Effect of F_IO_2 on Oxidative Stress during Interval Training at Moderate Altitude

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[1]Athlete Performance Laboratory, United States Olympic Committee, Colorado Springs, CO; [2]Department of Exercise Science, Health Promotion and Recreation, Colorado State University–Pueblo, Pueblo, CO; [3]The Orthopedic Specialty Hospital, Salt Lake City, UT; [4]Oxis Research, Portland, OR; and [5]Sport Science Department, USA Cycling, Colorado Springs, CO

ABSTRACT

WILBER, R. L., P. L. HOLM, D. M. MORRIS, G. M. DALLAM, A. W. SUBUDHI, D. M. MURRAY, and S. D. CALLAN. Effect of F_IO_2 on Oxidative Stress during Interval Training at Moderate Altitude. *Med. Sci. Sports Exerc.*, Vol. 36, No. 11, pp. 1888–1894, 2004. **Purpose:** To evaluate the effect of different fractions of inspired oxygen (F_IO_2) on oxidative stress during a high-intensity interval workout in trained endurance athletes residing at altitude. **Methods:** Subjects ($N = 19$) were trained male cyclists who were residents of moderate altitude (1800–1900 m). Testing was conducted at 1860 m (P_B 610–612 torr, P_IO_2 ~128 torr). Subjects performed three randomized, single-blind trials consisting of a standardized interval workout (6×100 kJ) while inspiring a medical-grade gas with F_IO_2 0.21 (P_IO_2 ~128 torr), F_IO_2 0.26 (P_IO_2 ~159 torr), and F_IO_2 0.60 (P_IO_2 ~366 torr). Serum lipid hydroperoxides (LOOH) and whole-blood reduced glutathione (GSH) were measured 60 min preexercise and immediately postexercise, and analyzed using standard colorimetric assays. Urinary malondialdehyde (MDA) and 8-hydroxydeoxyguanosine (8-OHdG) were measured 24 h preexercise and 24 h postexercise, and analyzed via HPLC and ELISA, respectively. **Results:** Compared with the control trial (F_IO_2 0.21), total time (min:s) for the 100-kJ work interval was faster (5% in F_IO_2 0.26; 8% in F_IO_2 0.60 ($P < 0.05$)) and power output (W) was higher (5% in F_IO_2 0.26, 8% in F_IO_2 0.60 ($P < 0.05$)) in the supplemental oxygen trials. There was a significant pre- versus postexercise main effect ($P < 0.05$) for LOOH and GSH; however, there were no significant differences in LOOH or GSH between the F_IO_2 trials. MDA and 8-OHdG were unaffected by either the interval training session or F_IO_2. **Conclusion:** Supplemental oxygen used in conjunction with high-intensity interval training at altitude ("live high + train low via supplemental O_2" (LH + TLO_2)) results in a significant improvement in exercise performance without inducing additional free radical oxidative stress as reflected in hematological and urinary biomarkers. **Key Words:** FREE RADICAL, REACTIVE OXYGEN SPECIES, HYPEROXIA, LIVE HIGH-TRAIN LOW, HYPOBARIC HYPOXIA

Live high–train low (LHTL) altitude training is used by contemporary elite athletes from several endurance sports in preparation for sea level competition (29). One method of LHTL allows athletes to live high in a natural, hypobaric hypoxic environment and train low in a simulated normoxic environment using supplemental oxygen for the purpose of simulating a $P_IO_2 \geq$ normoxia/sea level (159 torr) (7,17,31). Using supplemental oxygen in this manner is referred to as LH + TLO_2, which offers the practical advantage of allowing athletes to live in a natural, hypobaric hypoxic environment and effectively "train low" with minimal travel and inconvenience.

We have previously demonstrated that LH + TLO_2 results in significant increases in arterial oxyhemoglobin saturation (S_pO_2), oxygen uptake ($\dot{V}O_2$), and power output

(W), contributing to enhanced exercise performance (31). Based on our previous results, we have proposed the following as a potential mechanism for LH + TLO_2. Secondary to increments in F_IO_2 and P_IO_2, we saw an increase in whole body $\dot{V}O_2$, which in turn led to improvements in power output and exercise performance. $\dot{V}O_2$ was highly and significantly correlated ($r = 0.85$, $P < 0.05$) to power output, explaining approximately 72% of the total variability in power output. We speculated that enhanced F_IO_2 had minimal effect on cardiac output ($\dot{Q}$) based on our heart rate data, which showed that heart rate was similar between a normoxic cycling trial and two supplemental oxygen trials (F_IO_2 0.26/P_IO_2 ~159 torr; F_IO_2 0.60/P_IO_2 ~366 torr), and based on previous research by Peltonen et al. (23), who reported that during high-intensity cycling performed at sea level, stroke volume and $\dot{Q}$ were similar ($P > 0.05$) in hyperoxia (F_IO_2 0.32/P_IO_2 ~243 torr) and normoxia (F_IO_2 0.21/P_IO_2 ~159 torr). Results from earlier studies have also shown that heart rate, stroke volume, and cardiac output are unaffected during exercise in hyperoxia compared with normoxia (9,11). Rather, it appears that an increase in F_IO_2 may influence factors affecting oxygen extraction and utilization (a-v$O_{2\Delta}$). We previously demonstrated a significant correlation between S_pO_2 and $\dot{V}O_2$ (31). However, this correlation was relatively minor ($r = 0.28$, $P < 0.05$), suggesting that other factors affecting the a-v$O_{2\Delta}$ besides S_pO_2 and

