Exercise-Induced Oxidative Stress in Overload Training and Tapering

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ABSTRACT

VOLLAARD, N. B. J., C. E. COOPER, and J. P. SHEARMAN. Exercise-Induced Oxidative Stress in Overload Training and Tapering. *Med. Sci. Sports Exerc.*,Vol. 38, No. 7, pp. 1335–1341, 2006. Tapering can be an effective way of enhancing performance after a period of intensive training, but the mechanisms for this ergogenic effect are unclear. It was hypothesized that overload training will increase oxidative stress through an accumulative effect of repeated high-intensity exercise, whereas tapering will improve the antioxidant defense system and alleviate oxidative stress. **Purpose:** To study the oxidative stress response to overload training and tapering. **Methods:** A group of eight well-trained male endurance athletes (30 ± 6 yr; 73 ± 13 kg; 64 ± 6 mL·kg^{-1}·min^{-1}) performed two 4-wk periods of training in a crossover design. Each period included a 2-wk build-up phase followed either by 2 wk of training at the same load (control) or by a week with a 40% increase in training load (overload) preceding a week with a 60% reduction in training load (taper). Performance was monitored through weekly 15-min cycling time trials preceded by a 45-min preload at 70% Wmax. Blood samples were taken before and after the time trials and analyzed for oxidatively modified heme (OxHm), methemoglobin (metHb), and glutathione redox status. **Results:** Cycling time trials induced significant postexercise increases in levels of OxHm (+3.8%; $P < 0.001$) and oxidized glutathione (GSSG: +13.9%; $P < 0.05$) and decreases in metHb (−12.1%; $P < 0.001$), reduced glutathione (GSH: −14.4%; $P < 0.001$), and GSH/GSSG (−29.7%; $P < 0.001$). Tapering was shown to significantly increase performance (+4.9%; $P < 0.05$). Training modifications did not influence resting levels or exercise-induced changes of markers of oxidative stress. **Conclusion:** A short period of tapered training improves performance but does not seem to be associated with substantial changes in exercise-induced oxidative stress. **Key Words:** FREE RADICALS, ANTIOXIDANTS, OXIDATIVELY MODIFIED HEME, GLUTATHIONE, EXERCISE PERFORMANCE

Although elite athletes are under pressure to maintain a high level of performance over extended periods of time, peak performance in individual events is often only required at a limited number of major competitions. A reduction in training load (tapering) prior to these competitions has been proposed to be an effective way of improving performance after a period of heavy training (1,3,13,32), and anecdotal evidence suggests that tapering is in widespread use by athletes as an important part of prerace preparation. To date, many different taper regimes have been studied, and several have been observed to improve performance under laboratory conditions (1,3,11,14,16,32). Some progress has been made in defining the optimal reduction in training frequency, volume, and intensity of training during a taper, and the most favorable taper duration (18), but a poor understanding of the mechanisms of the performance enhancing effects of tapering holds back further advancements. Potential mechanisms underpinning tapering include increases in glycogen stores (20,22,25), hematological changes (17,31), neuromuscular changes (3,27), and psychological changes (9,10,19).

More recently it has been proposed that an enhancement of antioxidant defenses, or a reduction in the oxidative damage caused by a period of intensive training, might play a role in bringing about the performance enhancing effects of tapering (2,14). Physical exercise is associated with an increase in the production of reactive oxygen species (ROS), highly reactive substances that have the capacity to oxidatively modify numerous compounds in the body. A complex antioxidant defense system is in place to limit the potential negative effects of ROS, but despite this, increases in oxidatively modified biomarkers after exercise have been convincingly demonstrated (30). Although little is known about the effects of oxidative modifications on exercise

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performance, there is a possibility that prior oxidative damage caused by intensive training and/or damage occurring while exercising might affect performance. If this is the case, there are two implications for athletes aiming to optimize performance. Firstly, it seems inevitable that during training a certain level of oxidative stress will occur. Although this should not stop athletes from performing high-intensity work necessary for increasing fitness, if this oxidative stress is detrimental to performance the extent of the damage should be minimized before competition. This could be achieved by a period of tapering, during which the required recovery could take place. Secondly, oxidative stress during competition itself might impair performance, even if no oxidative damage is present at the onset of exercise. If this is the case, tapering might be beneficial by allowing time for the upregulation of levels of antioxidants prior to competition, thus minimizing the damage that free radicals might cause during exercise. It can be concluded that if exercise-induced oxidative stress negatively affects performance, a potential reduction of this effect associated with a period of reduced training could provide a feasible mechanism to explain the performance-enhancing effects of tapering.

