Blood Oxidative Stress Biomarkers: Influence of Sex, Exercise Training Status, and Dietary Intake

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ABSTRACT

Background: Sex and lifestyle factors are known to influence the oxidation of protein, lipids, and DNA. Biomarkers such as protein carbonyls (PC), malondialdehyde (MDA), and 8-hydroxydeoxyguanosine (8-OHdG) have been commonly used in an attempt to characterize the oxidative status of human subjects.

Objective: This study compared resting blood oxidative stress biomarkers, in relation to exercise training status and dietary intake, between men and women.

Methods: Exercise-trained and sedentary men and women (with normal menstrual cycles; reporting during the early follicular phase) were recruited from the University of Memphis, Tennessee, campus and surrounding community via recruitment flyers and word of mouth. Participants were categorized by sex and current exercise training status (ie, trained or untrained). Each completed a detailed 5-day food record of all food and drink consumed. Diets were analyzed for kilocalories and macro- and micronutrient (vitamins C, E, A) intake. Venous blood samples were obtained at rest and analyzed for PC, MDA, and 8-OHdG.

Results: In the 131 participants (89 men, of whom 74 were exercise trained and 15 untrained, and 42 women, of whom 22 were exercise trained and 20 untrained; mean [SD] age, 24 [4] years), PC did not differ significantly between trained men and women or between untrained men and women. However, trained participants had significantly lower plasma PC (measured in nmol · mg protein^{-1}) (mean [SEM] 0.0966 [0.0055]) than did untrained participants (0.1036 [0.0098]) ($P < 0.05$). MDA levels (measured in μmol · L^{-1}) were significantly lower in trained women (0.4264 [0.0559]) compared with trained men (0.6959 [0.0593]); in trained men and women combined (0.5621 [0.0566]) compared with untrained men and women combined (0.7397 [0.0718]); and in women combined (0.5665 [0.0611]) compared with men combined (0.7338 [0.0789]) ($P < 0.05$ for all comparisons). No significant differences were noted between any groups for 8-OHdG. Neither PC nor 8-OHdG were correlated to any dietary variable, with the exception of PC and percent of protein in untrained men ($r = 0.552$; $P = 0.033$). MDA was positively correlated to protein intake and negatively correlated to percent of carbohydrate and vitamin C intake, primarily in trained men ($P \leq 0.03$).

Conclusions: In this sample of young healthy adults, oxidative stress was lower in women than in men and in trained compared with untrained individuals, particularly regarding MDA. With the exception of MDA primarily in trained men, dietary intake did not appear to be correlated to biomarkers of oxidative stress. (*Gend Med.* 2008;5:218–228) © 2008 Excerpta Medica Inc.

Key words: free radicals, gender, exercise, nutrition.

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INTRODUCTION

The body's total antioxidant capacity serves to protect cells from excess production of reactive oxygen/nitrogen species (RONS).[1] Antioxidant capacity comprises endogenous (eg, uric acid, superoxide dismutase, catalase, glutathione peroxidase) and exogenous (eg, carotenoids, tocopherols, ascorbate, bioflavonoids) compounds. The exogenous antioxidants are consumed in the diet primarily from fruits and vegetables.[2] Because these exogenous compounds contribute to antioxidant capacity, dietary habits can alter an individual's susceptibility to oxidative damage.

Formation of RONS that exceed the body's antioxidant capacity has been termed *oxidative stress*.[3] RONS are produced in the body as the result of normal cellular metabolism as well as through exposure to a variety of environmental (eg, cigarette smoke, ozone, certain nutrients) and physiological (eg, physical and mental stress) challenges. The precise cellular damage and disease generation that may accompany oxidative stress are related specifically to which macromolecules (nucleic acid, protein, lipid) are targeted by RONS, the frequency and duration of attack, and the tissue-specific antioxidant defenses present.[4] For example, RONS reacting with DNA can produce extensive strand breakage and degradation of deoxyribose, an effect likely due to the formation of hydroxyl radicals.[5] Such changes may induce alterations in nucleotide bases and have the potential to be mutagenic, leading to disease over time. In this regard, the presence of 8-hydroxydeoxyguanosine (8-OHdG), an abnormal intermediate in nucleotide metabolism, is routinely assessed in urine and blood, whereby its presence indicates oxidative DNA damage. Biomarkers of protein oxidation have also been investigated and represent amino acid modifications such as the conversion of phenylalanine residues to o-tyrosine or tyrosine to dityrosine, as well as global modifications such as the conversion to carbonyl derivatives. Aromatic and sulfhydryl-containing residues are particularly susceptible to oxidation, often leading to loss of catalytic or structural function in the affected proteins, rendering them highly susceptible to proteolytic degradation.[6] Furthermore, increased levels of oxidized proteins have been associated with disease (eg, cancer, diabetes, cardiovascular disease).[7]