C_aO_2 also contributed to the observed increases in $\dot{V}O_2$ secondary to enhanced F_IO_2. We speculated that these additional factors may include changes in trans-capillary diffusion pressure, myoglobin-oxygen saturation, and/or plasma oxygen content (20,21), one or all of which may be favorably affected by an increase in F_IO_2.

Of concern, however, is the possibility that LH + TLO_2 may produce additional free radical oxidative stress and consequently impair positive training adaptations. The generation of free radicals, or reactive oxygen species (ROS), has been shown to increase during high-intensity aerobic exercise due to a 10- to 15-fold increase in oxygen consumption in combination with a 1–2% electron leakage from the electron transport system and subsequent reduction of molecular oxygen to the superoxide free radical (1,13). Additionally, free radicals may be produced during anaerobic exercise via transient periods of relative ischemia and reperfusion, thereby creating a burst of oxidative activity (1,13). Exercise-induced oxidative stress has been implicated in the damage of cellular membranes, increased cellular swelling, decreased cell membrane fluidity, DNA damage, and skeletal muscle protein changes, all of which can result in fatigue, DOMS, increased recovery time, and increased injury rate (1,24).

To our knowledge, only one previous study has examined the effect of supplemental oxygen on oxidative stress in well-trained athletes. Peltonen et al. (22) reported that serum and low-density lipoprotein (LDL)-cholesterol diene conjugation (DC) were not increased in male cyclists and triathletes who completed a maximal cycling exercise test in conditions of acute hyperoxia (F_IO_2 0.325, P_IO_2 ~245 torr) compared with normoxia.

We were compelled to evaluate oxidative stress in conditions of (a) a higher F_IO_2/P_IO_2, and (b) using a more comprehensive panel of biomarkers than previously used (22) in an effort to determine whether LH + TLO_2 might impose greater free radical oxidative stress during high-intensity training, and therefore require athletes using LH + TLO_2 to modify their use of supplemental oxygen in order to avoid underrecovery and overtraining.

Accordingly, the purpose of this investigation was to evaluate the acute effects of different fractions of inspired oxygen (F_IO_2 0.26 and 0.60) on free radical oxidative stress during a high-intensity interval workout in trained endurance athletes residing at altitude (that is, LH + TLO_2) using a series of hematological and urinary biomarkers. We hypothesized that the use of supplemental oxygen and accompanying increase in F_IO_2/P_IO_2 might produce additional and potentially detrimental oxidative stress (vs normoxia) due to greater mitochondrial oxidative flux.

METHODS

Subjects. Before subject recruitment, the procedures for this investigation were reviewed and approved by the institutional review board of the United States Olympic Committee (USOC) Sport Science and Technology Division. The subjects for this study were 19 trained male road cyclists, offroad cyclists, and triathletes who were residents
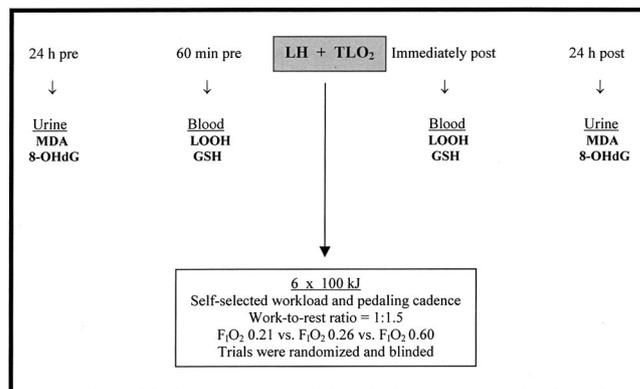
FIGURE 1—Experimental design showing the hematological and urine sampling schedule relative to the experimental exercise session. 8-OHdG, 8-hydroxydeoxyguanosine; GSH, reduced glutathione; LH + TLO_2, live high + train low via supplemental O_2; LOOH, lipid hydroperoxides; MDA, malondialdehyde.

of the Rocky Mountain region of Colorado (1800–1900 m) and had lived in that area for at least 2 yr. After being fully informed of the potential risks of the study, subjects were required to provide written consent verifying their willingness to participate. Subject characteristics (mean ± SD) are as follows: 30 ± 7 yr, 177 ± 5 cm, 69.8 ± 9.8 kg, and 9.1 ± 3.2% body fat. The subjects' maximal oxygen uptake (measured at 1860 m above sea level) was 4.09 ± 0.52 $L \cdot min^{-1}$, or 58.7 ± 3.8 $mL \cdot kg^{-1} \cdot min^{-1}$.