To date, only two studies have examined the antioxidant and oxidative stress response to tapering. In a study by Child et al. (2), a 7-d taper with an 85% reduction in training volume did not alter markers of antioxidant status or oxidative damage. However, training was only controlled for 1 wk prior to tapering, and the taper protocol failed to improve performance in a half-marathon. In this study the training load in the weeks prior to tapering may have been insufficient for tapering to induce a beneficial effect on either performance or oxidative stress. Conversely, after a 4-wk period of overload training, a 32% reduction in training load expressed in training impulse (TRIMP) during 2 wk was sufficient to improve antioxidant defenses in a study by Margaritis et al. (14). Although in this study the reduction in training load during tapering was lower than generally recommended (18), duathlon performance significantly improved compared with the overload period.

A clear understanding of the changes in antioxidant status and oxidative stress associated with tapering is still lacking. Thus, the present study examined whether increases (overload) and/or decreases (taper) in the training load of well-trained triathletes affect the levels of markers of oxidative stress and antioxidant defenses. In a crossover training study we investigated the effects of 1-wk periods of overload training (40% increase in TRIMP) and tapering (60% decrease in TRIMP) on resting levels and exercise-induced changes in oxidatively modified heme (OxHm, a recently characterized specific marker of damage to hemoglobin (29)), methemoglobin (metHb), and the ratio between reduced and oxidized glutathione (a frequently used marker for antioxidant status). If a reduction in oxidative damage and/or upregulation of the antioxidant defenses were associated with the increase in performance often observed after tapering, we would expect overload training to increase signs of oxidative stress, whereas tapering would attenuate such effects.

METHODS

Subjects. Ten well-trained male triathletes volunteered to take part in this study. Two subjects were unable to complete the study, one because of injury, the other because of time constraints. Characteristics of the remaining eight subjects are shown in Table 1. Study protocol and methods were approved by the ethical committee of the University of Essex, and each subject signed an informed consent prior to participation.

Preexperimental procedures. Two exhaustive incremental exercise tests were performed to determine heart rates corresponding to cycling and running lactate thresholds. The running test was conducted on a treadmill (HP Cosmos Quasar, Nussdorf, Germany), and consisted of a 3-min warm-up at 12 $km \cdot h^{-1}$, followed by 3-min incremental stages in which running speed was increased by 1 $km \cdot h^{-1}$, interspersed with 1-min breaks. A similar protocol was used for the cycling test (Lode Excalibur Sport, Groningen, the Netherlands), which started at 150 W and had 25-W increments. During both tests, blood samples for the measurement of blood lactate concentration (EBIO plus, Eppendorf, Hamburg, Germany) were obtained from a finger prick directly after each stage. From the resulting lactate curve, lactate threshold (LT) was determined as the intensity/heart rate (Polar, Kempele, Finland) at which lactate levels were 1 mM above the baseline.

A second exhaustive incremental cycling test was performed to determine cycling Wmax and $\dot{V}O_{2peak}$. The protocol involved a ramp starting at 150 W and continuously increasing by 30 $W \cdot min^{-1}$ to volitional exhaustion. $\dot{V}O_2$ was measured throughout the test using an online breath-by-breath gas analysis system with a Triple-V turbine for volume measurements (Oxycon Pro, Jaeger, Hoechberg, Germany).

Training program. An 8-wk crossover training program was designed, consisting of two 4-wk sections (Figure 1A). Each section comprised a 2-wk build-up phase followed either by an additional 2 wk at the same constant training load (control) or by a week of training at increased load (overload) preceding a week at decreased load (taper). The weekly schedule during the build-up and control phases consisted of six cycling or running sessions, with a minimum of three cycling sessions, and a rest day on the day before the performance trial. The sessions were divided into interval training (two sessions), distance training (three sessions), and a weekly performance trial. Although

TABLE 1. Subject characteristics (N = 8).

