Exercise and Mononuclear Cell DNA Damage: The Effects of Antioxidant Supplementation

G.W. Davison, C.M. Hughes, and R.A. Bell

The purpose of this investigation was to determine the effects of antioxidant supplementation on DNA damage following exercise. Fourteen subjects were randomly assigned to one of two groups and required to ingest either antioxidants (400 mg α-lipoic acid, 200 mg co-enzyme Q10, 12 mg manganese, 600 mg vitamin C, 800 mg N-acetyl cysteine, 400 µg selenium, and 400 IU α-tocopherol per day) or placebos for 7 d. Exercise increased DNA damage, PS, FRAP, and LDH (P < 0.05), but not selectively between groups. LDH and PS concentration decreased 1 h post-exercise (P < 0.05), while LH concentration decreased 1 h post-exercise in the antioxidant group only (P < 0.05). The antioxidant group had a higher concentration of LH (P < 0.05), perhaps due to a selective difference between groups post-exercise (P < 0.05). The main findings of this investigation demonstrate that exhaustive aerobic exercise induces DNA damage, while antioxidant supplementation does not protect against damage.

Key Words: free radicals, Comet assay, oxidative stress

It is widely accepted that an increase in oxygen consumption during exercise is associated with an increase in the formation of free radical species (25). “Free radicals” are described as highly reactive, reduced metabolites of oxygen that possess one or more unpaired electrons (39). A number of metabolic mechanisms of free radical formation during exercise have been identified, and include mitochondrial electron leakage, activation of xanthine oxidase, substrate autoxidation (21), intracellular calcium overload (20), and activation of NADPH oxidase (40). The activation of some of these mechanisms is thought to be largely dependent on the intensity of the exercise challenge. For example, it is suggested that due to an approximate 200-fold increase in O2 flux in skeletal muscle tissue at maximal exercise (22), there is a possibility that electrons might leak through rapidly respiring mitochondria and lead to the formation of free radical species such as quinone and superoxide (33).

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Research examining the effects of exercise on free radical production usually focus on the by-products of lipid and protein damage to quantify the extent of oxidative stress (11, 1). Recently, however, research has concentrated on deoxyribonucleic acid (DNA) as a marker of biochemical oxidation (17, 27), perhaps partially due to the fact that oxidative damage to DNA could be involved in the development of cancer (26). DNA modification and damage is mainly determined by using two different methodological techniques. 8-hydroxy-2′-deoxyguanosine (8OHdG) has been established as a biomarker of oxidative DNA modification (35, 36); however, this marker is regarded as indirect and relatively unreliable (41). The determination of DNA damage using the “Comet assay” or single cell gel-electrophoresis (SCG) assay is a relatively new, more sensitive, and stable method used to measure single or double DNA strand breaks in peripheral leukocytes (8). Indeed, various exercise studies have used the Comet assay technique to assess DNA damage in leukocytes with a profound degree of reproducibility (17, 30, 42). Leukocytes are the most popular systemic cell type used to determine DNA damage as the reticulocytes have no DNA. Moreover, the assessment of DNA damage in leukocytes is a relatively uncomplicated process, due to its ease of access, as opposed to the determination of DNA damage in tissues such as muscle, where biopsies can be painful and distressing for subjects.

It has been postulated that DNA damage caused by exercise and free radicals might be ameliorated by the actions of a variety of antioxidants (16, 40). The role of an antioxidant is to reduce the severity of damage to DNA and other molecules through the formation of a less dynamic radical or via the inhibition of a progressive cascading reaction (13). Few studies have used antioxidant supplements to examine whether DNA damage is attenuated following exercise, and of these studies most have shown a positive effect using a single antioxidant such as vitamin E (16, 38) or β-carotene (40). While supplementing with a single antioxidant and examining DNA damage following exercise could be beneficial, it might also be important to ingest a mixture of antioxidants due to their synergistic action. Indeed, Mastaloudis et al. (27) have recently shown that women are protected against DNA damage following exercise when vitamin C and E are ingested together. Therefore, the purpose of the present study is to ascertain whether DNA damage occurs in human peripheral blood mononuclear cells (PBMCs) following exhaustive aerobic exercise and, more importantly, to determine whether a novel mixture of antioxidants protect DNA from damage following exercise.