Preexperimental. Subjects completed a preexperimental testing session 7 d before the investigation. The first objective of this session was to collect descriptive data on the subjects including body composition (lean body mass, percent body fat), power output at blood lactate threshold (watts (W), total body weight ($W \cdot kg^{-1}$)), maximal oxygen uptake ($L \cdot min^{-1}$, $mL \cdot kg^{-1} \cdot min^{-1}$), and power at $\dot{V}O_{2max}$ (W, $W \cdot kg^{-1}$). The specific protocols for these tests have been previously described (30,32). Subjects inspired ambient room air (F_IO_2 ~0.21, P_B 610–612 torr, P_IO_2 ~128 torr) during all preexperimental tests.

The second objective of the preexperimental session was to have the subjects complete a habituation trial that simulated the experimental exercise bout (6 × 100 kJ). After fully recovering from the $\dot{V}O_{2max}$ test (as determined by heart rate, blood lactate, and RPE measurements), subjects completed the habituation trial while wearing the experimental equipment (breathing valve, noseclips, headgear, etc.) and while breathing ambient room air (F_IO_2 ~0.21, P_B 610–612 torr, P_IO_2 ~128 torr). This preexperimental habituation trial was designed to familiarize the subjects with the experimental equipment and procedures, and to reduce the potential of a learning effect.

Design. The experimental design is shown in Figure 1. The study required the subjects to perform a standardized exercise bout under three conditions: 1) F_IO_2 0.21 (P_B 610–612 torr, P_IO_2 ~128 torr), which served as the control/placebo trial; 2) F_IO_2 0.26, which at the elevation of our laboratory (1860 m, P_B 610–612 torr) yielded a P_IO_2 roughly equivalent to normoxia/sea level (~159 torr); and 3) F_IO_2 0.60, which served as a hyperoxic trial (P_B 610–612 torr, P_IO_2 ~366 torr).

The experimental trials were conducted at 7-d intervals in a randomized, single-blind fashion, and subjects were evaluated at the exact same time of day in each of the three trials. The weekly training regimen that took place between the experimental trials was controlled and documented, and subjects were required to refrain from high-intensity exercise in the 48 h before the experimental sessions. In general, the weekly training included 3 d of moderate-intensity, endurance/sublactate threshold cycle training (2.5–3.0 h per training session) and 1 d of high-intensity, $\geq$ lactate-threshold cycle interval training (1.0–1.5 h per training session). The day before the experimental LH + TLO_2 sessions consisted of 30–45 min of low-intensity cycling. The day after the experimental LH + TLO_2 sessions was a rest day, and no training was attempted. The sequence of the six training days and the experimental LH + TLO_2 session was kept consistent from week to week for each subject. The subjects did not do any LH + TLO_2 sessions during their nonexperimental training. The subjects were required to maintain consistency from week to week in their dietary intake and were required to refrain from ingesting antioxidant supplements (vitamins A, C, and E), alcohol, caffeine, and high-fat foods. Additionally, the subjects refrained from taking any antioxidant supplements for a minimum of 2 wk before the initial experimental session.

Experimental exercise bout. The experimental trials were conducted at the U.S. Olympic Training Center in Colorado Springs, CO, at an elevation of approximately 1860 m (P_B 610–612 torr, P_IO_2 ~128 torr). Upon reporting to the lab, subjects completed a standardized warm-up while inspiring ambient room air (F_IO_2 ~0.21, P_B 610–612 torr, P_IO_2 ~128 torr). Next, subjects were required to complete an interval workout consisting of 6 × 100 kJ performed on a bicycle ergometer (Lode Excalibur™; Groningen, The Netherlands) at a self-selected workload and pedaling cadence (Lode Excalibur set in "hyperbolic" mode, that is, cadence-independent mode) while breathing an experimental gas. The subjects were instructed to complete each work interval as they would normally do in a "typical training session," that is, complete the work interval as fast as possible without inducing premature fatigue. On average, the subjects completed the 6 × 100-kJ interval session at approximately 94% (F_IO_2 0.21), 101% (F_IO_2 0.26), and 107% (F_IO_2 0.21) of the preexperimental $\dot{V}O_{2max}$ (4.09 ± 0.52 L·min^{-1}) that was measured in ambient laboratory conditions (F_IO_2 ~0.21, P_B 610–612 torr, P_IO_2 ~128 torr). The bicycle ergometer was dynamically calibrated before the experimental exercise bout using the dynamometer and technique developed by Kyle (14).