Age (yr)	30 ± 6
Stature (m)	1.77 ± 0.08
Mass (kg)	72.5 ± 12.8
BMI ($kg \cdot m^{-2}$)	23.1 ± 2.7
Resting HR (bpm)	46 ± 11
Max HR (bpm)	183 ± 6
Cycling $\dot{V}O_{2peak}$ ($mL \cdot kg^{-1} \cdot min^{-1}$)	64.4 ± 6.0
Cycling power output at LT (W)	206 ± 27
Running speed at LT ($km \cdot h^{-1}$)	15.1 ± 1.2

Values are expressed as mean ± SD.

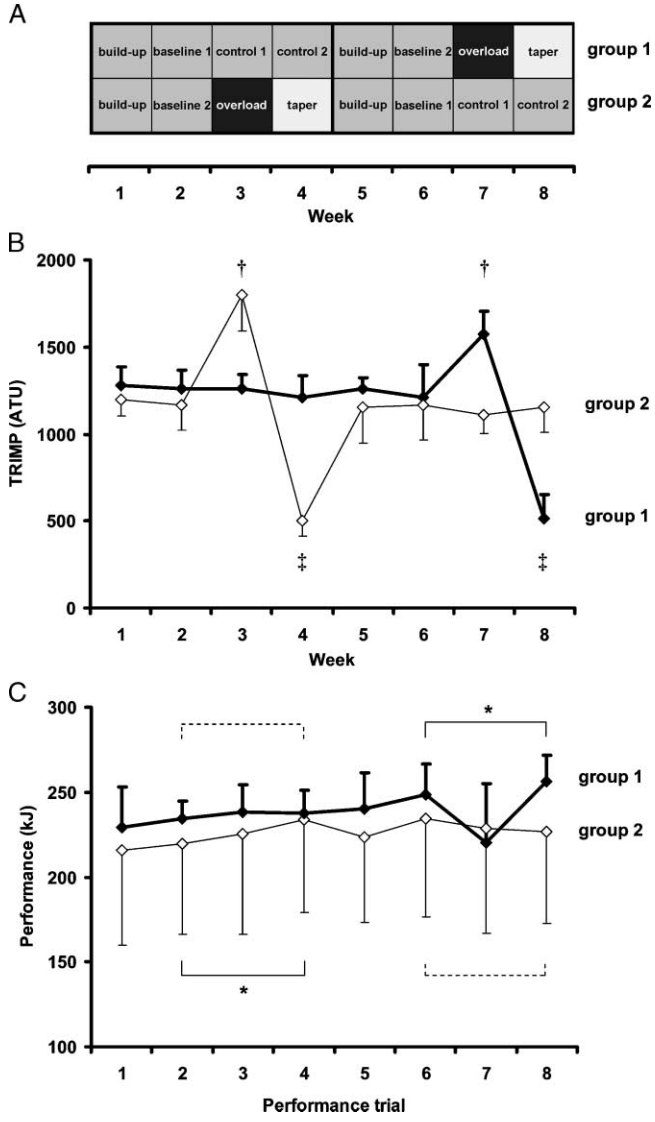

FIGURE 1—Training program, training load, and performance throughout the study. A) Build-up phases at the beginning of each 4-wk block were completed with baseline trials in weeks 2 and 6. Group 1 performed 2 wk of control training in weeks 3 and 4, overload training in week 7, and tapered training in week 8, whereas this order was reversed for group 2. B) TRIMP was constant throughout the build-up phases and control phases, but significantly increased by approximately 40% during overload training and decreased by approximately 60% during tapering. C) Performance data throughout the study for the subjects separated by group. The change in performance after a week of overload training followed by a week of tapering (4.9 ± 3.0%) was significant ($P < 0.05$) compared with the change in performance after 2 wk of control training (−0.9 ± 5.2%). Values are expressed as mean ± SD. † $P < 0.001$ for the increase in training load during overload training; ‡ $P < 0.001$ for the decrease in training load during tapering; *: $P < 0.05$ for the increase in performance after the combination of overload training and tapering (*solid lines*) compared with control training (*dashed lines*).

