Effects of endurance and high-intensity swimming exercise on the redox status of adolescent male and female swimmers

Athanasios Kabasakalis\textsuperscript{a}, George Tsalis\textsuperscript{a}, Ekaterini Zafrana\textsuperscript{b}, Demetrios Loupos\textsuperscript{a} & Vassilis Mougios\textsuperscript{a}

\textsuperscript{a} Physical Education and Sport Science, Aristotle University of Thessaloniki, Thessaloniki, Greece
\textsuperscript{b} St. Luke’s Hospital, Thessaloniki, Greece

Published online: 10 Jan 2014.


To link to this article: http://dx.doi.org/10.1080/02640414.2013.850595

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Effects of endurance and high-intensity swimming exercise on the redox status of adolescent male and female swimmers

ATHANASIOS KABASAKALIS1, GEORGE TSALIS1, EKATERINI ZAFRANA2, DEMETRIOS LOUPOS1, & VASSILIS MOUGIOS1

1Physical Education and Sport Science, Aristotle University of Thessaloniki, Thessaloniki, Greece and 2St. Luke’s Hospital, Thessaloniki, Greece

(Accepted 28 September 2013)

Abstract
Throughout adolescence, swimmers begin to carry out demanding endurance and high-intensity training sets, the effect of which on redox status is largely unknown. The aim of the present study was to investigate the effects of 2000-m continuous swimming and 6 × 50-m maximal swimming on the redox status of adolescent swimmers. Fifteen male and 15 female swimmers, aged 14–18 years, provided blood samples before, immediately after, 1 h after, and 24 h after each exercise for the determination of redox status parameters. Oxidative damage was short-lived and manifest as increases in 8-hydroxy-2′-deoxyguanosine (8-OHdG) 1 h after high-intensity exercise (39%, \( P < 0.001 \)) and in malondialdehyde immediately after both exercises (65%, \( P < 0.001 \)). Alterations in antioxidant parameters were sustained during recovery: reduced glutathione decreased 24 h post-exercise (11%, \( P = 0.001 \)), uric acid increased gradually after high-intensity exercise (29%, \( P < 0.001 \)) and bilirubin peaked 24 h post-exercise (29%, \( P < 0.001 \)). Males had higher 8-OHdG (49%, \( P = 0.001 \)) and uric acid (29%, \( P < 0.001 \)) concentrations than females. However, females showed higher values of malondialdehyde than males immediately post-exercise (30%, \( P = 0.039 \)), despite lower pre-exercise values. In conclusion, both endurance and high-intensity exercise perturbed the redox balance without inducing prolonged oxidative damage in trained adolescent male and female swimmers. These swimming training trials were not found to be detrimental to the redox homeostasis of adolescents.

Keywords: oxidative stress, swimming, training, 8-hydroxy-2′-deoxyguanosine, malondialdehyde, glutathione

Introduction
Physical exercise can affect the redox status in the human body (Bloomer, 2008). Several sources of free radicals during exercise have been proposed (Powers, Nelson, & Hudson, 2011), while, on the other hand, exercise can upregulate antioxidant mechanisms (Ji, 2008). Although reactive oxygen and nitrogen species (RONS) are useful as regulatory mediators in signalling processes, the implication of excessive RONS production in various diseases (Dröge, 2002) merits concern about the health of exercising humans. Findings regarding the effects of exercise on oxidative stress and antioxidant markers vary, as these effects are modulated by numerous factors. For example, a high training status enhances antioxidant protection (Finaud, Lac, & Filaire, 2006), females are considered to be more protected against oxidative stress than males (Tiidus, 2000), while exercise parameters, such as intensity and duration, affect RONS accumulation (Gomez-Cabrera, Viña, & Ji, 2009). Endurance exercise (mainly running and cycling) and high-intensity exercise (mainly resistance and sprint exercise) have been found to induce oxidative damage, although findings are at times inconsistent or equivocal (Bloomer, 2008; Finaud et al., 2006; Fisher-Wellman & Bloomer, 2009). Thus, new studies varying in exercise setting, sport discipline and training status continue to add valuable information on the topic.