In addition to DNA and protein oxidation, lipid peroxidation has been established as a major mechanism of cellular injury in humans.[8] Polyunsaturated lipids are especially susceptible to damage in an oxidizing environment and may react to form lipid peroxides. Lipid peroxides further react to form malondialdehyde (MDA), which can be measured in plasma and is routinely used for the purpose of estimating lipid-specific oxidative stress.[9] Although MDA has been suggested to be a practical indicator of oxidative stress for clinical investigation,[10] more specific lipid biomarkers, such as F_2-isoprostanes, have gained popularity in recent years.[11]

To date, a few studies have compared oxidative stress and antioxidant capacity between men and women.[12–15] Some studies have focused on exercise-induced oxidative stress rather than on resting levels,[1,3] and based on a PubMed search (using the terms *gender, sex, oxidative stress, exercise*), only one study has compared oxidative stress between exercise-trained and untrained men and women, reporting no significant sex or training status differences with regard to F_2-isoprostanes.[16] Moreover, most studies have been conducted using older populations,[6,12] often involving postmenopausal women and age-matched men, in an attempt to investigate the potential influence of estrogen on oxidative stress. Considering the available evidence, it appears that both men and women are susceptible to oxidative stress at rest[17] as well as in response to acute exercise.[3] The magnitude of exercise-induced oxidative stress appears similar between men and women, but resting preexercise concentrations of oxidative stress biomarkers may be lower in women, particularly with use of estrogen replacement therapy.[12]

Although acute exercise can stimulate an increase in RONS and subsequent oxidative stress, numerous studies have observed an adaptation in the body's antioxidant defense system as a result of aerobic[18,19] and anaerobic[20,21] exercise training. This upregulation in endogenous antioxidant defense is often associated with lower levels of

oxidative stress biomarkers.[18,22] However, as previously stated, a search of PubMed identified only one study comparing resting oxidative stress biomarkers between exercise-trained and untrained men and women.[16]

Aside from the ability of regular exercise training to enhance endogenous antioxidant defense, exogenous antioxidant intake also influences the degree of oxidative stress.[2,23] Although this has been reported in several studies using antioxidant supplements in both younger and older subjects, studies using whole food as the source of nutrients have primarily included older (aged >45 years) subjects.[24–26] To our knowledge, based on our literature search, no study has conducted correlation analyses between resting oxidative stress biomarkers and dietary nutrients in young, trained and untrained men and women. Therefore, the primary purpose of the present study was to compare resting oxidative stress biomarkers between young, exercise-trained and untrained men and women. A secondary purpose was to determine the relationship between oxidative stress biomarkers and selected dietary nutrients.

METHODS

Participants

Exercise-trained and sedentary men and women (with normal menstrual cycles; reporting during the early follicular phase) were recruited from the University of Memphis campus in Tennessee and surrounding community via recruitment flyers and word of mouth. The participants in this investigation were categorized by sex and current exercise training status (ie, trained or untrained). Trained individuals were those who actively participated in structured exercise for a minimum of 3 hours per week for at least 6 consecutive months prior to participation. All trained participants performed a combination of aerobic and anaerobic (primarily resistance) exercise. Untrained individuals were those who did not actively participate in structured exercise during the 6 months prior to participation and did not have a physically demanding job (eg, manual labor). Subjects were excluded if they self-reported cardiovascular, pulmonary, or metabolic disease on a health history questionnaire, or if they took nutritional supplements (eg, antioxidants) or medications (eg, anti-inflammatory or cardiovascular drugs, estrogen) that could have affected the oxidative stress markers being measured.

