 0.39$), PC ($R^2 = 0.13$), and TEAC ($R^2 = 0.11$). The addition of sex and estradiol added little to each model (increasing the R^2 only 0.02–0.04). Regression analysis to determine the contributions of premeal (fasting) TAG, sex, and TEAC on the TAG response to feeding noted that premeal (fasting) TAG best predicted the TAG response to feeding ($R^2 = 0.50$). The addition of TEAC ($R^2 = 0.54$) and TEAC + sex ($R^2 = 0.57$) to the model slightly improved its predictive power.

DISCUSSION

Data from the present investigation indicate that sex, but not exercise training status, influences postprandial oxidative stress. These findings are in reference to young men and women, performing primarily aerobic (women) or anaerobic (men) exercise, or no exercise at all. It should be highlighted that the sex effect seems mediated primarily by the TAG response to feeding, which is lower in women compared with men (on the basis of our AUC data) and is strongly related to fasting TAG levels. Hence, individuals with lower fasting TAG values may ultimately experience less oxidative stress after feeding, at least as measured using our chosen biomarkers. The TAG response to feeding contributes to a much greater degree to the variability in postprandial oxidative stress biomarkers than other proposed factors such as estradiol, exercise training status, and antioxidant capacity. Although previous studies have indicated an effect for all of these variables on attenuating oxidative stress in a fasted state, these contribute little to explaining the oxidative stress response to feeding. This is the first study to our knowledge to compare postprandial oxidative stress and TAG data between exercise-trained and untrained men and women. Our data indicate that women are less prone to postprandial oxidative stress compared with men, which may have health implications pertaining to

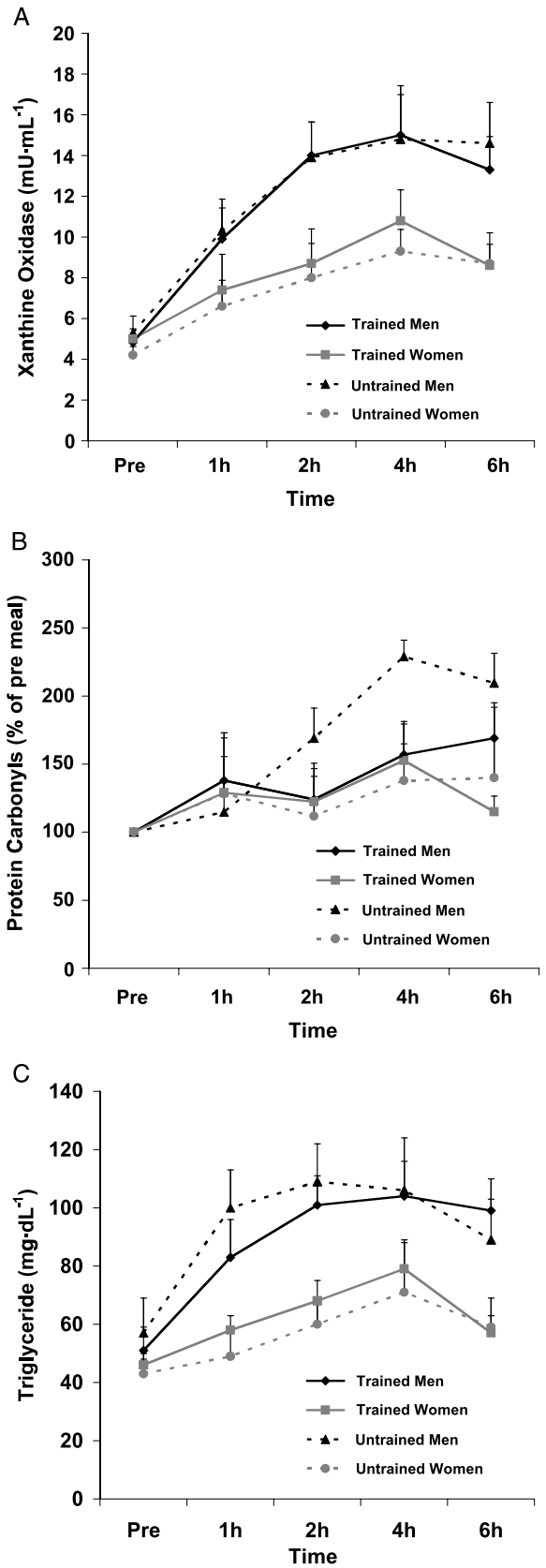

FIGURE 2—Plasma XO activity (A), PC (B), and serum TAG (C) before and after intake of a high-fat meal in exercise-trained and untrained men and women.