Swimming is a popular sport that requires sophisticated training for aerobic and anaerobic competition events (Maglischo, 2003). Swimming is unique among sports in combining factors such as the simultaneous contribution of arms and legs to propulsion, water immersion, and prone position (Aspenes & Karslen, 2012). Findings indicate increased oxidative damage in response to acute swimming exercise, more so in males than in females (Deminice et al., 2010; Inal, Akyüz, Turgut, & Gets rif, 2001; Sureda, Ferrer, Tauler, Tur, & Pons, 2008; Tauler et al., 2008). However, a
comprehensive view is lacking on the effects of endurance and high-intensity swimming sets used in everyday training on parameters of the redox status.

Adolescence in swimmers usually coincides with the onset of intensified systematic training, which is superimposed on the demands of maturation, rendering this a crucial period in a swimmer’s life (Lynn, 2006; Naughton, Farpour-Lambert, Carlson, Bradney, & Van Praagh, 2000). Research on the response of adolescents’ redox status to demanding exercise is limited. We believe this field is important from a health perspective, especially when it comes to exercise types commonly used in everyday training practice, for the following reason: If exercises performed regularly by adolescent athletes (as are the majority of competitive swimmers) disturb the redox status profoundly, then there should be considerable concern about the athletes’ redox homeostasis. If, however, these exercises elicit limited perturbations to the redox status, then one could dismiss concerns about detrimental oxidative damage caused by training.

Thus, the aim of the present study was to investigate and compare the effects of two widely used swimming training modules, one based on aerobic and another based on anaerobic energy metabolism, on oxidative damage and antioxidant parameters in male and female adolescent swimmers.

Materials and methods

Participants

Fifteen male swimmers, aged 15.4 (0.7) years (mean (μ) and SD (σ) throughout), and 15 female swimmers, aged 15.4 (1.1) years (range 14.3–16.5 and 14.1–17.6 years, respectively), took part in the study voluntarily. All swimmers had training ages of at least 5 years and participated in training daily. The athletes and their parents were informed orally and in writing about the details and potential risks of the experimental procedure, after which they provided written consent. Procedures were in accordance with the Helsinki declaration and were approved by the Institutional Review Board.

Exercise protocols

The swimmers took part in two exercise sessions of similar duration (25.5–29 min), each on a different day. The 2 days were spaced one week apart during the mesocycle of their general preparation. The sessions took place in a heated indoor 50-m swimming pool, 2 days after the swimmers’ last training session. One session included the T-2000 swimming test, which is 2000 m of continuous freestyle swimming at the fastest possible steady pace. This test is used in training practice to estimate aerobic capacity (Maglischo, 2003). The other session included a set of six 50-m maximal freestyle swimming bouts, one every 5 min, with passive recovery in between. The swimmers started each bout with a push-off from the wall inside the pool. This set is one of the most popular for the improvement of anaerobic capacity and, specifically, peak lactate production capacity (Maglischo, 2003). The swimmers executed the two trials in a random and counterbalanced order. Time was recorded with electronic stopwatch. Each trial was preceded by light, 10-min long swimming warm-up.

Dietary control and anthropometric measurements

Before the first session, the participants were asked to record their food intake for 4 days around the session, that is, 2 days before the session, the day of the session and the next day up until the 24-h post-exercise sampling. Then, they were asked to have the exact same food intake around the second session. The participants did not take any nutritional supplements or medication during the study.

Three days before the first session, weight and height were measured to calculate body mass index. In addition, body fat was estimated by measuring 4-terminal bioelectrical impedance through a Bodystat 1500 apparatus (Douglas, UK).

Blood sampling

Four 4-ml blood samples were obtained in each session from an antecubital vein. The first sample was obtained pre-exercise (after warm-up), the second immediately post-exercise, the third 1 h post-exercise and the fourth 24 h post-exercise. Blood was immediately dispensed into an EDTA-containing test tube, which was centrifuged at 1500 g, 4°C, for 2 min. The supernatant plasma was collected and stored in aliquots at −80°C. After removing the leukocytes from the top of the sediment by aspiration, we washed the remaining erythrocytes three times, each time by adding cold saline, mixing well, centrifuging at 3000 g, 4°C, for 3 min and discarding the supernatant. In the end, the washed erythrocytes were stored at −80°C until analysis.