During the initial screening visit in the University Cardiorespiratory/Metabolic Laboratory, an exercise questionnaire was administered, which included the type, duration, frequency, and intensity (using the rating of perceived exertion scale) of both aerobic and anaerobic exercise. It also asked how long participants have consistently been engaged in these types of exercise. The answers were reviewed in detail with each subject by a research assistant, and we then computed the total volume of exercise for each by simply adding the number of minutes of exercise performed as well as how long participants had been performing this activity. This assessment was specific to formal "exercise," and general "physical activity" was not included in our total assessment of trained individuals. Participants claiming to be untrained were excluded if they reported any formal exercise or if they had a physically demanding job. At the initial visit, an explanation of dietary records was also performed. All experimental procedures were performed in accordance with the Helsinki Declaration and approved by the University of Memphis Human Subjects Review Board. Participants provided both oral and written consent.

Blood Collection and Analysis

Approximately 1 week after the initial screening visit, participants reported to the laboratory in an 8-hour postabsorptive (ie, fasted) state. Women reported during the early follicular phase of their menstrual cycle (days 1–5; based on self-report) to avoid potential elevations in estrogen, which may have influenced the dependent variables. In addition, women could not be using oral contraceptives at the time of the study. Participants were instructed not to perform any strenuous physical activity during the 48 hours prior to blood collection, because this may have altered the biomarkers of interest. Participants verified compliance to this recommendation. Venous blood samples were collected by a phlebotomist (R.J.B.) via needle and

Vacutainer (Becton-Dickinson and Company, Franklin Lakes, New Jersey) after a 10-minute quiet rest period. Blood used for analysis of protein carbonyls (PC) and MDA was collected into Vacutainer tubes containing EDTA, immediately processed to obtain plasma, and stored in separate aliquots at –80°C until analyzed. Blood used for the analysis of 8-OHdG was collected into Vacutainer tubes containing no additive, allowed to clot at room temperature for 30 minutes, processed for serum, and stored at –80°C until analyzed.

PC was analyzed in plasma using an enzyme-linked immunosorbant assay (ELISA) procedure described by the manufacturer (Zenith Technologies, Dunedin, New Zealand). The intra- and interassay coefficient of variation was 4.6% and 6.4%, respectively. MDA was analyzed in plasma using a commercially available colorimetric assay (Northwest Life Science Specialties, LLC, Vancouver, Washington), using the modified method described by Jentzsch et al.[27] The intra- and interassay coefficient of variation was 4.1% and 5.7%, respectively. 8-OHdG was analyzed in serum using a high-sensitivity ELISA procedure described by the manufacturer (Japan Institute for the Control of Aging, Fukuroi, Japan; Genox Corporation, Baltimore, Maryland). The intra- and interassay coefficient of variation was 3.5% and 4.3%, respectively. To ensure quality control, both high- and low-calibrated controls were used in each ELISA procedure. Assays were performed in duplicate on first thaw.

Dietary Records

All participants completed detailed daily food records in which they were instructed to record all food and drink consumed for the 5 consecutive days before providing a blood sample. This period included 2 weekend days and 3 weekdays, with few exceptions. During this period of recording, participants were reminded via phone, e-mail, or personal contact to continue their normal diets and to record all food and drink consumed. Participants were provided with detailed written instructions (complete with examples) on how to complete the food records, in addition to personal instruction using food models (as necessary) during their initial visit to the laboratory. Participants were provided with 5 copies of a standard food recording form, which included the time food was consumed, the specific food, how food was prepared, the quantity consumed, kilocalories if known, and comments. When records were returned, a research assistant reviewed each entry with the participants to ensure accuracy. Records were analyzed for intake of kilocalories, protein, carbohydrate, fat, vitamin C, vitamin E, and vitamin A using commercially available software (Diet Analysis Plus, version 4.0, ESHA Research, Salem, Oregon). The antioxidant nutrients (vitamins C, E, A) were selected because they have been previously reported to potentially influence oxidative stress biomarkers.[16,28,29]

Statistical Analysis

Participant characteristic (mean [SD]) and dietary data (mean [SEM]) were computed for all groups. Oxidative stress biomarkers were analyzed using a 2 (sex) × 2 (training status) analysis of variance. When appropriate, a Tukey post hoc test was used. Oxidative stress biomarker data are presented as mean (SEM). Pairwise correlations for each oxidative stress biomarker and all dietary variables were computed. Multiple stepwise forward regression analysis was performed to determine the contributions of the following potential predictor variables on our oxidative stress biomarkers: sex, training status, and antioxidant vitamins (C, E, A). Only predictor variables with significant F distributions were considered in the model, using the probabilities of 0.25 and 0.10 to enter and exit, respectively. Variables were checked for colinearity before inclusion in the model. All analyses were performed using JMP statistical software, version 4.0 (SAS Institute Inc., Cary, North Carolina). Statistical significance was set at $P \leq 0.05$.