All experimental gas mixtures were classified as medical grade, and the oxygen concentrations were verified in our laboratory preexperimentally using a zirconium oxide oxygen analyzer (Applied Electrochemistry Industries, Pittsburgh, PA). Subjects were blinded to F_IO_2. The experimental gas mixture was introduced manually to the subject using the following components in series: 1) a 14,000-kPa high pressure cylinder containing a certified medical-grade gas mixture comprised of 21%, 26%, or 60% oxygen, balance nitrogen (Kanox, Hutchinson, KS); 2) a 1.0-m long, 13-mm ID Tygon tube plus compatible high pressure couplers and adapters plus a 50-cm, 25-mm ID polyvinylchloride tube; 3) a 120-L neoprene meteorological balloon (Warren Collins, Braintree, MA), which served as the gas reservoir; 4) a low turbulence, 32-mm ID three-way valve (Warren Collins, Braintree, MA); 5) a 1.0-m long, 38-mm ID respiratory tube (SensorMedics, Yorba Linda, CA); and 6) a model 2700 two-way nonrebreathing valve (Hans Rudolph, Kansas City, MO), mouthpiece and noseclips. The two-way nonrebreathing valve, mouthpiece and noseclips were removed from the subjects during the recovery intervals at which time they breathed ambient room air (F_IO_2 ~0.21, P_B 610–612 torr, P_IO_2 ~128 torr) and pedaled easily at a workload of 50–75 W. Removal of the nonrebreathing valve and the cessation of experimental gas inhalation was done to mimic the conditions (i.e., fluid intake, verbal communication with coach, removal of saliva and perspiration from the facial area, etc.) that athletes experience during the recovery period of a typical interval workout using supplemental oxygen. The work:recovery ratio for the 6 × 100-kJ interval session was 1:1.5.

A laptop computer was interfaced electronically with the bicycle ergometer and positioned within easy reach of the subject. An in-house–generated computer software program allowed the subject to manipulate the workload during the work interval. The subjects were allowed to self-select their workload and pedaling cadence during the exercise bout, but that information was concealed from them. Verbal encouragement was not provided in order to prevent bias due to potential intertrial inconsistency. Accumulated work (kJ) was measured and displayed on the laptop computer and was visible to the subjects at all times; therefore, verbal updates of progress were not necessary.

Biomarkers of oxidative stress and endogenous antioxidant status. Lipid hydroperoxides (LOOH), a biomarker of lipid peroxidation, was measured from venous blood samples taken 60 min preexercise and immediately postexercise (Fig. 1). Venous blood samples (~10 mL) were drawn from an antecubital vein with the subject in a seated and upright position using a Vacutainer™ collection tube containing sodium heparin. The collection tube was chilled on ice for 5 min, then centrifuged for 10 min at 1500 × g. Upon completion of centrifugation, a 1.0-mL serum aliquot was transferred to a sterile specimen tube and stored at −70°C for subsequent analysis. Analysis of triplicate serum LOOH samples was done via a colorimetric assay using a commercial assay kit (LPO-CC) from Kamiyama Boimedical Company (Kamiya Biomedical Company, Seattle, WA). This assay is based on the reduction of lipid hydroperoxides, in the presence of hemoglobin, to hydroxyl derivatives (18). Inter- and intra-assay variability for this procedure was 1–2 and 1–4%, respectively.

Reduced glutathione (GSH), a biomarker of the endogenous glutathione peroxidase (GPx) antioxidant system, was measured from venous blood samples taken 60 min preexercise and immediately postexercise (Fig. 1). Venous blood samples (~ 10 mL) were drawn from an antecubital vein with the subject in a seated and upright position using a Vacutainer™ collection tube containing sodium heparin. After mixing via

gentle inversion, 250 μL of whole blood was immediately transferred to a sterile specimen tube and stored at −70°C for subsequent analysis. Analysis of triplicate GSH samples was done via a colorimetric assay using Ellman's reagent (5, 5'-dithiobis-2-nitrobenzoic acid, or DTNB), which reacts with GSH to form a spectrophotometrically detectable product at 412 nm (27). Inter- and intra-assay variability for this procedure was 1–3 and 1–9%, respectively. Final values for GSH were corrected for red cell lysate hemoglobin concentration (nmol GSH·mg hemoglobin^{-1}).

Malondialdehyde (MDA), a biomarker of lipid peroxidation, was measured from urine samples collected 24 h preexercise and 24 h postexercise (Fig. 1). Upon collection of the urine samples in a sterile collection container, 1.5 mL of urine was transferred to a sterile specimen tube and stored at −70°C for subsequent analysis. Analysis of triplicate MDA samples was performed using thiobarbituric acid (TBA) as a color indicator in conjunction with HPLC analyses (12). A 1.0-mL urine sample was defrosted and added to a clean glass tube containing 0.05 mL of 0.0227 M butylated hydroxytoluene (BHT) and 1.5 mL of 0.5% thiobarbituric acid. The tube was then capped, vortexed, and incubated at 95°C for 40 min. After cooling, 1.5 mL of chloroform was added and centrifuged for 10 min at 4500 × g. The upper aqueous layer was collected for HPLC analysis using a YMC A-303 ODS (5 μg, 250 × 4.6 mm) column. The concentrations of MDA were determined from a standard curve. Inter- and intra-assay variability for this procedure was 1–3 and 1–10%, respectively. Final values for MDA were corrected for urinary creatinine concentration (nmol MDA·mg^{-1} creatinine), which was determined using a standard colorimetric assay kit (Sigma Diagnostics, St. Louis, MO).