For lactate determination (measured as an index of exercise intensity), 14 μL of capillary blood was obtained from a fingertip immediately before and after the T-2000 test. Likewise, capillary blood was obtained immediately before and at 2, 4, 6 and 8 min after the 6 × 50-m trial so as to record the peak post-exercise value. Blood was mixed immediately with 140 μL of 0.3 mol · L⁻¹ HClO₄ and stored at −80°C until analysis.
**Biochemical analysis**

We determined the following six markers of the redox status: 8-hydroxy-2′-deoxyguanosine (8-OHdG), malondialdehyde, protein carbonyls (PC), reduced glutathione (GSH), uric acid and bilirubin. 8-OHdG is an index of DNA oxidative damage, as it is formed by reaction of the hydroxyl radical with guanine (Valavanidis, Vlachogianni, & Fiotakis, 2009). Malondialdehyde is an index of lipid peroxidation, as it is derived by peroxidation of polyunsaturated fatty acids (Del Rio, Stewart, & Pellegrini, 2005). PCs, an index of protein peroxidation, are produced on amino acid side chains when they are oxidised (Dalle-Donne, Rossi, Giustarini, Milzani, & Colombo, 2003). GSH, a tripeptide with a reducing sulphydryl group on its cysteine residue, is a marker of antioxidant capacity (Pastore, Federici, Bertini, & Piemonte, 2003). Finally, uric acid, a product of purine catabolism, and bilirubin, a product of heme catabolism, are considered antioxidant capacity markers, as they reduce RONS (Glantzounis, Tsimoyannis, Kappas, & Galaris, 2005; Vitek & Schwertner, 2007). All markers were measured in plasma except GSH, which was measured in erythrocytes, as described below.

8-OHdG was measured through an enzyme immunoassay (Cayman, Ann Arbor, MI, USA). The coefficient of variation (CV) of the method was 6.3%. Malondialdehyde was measured spectrophotometrically, according to Gérard-Monnier et al. (1998), with a CV of 7.2%. Total plasma protein was assayed calorimetrically by the use of kits from Spinreact (Girona, Spain), in order to express PC per mg protein. PCs were measured spectrophotometrically according to the method of Reznick and Packer (1994), as modified by Patsoukas et al. (2004), with a CV of 5.5%. Uric acid and bilirubin were measured spectrophotometrically by the use of kits from Spincom (Girona, Spain), with CV of 1.1% and 2.9%, respectively.

For the determination of GSH, an aliquot of the washed erythrocytes was thawed and diluted with an approximately equal volume of distilled water. Haemoglobin was first measured in this erythrocyte lysate with a kit from Spinreact in order to express GSH per g of haemoglobin. GSH was then measured in the remaining lysate spectrophotometrically according to the method of Reddy, Murthy, Krishna, and Prabhakar (2004), except that deproteinisation was achieved by ultrafiltration rather than acid precipitation, since we found that the latter caused extensive oxidation of GSH, as also reported by Rossi et al. (2002). Thus, the lysate was passed through a Microcon filter (Millipore, Billerica, MA, USA), by centrifugation at 14,000 g, 6°C, for 1 h, and GSH was measured in the filtrate. The CV of the method was 1.2%.

Finally, lactate was measured spectrophotometrically in the supernatant after centrifugation of the haemolysates described above at 1500 g for 5 min, according to an enzymic method from Sigma Diagnostics (data sheet of product number L3916, lactic dehydrogenase).

**Statistical analysis**

Descriptive data are reported as means (x) and SD (s). Student’s t-test was used to compare males and females regarding the anthropometric characteristics and performance at each exercise trial. Three-way ANOVA (trial × time × sex) with repeated measures on trial and time, followed by Duncan post-hoc test where justified, was used for the analysis of the biochemical data. Pearson’s correlation analysis was performed for all studied parameters. The level of statistical significance was set at α = 0.05.

**Results**

Anthropometric characteristics of the participants are presented on Table I. Swimming time at the 2000-m bout (in min:s) was 26:30 (1:21) for males and 28:38 (1:48) for females. Mean swimming time at each of the six 50-m bouts was 30.65 (1.79) and 33.76 (2.24) s, respectively. The corresponding swimming velocities were 1.26 (0.06) and 1.17 (0.07) m·s⁻¹ at the endurance trial; and 1.64 (0.09) and 1.49 (0.09) m·s⁻¹ at the high-intensity trial, males being faster than females in both trials (P < 0.001).

Blood lactate results are presented in Table II. We found significant main effects of trial (P < 0.001), time (P < 0.001) and sex (P = 0.020), as well as a significant interaction of trial and time (P < 0.001). Combined with the results of the post-hoc test, as marked on the Table, these findings show that lactate increased with exercise, was higher after the 6 × 50-m compared to the 2000-m trial, and was higher in males than females post-exercise.

