No Indications of Persistent Oxidative Stress in Response to an Ironman Triathlon

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ABSTRACT

NEUBAUER, O., D. KÖNIG, N. KERN, L. NICS, and K.-H. WAGNER. No Indications of Persistent Oxidative Stress in Response to an Ironman Triathlon. *Med. Sci. Sports Exerc.*, Vol. 40, No. 12, pp. 2119–2128, 2008. **Introduction**: Training for and competing in ultraendurance exercise events is associated with an improvement in endogenous antioxidant defenses as well as increased oxidative stress. However, consequences on health are currently unclear. **Purpose**: We aimed to examine the impact of training- and acute exercise-induced changes in the antioxidant capacity on the oxidant/antioxidant balance after an ironman triathlon and whether there are indications for sustained oxidative damage. **Methods**: Blood samples were taken from 42 well-trained male triathletes 2 d before an ironman triathlon, then immediately postrace, 1, 5, and 19 d later. Blood was analyzed for conjugated dienes (CD), malondialdehyde (MDA), oxidized low-density lipoprotein (oxLDL), oxLDL:LDL ratio, advanced oxidation protein products (AOPP), AOPP:total protein (TP) ratio, Trolox equivalent antioxidant capacity (TEAC), uric acid (UA) in plasma, and activities of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and catalase (CAT) in erythrocytes. **Results**: Immediately postrace, there were significant increases in CD, AOPP, TEAC, UA (for all $P < 0.001$), and AOPP:TP ($P < 0.01$). MDA rose significantly ($P < 0.01$) 1 d postrace, whereas CD ($P < 0.01$), AOPP ($P = 0.01$), AOPP:TP ($P < 0.05$), and TEAC ($P < 0.001$) remained elevated. OxLDL:LDL trended to increase, whereas oxLDL significantly ($P < 0.01$) decreased 1 d postrace. Except for GSH-Px ($P = 0.08$), activities of SOD ($P < 0.001$) and CAT ($P < 0.05$) significantly decreased postrace. All oxidative stress markers had returned to prerace values 5 d postrace. Furthermore, several relationships between training status and oxidative stress markers, TEAC, and antioxidant enzyme activities were noted. **Conclusions**: This study indicates that despite a temporary increase in most (but not all) oxidative stress markers, there is no persistent oxidative stress in response to an ironman triathlon, probably due to training- and exercise-induced protective alterations in the antioxidant defense system. **Key Words:** ULTRAENDURANCE EXERCISE, LIPID PEROXIDATION, PROTEIN OXIDATION, PLASMA ANTIOXIDANT CAPACITY, ANTIOXIDANT ENYZMES

There is overwhelming evidence that physical activity harvests many beneficial physiological effects that improve physical fitness and play a major role in the prevention of various chronic disease states (30). Research has even shown an increased life expectancy in former top-level athletes including long distance runners and cross-country skiers (but not ultraendurance athletes) (14,32). However, some empirical as well as epidemiologic data, recently reviewed by Knez et al. (10), paradoxically suggest that an exceptionally high volume of exercise is associated with an increased risk of developing cardiovascular disease (15). Oxidative stress is proposed to be one of the main potential mechanisms that, at least partly, might offset the positive outcome imparted by regular physical training (6,10), probably due to the increased oxidation of plasma lipoproteins and the consequent hypothesized contribution to atherosclerosis (10,34). Thus, concerns have arisen about the growing number of athletes engaged in ultraendurance sports because extremely demanding exercise such as an ironman triathlon is associated with an increased formation of reactive oxygen species (ROS). Probable mechanisms for increased ROS production during strenuous aerobic exercise include inadequate electron transfer through the mitochondrial respiratory chain during oxygen metabolism, inflammatory responses, increased xanthine oxidase activity triggered by transient hypoxic conditions (that even may occur during predominantly aerobic exercise caused by blood-redistribution), and autoxidation of haem proteins (6,13,39).