subjects were allowed to have between one and three running sessions a week, they were required to maintain the same program throughout the build-up and control weeks. During the overload week, training load (TRIMP) was significantly increased through a larger training volume (min). During the taper week, training volume was progressively decreased by, on average 50, 64, and 68% on days 1, 2, and 4, respectively, and additional rest days were scheduled on days 3 and 5. Training intensities (target heart rates) were unchanged during the overload and taper weeks. Subjects were randomly assigned to a group performing the overload/taper section first, and a group performing control training first. Of the eight subjects who completed the study, four subjects performed control training first (group 1), whereas the other four subjects performed the overload and taper training first (group 2).

Prescribed intensities for each session were regulated and monitored using heart rate data. Training load was calculated as a training impulse (TRIMP) and expressed in arbitrary training units (ATU), as described by Morton et al. (15). TRIMP for each 15-s section of a session was calculated as the product of time (T) in minutes, the exercise heart rate ratio (HR_{ratio}), and an intensity-weighting factor (Y) (equations 1–3). The value for α in equation 2 was taken as 1.92 for men and 1.67 for women (15). TRIMP for each session was calculated as the sum of all 15-s sections. Average weekly TRIMP for the two groups of subjects are illustrated in Figure 1B.

$$\text{HRratio} = (\text{HRexercise} - \text{HRrest})/(\text{HR}_{max} - \text{HRrest}) \quad [1]$$

$$Y = e^{\alpha} * \text{HR}_{ratio} \quad [2]$$

$$\text{TRIMP} = T * \text{HRratio} * Y \quad [3]$$

Performance trials. Weekly cycling performance trials were used to monitor performance throughout the study. The trials consisted of a 45-min preload at an intensity of 70% of Wmax, followed by a 15-min time trial in which the subjects were asked to perform as much work as possible (12). During the preload, the electrically braked ergometer (Lode Excalibur Sport, Groningen, the Netherlands) was in pedal-rate–independent mode, whereas after 45 min the ergometer was switched to linear mode, allowing the subjects to pace themselves through varying their cadence. The linear factor (L) was chosen to produce a power output corresponding to 80% Wmax at a pedal rate of 90 rpm (equation 4).

$$L = W/(\text{rpm}^2) \quad [4]$$

Performance was expressed as the amount of work (kJ) performed during the time trial. To ensure equal conditions for each trial, no encouragement was given, and subjects were blinded from information on power output, heart rate, and pedal frequency. Time was called out after each minute and 30 s before the end of the trial. Each subject performed a total of nine performance trials: one at the start of the training program (familiarization trial) and one at the end of each of the eight training weeks. Data from the performance trial in week 2 were used as first baseline data (baseline 1). Subjects in group 1 then performed control

trial 1 and control trial 2 in weeks 3 and 4, respectively, while subjects in group 2 performed the overload trial and taper trial. After 2 wk of regular training, a second baseline trial was performed in week 6 (baseline 2), followed by the overload trial and taper trial in weeks 7 and 8 for the subjects of group 1 and control trial 1 and control trial 2 for the subjects in group 2 (Figure 1A).

Blood measurements. Blood samples were taken at the performance trials after the build-up phase (baseline trials in weeks 2 and 6) and after trials following weeks of control training, overload training, and tapering (weeks 3 and 4 and weeks 7 and 8). Pre- and postexercise samples were drawn from an antecubital vein into a 6-mL Vacutainer EDTA whole-blood tube (Becton-Dickinson, Oxford, UK). Part of the whole blood was transferred into microcentrifuge tubes and quartz EPR tubes and directly frozen in liquid nitrogen. For the analysis of total glutathione (TGSH) and oxidized glutathione (GSSG), directly after taking the blood sample, 0.75 mL 6% TCA or 0.75 mL 6% TCA with 40-mM NEM was added to 0.50 mL of whole blood, respectively, followed by vortex mixing. After incubation on ice for 10 min, samples were centrifuged at 13,500 $\times$ *g* for 6 min at 4°C. Clear supernatant was frozen in liquid nitrogen. Until further analysis, microcentrifuge tubes were stored at −80°C, and EPR tubes in liquid nitrogen (77K). The remainder of the whole blood was used to determine hematocrit and hemoglobin levels (HemoCue B-Hemoglobin, Derbyshire, UK).