Data on the oxidative damage and antioxidant parameters are presented in Tables III and IV, respectively. Main effects of time and sex and an interaction of trial and time were found for 8-OHdG (P < 0.001, Table I. Anthropometric characteristics (x and s) of the swimmers.

<table>
<thead>
<tr>
<th></th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>67.1 (5.8)</td>
<td>58.6 (4.7)</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.75 (0.07)</td>
<td>1.66 (0.05)</td>
</tr>
<tr>
<td>BMI (kg · m⁻²)</td>
<td>22.1 (2.0)</td>
<td>21.2 (1.2)</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>7.2 (2.3)</td>
<td>17.9 (4.1)</td>
</tr>
</tbody>
</table>

Notes: BMI, body mass index; *significantly different from males (P ≤ 0.001).
P = 0.001 and P < 0.001, respectively). Malondialdehyde exhibited a main effect of time (P < 0.001) and an interaction of time and sex (P = 0.019). For GSH, a main effect of time was found (P = 0.003). Main effects of trial, time and sex, and an interaction of trial and time were found for uric acid (P < 0.001). Finally, bilirubin exhibited a main effect of time (P < 0.001) and an interaction of trial and time (P = 0.039).

According to the most important results of the post-hoc test performed on the aforementioned redox status markers, 8-OHdG was higher immediately after than 24 h after the 2000-m trial (P = 0.009), also being higher 1 h after the 6 × 50-m trial than pre-, post- and 24 h post-exercise (P < 0.001) and higher 1 h after the 6 × 50-m trial compared to the 2000-m trial (P = 0.044). Immediately after exercise, malondialdehyde was higher than pre-, 1 h post- and 24 h post-exercise (P < 0.001, P = 0.003 and P < 0.001, respectively), while the 1 h post-exercise value was higher than baseline (P = 0.007). Additionally, malondialdehyde in females was higher than in males immediately after exercise (P = 0.039). GSH 24 h post-exercise was lower than at baseline (P = 0.002) and 1 h post-exercise (P = 0.025). Uric acid was higher 1 h and 24 h after the 6 × 50-m trial compared to pre-exercise and compared to the corresponding values after the 2000-m trial (P < 0.001). Bilirubin

### Table II. Blood lactate concentration (x and s) pre- and post-exercise in the two trials for male (M) and female (F) swimmers.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Trial</th>
<th>Sex</th>
<th>Pre</th>
<th>Immediately post</th>
<th>1 h post</th>
<th>24 h post</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactate (mmol · L⁻¹)</td>
<td>2000 m</td>
<td>M</td>
<td>3.3 (1.2)</td>
<td>6.1 (1.6)ᵃᵇᶜ</td>
<td>11.1 (2.7)ᵈ</td>
<td>9.9 (2.7)ᵃᵇᶜ</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>2.4 (0.9)</td>
<td>5.4 (1.0)ᵃᵇᶜ</td>
<td>8.3 (2.0)ᵈ</td>
<td>7.0 (2.7)ᵃᵇᶜ</td>
</tr>
<tr>
<td></td>
<td>6 × 50 m</td>
<td>M</td>
<td>3.3 (1.1)</td>
<td>18.7 (3.1)ᵃᵇ</td>
<td>15.2 (7.5)ᵃᵇ</td>
<td>10.6 (3.6)ᶜ</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>2.6 (0.9)</td>
<td>16.0 (4.3)ᵇ</td>
<td>9.6 (4.2)ᵃᵇ</td>
<td>6.9 (3.0)ᶜ</td>
</tr>
</tbody>
</table>

Notes: ˢSignificantly different from post-exercise; ˢSignificantly different from pre-exercise; ˢSignificantly different from 6 × 50 m according to Duncan pairwise tests (P ≤ 0.020).