8-hydroxydeoxyguanosine (8-OHdG), a marker of DNA peroxidation, was measured from urine samples collected 24 h preexercise and 24 h postexercise (Fig. 1). Upon collection of the urine samples in a sterile collection container, 1.5 mL of urine was transferred to a sterile specimen tube and stored at −70°C for subsequent analysis. Analysis of triplicate 8-OHdG samples was made using an ELISA assay kit (Japan Institute for the Control of Aging, Fukuroi, Japan) according to the methodology of Toyokuni et al. (28). Inter- and intra-assay variability for this procedure was 1–5 and 1–17%, respectively. Final values for 8-OHdG were corrected for urinary creatinine concentration (ng 8-OHdG·mg^{-1} creatinine), which was determined using a standard colorimetric assay kit (Sigma Diagnostics).

Statistical analysis. A single-factor (F_IO_2) univariate repeated measures ANOVA was used to evaluate mean differences between the three F_IO_2 trials in average total time and average power output produced in the 6 × 110-kJ exercise bout. A two-factor (F_IO_2 × time) univariate repeated measures ANOVA was used to evaluate mean differences in the serum and urinary biomarkers of oxidative stress between the three F_IO_2 trials. If a significant F-ratio was indicated by the ANOVA, a Tukey HSD *post hoc* test was used to identify significant differences between the individual means. A probability level of 0.05 was established *a priori* to define statistical significance for all anal-
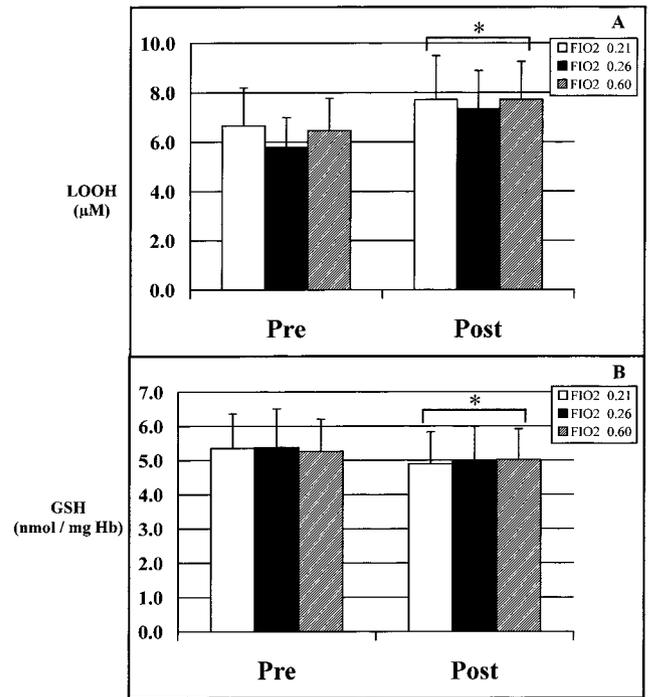
FIGURE 2—Response of serum LOOH (A) and whole-blood GSH (B) relative to a 6 × 100-kJ bicycle ergometer interval session performed by trained cyclists at moderate altitude (1860 m) with F_IO_2 0.21 (P_IO_2 ~128 torr), F_IO_2 0.26 (P_IO_2 ~159 torr), and F_IO_2 0.60 (P_IO_2 ~366 torr). GSH, reduced glutathione; LOOH, lipid hydroperoxides. Values are the group mean ± SD; * significant main effect pre- versus postexercise ($P < 0.05$).

yses. Data are reported as the group mean ± SD unless otherwise noted.

RESULTS

Average total time (min:s) for the 100-kJ work interval in the control trial (F_IO_2 0.21/P_IO_2 ~ 128 torr) was 6:17 ± 0:58. Compared with the control trial, average total time for the 100-kJ work interval was 5% faster in the F_IO_2 0.26/P_IO_2 ~ 159 torr trial (5:58 ± 0:55) and 8% faster in the F_IO_2 0.60/P_IO_2 ~ 366 torr trial (5:45 ± 0:51, $P < 0.05$). The intraclass reliability coefficient for average total time was 0.978. Consistent with the improvements in average total time for the 100-kJ work interval were increments in average power output (W) that were equivalent to 5% in the F_IO_2 0.26/P_IO_2 ~ 159 torr trial (279 ± 41 W) and 9% in the F_IO_2 0.60/P_IO_2 ~ 366 torr trial (290 ± 43 W, $P < 0.05$) compared with the F_IO_2 0.21/P_IO_2 ~ 128 torr trial (265 ± 41 W).