Levels of oxidatively modified heme (OxHm) were determined using an HPLC assay (29). Whole blood was diluted 10 times with water and centrifuged at 9400 $\times$ *g* for 10 min, after which supernatant was transferred to a microcentrifuge tube with a 0.22-μm cellulose acetate membrane microcentrifuge filter (Sigma-Aldrich, Poole, Dorset, UK) and centrifuged at 850 $\times$ *g* for 10 min. Samples were then transferred to a vial for reverse-phase HPLC analysis (Agilent HP1100 HPLC fitted with a diode array spectrophotometer). A Zorbax StableBond 300 C3 250 $\times$ 4.6-mm column fitted with a 12 $\times$ 4.6-mm guard column was used. Solvents consisted of 0.1% trifluoroacetic acid dissolved in water or acetonitrile. An initial 35% proportion of 0.1% TFA in acetonitrile increased to 37% after 10 min, to 40% after 15 min, and to 43% after 16 min. The remainder was 0.1% TFA in water. Flow rate was set at 1 $mL \cdot min^{-1}$, and temperature was 25°C. Heme B was determined from the 14.6-min eluant. Integrated area under the curve of the chromatogram at 630 nm was converted to a concentration of heme B by comparison with a heme B standard of known concentration measured at 430 and 630 nm (ϵ 430 nm: 133,000 $M^{-1} \cdot cm^{-1}$). OxHm was determined from the 8.3- to 8.4-min eluant by drawing a baseline from the troughs on either side of the cluster of three peaks with elution times between 8.0 and 8.7 min, after which the two peaks on the right were isolated with a drop line. Of these two peaks, the integrated area under the curve of the chromatogram at 400 nm was converted to a concentration of OxHm using an extinction coefficient of 76,000 $M^{-1} \cdot cm^{-1}$. OxHm was expressed as a percentage of heme B.

Methemoglobin levels (MetHb) were determined by electron paramagnetic resonance spectroscopy (EPR) as described by Svistunenko et al. (26). Spectra of blood samples were determined using a Bruker EMX spectrometer with an Oxford Instruments liquid helium system and a high-quality spherical Bruker resonator SP9703. Operating conditions were: frequency, 9.4692 GHz; power, 3.181 mW; conversion, 81.920 ms; time constant, 81.920; sweep time, 167.772 s; temperature, 10 K; scans, 1; modulation frequency, 100 kHz; modulation amplitude, 5 G; center field, 2500 G; sweep width, 3800 G. Peak-to-trough distance of the signal between 1100 and 1200 G was determined and compared against a standard of known concentration to determine MetHb concentration. Levels were expressed as a percentage of total hemoglobin.

Total glutathione (TGSH) was determined using a spectrophotometrical assay. After thawing, 0.80 mL of 200-mM sodium phosphate buffer (pH 7.6) was added to 0.20 mL of sample and vortex-mixed. To this was added 0.10 mL of 1-mM NADPH with 15 $U \cdot mL^{-1}$ of glutathione reductase solution in 200-mM sodium phosphate buffer (pH 7.6). Samples were vortex-mixed and incubated at room temperature for 15 min to reduce all present GSSG. The enzyme was subsequently inhibited by adding 0.20 mL of 1-M HCl. To the 0.50 mL sample was then added 0.50 mL of 200-μM DTNB in 200-mM sodium phosphate buffer (pH 7.6). Absorbance of the sample was read from 400 to 550 nm (Cary 5E, Varian, Walton-on-Thames, UK). Absorption at 550 nm was subtracted from the value at 412 nm as a baseline correction, and the resulting value was used to determine the TGSH concentration using a standard curve. Standard curves were constructed using GSH standards dissolved in phosphate-buffered saline (PBS), with a range of 0–2 mM.