### Table III. Oxidative damage parameters (x and s) pre- and post-exercise in the two trials for male (M) and female (F) adolescent swimmers.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Trial</th>
<th>Sex</th>
<th>Pre</th>
<th>Immediately post</th>
<th>1 h post</th>
<th>24 h post</th>
</tr>
</thead>
<tbody>
<tr>
<td>8-OHdG (ng · mL⁻¹)</td>
<td>2000 m</td>
<td>M</td>
<td>10.6 (3.5)</td>
<td>11.9 (2.9)ᵃᵈ</td>
<td>11.1 (2.7)ᵈ</td>
<td>9.9 (2.7)ᵃᵇᶜ</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>7.1 (2.4)</td>
<td>9.0 (3.1)ᵃᵈ</td>
<td>8.3 (2.0)ᵈ</td>
<td>7.0 (2.7)ᵃᵇᶜ</td>
</tr>
<tr>
<td></td>
<td>6 × 50 m</td>
<td>M</td>
<td>11.3 (3.2)</td>
<td>11.1 (3.2)</td>
<td>15.2 (7.5)ᵃᵇ</td>
<td>10.6 (3.6)ᶜ</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>6.6 (2.6)</td>
<td>7.2 (3.3)</td>
<td>9.6 (4.2)ᵃᵇ</td>
<td>6.9 (3.0)ᶜ</td>
</tr>
<tr>
<td>MDA (nmol · mL⁻¹)</td>
<td>2000 m</td>
<td>M</td>
<td>0.49 (0.33)</td>
<td>0.67 (0.42)ᵃᵇᶜ</td>
<td>0.65 (0.26)</td>
<td>0.55 (0.50)b</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>0.44 (0.28)</td>
<td>0.88 (0.52)ᵃ</td>
<td>0.57 (0.34)ᵃᵇ</td>
<td>0.49 (0.30)b</td>
</tr>
<tr>
<td></td>
<td>6 × 50 m</td>
<td>M</td>
<td>0.57 (0.16)</td>
<td>0.74 (0.32)ᵃᵃ</td>
<td>0.70 (0.23)</td>
<td>0.53 (0.23)b</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>0.46 (0.19)</td>
<td>0.96 (0.73)ᵃ</td>
<td>0.71 (0.73)ᵃᵇ</td>
<td>0.55 (0.24)b</td>
</tr>
<tr>
<td>PC (nmol · mg⁻¹ protein)</td>
<td>2000 m</td>
<td>M</td>
<td>0.52 (0.15)</td>
<td>0.45 (0.14)</td>
<td>0.53 (0.17)</td>
<td>0.49 (0.15)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>0.50 (0.15)</td>
<td>0.48 (0.14)</td>
<td>0.47 (0.11)</td>
<td>0.51 (0.14)</td>
</tr>
<tr>
<td></td>
<td>6 × 50 m</td>
<td>M</td>
<td>0.52 (0.16)</td>
<td>0.52 (0.14)</td>
<td>0.51 (0.16)</td>
<td>0.52 (0.15)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>0.49 (0.13)</td>
<td>0.48 (0.13)</td>
<td>0.49 (0.17)</td>
<td>0.53 (0.16)</td>
</tr>
</tbody>
</table>

Notes: 8-OHdG, 8-hydroxy-2′-deoxyguanosine; MDA, malondialdehyde; PC, protein carbonyls; ˢSignificantly different from pre-exercise; ˢSignificantly different from post-exercise; ˢSignificantly different from 6 × 50 m time point; ˢSignificantly different from females (P < 0.05).