Materials and Methods

Subject Characteristics

Fourteen (n = 14) apparently healthy, male subjects provided written informed consent before participating in this study. See Table 1 for subject characteristics. Subjects had no physician-diagnosed diseases or ailments assessed by a medical history questionnaire. All were non-smokers and free from any form of antioxidant supplementation (e.g., vitamin C, vitamin E, or carotenoids) or other supplement use (e.g., creatine). The local medical research ethics committee (University of Ulster) granted approval for this study.
Experimental Design

Each subject attended the human performance laboratory at the University of Ulster precisely 1 wk before exercise to be familiar with the incremental testing protocol. Subjects were randomly assigned in a double-blind fashion to one of two treatment groups, placebo (n = 7) or antioxidant (n = 7). Each subject was required to ingest 4 capsules per day for 7 d of either the placebo or antioxidant cocktail (Cultech Limited, Wales, UK). In total, the antioxidant cocktail group ingested 400 mg alpha-lipoic acid, 200 mg co-enzyme Q10, 12 mg manganese, 600 mg vitamin C, 800 mg N-acetyl cysteine, 400 μg selenium, and 400 IU of natural vitamin E per day. Subjects were instructed to refrain from exercise and alcohol for 48 h before experimental testing and to maintain their usual dietary pattern. Dietary composition in the 72 h before exercise testing was recorded by means of a food diary and assessed using a commercially available nutritional assessment package (Balanced Diet, version 1.12). In addition, a pre-supplementation blood sample was obtained to determine the difference in antioxidant capacity of the plasma between groups.

Experimental Testing

On arrival at the laboratory, subject body mass and stature were determined according to standard procedures. All subjects were subsequently required to complete a treadmill test to volitional exhaustion. The protocol was specifically designed to be progressive and incremental to elicit VO$_{2\text{max}}$. Treadmill speed was set at 10 km/h with a 1% rise in elevation at 1-min intervals until volitional fatigue. Validation of

Table 1  Subject Characteristics and Post-Exercise Data for Antioxidant and Placebo Groups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Antioxidant (n = 7)</th>
<th>Placebo (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>22.3 ± 4.2</td>
<td>22.3 ± 4.1</td>
</tr>
<tr>
<td>Stature (cm)</td>
<td>177.5 ± 6.7</td>
<td>181.0 ± 6.5</td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td>74.5 ± 10.1</td>
<td>80.7 ± 12.5</td>
</tr>
<tr>
<td>VO$_{2\text{max}}$ (mL kg$^{-1}$ min$^{-1}$)</td>
<td>58.93 ± 6.45</td>
<td>54.36 ± 8.31</td>
</tr>
<tr>
<td>HR$_{\text{max}}$ (b/min)</td>
<td>194 ± 7.77</td>
<td>187 ± 7.77</td>
</tr>
<tr>
<td>Time to exhaustion (min)</td>
<td>12.18 ± 1.5</td>
<td>10.43 ± 2.5</td>
</tr>
<tr>
<td>Estimated total calorie expenditure (kcal)</td>
<td>215 + 26</td>
<td>193 + 27</td>
</tr>
</tbody>
</table>

Note. All values are means ± standard deviation. VO$_{2\text{max}}$ (mL kg$^{-1}$ min$^{-1}$), maximum oxygen uptake per minute; HR$_{\text{max}}$, maximal heart rate; kcal, kilocalories.
VO_{2\text{max}} \text{ was obtained if the respiratory exchange ratio (RER) was greater than 1.15 arbitrary units at the termination of test, had a plateau in the oxygen uptake/exercise intensity relationship (< 2 mL \cdot kg^{-1} \cdot \text{min}^{-1}), and a heart rate value to within 10 beats/min of age-predicted maximum (220 – age). Throughout the test, breath-by-breath oxygen uptake was recorded using an on-line gas analysis system (Quinton Instrument, Seattle, WA), in conjunction with heart rate via a portable heart rate short-angled telemetry device (Polar sport tester, Kempele, Finland). Tests were carried out in the morning following a standardized 12 h overnight fast.