Nevertheless, research in the area of exercise-induced oxidative stress has lead to controversial results, and to date there is little conclusive information. For example, it remains unclear whether the exercise-induced production of free radicals results in persisting oxidative stress responses and adverse effects on health such as LDL oxidation (6,10,36,39). Training appears to lead to adaptations of the endogenous antioxidant defense system (11,25,28); however, it is unknown whether these up-regulated protective mechanisms are sufficient to prevent cumulative oxidative stress and oxidative damage. Moreover, the lack of consensus most likely

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TABLE 1. Characteristics of the study participants and their performance in the Ironman triathlon.

Age (yr)	35.3 ± 7.0
Height (cm)	180.6 ± 5.6
Weight (kg)	75.1 ± 6.4
BMI ($kg \cdot m^{-2}$)	23.0 ± 1.2
Percentage of body fat (%)	11.8 ± 4.1
Cycling $\dot{V}O_{2peak}$ ($mL \cdot kg^{-1}\ min^{-1}$)	56.6 ± 6.2
Peak PO (W_{peak})	357.9 ± 50
Relative peak PO ($W_{peak} \cdot kg^{-1}$)	4.8 ± 0.5
IAT (W)	219.4 ± 43.5
Relative IAT ($W \cdot kg^{-1}$)	2.9 ± 0.5
Power output at 2 mmol blood lactate·L^{-1} ($W \cdot kg^{-1}$)	193.6 ± 46.5
Relative PO at 2 mmol blood lactate·L^{-1} ($W \cdot kg^{-1}$)	2.6 ± 0.5
Power output at 3 mmol blood lactate·L^{-1} ($W \cdot kg^{-1}$)	237.1 ± 46.0
Relative PO at 3 mmol blood lactate·L^{-1} ($W \cdot kg^{-1}$)	3.1 ± 0.6
Training over a period of 6 months before the Ironman triathlon	
Weekly net endurance exercise time (WNET) ($h \cdot wk^{-1}$)	10.7 ± 2.6
Swim training ($km \cdot wk^{-1}$)	4.8 ± 2.2
Cycle training ($km \cdot wk^{-1}$)	144.0 ± 52.1
Run training ($km \cdot wk^{-1}$)	36.4 ± 10.6
Performance in the Ironman triathlon	
Total race time (h:min:s)	10:51:52 ± 01:01:22
3.8-km swim time (h:min:s)	01:09:51 ± 00:10:28
180-km cycle time (h:min:s)	05:28:21 ± 00:29:08
42.2-km run time (h:min:s)	04:08:26 ± 00:31:36
Training after completion of the Ironman triathlon until 19 d postrace	
WNET ($h \cdot wk^{-1}$)	4.2 ± 2.4
Swim training ($km \cdot wk^{-1}$)	2.1 ± 2.2
Cycle training ($km \cdot wk^{-1}$)	66.3 ± 53.8
Run training ($km \cdot wk^{-1}$)	12.3 ± 11.5

Data are presented as mean ± SD, $N = 42$.

also originates from the diversity of study designs and methodological approaches that are used to induce and measure oxidative stress (9,11,13). In particular, different durations, intensities, and types of exercise probably contribute to inconsistencies even among the few studies that have examined oxidative stress specifically in competitors of ultraendurance races such as long-distance triathlons (7,11,17,23) or ultramarathon running (19,22).

The data presented here are part of a larger study that aimed to get a broader picture of certain stress responses to vigorous aerobic exercise in a large cohort of athletes and to explore hypothesized associations between oxidative, muscular, cardiac (12), inflammatory, immunoendocrine stress, and genome stability. The primary aim of the present study was to comprehensively quantify antioxidant and oxidative stress responses to an ironman triathlon. Of utter importance, we monitored these responses 19 d into recovery to verify whether there are indications of delayed onset of oxidative stress, sustained oxidative damage, and health consequences. Furthermore, the relevance of training status on the magnitude of oxidative stress markers and the antioxidant capacity was examined. We hypothesized that even small differences in training levels within a large group of well-trained athletes would affect the changes of oxidative stress and endogenous antioxidant variables after an acute bout of ultraendurance exercise. Finally, due to recent inconsistent outcomes that were probably related with diverse analytical approaches, we aimed to particularize the damage on blood cell components and blood lipids by using various markers to detect different phases of lipid peroxidation as well as protein oxidation.