Oxidized glutathione (GSSG) was determined using a method modified from Sacchetta et al. (23). After thawing, 0.80 mL of 180-mM NaOH with 200-mM CAPS was added to the 0.20-mL sample, vortex-mixed, and incubated at room temperature for 15 min. To the 0.50-mL sample was then added 0.35 mL of 200-mM NaH_2PO_4 in a 1.5-mL plastic microcuvette. Directly before measuring sample-absorbance, 0.25 mL of 1-mM NADPH in 200-mM sodium phosphate buffer (pH 7.6) and 0.25 mL of 400-μM DTNB with 3 $U \cdot mL^{-1}$ of glutathione reductase in 200-mM sodium phosphate buffer were added to the sample. The resulting chain reaction reduces GSSG to GSH, which reacts with DTNB to reproduce GSSG along with 2-nitro-5-thiobenzoic acid (TNB), which has a high extinction coefficient at 412 nm. The spectrophotometer was set to measure absorbance from 400 to 450 nm every 30 s for 90 s. The reaction rate, which is directly dependent on the concentration of GSSG, was determined by measuring the increase in absorbance at 412 nm caused by the production of TNB. The slope of the regression equation of the increase in absorbance over time was used to determine GSSG concentration from a standard curve. Standard curves were constructed using GSSG standards dissolved in phosphate-buffered saline (PBS), with a range of 0–80 μM.

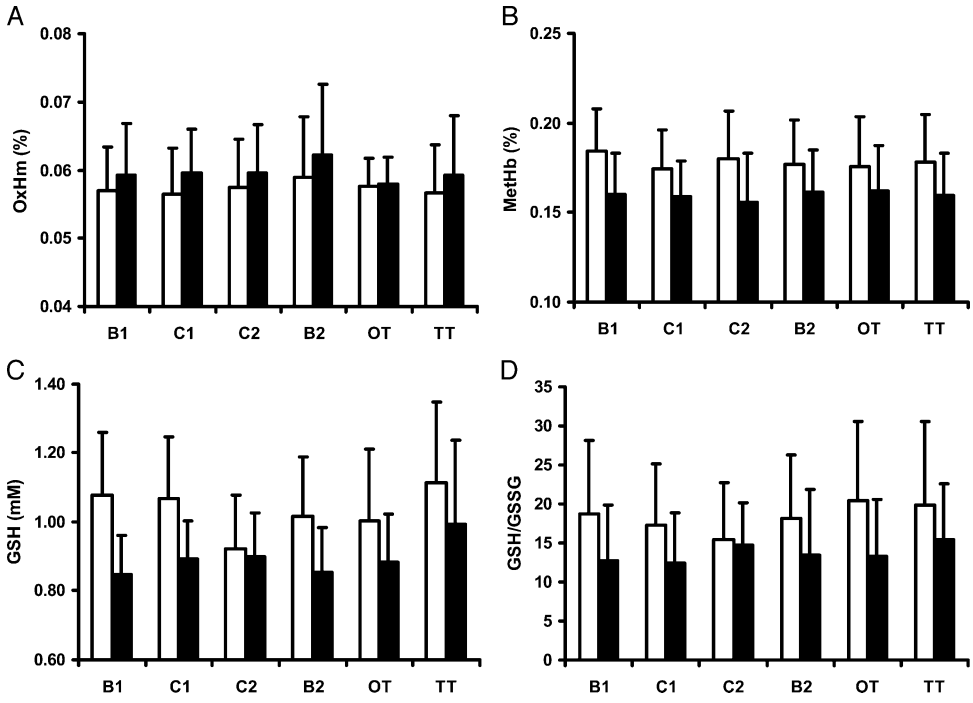

FIGURE 2—Effects of training modifications on markers of oxidative stress. Consistent changes from pre- to postexercise were observed for A) OxHm ($P < 0.001$); B) metHb ($P < 0.001$); C) GSH ($P < 0.001$); and D) GSH/GSSG ($P < 0.001$). Training modifications did not influence resting levels or exercise-induced changes of these markers. *Light columns*, preexercise; *dark columns*, postexercise; C1 and C2, control trials; OT, overload trial; TT, taper trial; B1 and B2, corresponding baseline trials. Values are expressed as mean ± SD.

GSSG was expressed in GSH equivalents. Levels of reduced glutathione (GSH) were calculated by subtracting GSSG from TGSH. Glutathione redox status was expressed as GSH/GSSG.