Blood Sampling

Venous blood was collected from a prominent forearm vein in a supine position at pre-supplementation, pre-exercise, immediately post-exercise, and 1 h post-exercise using the Vacutainer system (Becton-Dickinson, Oxford, UK). Whole blood was drawn into either 4 mL di-potassium ethylene diamine tetra-acetic acid (EDTA) or 6 mL serum separation tubes (SST). EDTA and SST tubes (once clotted) were centrifuged (Heraeus Sepatech Centrifuge 17RS, Osterode/Harz, Germany) at 3000 rpm (RCF = 996 g) for 10 min at 4°C. Aliquots of plasma and serum [for lactate dehydrogenase (LDH), ferric reducing antioxidant power (FRAP), lipid hydroperoxides (LH), and protein sulphydryls (PS)] were stored for a maximum of 6 wk at –80°C. DNA damage in PBMCs using the Comet assay was examined on the day of each test.

Biochemical Indices

Deoxyribonucleic Acid (DNA). DNA damage was measured in human PBMCs using a modified Comet assay (29). PBMC separation from whole blood samples was performed using Histopaque-1077 (Sigma-Aldrich, St. Louis, MO). Briefly, 3.0 mL of whole blood was layered on to 3.0 mLs of Histopaque and centrifuged for 30 min (3500 rpm, RCF = 1356 g at room temperature). The opaque mononuclear cell layer was aspirated and washed in PBS. The Comet assay was carried out on the prepared cells. Fully frosted slides (Richards Supply Co. Ltd., London) were covered with 100 μl of 0.5% Normal Melting Point Agarose (Sigma-Aldrich) and allowed to solidify under a coverslip. Next, 50 μl of cells (1 × 10^6) were mixed with 50 μl of Low Melting Point Agarose and allowed to solidify. The cover slips were removed and the slides placed in lysis buffer (2.5M NaCl, 100 mM NaEDTA, 10 mM Tris, 1% Triton X, and 10% DMSO, pH 10), for 1 h at 4°C. The slides were removed from the lysis solution and placed in a horizontal electrophoresis unit containing electrophoresis buffer (300 mM NaOH, 1 mM EDTA, pH 12.5) for 20 min, to allow the DNA to unwind. Electrophoresis (2V, 300 mA) was then performed for 20 min at room temperature. During this process, broken strands of DNA move through the agarose and are deposited to one side, forming the comet “tail.” The slides were removed from the electrophoresis tank and washed with neutralizing solution (0.4 M Tris, pH 7). The slides were then stained with 50 μl of ethidium bromide and covered with a coverslip. All steps were carried out under yellow light to prevent further DNA damage. A random sample of 50 cells from each slide was analyzed by image analysis using a Hewlett-Packard VGA monitor and Fenestra Comet software program (version 2.2). Observations were
made at magnification 400x using an epifluorescent microscope (Olympus BH2). DNA damage was quantified using tail moment (percentage of DNA in tail × tail length). Mean and standard deviation of the tail moment were calculated from each sample.

**Lipid Hydroperoxides (LH).** Serum LH was measured spectrophotometrically using the method of Wolff (43). Briefly, this ferrous iron/xylenol orange (FOX) assay quantifies the susceptibility to iron-induced LH formation in blood. The presence of iron ions in the assay protocol might therefore, yield slightly higher LH values compared with other methods.