### Table IV. Antioxidant parameters (x and s) pre- and post-exercise in the two trials for male (M) and female (F) adolescent swimmers.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Trial</th>
<th>Sex</th>
<th>Pre</th>
<th>Immediately post</th>
<th>1 h post</th>
<th>24 h post</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSH (μmol · g⁻¹ Hb)</td>
<td>2000 m</td>
<td>M</td>
<td>6.51 (3.20)</td>
<td>5.31 (1.02)</td>
<td>6.01 (2.10)</td>
<td>5.03 (1.10)ᵃᵇᶜ</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>5.51 (1.09)</td>
<td>5.32 (0.88)</td>
<td>5.56 (1.10)</td>
<td>5.37 (0.77)ᵃᵇᶜ</td>
</tr>
<tr>
<td></td>
<td>6 × 50 m</td>
<td>M</td>
<td>5.63 (1.52)</td>
<td>5.75 (1.22)</td>
<td>6.05 (1.98)</td>
<td>5.35 (1.30)ᵃᵇᶜ</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>5.87 (1.67)</td>
<td>6.14 (2.13)</td>
<td>5.42 (0.81)</td>
<td>5.29 (0.79)ᵃᵇᶜ</td>
</tr>
<tr>
<td>Uric acid (mmol · L⁻¹)</td>
<td>2000 m</td>
<td>M</td>
<td>0.36 (0.07)</td>
<td>0.37 (0.06)</td>
<td>0.36 (0.05)ᵈ</td>
<td>0.38 (0.08)ᵈ</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>0.29 (0.08)</td>
<td>0.29 (0.06)</td>
<td>0.29 (0.05)ᵈ</td>
<td>0.29 (0.09)ᵈ</td>
</tr>
<tr>
<td></td>
<td>6 × 50 m</td>
<td>M</td>
<td>0.37 (0.07)</td>
<td>0.40 (0.07)ⁿ</td>
<td>0.48 (0.11)ᵃᵇ</td>
<td>0.47 (0.11)ᵃᵇ</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>0.28 (0.09)</td>
<td>0.33 (0.10)ⁿ</td>
<td>0.34 (0.10)ᵃᵇ</td>
<td>0.36 (0.09)ᵃᵇ</td>
</tr>
<tr>
<td>Bilirubin (mmol · L⁻¹)</td>
<td>2000 m</td>
<td>M</td>
<td>24.8 (11.4)</td>
<td>21.5 (8.7)ᵈ</td>
<td>17.7 (7.8)</td>
<td>27.2 (15.3)ᵃᵈ</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>19.9 (13.6)</td>
<td>18.8 (11.5)ᵈ</td>
<td>16.2 (6.9)</td>
<td>21.3 (16.0)ᵃᵈ</td>
</tr>
<tr>
<td></td>
<td>6 × 50 m</td>
<td>M</td>
<td>23.7 (10.3)</td>
<td>29.3 (14.8)</td>
<td>18.3 (10.1)ᵇ</td>
<td>36.1 (18.6)ᵇᵃᵇᶜ</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>21.4 (15.6)</td>
<td>25.6 (21.4)</td>
<td>15.4 (12.5)ᵇ</td>
<td>30.7 (17.5)ᵇᵃᵇᶜ</td>
</tr>
</tbody>
</table>

Notes: GSH, reduced glutathione; Hb, haemoglobin; ˢSignificantly different from pre-exercise; ˢSignificantly different from post-exercise; ˢSignificantly different from 1 h post-exercise; ˢSignificantly different from the respective 6 × 50-m time point (P < 0.05).
was higher 24 h after the 6 × 50-m trial compared to pre-, immediately post- and 1 h post-exercise (P < 0.001, P = 0.037 and P < 0.001, respectively) and higher than the corresponding value after the 2000-m trial (P = 0.045).

Several correlations were found between the studied parameters, but they were either weak or sparse and not consistent across trials, time points or sexes (data not shown).

Discussion

In the present study, we examined the effects of two swimming training trials (2000-m continuous swimming at the fastest possible steady pace and 6 × 50-m maximal swimming every 5 min) on the redox status of adolescent male and female swimmers. These trials are used to enhance and evaluate the oxygen and the lactate energy systems, respectively, and are widely used in the training routine of competitive swimmers (Maglischo, 2003). The study of exercise modalities used in the daily practice of adolescent athletes is important, as it is reasonable to assume that such modalities play a bigger role in the athletes' homeostasis than exercises used mainly in laboratory settings to assess an athlete's status.

The blood lactate values after the 2000-m trial indicate that energy production was based mainly on aerobic metabolism (Wells, Selvadurai, & Tein, 2009). The high values found after the 6 × 50-m trial confirm the major contribution of anaerobic metabolism to energy production (Maglischo, 2003). The effect of lactate produced during exercise on the redox status is rather unclear, as, on the one hand, it has been found to act as an antioxidant (Groussard et al., 2000), while, on the other, the drop in pH accompanying lactate production has been reported to increase oxidative stress (Bailey et al., 2004).

Oxidative damage after the swimming sessions was evident in DNA and lipids, but not in proteins. However, the damage observed was transient. As far as DNA damage is concerned, 8-OHdG peaked immediately after the 2000-m trial and 1 h after the 6 × 50-m trial, and was back at baseline 24 h after both exercises. Other studies have indicated a stability of 8-OHdG values from pre- to post-exercise in participants of different training status and in various exercise modalities (Bloomer, Fry, Falvo, & Moore, 2007; Bloomer, Goldfarb, & McKenzie, 2006; Bloomer, Goldfarb, Wideman, McKenzie, & Consitt, 2005; Inoue, Mu, Sumikawa, Adachi, & Okochi, 1993), as well as in adolescent athletes (Hamurcu, Saritas, Baskol, & Akpinar, 2010). DNA repair mechanisms probably contribute partially to the stability of 8-OHdG concentrations (Radák, Kumagai, Nakamoto, & Goto, 2007). To our knowledge, the only study (besides ours) showing an increase in 8-OHdG is that by Tsakiris, Parthimos, Parthimos, Tsakiris, and Schulpis (2006), in the serum of adolescents after a 90-min basketball training session. Regarding lipid peroxidation, malondialdehyde peaked immediately after both exercises and then gradually returned to baseline. Mixed results on malondialdehyde kinetics after exercise have been found in studies encompassing miscellaneous exercise settings (Alessio et al., 2000; Bloomer et al., 2005; Bloomer, Falvo, et al., 2006; Fatouros et al., 2010; Muñoz et al., 2010). On the basis of the finding of the present and the aforementioned studies, the post-exercise malondialdehyde kinetics seems to depend on exercise type and parameters.