MATERIALS AND METHODS

Subjects. The study population comprised 48 nonprofessional well-trained healthy male triathletes who participated in the 2006 Ironman Austria; 42 of them completed the study and were included in the statistical analysis. The subjects were recruited from all over Austria half a year before the event. They were informed about the purpose and the risks of the study before they provided written informed consent. The Ethics Committee of the Medical University of Vienna approved the study. The characteristics of the subjects and their performance in the ironman triathlon are shown in Table 1.

Study design. All participants of the study were physically fit, free of acute or chronic illnesses, within a normal range of body mass index and nonsmokers. Furthermore, they were not taking prescribed medication and avoided taking more than 100% of RDA of antioxidants (as sup plements in addition to their normal dietary intake) in the 6 wk before the race and until the final blood sampling 19 d postrace. Subjects were required to complete a medical and health screening, a food frequency, a supplementation questionnaire, and 24-h dietary recalls before each blood sampling, and they had to document their training in the 6 months before the ironman triathlon and thereafter until the end of the study (Table 1). Blood samples were taken 2 d prerace, immediately (within 20 min), 1, 5, and 19 d postrace. The athletes abstained from intense exercise 48 h before spiroergometry testing and before each blood sampling

TABLE 2. Plasma values of biochemical variables.

	PRE	POST	1 d POST	5 d POST	19 d POST	Time Effect (P)
UA ($\mu mol \cdot L^{-1}$)	311 ± 54	465 ± 92***	422 ± 63***	338 ± 62***	328 ± 53***	<0.001
TC ($mmol \cdot L^{-1}$)	5.1 ± 0.9	5.1 ± 1.0	4.2 ± 0.8***	4.7 ± 0.8***	5.1 ± 0.9	<0.001
HDL ($mmol \cdot L^{-1}$)	1.9 ± 0.4	2.0 ± 0.4*	1.9 ± 0.4	1.8 ± 0.5	1.7 ± 0.4*	<0.001
LDL ($mmol \cdot L^{-1}$)	2.8 ± 0.8	2.4 ± 0.8***	1.9 ± 0.7***	2.5 ± 0.6***	2.9 ± 0.8	<0.001
VLDL ($mmol \cdot L^{-1}$)	0.4 ± 0.2	0.7 ± 0.3*	0.4 ± 0.3	0.4 ± 0.2	0.4 ± 0.2	<0.001
TG ($mmol \cdot L^{-1}$)	0.9 ± 0.4	1.6 ± 0.6***	0.9 ± 0.6	0.9 ± 0.4	0.9 ± 0.4	<0.001

Values are presented as mean ± SD; $N = 42$.
* Significantly different from prerace values, $P < 0.05$.
** Significantly different from prerace values, $P < 0.01$.
*** Significantly different from prerace values, $P < 0.001$.
HDL, high-density lipoprotein; LDL, low-density lipoprotein; VLDL, very low density lipoprotein; PRE, 2 d prerace; POST, immediately postrace; UA, uric acid; TC, total cholesterol; 1 d POST, 1 d postrace; 5 d POST, 5 d postrace; 19 d POST, 19 d postrace.

TABLE 3. Plasma values of oxidative stress markers.