**Lactate Dehydrogenase (LDH) and Ferric Reducing Antioxidant Power (FRAP).** LDH and FRAP were measured on an automated Hitachi 912 analyzer (Roche Diagnostics, Lewes, UK). Spectrophotometric readings were recorded at 340 nm and completed in duplicate to ensure reliability and reproducibility (4). In contrast to other tests of total antioxidant power, the FRAP assay is a direct test of “total antioxidant power.” The FRAP assay uses antioxidants as reductants in a redox-linked colorimetric method, employing an easily reduced oxidant present in stoichiometric excess (4). The FRAP assay was chosen as it provides an individual measure of total antioxidant activity in biological fluids.

**Protein Sulphydryls (PS).** PS were measured on a Cobas Fara analyser (Roche Diagnostics) using the method of Koster et al. (23) with minor modification. Briefly, reagent one was made by dissolving 13.97 g of dipotassium hydrogen phosphate and potassium dihydrogen phosphate in distilled water at a pH of 7.4 to make a 0.1 M phosphate buffer solution. For the second reagent, 79.26 mg of 5,5'-dithio-bis-2 nitrobenzoic acid (DTNB), was added to 100 mL of 0.1 M phosphate buffer.

**Packed Cell Volume (PCV) and Hemoglobin (Hb).** PCV and Hb concentration was measured on whole blood to correct for acute-exercise induced plasma volume shifts (corrected for all time periods) using the equations of Dill and Costill (12). PCV (percent) was measured using the standard microcapillary reader technique, and corrected by 1.5% for plasma trapped within erythrocytes (9). Hemoglobin (g/dL) was measured using a β-hemoglobin photometer (Hemocue Ltd., Angelholm, Sweden).

**Statistical Analysis**

A power of the test calculation was performed using the equations of Altman (2). Statistical analysis was performed using the SPSS social statistics software package, (version 11.0, SPSS, Inc., Surrey, UK). Data were analyzed using parametric statistics following mathematical confirmation of a normal distribution by repeated Kolmogorov-Smirnov tests. Physical characteristics, dietary profiles, estimated calorie expenditure, exercise time, respiratory and cardiovascular post-exercise data were analyzed using independent sample *t*-tests. Resting and exercise data were analyzed using a two-way split plot \([A \times (B)]\) mixed analysis of variance (ANOVA) which incorporated one between (group: placebo and antioxidant) and one within (state: pre-exercise vs. post-exercise vs. 1 h post-exercise) subjects factor. Following a significant interaction effect (state × group), within subject factors were analyzed using Bonferroni-corrected paired sample *t*-tests. Between subject differences were analyzed using a one-way ANOVA with a posteriori Tukey Honestly Significant
DNA Damage and Antioxidant Supplementation

Difference test. The two-way ANOVA can provide main effects for state and group and interaction effects for state × group. A main effect for state indicates a difference ($P < 0.05$) between rest vs. exercise vs. 1 h post-exercise (pooled antioxidant and placebo values), a main effect for group indicates a difference ($P < 0.05$) between antioxidant and placebo groups (pooled rest, exercise, and 1 h post-exercise values), and an interaction effect (state × group) indicates a difference ($P < 0.05$) within or between groups as a function of state. The alpha was established at $P < 0.05$ (95% CI) and all values are reported as a mean ± standard deviation.

Results

Dietary Status and Maximal Exercise

There was no significant difference in caloric intake and macronutrient composition between groups. VO$_{2\text{max}}$, exercise time to exhaustion, maximal heart rate, and estimated total calorie expenditure did not differ between groups as observed in Table 1. Oxygen uptake, however, increased as a function of aerobic exercise (pooled antioxidant and placebo data, $P < 0.05$).