ANOVA revealed a significant ($P < 0.05$) pre- versus postexercise main effect for serum LOOH (Fig. 2A). LOOH was 16%, 27%, and 19% higher in the F_IO_2 0.21, F_IO_2 0.26, and F_IO_2 0.60 trials, respectively, immediately after the 6 × 100-kJ interval session compared with 60 min preexercise. However, there were no significant differences in LOOH between the varying F_IO_2 concentration trials either before or immediately after the 6 × 100-kJ training session (Table 1). Similar results were seen for whole-blood GSH. There was a significant ($P < 0.05$) pre- versus postexercise main effect for GSH, which was 8%, 8%, and 5% lower in the

TABLE 1. Hematological (LOOH, GSH) and urinary (MDA, 8-OHdG) oxidative stress markers relative to a 6 × 100-kJ bicycle ergometer interval session performed by trained cyclists at moderate altitude (1860 m) with F_IO_2 0.21 (P_IO_2 ~128 torr), F_IO_2 0.26 (P_IO_2 ~159 torr), and F_IO_2 0.60 (P_IO_2 ~366 torr); Postexercise samples for LOOH and GSH were taken within 3 min of exercise.

	Time	F_IO_2 0.21	F_IO_2 0.26	F_IO_2 0.60
LOOH* (μM)	60 min pre	6.66 ± 1.83	5.78 ± 1.67	6.47 ± 1.31
	post	7.71 ± 1.79	7.34 ± 1.52	7.71 ± 1.52
GSH* (nmol·mg Hb)	60 min pre	5.35 ± 1.01	5.38 ± 1.24	5.26 ± 0.94
	post	4.90 ± 1.07	4.95 ± 1.06	5.02 ± 1.26
MDA (nmol·mg^{-1} creatinine)	24 h pre	0.98 ± 0.42	1.03 ± 0.51	1.05 ± 0.49
	24 h post	0.97 ± 0.59	0.94 ± 0.30	0.92 ± 0.32
8-OHdG (ng·mg^{-1} creatinine)	24 h pre	5.65 ± 2.31	7.11 ± 2.48	7.09 ± 4.12
	24 h post	7.59 ± 3.96	7.52 ± 4.76	7.58 ± 2.79

* Significant main effect pre- vs postexercise ($P < 0.05$).
8-OHdG, 8-hydroxydeoxyguanosine; GSH, reduced glutathione; Hb, hemoglobin; LOOH, lipid hydroperoxides; MDA, malondialdehyde.
Values are the group mean ± SD.

F_IO_2 0.21, F_IO_2 0.26, and F_IO_2 0.60 trials, respectively, 60 min after the 6 × 100-kJ interval session compared with 60 min preexercise (Fig. 2B). However, there were no significant differences in GSH between the three F_IO_2 concentration trials either before or immediately after the 6 × 100-kJ training session (Table 1).

Urinary MDA was not affected by either the interval training session or F_IO_2. There were no significant differences between the varying F_IO_2 concentration trials either before or after the 6 × 100-kJ training session (Fig. 3A). MDA was 1%, 9%, and 12% lower in the F_IO_2 0.21, F_IO_2 0.26, and F_IO_2 0.60 trials, respectively, 24 h after the 6 × 100-kJ interval session compared with 24 h preexercise, but these decrements were not statistically significant ($P > 0.05$) (Table 1). Likewise, there were no significant differences in urinary 8-OHdG between the three F_IO_2 concentration trials either before or after the 6 × 100-kJ training session (Fig. 3B). 8-OHdG was 34%, 6%, and 7% higher in the F_IO_2 0.21, F_IO_2 0.26, and F_IO_2 0.60 trials, respectively, 24 h after the 6 × 100-kJ interval session compared with 24 h preexercise; however, these increments were not statistically significant ($P > 0.05$) (Table 1).

DISCUSSION

We evaluated free radical oxidative stress during a typical training session of LH + TLO$_2$, an altitude training strategy whereby athletes live high in a natural, hypobaric hypoxic environment and "train low" in a simulated normoxic environment using supplemental oxygen. We demonstrated that the use of supplemental oxygen (F_IO_2 0.26/P_IO_2 ~159 torr and F_IO_2 0.60/P_IO_2 ~366 torr) during a high-intensity interval workout by trained cyclists at moderate altitude (1860 m) results in a significant improvement in exercise performance *without* inducing additional oxidative stress as reflected by several hematological and urinary biomarkers. These results were contrary to our initial hypothesis, which postulated that the use of supplemental oxygen and accompanying increase in F_IO_2/P_IO_2 might produce additional and potentially detrimental oxidative stress (vs normoxia) due to greater mitochondrial oxidative flux. Our findings are novel in that they provide objective data regarding the relative

absence of additional oxidative stress in LH + TLO$_2$, thereby building on our previous findings (31) and supporting the use of LH + TLO$_2$ as a safe, practical, and effective altitude training method.