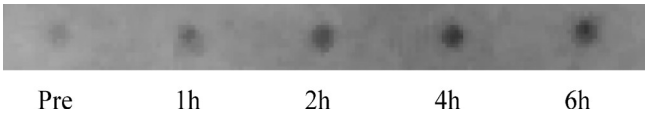

FIGURE 3—Representative sample of PC dot blot before and after intake of a high-fat meal.

the development of oxidative stress-related disease. Future study is needed to investigate this possibility.

It should be noted that although we report an influence of sex on postprandial oxidative stress, it is possible that our results are somewhat confounded by the type of exercise performed by our men and women. That is, women performed predominantly aerobic exercise, whereas men performed predominantly anaerobic exercise. It has recently been reported that anaerobic exercise can lower the postprandial TAG response to high-fat feeding (39) and may have an influence on postprandial oxidative stress. Therefore, it is likely that regular performance of both exercise types may influence postprandial oxidative stress. However, it is possible that aerobic training imparts more of an influence on postprandial oxidative stress than does anaerobic training, which could be one explanation for why women exhibited an attenuated TAG and oxidative stress response to feeding. If this were true, a homogenous sample of aerobically trained men and women may be needed to more specifically test the influence of training status on postprandial oxidative stress. However, it should be understood that sampling a homogenous population of aerobically trained individuals may not be representative of the training habits of most individuals. That is, most people perform a combination of aerobic and anaerobic work. Therefore, such data may not be able to be generalized to the population at large and were our rationale for including men and women who performed a combination of exercise types. It is important to note that our data demonstrating an impact of sex on postprandial oxidative stress are relative to both trained and untrained men and women. Therefore, even if the type of exercise influenced our results related to our trained subjects, we also noted differences in postprandial oxidative stress between untrained men and women.

On the basis of our findings and given the potential confounding effect of exercise type (as discussed above), we reject our hypothesis that exercise-trained subjects would experience an attenuated oxidative stress response after feeding compared with untrained subjects. This is evidenced by statistically insignificant differences being noted between trained and untrained subjects and further supported by our small effect sizes for all variables ($\leq$0.18). The difference in exercise training status between the two groups was demonstrated by an analysis of self-reported physical activity data. Trained men and women performed aerobic and/or anaerobic exercise multiple hours per week and greater amounts of moderate, hard, and total activities during the week leading up to the test day compared with untrained subjects. We believed that this level of training

would have been sufficient to induce adaptations in both blood antioxidant capacity and TAG processing. However, as mentioned above, it is possible that aerobic training may be more influential in relation to these variables. It is also possible that a higher volume and/or intensity of exercise is needed to significantly increase antioxidant enzyme activity and influence postprandial oxidative stress.

Related to antioxidant status, no statistically significant differences were noted in antioxidant enzyme activity between men and women in the present study (Table 3) despite differences in oxidative stress biomarkers. Therefore, we believe that other factors are responsible for the differences in oxidative stress, perhaps antioxidants such as glutathione (4,12) and vitamin E (12), which have been previously been reported to be higher in women compared with men, but are not measured in the present study. Aside from sex effects, a training status effect was noted for TEAC, with trained subjects having higher overall values than untrained subjects. However, as with men and women, no statistically detected differences were noted between trained and untrained subjects for any of the antioxidant enzymes. Perhaps greater differences in these enzymes between trained and untrained subjects would be needed to discern a training status effect for the postprandial oxidative stress biomarkers. Although it is possible that a larger sample size may be needed to detect differences in antioxidant enzyme activity in a sample of young men and women, effect size calculation indicate only small effects for trained compared with untrained subjects in relation to antioxidant enzyme activity ($\leq$0.27). On the basis of these findings, it is more likely that altering the exercise type, volume, and/or intensity would have a greater influence on our findings as opposed to simply increasing our sample size. Of course, it is possible that our sample was unique and failed to respond in terms of increased antioxidant enzyme activity. Additional study is needed to replicate our findings.