Pre-Supplementation

Pre-supplementation FRAP concentration for the antioxidant group was 1115.17 ± 144.85 μmol/L and 1141.29 ± 42.44 μmol/L for the placebo group. Pre-supplementation PS concentration for the antioxidant group was 358.77 ± 16.33 μmol/L and 395.40 ± 31.13 μmol/L for the placebo group. There was no significant difference between groups for any biological variable.

Oxidative Stress and Antioxidants

Figure 1 represents an increase in PBMC DNA damage as a consequence of strenuous exercise (pooled antioxidant and placebo values, 0.34 ± 0.20, 0.68 ± 0.34, 0.73 ± 0.49 for rest, post-exercise, and 1 h post-exercise, respectively, $P < 0.05$). Typical rest and immediately post-exercise Comet images are shown in Figure 2. These images demonstrate an increase in PBMC DNA within the comet tail, indicating an increase in PBMC DNA damage following exercise. PBMC DNA data for the antioxidant and placebo groups are shown in Table 2. This damage was not selectively different between antioxidant and placebo groups (power = 0.27).

Strenuous aerobic exercise increased the venous concentration of all blood biomarkers (pooled antioxidant and placebo data, $P < 0.05$), apart from LH as shown in Table 2. There was a decrease in LDH and PS concentration from exercise to 1 h post-exercise (pooled antioxidant and placebo data, $P < 0.05$), and a selective decrease in LH concentration 1 h post-exercise in the antioxidant group ($P < 0.05$ vs. exercise). The antioxidant group had a higher concentration of LH (pooled rest, exercise and 1 h exercise data, $P < 0.05$ vs. placebo), perhaps due to a selective difference between groups post-exercise ($P < 0.05$ vs. placebo). No selective differences between groups were observed for FRAP (power = 0.1), LDH (power = 0.3), or PS (power = 0.05) concentration.
Figure 1—Oxidation of PBMC DNA over time; main effect for state (pooled antioxidant and placebo values, $P < 0.05$).

![Figure 1](image1)

**Discussion**

Strong experimental evidence indicates that strenuous aerobic exercise can increase by-products of protein and lipid oxidation. Using the Comet assay, which is a sensitive technique for measuring DNA damage in cells (29), the present study demonstrates that exercise can cause PBMC DNA damage, and that supplementation with a cocktail of antioxidants appears to have no effect on DNA damage. Lactate dehydrogenase increased following exercise, which might be the result of sarcolemmal membrane rupture, perhaps indicating the strenuous nature of the exercise. Plasma antioxidant concentration increased following exercise, arguably
Table 2  Biochemical Indices at Rest, Exercise, and 1 h Post Exercise for the Antioxidant and Placebo Groups

<table>
<thead>
<tr>
<th></th>
<th>Antioxidant group (n = 7)</th>
<th>Placebo group (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rest</td>
<td>Exercise</td>
</tr>
<tr>
<td>DNA (tail moment)</td>
<td>0.28 ± 0.17</td>
<td>0.40 ± 0.22</td>
</tr>
<tr>
<td></td>
<td>Main effect for state</td>
<td></td>
</tr>
<tr>
<td>LH (μmol/L)</td>
<td>1.15 ± 0.17</td>
<td>1.23 ± 0.17†</td>
</tr>
<tr>
<td></td>
<td>Main effect for group</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Interaction effect for state × group</td>
<td></td>
</tr>
<tr>
<td>PS (μmol/L)</td>
<td>348 ± 25.4</td>
<td>403.8 ± 20</td>
</tr>
<tr>
<td></td>
<td>Main effects for group and state</td>
<td></td>
</tr>
<tr>
<td>FRAP (μmol/L)</td>
<td>1036 ± 128</td>
<td>1164 ± 173</td>
</tr>
<tr>
<td></td>
<td>Main effect for state</td>
<td></td>
</tr>
<tr>
<td>LDH (IU/L)</td>
<td>341.3 ± 26</td>
<td>483.7 ± 87</td>
</tr>
<tr>
<td></td>
<td>Main effect for state</td>
<td></td>
</tr>
</tbody>
</table>