We were compelled to conduct this investigation based on the concern that LH + TLO$_2$ might produce additional free radical oxidative stress and consequently impair positive training adaptations. The generation of reactive oxygen species (ROS) increases during high-intensity aerobic exercise (1,13) and has been associated with damage of cellular membranes, increased cellular swelling, decreased cell membrane fluidity, DNA damage, and skeletal muscle protein changes, all of which can result in fatigue, DOMS, increased recovery time, and increased injury rate (1,24). Thus, our concern was that the use of LH + TLO$_2$ might produce additional mitochondrial oxidative flux and consequently result in an increased level of ROS. We believed it was imperative to determine whether LH + TLO$_2$ might impose greater free radical oxidative stress, and therefore require athletes using LH + TLO$_2$ to modify their use of supplemental oxygen in order to avoid underrecovery and overtraining.

The results of the current investigation did not support our original hypothesis regarding greater free radical oxidative stress in the supplemental oxygen trials. Compared with the control trial (F_IO_2 0.21/P_IO_2 ~ 128 torr), average total time (min:s) for the 100-kJ work interval was faster (5% in F_IO_2 0.26/P_IO_2 ~ 159 torr; 8% in F_IO_2 0.60/P_IO_2 ~ 366 torr ($P < 0.05$)), and average power output (W) was higher (5% in F_IO_2 0.26/P_IO_2 ~ 159 torr, 9% in F_IO_2 0.60/P_IO_2 ~ 366 torr ($P < 0.05$)) in the supplemental oxygen trials. Despite the enhanced exercise performance demonstrated in the supplemental oxygen trials, oxidative stress was not different in the supplemental
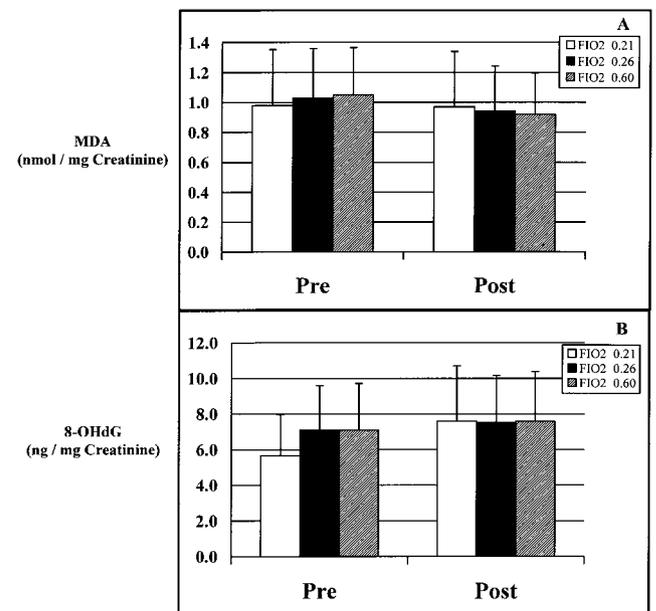

FIGURE 3—Response of urinary MDA (A) and urinary 8-OHdG (B) relative to a 6 × 100-kJ bicycle ergometer interval session performed by trained cyclists at moderate altitude (1860 m) with F_IO_2 0.21 (P_IO_2 ~128 torr), F_IO_2 0.26 (P_IO_2 ~159 torr), and F_IO_2 0.60 (P_IO_2 ~366 torr). 8-OHdG, 8-hydroxydeoxyguanosine; MDA, malondialdehyde. Values are the group mean ± SD.

oxygen trials compared with the control trial. There was a significant ($P < 0.05$) pre- versus postexercise main effect for serum lipid hydroperoxides (LOOH) and whole-blood reduced glutathione (GSH); specifically, LOOH increased and GSH decreased as a result of the 6×100-kJ interval session across all three F_IO_2 trials (Fig. 2). However, there were no significant differences in LOOH or GSH between the F_IO_2 trials, either before or immediately after the 6×100-kJ training session (Table 1). Additionally, urinary MDA and urinary 8-hydroxydeoxyguanosine (8-OHdG) were not affected by either the interval training session or F_IO_2 (Fig. 3) as there were no significant differences in MDA or 8-OHdG between the F_IO_2 trials either before or after the 6×100-kJ training session (Table 1).

To our knowledge, only one previous study has examined the effect of supplemental oxygen on oxidative stress in well-trained athletes. Peltonen et al. (22) reported that serum and LDL-cholesterol DC were not increased in male cyclists and triathletes who completed a maximal cycling exercise test in conditions of acute hyperoxia (F_IO_2 0.325, P_IO_2 $\sim$245 torr) compared with normoxia. Thus, although our investigation evaluated oxidative stress using a different/higher experimental F_IO_2/P_IO_2, as well as a more comprehensive panel of biomarkers, our findings are in general agreement with those of Peltonen et al. (22).