With reference to protein peroxidation, the absence of changes in the plasma concentration of PC may indicate either a low sensitivity of this marker to the exercise protocols utilised or an adequate antioxidant protection of proteins in trained swimmers. The PC concentration has been found to remain unchanged after anaerobic exercise (Bloomer, Falvo, et al., 2006) and even after extremely demanding exercise bouts like ultramarathon swimming (Kabasakalis et al., 2011) and ultramarathon running (Quindry et al., 2008). However, other studies have found PC values to increase after exercise (Bloomer, Davis, Consitt, & Wideman, 2007; Hudson et al., 2008; Kyparos, Salonikidis, Nikolaidis, & Kouretas, 2007; Kyparos, Vrabs, Nikolaidis, Riganas, & Kouretas, 2009; Nikolaidis, Kyparos, et al., 2007) or have produce mixed results depending on type of exercise (Alessio et al., 2000; Bloomer et al., 2005). Therefore, the effect of exercise on the circulating PC concentration remains unclear.

Coming to the antioxidant parameters, the decrease in erythrocyte GSH 24 h post-exercise is possibly due to an increased consumption of this important antioxidant during recovery in defence against an exercise-induced increase in RONS such as hydrogen peroxide (Camera & Piccardo, 2002). In studies where GSH has been measured in either whole blood or erythrocytes after endurance or high-intensity exercise, it has been found to either remain stable (Camus et al., 1994; Tauler et al., 2006) or, in most cases, decrease (Bloomer et al., 2005; Dufaux, Heine, Kothe, Prinz, & Rost, 1997; Elokda, Shields, & Nielsen, 2005; Goldfarb, Patrick, Bryer, & You, 2005; Laaksonen et al., 1999; Michailidis et al., 2007; Steinberg, Delliaux, & Jammes, 2006). The latter findings probably underpin the implication of GSH in reinstating redox homeostasis post-exercise. Delayed decreases in GSH have been found in other studies too, as in those by Lee et al. (2002) and Nikolaidis, Paschalis, et al. (2007).

The 6 × 50-m trial caused an increase in plasma uric acid. This is probably due to the increased...
breakdown of adenine during intense interval exercise (Mougios, 2006). The increased concentration of uric acid raises the possibility of increased RONS production (Nies & Simon, 2007), to the extent that xanthine oxidase overwhelmed xanthine dehydrogenase. On the other hand, the increased concentration of uric acid may have contributed to the antioxidant defence during recovery (Hellsten, Sjödin, Richter, & Bangsbo, 1998), as it possesses important antioxidant properties (Glantzouinis et al., 2005). Increased uric acid is a common finding after anaerobic (Demini et al., 2011; Groussard et al., 2003) and aerobic exercise (Aguiló et al., 2005; Briviba et al., 2005; Chevion et al., 2003; Neubauer, König, Kern, Nics, & Wagner, 2008), although no change was found in the present study after endurance exercise. There is evidence that exercise intensity is a more important determinant of the uric acid response than total work output (Green & Fraser, 1988).

The delayed peak found in the bilirubin concentration 24 h post-exercise (mainly after the 6 × 50-m trial) is in agreement with findings by other researchers (Neubauer et al., 2010; Paschal et al., 2007; Theodorou et al., 2010). Bilirubin has been found not only to increase (Benitez et al., 2002; Caimi, Canino, Amodeo, Montana, & Presti, 2009; Chevion et al., 2003; Rokitzki et al., 1994; Skenderi et al., 2008) but also to remain unchanged (Briviba et al., 2005; Tauler et al., 2003) after aerobic exercise. Being a product of haem catabolism, bilirubin can increase after exercise as a result of haemolysis (Skenderi et al., 2008). Naturally, significant haemolysis can occur in sports involving repeated foot striking on the ground, as in running. However, according to Shaskey and Green (2000), haemolysis can occur during exercise because of other factors as well. Indeed, data indicate haemolysis after endurance swimming (Selby & Eichner, 1986). In our study, however, the biphasic response of bilirubin to exercise (that is, decrease after 1 h and increase after 24 h) renders it difficult to suggest any change in haemolytic rate. In any event, the increased bilirubin concentration during recovery should be acknowledged as contributing notably to the antioxidant defence (Vitek & Schwertner, 2007).