Intraassay coefficients of variation (CV) were determined as 6.0% for OxHm, 3.3% for metHb, 1.5% for TGSH, and 13.0% for GSSG.

Statistical analyses. All data are presented as mean ± standard deviation (SD). To compare overload training and tapering to control training, change scores for resting levels and exercise-induced changes in levels of OxHm, metHb, GSH, GSSG and GSH/GSSG, and for performance, were calculated as the difference from baseline trials to control trial 1 or control trial 2, and the difference from baseline to overload or tapering. The Kolmogorov–Smirnov test was used to check distributions of change scores for normality. Because the change scores were not normally distributed for all samples ($P < 0.05$), differences were tested using the nonparametric Wilcoxon's signed rank test. Change scores of overload training were compared with change scores of control 1, and change scores of tapering were compared with change scores of control 2. With eight subjects and a power of 80%, the detectable effect size was 1.2 × SD.

Data from the six trials performed by the eight subjects were pooled to establish the effect of exercise on levels of markers of oxidative stress, using paired-samples *t*-tests. A P value of < 0.05 was considered statistically significant.

RESULTS

Performance data from the trials throughout the study are displayed in Figure 1C. Compared with the first baseline trial (week 2), performance of subjects in group 1 was increased by 1.8 ± 2.7% in control trial 1 and by 1.5 ± 1.3% in control trial 2. Subjects in group 2 increased performance by 2.2 ± 9.4% after overload training and by 6.6 ± 1.3% after the combination of overload tapering and tapering. Following the 2-wk "wash-out" period (weeks 5 and 6), subjects in group 1 decreased performance by 11.1 ± 13.4% after overload training but increased performance by 3.2 ± 3.5% after the combination of overload training and tapering compared with baseline 2. Subjects in group 2 decreased performance by 3.0 ± 6.7% in control trial 1 and by 3.2 ± 6.8% in control trial 2 compared with baseline 2. Combined data of the two groups indicated that the mean increase in performance from the baseline trial to the taper trial (4.9 ± 3.0%) was significant ($P < 0.05$) compared with the mean change in performance from the baseline trial to control trial 2 (−0.9 ± 5.2%). Six out of eight subjects experienced peak performance after tapering. Variation in response to overload training was large, but the mean change was not significantly different from control trial 1 (Figure 1C).

Pooled data revealed a small but significant exercise-induced increase in levels of OxHm (+3.8%; $P < 0.001$) and moderate changes in GSH (−14.4%; $P < 0.001$), GSSG (+13.9%; $P < 0.05$), and GSH/GSSG (−29.7%; $P < 0.001$), indicating the occurrence of oxidative stress in the performance trials. MetHb levels significantly dropped as a result of exercise (−12.1%; $P < 0.001$). Training modifications had no substantial effects on exercise-induced changes of markers of oxidative stress or antioxidant status (Figure 2).

DISCUSSION

Although trained endurance athletes need a substantial volume of high-intensity training to improve or even to maintain their performance level, a short period of reduced

training load may bring about beneficial effects. In the present study, a 1-wk taper period involving a 60% reduction in training load improved cycling performance in a 15-min time trial by, on average, approximately 5%, a finding that is in line with other studies that have examined the effects of tapering on performance (1,3,11,14,16,32). To our knowledge, the present study was the first to make use of a crossover design to examine the effectiveness of tapering, enabling us to compare tapering with a period of control training while diminishing the influence of order effects.

The main aims of the present study were to examine whether training modifications like overload training and tapering affected exercise-induced oxidative stress and whether such potential changes in turn affected exercise performance. Evidence for the occurrence of oxidative stress during the performance trials was provided by the postexercise increase in OxHm levels and the drop in the ratio GSH/GSSG. We have recently characterized OxHm as an iron chlorin species that is exclusively formed in erythrocytes through the reaction of hemoglobin with peroxides (29). The present study supports our previous findings showing an increase in OxHm under situations of acute oxidative stress (29). However, the lack of changes in resting OxHm levels and exercise-induced changes in OxHm suggests that whereas acute exercise poses a stress to the body, repeated high-intensity exercise is not associated with accumulative oxidative damage to hemoglobin. Similarly, glutathione redox status was not affected by training modifications, indicating that trained endurance athletes rapidly recover from oxidative stress even under conditions of increased training loads and do not require a taper period for this purpose.