Note. Values are means ± standard deviation. DNA, deoxyribonucleic acid; LH, lipid hydroperoxides; PS, protein sulphydryls; FRAP, ferric reducing antioxidant power; LDH, lactate dehydrogenase; ‡ Within-group difference as a function of state (P < 0.05). † Between-group difference (P < 0.05).
as a response to an increase in free radical concentration and concomitant host response. Our data corroborate previous findings of the increase in DNA damage as a result of strenuous aerobic exercise. For example, Hartmann et al. (18) observed an increase in DNA damage in PBMCs after a single bout of exhaustive treadmill running, while Tsai et al. (42) used a 42-km marathon race to show a significant degree of DNA damage in peripheral immunocompetent cells. Mastaloudis et al. (27) more recently observed an increase in DNA damage in PBMCs following a 50-km ultramarathon. As in previous studies in the area, we chose to examine DNA damage in circulating leukocytes, although a limitation of this is that it is difficult to determine whether the DNA damage observed is typical of that within skeletal muscle (27). Until a study is completed measuring DNA damage in both myocytes and leukocytes, we can only speculate on the significance of exercise-induced DNA damage and the associated mechanisms involved. The mechanism(s) associated with DNA damage during aerobic exercise is presently unclear and might be multifold (17, 32). Previous work postulates that the observed damage to DNA is associated with an exercise-induced free radical concentration (18, 32, 42).

Oxygen free radicals can be derived via “leakage” from the ubiquinone-cytochrome b region of the mitochondrial electron transport chain during increased oxygen flux brought about by exercise (10), and importantly, Loft et al. (25) have demonstrated that oxidative DNA damage correlates with oxygen consumption in humans. Given that we observed an increase in systemic oxygen flux as a result of exercise, it is tempting to speculate that the primary source of the free radicals is the mitochondria. It is important to consider the limited superoxide diffusibility across the mitochondrial and outer cellular membrane, while the presence of both manganese and copper-zinc superoxide dismutase inside the cell largely precludes a mitochondrial origin of superoxide (11). Superoxide, however, is converted to hydrogen peroxide by superoxide dismutase, which can easily diffuse across sarcolemmal membranes into extracellular compartments (15) where it aids in the generation of other highly toxic free radical species that could have the potential to infiltrate leukocytes to cause damage to DNA. In addition, we have previously reported an increased detection of systemic lipid-derived alkoxyl free radicals using a similar exhaustive aerobic exercise protocol (11), and it might be possible due to their greater half-life, for these particular radicals to also cross leukocyte membranes and induce damage.

An alternative mechanism involves the generation of free radical species in extra-vascular compartments due to myofibril damage with inflammatory cell infiltrations and subsequent activation of circulating phagocytes (42). Superoxide and hypochlorous radicals are produced via this mechanism (31, 32) and could permeate into peripheral lymphocytes leading to modification of DNA (42). In our study, however, the venous concentration of lactate dehydrogenase increased as a function of exercise, suggesting increased sarcolemmal permeability, which of course during exercise predominately rises from sarcolemmal membrane rupture (19).

Moreover, intense maximal aerobic exercise could theoretically render the muscle ischemic, and ischemic tissues might experience increased oxidant concentration on reperfusion via a xanthine oxidase mechanism (14). As catecholamine concentration has also been shown to increase during maximal exercise (28), the
possibility of superoxide generation as a consequence of catecholamine autoxidation cannot be excluded (5).

DNA damage could also occur as a result of the interaction between by-products of lipid peroxidation and deoxynucleosides (6). Although lipid hydroperoxides did not increase significantly following exercise, we cannot exclude the possibility that lipid peroxidation did occur, which might have in part contributed to the overall increase in DNA damage. The increased activity observed in lipid hydroperoxide concentration post-exercise in the antioxidant group partially supports this observation.