Related to the TAG processing, regular endurance training results in increased TAG utilization (6) mediated primarily by training volume (21). As with antioxidant enzyme activity, it is possible that the exercise training performed by our subjects was insufficient in volume and/or intensity to demonstrate a training status effect for TAG. Likewise, it is possible that a greater emphasis on aerobic training may have more favorably influenced our results. Finally, it should be noted that subjects in all groups were young and otherwise healthy, with fasting TAG values <150 $mg \cdot dL^{-1}$, with no group differences noted. It is possible that significant differences would have been noted in TAG response values between trained and untrained subjects if greater disparity was apparent in fasting TAG values on the basis of our findings that premeal (fasting) TAG best predicted the TAG response to feeding ($R^2 = 0.50$).

To avoid any alterations in postprandial TAG and oxidative stress values related to premeal strenuous physical activity (19), subjects in this study were told to abstain from strenuous exercise during the 24-h period immediately preceding the test meal. Upon interview at the time of testing, subjects indicated that they were compliant with this premeal requirement. Although we are confident of this, it should be noted that noncompliance to this important instruction could have influenced our findings.

Women have been noted as having lower fasting TAG than men and may process TAG more efficiently than men (7). Our data agree with these observations because women experienced lower postprandial hypertriglyceridemia compared with men (Fig. 2C). Although this finding may be related to serum estrogen (33,37), our data refute this notion. Serum estradiol, which was low in all groups because of women reporting during the early follicular phase of their menstrual cycle, failed to explain any significant degree of variability in the postprandial TAG data. Rather, fasting TAG explained the majority of the variability in the TAG response to feeding. Lipoprotein lipase activity, the rate-limiting enzyme for serum TAG removal, may be greater in women than in men (30), which may also help to explain our findings.

In the present study, all measures of oxidative stress were strongly related to the serum TAG response to feeding. Postprandial hypertriglyceridemia leads to neutrophilia (34) and is positively correlated with leukocyte superoxide anion production (1). In support of these findings, blood lipids contribute to platelet-derived superoxide anion production, via the activation of phospholipase A2 and NADH/NADPH enzymes (28). Moreover, increased superoxide in the presence of nitric oxide has been shown to generate the toxic reaction product peroxynitrite (8), which has the potential to increase lipid peroxidation and to cause posttranslational modification to proteins (23).

Our data agree with these findings in that the increase in postprandial TAG was related to the increase in XO, as well as the other oxidative stress biomarkers. XO is used as a marker of oxidative stress (14) because this enzyme is a ubiquitous generator of radical species, possibly leading to superoxide radical. Although in aqueous solutions, the superoxide radical is poorly reactive, it can be easily converted into H_2O_2 through the activity of SOD. As with superoxide anion, much of the cytotoxic effects of H_2O_2 are due to its conversion into the hydroxyl radical, with the potential for subsequent damage to various cellular structures, including proteins and lipids. Our data for PC and MDA, in relation to both XO and H_2O_2, support this notion. This increased oxidative stress, coupled with hypertriglyceridemia, has a direct detrimental effect on endothelial function and plays an important role in the development of atherosclerotic disease (1,10). As such, identification of effective methods of attenuating postprandial TAG and subsequent oxidative stress may be associated with a reduction in the risk of oxidative stress-related dysfunction and disease.

In conclusion, we report that sex, but not exercise training status, influences postprandial oxidative stress.

Within this context, the TAG response to feeding, which is lower in women compared with men, seems most influential. Hence, minimizing the TAG response to feeding may be the most important consideration in attenuating postprandial oxidative stress. This may involve drug, nutrient, and/or lifestyle intervention (e.g., dietary and weight loss) aimed at either decreasing fasting TAG and/or reducing the TAG response after feeding. Such adaptations may allow for lower postprandial oxidative stress, which may be associated with lower incidence of oxidative stress-related disease. This has particular relevance for individuals who are prone to an exacerbation in postprandial oxidative stress, such as patients with diabetes and those with atherosclerotic disease.

Funding for this work was provided by The University of Memphis. The authors declare that they have no competing interests. The results of the present study do not constitute endorsement by ACSM.