Based on our results, and within the limitations of the current investigation, we propose three potential interrelated physiological reasons as to why we did not observe significantly greater oxidative stress in the supplemental oxygen trials. First, it is possible that the generation of ROS is not driven exclusively or primarily by an increase in mitochondrial oxidative flux, that is, there is no mass action effect of oxygen on free radical generation (2,4,5,6). Our data tended to support this proposition. Whole-body $\dot{V}O_2$ was 7% and 14% higher in the F_IO_2 0.26 and F_IO_2 0.60 trials, respectively (31), compared with the control trial (F_IO_2 0.21), yet we did not observe any significant difference in oxidative stress in the supplemental oxygen trials as measured in a number of hematological and urinary biomarkers. Several mechanisms have been proposed as contributing to free radical production independent of mitochondrial oxidative flux. Catecholamine and stress hormone auto-oxidation may contribute to ROS generation in conjunction with high-intensity exercise (10). Another source of free radical production may be related to transition metals such as iron (ferritin iron), which are capable of catalyzing several reactions that produce ROS (25). In addition, there is strong evidence to support the concept that ischemia-reperfusion is significantly related to increased free radical generation during high-intensity exercise (2,3).

Second, it is possible that the well-trained endurance athletes who were evaluated in our investigation had developed a relatively robust endogenous antioxidant capacity as a result of several years of resolute training. It has been suggested that well-trained endurance athletes have an enhanced antioxidant capacity compared with untrained individuals, perhaps due to a more responsive GSH and/or glutathione peroxidase (GPx) antioxidant system (26). In-

deed, several previous studies conducted at sea level have demonstrated that GSH and/or GPx activity were not altered in well-trained endurance athletes in response to a simulated duathlon (19), an "Ironman" triathlon race (15), a half-marathon race (8), or the initial stage of a professional cycling tour (16). It was not the purpose of our investigation to compare the oxidative stress and endogenous antioxidant response of our well-trained subjects with that of age-matched untrained individuals. Nevertheless, it is possible that the well-trained endurance athletes in the current investigation had developed a relatively robust endogenous antioxidant capacity, which in turn may explain in part why we did not observe significantly greater oxidative stress in the supplemental oxygen trials as we had originally hypothesized.

A third reason that serves to explain our findings is related to, and expands upon, the second rationale just described. It has been proposed that exercise-induced oxidative stress may be attenuated by either intermittent hypoxia via high-intensity endurance training or by chronic hypobaric hypoxia (altitude), both of which produce a decrease in mitochondrial PO_2. It is believed that this decrease in mitochondrial PO_2 leads to an increase in the generation of mitochondrial and/or systemic ROS (2,4–6). Bailey et al. (4,5) postulated that this increase in ROS is due more to the decrease in mitochondrial PO_2 than the increase in mitochondrial O_2 flux *per se*. ROS in turn act as secondary messengers to initiate several positive functional adaptations in response to the mitochondrial hypoxia that is experienced during high-intensity endurance training and/or hypobaric hypoxia (altitude) (4–6). Some of these functional adaptations include increased pulmonary ventilation ($\dot{V}_E$) and heart rate, as well as enhanced erythrocyte and hemoglobin synthesis. In addition, it is believed that the endogenous antioxidant system is enhanced via the ROS second messenger sequence (4–6). The endurance athletes in our investigation had completed high-intensity training sessions on a regular basis over several years. They were also long-time residents of moderate altitude (1800–1900 m). Given this combination of chronic hypobaric hypoxia (altitude) and exercise-induced hypoxia, it is possible that ROS-initiated adaptations to the endogenous antioxidant system as proposed by Bailey et al. (4,5) and others (6) may help explain why we did not observe an increase in free radical oxidative stress in the supplemental oxygen trials among the endurance athletes evaluated in our study.

In summary, we evaluated free radical oxidative stress during a typical training session of LH + TLO₂, an altitude training strategy whereby athletes live high in a natural, hypobaric hypoxic environment and "train low" in a simulated normoxic environment using supplemental oxygen. We were compelled to conduct this investigation based on the concern that LH + TLO₂ might produce additional free radical oxidative stress and consequently impair positive training adaptations. We demonstrated that the use of supplemental oxygen (F_IO_2 0.26/P_IO_2 $\sim$159 torr and F_IO_2 0.60/P_IO_2 $\sim$366 torr) during a high-intensity interval workout by trained cyclists at moderate altitude (1860 m) results

in a significant improvement in exercise performance *without* inducing additional oxidative stress as reflected by several hematological (LOOH, GSH) and urinary (MDA, 8-OHdG) biomarkers. These results were contrary to our initial hypothesis, which postulated that the use of supplemental oxygen and accompanying increase in F_IO_2/P_IO_2 might produce additional and potentially detrimental oxidative stress (vs normoxia) due to greater mitochondrial oxidative flux. Our findings suggest that $LH + TLO_2$ results in a significant improvement in exercise performance without inducing additional free radical oxidative stress in well trained endurance athletes residing at moderate altitude, thereby supporting the use of $LH + TLO_2$ as a safe, practical, and effective altitude training method. However, it should be noted that the current investigation focused strictly on the acute effects of a single bout of $LH + TLO_2$. Given that athletes who employ this method of altitude training typically complete a $LH + TLO_2$ training session on a 10- to 14-d cycle for several weeks, it is important to investigate and understand the cumulative effects of several $LH + TLO_2$ workouts on oxidative stress and the potential negative impact it may have on training progression. Studies designed to answer this important question are warranted.

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