Lipid hydroperoxides are considered to be the first major by-products of lipid oxidative metabolism (3), and are formed by the abstraction of a hydrogen atom from a fatty acid side chain, usually by aqueous peroxy radicals (15). Although our data would seem to demonstrate that supplementing with a cocktail of antioxidants can selectively decrease lipid hydroperoxide concentration 1 h post-exercise, one cannot conclude that this combination of antioxidants is effective in attenuating lipid peroxidation, as the drop in lipid hydroperoxides 1 h post-exercise could simply be due to an increase in clearance from the circulation.

Plasma protein sulphhydril content and total antioxidant power of the plasma was shown to increase as a function of exhaustive aerobic exercise. Pincemail et al. (34) have previously demonstrated an increase in α-tocopherol mobilization as a result of intensive exercise, suggesting that the venous rise might be due to a lipolysis-dependent effect. In addition, Rajguru et al. (37) reported an animal study in which the swim exercise to exhaustion increased plasma sulphhydrils, while Child et al. (7) have shown an increase in serum antioxidant capacity following a simulated half-marathon race. Due to the fact that antioxidant mobilization increased in the present study, this would seem to support the notion that the primary cause of the exercise-induced damage to PBMC DNA observed is free radical mediated.

Surprisingly, lipid hydroperoxide concentration was found to be higher following antioxidant supplementation compared to placebo. Following the ingestion of a high dose of multivitamins, Hartmann et al. (16) observed similar damage to DNA, and suggested that the combination of vitamins and trace metals caused an oxidative reaction in vivo. The antioxidant mixture in our study, however, differed from that used by Hartmann et al. (16), and did not contain iron or copper, thus the reason for the observed effect of antioxidant supplementation on lipid oxidation is unclear and warrants further investigation.

Antioxidant supplementation has been associated with a decrease in DNA damage in a number of studies (16, 27, 38, 40). In our study, however, antioxidant supplementation did not selectively inhibit DNA damage induced by the strenuous aerobic exercise. One explanation could be associated with inappropriate amounts of ingested antioxidants, thus rendering the defenses incapable of combating an increase in exercise-induced oxidative stress. Levine et al. (24), however, has demonstrated that leukocyte cells are saturated with ascorbic acid at doses of 200 mg. The ingested dose of ascorbic acid in the present study was 600 mg per day; therefore one would expect leukocyte ascorbic acid saturation and inhibition of DNA damage to occur as a result of antioxidant supplementation. Moreover, Benzie and Strain (4) measured the plasma antioxidant power of 68 apparently healthy adults and report an average population value for FRAP of 1035 μM. Since the mean FRAP concentration was slightly higher for both the antioxidant (1115.17
μM) and placebo (1141.29 μM) groups pre-supplementation, it might have been possible that the intra- and extra-cellular compartments were already saturated with potent antioxidants, thus explaining the lack of a treatment effect. One other potential explanation for the lack of a selective difference for antioxidant supplementation between groups could be due to the consequence of limited statistical power. Retrospective calculation of power (power = 0.27) shows that for a possible interaction effect to occur as a result of antioxidant supplementation, any future study using the same methodological design would need more than double the existing subject number. Although we performed calculations of power before the study began, calculating the power of the test retrospectively highlights the inherent weakness of performing such calculations prospectively.

In conclusion, this study has demonstrated that exhaustive aerobic exercise can induce damage to PBMC DNA, perhaps by an increase in reactive oxygen species generation. The clinical significance of this DNA oxidation brought about by strenuous aerobic exercise is currently obscure and warrants further investigation; it has been suggested, however, that low levels of exercise-induced DNA damage might be beneficial by up-regulating DNA repair enzymes (27). Supplementation with a novel antioxidant combination has no effect on DNA damage and this could be attributable in part to saturated plasma antioxidant levels or insufficient power.

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References