REFERENCES

1. Bae JH, Bassenge E, Kim KB, et al. Postprandial hypertriglyceridemia impairs endothelial function by enhanced oxidant stress. *Atherosclerosis*. 2001;155(2):517–23.
2. Blendea MC, Bard M, Sowers JR, Winer N. High-fat meal impairs vascular compliance in a subgroup of young healthy subjects. *Metabolism*. 2005;54(10):1337–44.
3. Bloomer RJ, Goldfarb AH. Anaerobic exercise and oxidative stress: a review. *Can J Appl Physiol*. 2004;29(3):245–63.
4. Borras C, Sastre J, Garcia-Sala D, Lloret A, Pallardo FV, Vina J. Mitochondria from females exhibit higher antioxidant gene expression and lower oxidative damage than males. *Free Radic Biol Med*. 2003;34(5):546–52.
5. Ceriello A, Taboga C, Tonutti L, et al. Evidence for an independent and cumulative effect of postprandial hypertriglyceridemia and hyperglycemia on endothelial dysfunction and oxidative stress generation: effects of short- and long-term simvastatin treatment. *Circulation*. 2002;106(10):1211–8.
6. Cohen JC, Noakes TD, Benade AJ. Postprandial lipemia and chylomicron clearance in athletes and in sedentary men. *Am J Clin Nutr*. 1989;49(3):443–7.
7. Couillard C, Bergeron N, Prud'homme D, et al. Gender difference in postprandial lipemia: importance of visceral adipose tissue accumulation. *Arterioscler Thromb Vasc Biol*. 1999;19(10):2448–55.
8. Csont T, Bereczki E, Bencsik P, et al. Hypercholesterolemia increases myocardial oxidative and nitrosative stress thereby leading to cardiac dysfunction in apoB-100 transgenic mice. *Cardiovasc Res*. 2007;76(1):100–9.
9. Dalle-Donne I, Rossi R, Colombo R, Giustarini D, Milzani A. Biomarkers of oxidative damage in human disease. *Clin Chem*. 2006;52(4):601–23.
10. de Koning EJ, Rabelink TJ. Endothelial function in the postprandial state. *Atheroscler Suppl*. 2002;3(1):11–6.
11. Finaud J, Lac G, Filaire E. Oxidative stress: relationship with exercise and training. *Sports Med*. 2006;36(4):327–58.
12. Goldfarb AH, McKenzie MJ, Bloomer RJ. Gender comparisons of exercise-induced oxidative stress: influence of antioxidant supplementation. *Appl Physiol Nutr Metab*. 2007;32(6):1124–31.
13. Halliwell B, Cross CE. Oxygen-derived species: their relation to human disease and environmental stress. *Environ Health Perspect*. 1994;102(Suppl 10):5–12.
14. Hellsten Y. The role of xanthine oxidase in exercise. In: Sen CK, Packer L, Hanninen O, editors. *Handbook of Oxidants and Antioxidants in Exercise*. 1st ed. Amsterdam (the Netherlands): Elsevier; 2000. p. 153–76.
15. Ide T, Tsutsui H, Ohashi N, et al. Greater oxidative stress in healthy young men compared with premenopausal women. *Arterioscler Thromb Vasc Biol*. 2002;22(3):438–42.
16. Jentzsch AM, Bachmann H, Furst P, Biesalski HK. Improved analysis of malondialdehyde in human body fluids. *Free Radic Biol Med*. 1996;20(2):251–6.
17. Ji LL. Exercise-induced modulation of antioxidant defense. *Ann N Y Acad Sci*. 2002;959:82–92.
18. Ji LL, Gomez-Cabrera MC, Vina J. Exercise and hormesis: activation of cellular antioxidant signaling pathway. *Ann N Y Acad Sci*. 2006;1067:425–35.
19. Katsanos CS, Moffatt RJ. Acute effects of premeal versus postmeal exercise on postprandial hypertriglyceridemia. *Clin J Sport Med*. 2004;14(1):33–9.
20. Kayali R, Cakatay U, Tekeli F. Male rats exhibit higher oxidative protein damage than females of the same chronological age. *Mech Ageing Dev*. 2007;128(5–6):365–9.
21. LeMura LM, von Duvillard SP, Andreacci J, Klebez JM, Chelland SA, Russo J. Lipid and lipoprotein profiles, cardiovascular fitness, body composition, and diet during and after resistance, aerobic and combination training in young women. *Eur J Appl Physiol*. 2000;82(5–6):451–8.
22. Mc Clean CM, Laughlin JMc, Burke G, et al. The effect of acute aerobic exercise on pulse wave velocity and oxidative stress following postprandial hypertriglyceridemia in healthy men. *Eur J Appl Physiol*. 2007;100(2):225–34.
23. Pacher P, Schulz R, Liaudet L, Szabo C. Nitrosative stress and pharmacological modulation of heart failure. *Trends Pharmacol Sci*. 2005;26(6):302–10.
24. Powers SK, Ji LL, Leeuwenburgh C. Exercise training-induced alterations in skeletal muscle antioxidant capacity: a brief review. *Med Sci Sports Exerc*. 1999;31(7):987–97.
25. Pruessner JC, Kirschbaum C, Meinlschmid G, Hellhammer DH. Two formulas for computation of the area under the curve represent measures of total hormone concentration versus time-dependent change. *Psychoneuroendocrinology*. 2003;28(7):916–31.
26. Radak Z, Taylor AW, Ohno H, Goto S. Adaptation to exercise-induced oxidative stress: from muscle to brain. *Exerc Immunol Rev*. 2001;7:90–107.
27. Rice-Evans CA. Measurement of total antioxidant activity as a marker of antioxidant status *in vivo*: procedures and limitations. *Free Radic Res*. 2000;33(Suppl):S59–66.
28. Sanguigni V, Pignatelli P, Caccese D, et al. Increased superoxide anion production by platelets in hypercholesterolemic patients. *Thromb Haemost*. 2002;87(5):796–801.
29. Sies H, Stahl W, Sevanian A. Nutritional, dietary and postprandial oxidative stress. *J Nutr*. 2005;135(5):969–72.
30. St-Amand J, Despres JP, Lemieux S, et al. Does lipoprotein or hepatic lipase activity explain the protective lipoprotein profile of premenopausal women? *Metabolism*. 1995;44(4):491–8.
31. Tsai WC, Li YH, Lin CC, Chao TH, Chen JH. Effects of oxidative stress on endothelial function after a high-fat meal. *Clin Sci (Lond)*. 2004;106(3):315–9.
32. Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. *Int J Biochem Cell Biol*. 2007;39(1):44–84.
33. van Beek AP, de Ruijter-Heijstek FC, Erkelens DW, de Bruin TW. Menopause is associated with reduced protection from postprandial lipemia. *Arterioscler Thromb Vasc Biol*. 1999;19(11):2737–41.