	PRE	POST	1 d POST	5 d POST	19 d POST	Time Effect (*P*)
CD ($\mu g \cdot mL^{-1}$)	3.85 ± 1.27	7.35 ± 1.54***	4.36 ± 1.30**	4.03 ± 1.16	3.78 ± 0.91	<0.001
MDA ($\mu mol \cdot L^{-1}$)	2.39 ± 0.49	2.56 ± 0.67	2.60 ± 0.73**	2.39 ± 0.68	2.42 ± 0.78	0.092
OxLDL ($U \cdot L^{-1}$)	35.8 ±13.7	31.3 ± 12.4*	29.3 ± 13.6**	32.3 ± 11.3	36.9 ± 13.6	<0.001
OxLDL:LDL rat. ($U \cdot mmol \cdot L^{-1}$)	13.3 ± 5.3	13.8 ± 6.2	14.4 ± 6.1	12.1 ± 5.2	13.1 ± 4.9	0.363
AOPP ($\mu mol \cdot L^{-1}$)	39.8 ± 8.4	49.9 ± 14.8***	48.1 ± 17.1**	39.9 ± 6.7	41.7 ± 8.9	<0.001
AOPP:TP ($\mu mol \cdot g \cdot dL^{-1}$)	5.28 ± 1.04	6.34 ± 1.88**	6.12 ± 1.97*	5.47 ± 1.05	5.91 ± 1.45	0.005

Values are presented as mean ± SD; $N = 42$.
* Significantly different from prerace values, $P < 0.05$.
** Significantly different from prerace values, $P < 0.01$.
*** Significantly different from prerace values, $P < 0.001$.
CD, conjugated dienes; MDA, malondialdehyde; oxLDL, oxidized LDL; AOPP, advanced oxidation protein products; TP, total protein.

(except the ironman itself). The subjects had fasted overnight before the 2-d prerace and the 5- and 19-d postrace blood samples, but on race day and 1 d postrace, they were allowed to drink and eat *ad libitum*, and the quantities of intake were recorded. After the triathlon, the athletes performed "recovery" training that was of moderate intensity and duration until the end of the study (Table 1).

Race conditions. The ironman triathlon was held in Klagenfurt, Austria, on July 16, 2006, and consisted of a 3.8-km swim, followed by 180-km cycling and 42.2-km running. When the race started at 7:00 a.m., the air temperature and relative humidity were 15°C and 77%, with the lake temperature at 25°C. Between 4:00 and 5:00 p.m., respectively, by finishing time (median time for subjects approximately 5:43 p.m.), air temperature reached a maximum and was 27.2°C, and relative humidity had decreased to 36% (data provided by the Carinthian Centre of the Austrian Central Institute for Meteorology and Geodynamics).

$\dot{V}O_{2peak}$ testing protocol. The triathletes were tested 3 wk before the race on a cycle ergometer (Ergometrics 900, Sensormedics GmbH, Höchberg, Germany). The maximal test protocol started at an initial intensity of 50 W, followed by 50-W increments every 3 min until exhaustion. During the test oxygen and carbon dioxide fractions (via Sensormedics 2900 Metabolic measurement cart), power output (PO), heart rate, and ventilation were recorded continuously. Earlobe blood samples for the measurement of the lactate concentration were taken at the beginning and at the end of each stage to determine performance parameters including the individual anaerobic threshold (IAT) (31).

Blood sampling. Each blood sample was collected into heparin, ethylenediaminetetraacetic acid, or serum vacutainers (Vacuette, Greiner, Austria). A field laboratory was installed at the race to ensure the appropriate collection of the first three blood samples. The blood was immediately cooled to 4°C and plasma or the serum separated at 1711*g* for 20 min at 4°C. Aliquots were immediately frozen at −80°C. Whole blood was taken for the hematological profile, and erythrocytes were also collected and frozen in aliquots at −80°C. All samples were analyzed within 6 months.

Hematological profile. The hematological profile was assessed with an MS4 Hematology 3-Part-Differential-Analyzer (Melet Schloesing Laboratories, Maria Enzersdorf, Austria). Exercise-induced changes in plasma volume were calculated (5) until 5 d postrace to assess expansion of plasma volume, which persists for 3 to 5 d after the cessation of demanding exercise (33). All results are reported adjusted for these changes, except for Trolox equivalent antioxidant capacity (TEAC) and ratios of oxLDL:LDL and AOPP:TP. For these indices, we used the data uncorrected for changes in plasma volume to consider their actual concentration to which the body responds.