RESULTS

This investigation included 131 individuals (89 men, of whom 74 were exercise trained and 15 untrained, and 42 women, of whom 22 were exercise trained and 20 untrained). Participants were young (mean age, 24 [4] years), and of nor-

mal body weight and percent body fat, which was expected for trained and untrained men and women. Participant descriptive characteristics and anthropometric measurements are shown in **Table I.**

Dietary intake appeared normal based on sex and training status (**Table II**), with no significant differences noted between groups, except for higher protein intake in trained men ($P < 0.05$).

Protein Carbonyls

PC levels did not differ significantly between trained men and women or between untrained men and women. However, when men and women were combined, mean PC levels (in nmol · mg protein^{-1}) of trained participants (0.0966 [0.0055]) were significantly lower than those of untrained participants (0.1036 [0.0098]) ($P < 0.05$) (**Table III**). No significant correlations were noted for any of the dietary variables and PC, except for untrained men and percent of protein ($r = 0.552$; $P = 0.033$). Significant F distributions were only present for sex and training status when considering predictor variables in the regression model. Sex explained the greatest amount of variability in PC ($R^2 = 0.021$); the addition of training status added little to the model ($R^2 = 0.037$).

Malondialdehyde

As shown in **Table III**, mean MDA levels (in μmol · L^{-1}) were significantly lower in trained women (0.4264 [0.0559]) compared with trained men (0.6959 [0.0593]); in trained men and women combined (0.5621[0.0566]) compared with untrained men and women combined (0.7397 [0.0718]); and in women combined (0.5665 [0.0611]) compared with men combined (0.7338 [0.0789]) ($P < 0.05$ for all comparisons). Significant correlations were noted for certain dietary variables, in particular the macronutrients and vitamin C ($P \leq 0.03$) (**Table IV**). Significant F distributions were only present for sex and vitamin C when considering predictor variables in the regression model. Vitamin C explained the greatest amount of variability in MDA ($R^2 = 0.062$), with the addition of sex increasing the R^2 to 0.138.

8-Hydroxydeoxyguanosine

8-OHdG levels were not significantly different between any groups (**Table III**). No significant correlations were noted between the dietary variables and 8-OHdG (data not shown). No significant F distributions were noted when considering predictor variables in the regression model for 8-OHdG.

Table I. Descriptive characteristics of exercise-trained and untrained men and women. Data are presented as mean (SD).

	Trained		Untrained	
Characteristic	Men (n = 74)	Women (n = 22)	Men (n = 15)	Women (n = 20)
Age, y	24 (3)	23 (3)	24 (5)	22 (7)
Height, cm	177 (5)*	164 (5)	178 (6)*	165 (4)
Weight, kg	83 (10)*	61 (8)	84 (8)*	64 (10)
BMI, kg · m^{-2}	26.5 (3)	22.7 (2)	26.5 (3)	23.5 (3)
Body fat, %	12 (4)†	16 (5)‡	18 (5)	25 (7)
Hours · wk^{-1} total exercise	5 (2)§	4 (2)§	NA	NA
Hours · wk^{-1} aerobic exercise	2 (2)§	2 (2)§	NA	NA
Hours · wk^{-1} anaerobic exercise	3 (2)§	2 (2)§	NA	NA
Years exercising	4 (2)§	4 (2)§	NA	NA

BMI = body mass index; NA= not available.
*$P < 0.05$ versus trained and untrained women.
†$P < 0.05$ versus untrained men and women.
‡$P < 0.05$ versus untrained women.
§$P < 0.05$ for all exercise data values for trained versus untrained participants. No differences were noted between trained men and trained women.

Table II. Dietary data from exercise-trained and untrained men and women. Data are presented as mean (SEM).