The findings that the 8-OHdG concentration returned to baseline 1 h after the 2000-m trial and 24 h after the 6 × 50-m trial and that the malondialdehyde concentration was back at baseline throughout recovery indicate that exercise-induced oxidative damage to DNA and lipids was rather short-lived in the trained male and female adolescent swimmers of this study. The short duration of oxidative damage could be attributed to either a low potency of the swimming trials to induce prolonged oxidative stress or training adaptations of antioxidant mechanisms already acquired by the athletes (Finaud et al., 2006). In addition, it should be taken into account that, during swimming, core body temperature is altered only modestly and returns promptly to normal levels after exercise because of the thermoregulatory capacity of water (Holmér & Bergh, 1974). This may prevent a major perturbation of the redox status, as body temperature can affect the redox status parameters (Mestre-Alfaro et al., 2012). The findings of the present study could support the hypothesis of hormesis, according to which exercise induces a transient oxidative stress that provokes positive adaptations of the antioxidant capacity in the long run (Radak, Chung, & Goto, 2008). In our view, swimming training should not be regarded as oxidatively damaging. Therefore, in terms of redox status, swimming training could be safely recommended and executed, as long as it is well planned and the redox status is frequently monitored. Safe training and regular monitoring are essential for preserving athletes’ health, as detrimental perturbations of the redox status are associated with serious health consequences (Dröge, 2002). Moreover, athletes should avoid such perturbations to prevent adverse effects on performance (Reid, 2008).

As far as the comparison of endurance and high-intensity exercise is concerned, the higher values of 8-OHdG after the 6 × 50-m trial compared to the 2000-m trial possibly reflect the importance of exercise intensity in determining the magnitude of DNA damage. Conversely, the absence of differences between exercises with regard to malondialdehyde could mean that lipid peroxidation is independent of exercise parameters, at least in exercises within the same sport discipline. Likewise, in a study by Hoffman et al. (2007) that included low- and high-intensity squatting, malondialdehyde increased with exercise independent of intensity. The PC and GSH responses to the two exercises were also not different, although Gohil, Viguie, Stanley, Brooks, and Packer (1988) and Nikolaidis et al. (2006) found different GSH levels according to duration and/or intensity after exercises other than swimming. By contrast, the plasma uric acid and bilirubin responses differed between trials, as the former increased only after 6 × 50-m trial, while the latter increased more after the 6 × 50-m than the 2000-m trial. In summary, plasma 8-OHdG, uric acid and bilirubin were affected by swimming intensity, whereas malondialdehyde, PC and GSH were not. Exercise intensity seems to be a key factor in exercise-induced perturbations of redox status (Bloomer, 2008). This possibly highlights the role of certain RONS production mechanisms, such as the one involving xanthine oxidase (Sachdev & Davies, 2008), a notion supported by the increase of uric acid after the high-intensity trial in the present study.
Finally, regarding sex-dependent differences, we are not aware of any other study comparing male and female adolescents in terms of 8-OHdG values. Therefore, we can only contrast our finding of higher overall 8-OHdG concentration in males than females to the finding of similar values in adult male and female athletes in a study by Bloomer and Fisher-Wellman (2008). Of note is that females had higher malondialdehyde values than males immediately after both trials. Based on this, we could hypothesise that adolescent females are more susceptible to exercise-induced lipid peroxidation than males. By contrast, in a study by Tauler et al. (2008), who studied the induced lipid peroxidation than males. Considering the adaptations to regular exercise and the hypothesis of hormesis, we conclude that, although swimming exercise partially perturbed the redox homeostasis of adolescent males and females, it was not harmful to their redox status in the long run.

Acknowledgements

The authors acknowledge the contribution of St. Luke’s Hospital and the Bodossakis Foundation to the completion of this study.

References


**Swimming effects on redox status**