34. van Oostrom AJ, Sijmonsma TP, Verseyden C, et al. Postprandial recruitment of neutrophils may contribute to endothelial dysfunction. *J Lipid Res*. 2003;44(3):576–83.
35. Vina J, Sastre J, Pallardo FV, Gambini J, Borras C. Role of mitochondrial oxidative stress to explain the different longevity between genders: protective effect of estrogens. *Free Radic Res*. 2006;40(12):1359–65.
36. Vollaard NB, Shearman JP, Cooper CE. Exercise-induced oxidative stress: myths, realities and physiological relevance. *Sports Med*. 2005;35(12):1045–62.
37. Westerveld HT, Kock LA, van Rijn HJ, Erkelens DW, de Bruin TW. 17β-Estradiol improves postprandial lipid metabolism in postmenopausal women. *J Clin Endocrinol Metab*. 1995;80(1):249–53.
38. Whaley MH, editor. *ACSM's Guidelines for Exercise Testing and Prescription*. 7th ed. Philadelphia, Lippincott Williams & Wilkins; 2005. pp. 205–31.
39. Zafeiridis A, Goloi E, Petridou A, Dipla K, Mougios V, Kellis S. Effects of low- and high-volume resistance exercise on postprandial lipaemia. *Br J Nutr*. 2007;97(3):471–7.
40. Zilversmit DB. Atherogenesis: a postprandial phenomenon. *Circulation*. 1979;60(3):473–85.