The observed lack of changes in resting levels and exercise-induced changes in levels of markers of oxidative stress is in line with the studies by Child et al. (2) and Margaritis et al. (14), in which tapering failed to affect malondialdehyde and TBARS, respectively. However, in contrast to our findings, Margaritis et al. reported that after overload training, tapering was associated with small but significant changes in antioxidant status, including decreases in resting GSH and GSSG levels, superoxide dismutase (SOD) activity, and plasma total antioxidant capacity (TAC), a reduction in the exercise-induced decrease in GSH and vitamin E levels, and a greater increase in postexercise plasma TAC and glutathione peroxidase (GPX) activity (14). The reason for this disparity is unclear but may be related to differences in the training programs that were followed. In the study by Margaritis et al., subjects performed 4 wk of overload training followed by 2 wk of tapering, whereas in the present study, 1 wk of overload training was followed by 1 wk of tapering. Furthermore, the 32% reduction in training load in TRIMP from overload training to tapering in the study by Margaritis et al. was far lower than the 70% reduction from overload training to tapering in the present study, and did not appear to be different from the subjects' regular training load as followed prior to overload training. Unfortunately, Margaritis et al. did not compare tapering with control training, so it remains unclear whether their observed results should be ascribed to the 4 wk of overload training or to tapering. Although there are discrepancies between studies using different training programs in the observed effects of tapering on antioxidant status, it seems that the potential changes are not associated with a reduction of exercise-induced oxidative stress.

The hypothesis that tapering may improve performance through a mechanism involving reduced oxidative stress is based on the theory that an increase in the production of ROS with exercise may be harmful to performance. This theory appears to be related to the links between oxidative stress, disease, and aging. However, after nearly three decades of research investigating exercise-induced oxidative stress, there is still no evidence for any detrimental effects of the ROS produced with exercise, other than the small to moderate increase in levels of oxidatively modified biomarkers that is often observed with exercise (30). From the widely used model that proposes that ROS produced with exercise can overwhelm the antioxidant defenses (7,21,24), it would logically follow that exercise of increasingly high intensity and duration should lead to an exponential increase in oxidative damage, but such an effect has not been shown. Conversely, comparable increases in levels of markers of oxidative stress are observed with exercise of a wide range of intensities and durations (30). Furthermore, although exercise training enhances antioxidant defenses (5,8,28), this does not attenuate exercise-induced oxidative stress in highly trained athletes. This is despite scope for further enhancement, as antioxidant defenses in skeletal muscle are relatively low compared with other tissues (4,6). Thus, it seems that antioxidant defenses are fully capable of regulating the extent of oxidative stress. This suggests that rather than being a negative side effect of exercise, the increase in free radical production may serve hitherto unknown physiological functions. The findings of the present study that neither overload training nor tapering seem to affect exercise-induced oxidative stress are in line with this notion. In this light it seems that rather than looking for ways to minimize the signs of exercise-induced oxidative stress, research efforts should be diverted to examining the physiological functions in which ROS are involved.

Concerning the mechanisms of the commonly observed increase in performance after tapering, it is worth noting that regardless of study design, experiments investigating the effects of training modifications on exercise performance will inevitably suffer from methodological difficulties involving the lack of blinding of treatment. Trained endurance athletes are generally aware of the potential ergogenic effects of tapering but cannot practically be prevented from knowing whether training volume is increased or decreased during a study. Although the literature investigating placebo effects and/or expectancy effects on exercise performance is in its infancy, it cannot be discounted that such phenomena may account for at least part of the increase in performance observed in some laboratory studies in which full blinding of participants is not achievable. Although this

does not seem to have been acknowledged previously, expectancy effects may provide a credible mechanism for the observed beneficial effects of tapering, which requires further investigation.

In conclusion, using a crossover design we have provided further evidence that a short taper can be beneficial to performance. However, neither tapering nor overload training appear to affect the extent of exercise-induced oxidative stress experienced by endurance-trained athletes.

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