	Trained		Untrained	
Variable	Men (n = 74)	Women (n = 22)	Men (n = 15)	Women (n = 20)
Kilocalories	2696 (90)	1810 (164)	1967 (129)	1876 (166)
Protein, g	141 (7)*	81 (7)	68 (5)	64 (5)
Protein, %	22 (1)	17 (1)	14 (1)	14 (1)
Carbohydrate, g	323 (12)	234 (21)	251 (17)	242 (22)
Carbohydrate, %	47 (1)	49 (3)	53 (3)	52 (1)
Fat, g	94 (4)	66 (6)	72 (7)	71 (8)
Fat, %	31 (1)	31 (1)	33 (2)	34 (1)
Vitamin C, mg	132 (15)	146 (48)	45 (10)	73 (10)
Vitamin E, mg	10 (1)	6 (1)	4 (1)	6 (1)
Vitamin A, RE	1110 (63)	880 (133)	539 (147)	832 (125)

RE = retinol equivalent.
*$P < 0.05$ versus all other groups.

DISCUSSION

The present study was designed to compare resting oxidative stress biomarkers between young, exercised-trained and untrained men and women, and to determine the relationship between these biomarkers and selected dietary nutrients. We utilized a subject population comprised of young (aged 18–30 years), healthy individuals, most of whom had normal body weight (body mass index [BMI] <25 kg · m^{-2}) and normal body fat (men <20%, women <30%). Participants also confirmed consumption of their regular diet over the reporting period.

Our data indicate that for both PC and MDA, values were significantly lower in trained compared with untrained participants. These results are in agreement with previous reports[30–32] and were not surprising, based on findings of an upregulation in the body's antioxidant defense system as an adaptation to regular exercise training.[19,33] In response to repeated increases in RONS production via acute exercise bouts, the body adapts to counteract the effects of the exercise stress in an attempt to prepare for future RONS attack. This is evidenced by previous reports of an

Table III. Resting blood oxidative stress biomarkers of exercise-trained and untrained men and women. Data are presented as mean (SEM).

	Trained			Untrained			Combined	
Variable	Men (n = 74)	Women (n = 22)	Combined (n = 96)	Men (n = 15)	Women (n = 20)	Combined (n = 35)	Men (n = 89)	Women (n = 42)
Protein carbonyls, nmol · mg protein^{-1}	0.0948 (0.0055)	0.0985 (0.0056)	0.0966 (0.0055)*	0.0965 (0.0088)	0.1106 (0.0112)	0.1036 (0.0098)	0.0957 (0.0072)	0.1045 (0.0135)
MDA, μmol · L^{-1}	0.6959 (0.0593)†	0.4264 (0.0559)	0.5621 (0.0566)*	0.7734 (0.0988)	0.7082 (0.0466)	0.7397 (0.0718)*	0.7338 (0.0789)‡	0.5665 (0.0611)
8-OHdG, ng · mL^{-1}	5.5272 (0.3752)	4.9622 (0.5609)	5.2448 (0.4681)	6.5782 (0.7874)	4.9316 (0.4187)	5.7607 (0.6157)	6.0590 (0.5795)	4.9510 (0.4882)

MDA = malondialdehyde; 8-OHdG = 8-hydroxydeoxyguanosine.
*$P < 0.05$ versus untrained combined men and women.
†$P < 0.05$ versus trained women.
‡$P < 0.05$ versus women combined.

Table IV. Correlation matrix for malondialdehyde and dietary variables of exercise-trained and untrained men and women.

	Trained			Untrained			Combined	
Variable	Men (n = 74)	Women (n = 22)	Combined (n = 96)	Men (n = 15)	Women (n = 20)	Combined (n = 35)	Men (n = 89)	Women (n = 42)
Kilocalories	0.08	0.04	0.21	–0.23	0.17	–0.04	0.01	0.15
Protein, g	0.43*	0.07	0.48*	0.08	0.05	0.07	0.30*	–0.17
Protein, %	0.53*	0.12	0.50*	0.28	–0.30	0.07	0.41*	–0.34*
Carbohydrate, g	–0.24	0.09	–0.05	–0.03	0.26	0.09	–0.23	0.17
Carbohydrate, %	–0.45*	0.19	–0.33*	0.21	0.45	0.26	–0.28*	0.22
Fat, g	0.13	–0.02	0.24*	–0.15	0.04	–0.07	0.06	0.13
Fat, %	0.13	–0.04	0.10	0.02	–0.35	–0.11	0.11	0.03
Vitamin C, mg	–0.37*	0.26	–0.25*	0.14	0.02	0.04	–0.33*	–0.14
Vitamin E, mg	–0.26	–0.24	–0.14	0.16	–0.04	0.02	–0.24	0.02
Vitamin A, RE	–0.21	0.07	–0.11	0	0.17	0.02	–0.19	0.04

RE = retinol equivalent.
*$P \leq 0.03$.

increase in antioxidant enzymes and glutathione,[19] as well as lower levels of resting and exercise-induced oxidative stress in trained compared with untrained individuals.[34]

We also found significantly lower levels of MDA in women than in men, which is in agreement with previous studies measuring MDA.[9,35,36] Although lower resting MDA in women may be due in part to higher estrogen concentration, which is known to possess antioxidant properties in vitro[37] and is involved in the upregulation of endogenous antioxidants,[38] we do not believe this was a major factor influencing our findings. This is supported by research by Chung et al,[39] who noted no difference in oxidative stress during the follicular and luteal phases of the menstrual cycle, despite estrogen being significantly higher during the luteal phase. Although we did not measure estrogen levels in our participants, we controlled the time of blood sample collection by having women report during the first few days of their menstrual cycle. Therefore, estrogen levels in the women were likely very low, which was also reported by Chung et al in women tested during this phase of the menstrual cycle. At such low levels, it is unlikely that estrogen had any significant effect on our biomarkers; however, the lack of direct measurement of circulating estrogen was a limitation of our study design.

It is possible that factors aside from estrogen, such as higher levels of vitamin E and glutathione, may help to explain the lower oxidative stress in women compared with men, a finding that our research group has recently reported.[35] But caution should be used when considering this possibility, because we did not actually measure these variables in the present study. Our failure to directly measure various markers of antioxidant status within the present design is another limitation of this study. Future research within this area should consider inclusion of biomarkers specific to antioxidant capacity. These may include enzymatic activity (eg, superoxide dismutase, catalase, glutathione peroxidase) and glutathione status, as well as global markers of antioxidant status such as the Trolox-equivalent antioxidant capacity assay, the oxygen radical absorbance capacity assay, the ferric reducing ability of plasma assay, and the total radical-trapping antioxidant parameter assay. These additional biomarkers may provide a more detailed analysis of the oxidative status of trained and untrained men and women.

In the present study, we found no significant difference between sex and training status for 8-OHdG. This could potentially be explained by our subject population (ie, all participants were young, healthy individuals), and therefore, resting levels of 8-OHdG were relatively low (normal

serum range, 5–8 ng · mL^{-1}; Japan Institute for the Control of Aging or Genox Corporation). It is possible that DNA is better protected against oxidative stress than are lipids and proteins, perhaps because DNA can undergo rapid repair once oxidized.[40]

Our findings extend the previous work of Watson et al,[16] who reported no significant sex or exercise training status differences with regard to F_2-isoprostanes in a sample of young (aged 18–35 years) subjects. It is possible that the conflicting results between the present study (as well as others that agree with our findings[31,32]) and those of Watson's group could be due to the greater specificity of the F_2-isoprostane assay compared with that of MDA or other previously used biomarkers, such as thiobarbituric acid reactive substances or expired pentane. Aside from the chosen biomarker of lipid peroxidation, both the present study and that of Watson's group were similar in that all subjects were young (aged 18–35 years), exercise-trained (≥3 hours per week) or untrained nonsmokers who did not use antioxidant supplements. Although the Watson et al study used only 1 biomarker of oxidative stress (F_2-isoprostanes), these investigators included several markers of antioxidant status, noting higher α-tocopherol and β-carotene in trained compared with untrained subjects (when men and women were combined). Lastly, although these investigators measured the subjects' 4-day dietary intake, dietary variables were not included in any correlation analysis with either the blood oxidative stress or antioxidant biomarkers. We believe that in addition to including lipid, protein, and DNA biomarkers of oxidative stress, which were measured in the present study, our work further expands on Watson et al's research by including the correlation analysis with these biomarkers and dietary variables.

Dietary intake has been noted to have an influence on the degree of oxidative stress,[2] but previous studies investigating the effects of dietary intake on markers of oxidative stress have utilized subject populations who were more prone to oxidative damage because of increased age and presence of disease. Under these circumstances, Thompson et al[25] found that altering dietary intake to favor ingestion of antioxidant rich fruits and vegetables reduced oxidative stress in human subjects. However, the results were not uniform, with significant changes only occurring in those subjects whose baseline oxidative stress levels were above the median (subjects were stratified based on resting oxidative stress levels), suggesting that the antioxidant effects of dietary nutrients may be greater in individuals already possessing elevated basal levels of oxidative stress. This appears logical, because a currently undefined basal level of oxidative stress is believed to be necessary for proper physiological function, and any reduction beyond that threshold may not be conducive to optimal health.[17] Thompson et al[26] also reported a significant reduction versus baseline in 8-iso-PGF2α (a marker of lipid peroxidation) in women after ingestion of a high fruit and vegetable diet ($P < 0.05$). Again, reductions were dependent on the subject's baseline values of oxidative stress, which may have been elevated due to the mean age (48 years) and overweight status (median BMI = 26.6 kg · m^{-2}) of the population, before diet intervention.

We noted no significant correlations between dietary nutrients and PC (except for untrained men and percent of protein) or 8-OHdG. It is possible that our participants, because they were young, healthy, and of normal weight, presented with low levels of oxidative stress. Therefore, dietary intake had little influence on these measured biomarkers. Our findings are in agreement with Moller et al,[41] who observed no change in DNA oxidation after dietary intervention in young (aged 20–36 years), normal-weight men and women. It is interesting to note, for example, that despite vitamin C intake being well below the recommended intake in our sample of untrained men, this was not correlated with any of our oxidative stress biomarkers, nor were the biomarkers significantly elevated in these men. Therefore, based on our current work, it appears that young, otherwise healthy individuals present with relatively low levels of oxidative stress biomarkers, which may not be significantly influenced by a "less than adequate" dietary intake. This also underscores the complexity of the antioxidant/oxidant system and indicates the need for multi-

ple analytical measures to be included within research designs of in vivo oxidative stress, to aid in a full understanding of the findings.

We did note that MDA was significantly positively correlated to protein intake and negatively correlated to both percent of carbohydrate and vitamin C intake, primarily in trained men (**Table IV**). However, despite being statistically significant, these correlations were relatively weak. It is possible that we noted statistical significance in our trained men because of the larger sample size in this group, in comparison to the other 3 groups. It should be noted that other than the significant correlations previously discussed, no other correlations were statistically significant. Therefore, we are doubtful that the addition of more samples within the other groups would have led to additional findings of significant correlations. A sample size calculation was not performed a priori, which is a limitation of this work.

Although somewhat controversial within the scientific community,[42] protein consumption beyond the recommended dietary intake is common in trained individuals, to meet the body's heightened demand for this macronutrient as a result of regular exercise training. It is possible that our participants' excess protein intake could have led to an increase in lipid peroxidation, because increased superoxide production has been reported after consumption of a high-protein meal.[43] However, further research is needed before conclusions can be inferred.

Vitamin C was negatively correlated to MDA, suggesting that higher intake of vitamin C may better protect against lipid peroxidation. The correlations appear to be sex specific, suggesting that perhaps diet may have more of an effect on men than on women in relation to oxidative stress. This could partially be explained by the higher resting MDA levels observed in men compared with women. Consistent with other findings, if oxidative stress levels are already low, which is the case with young and healthy women, additional antioxidant nutrient intake may not significantly influence oxidative stress biomarkers.[28,41]

CONCLUSIONS

The results of the present study indicate that in these young, healthy individuals: 1) MDA was lower in women compared with men; and 2) PC and MDA were lower in trained compared with untrained individuals. In addition, with the exception of MDA, primarily in trained men, dietary intake did not appear to be correlated to the biomarkers of oxidative stress. Future research may consider using longer periods of dietary reporting, in addition to more biomarkers of oxidative stress and antioxidant capacity, in an attempt to best characterize the oxidative status of exercise-trained men and women with regard to their habitual nutrient intake.

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