Plasma concentration of lipoproteins and biochemical variables. Concentrations of total cholesterol (TC), HDL, triglycerides (TG), total protein (TP), and uric acid (UA) were measured using an automatic analyzer (Vitros DT 60 II module, Ortho-clinical Diagnostics, Germany). Levels of VLDL and LDL were calculated (VLDL = TG/2.2; LDL = TC − HDL − VLDL).

Plasma concentrations of markers of oxidative stress. Malondialdehyde (MDA) and conjugated dienes (CD) were both detected with high-performance liquid chromatography (HPLC) as reported previously (29). Oxidized LDL (oxLDL) concentrations were measured using an enzyme-linked immunosorbent assay (ELISA) kit (Mercodia AB, Uppsala, Sweden). Advanced oxidation protein products (AOPP) were determined via a colorimetric assay kit (Immundiagnostik AG, Bensheim, Germany). For both

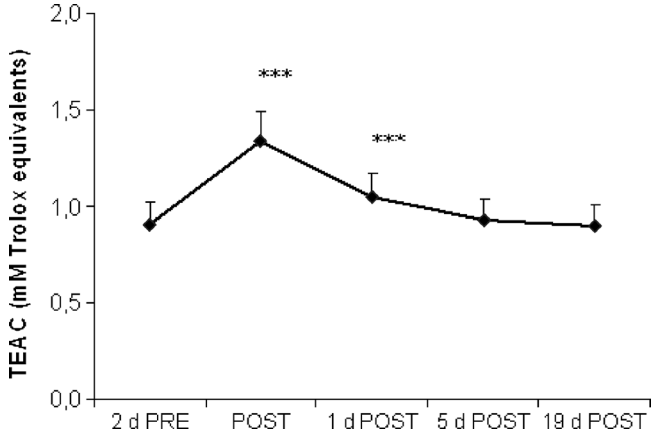

FIGURE 1—Changes in Trolox equivalent antioxidant capacity (TEAC) 2 d prerace (PRE) and immediately, 1, 5, and 19 d postrace (POST). Data are mean ± SD; $N = 42$. ***Change was significantly different from prerace values ($P < 0.001$).

TABLE 4. Plasma values of antioxidant erythrocyte enzymes.

	PRE	POST	1 d POST	5 d POST	19 d POST	Time Effect (*P*)
SOD (IU·g^{-1} Hb)	1813 ± 257	1695 ± 156***	1821 ± 194	1769 ± 177***	1722 ± 159***	<0.001
GSH-Px (IU·g^{-1} Hb)	22.5 ± 9.9	22.3 ± 9.7	21.7 ± 9.2	22.9 ± 9.4	22.0 ± 9.5	0.110
CAT (IU·g^{-1} Hb)	282 ± 59	271 ± 56*	265 ± 55***	273 ± 57	266 ± 47**	0.036

Values are presented as mean ± SD; $N = 42$.
* Significantly different from prerace values, $P < 0.05$.
** Significantly different from prerace values, $P < 0.01$.
*** Significantly different from prerace values, $P < 0.001$.
SOD, superoxide dismutase; GSH-Px, glutathione peroxidase; CAT, catalase; PRE, 2 d prerace; POST, immediately postrace; 1 d POST, 1 d postrace; 5 d POST, 5 d postrace; 19 d POST, 19 d postrace.

oxLDL and AOPP, absorbance of samples and standards were read with a Fluostar Optima microplate reader (BMG labtechnologies, Germany), and all measures were made in duplicate.

Activities of antioxidant erythrocyte enzyme and antioxidant capacity of plasma. Erythrocyte activities of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and catalase (CAT) were determined using methods reported previously (1,3,40). Briefly, the principles of these methods were as follows: SOD activity was defined via its inhibition of the auto-oxidation of 1,2,3-trihydroxybenzol (pyrogallol) in the presence of superoxide anion (O_2^-), GSH-Px activity was defined in proportion to the oxidation of $NADPH_2$ to $NADP^+$, and CAT activity was measured by the rate of breakdown of hydrogen peroxide (H_2O_2). Trolox equivalent antioxidant capacity (TEAC) of plasma was analyzed photometrically as described previously (36).

Data analysis. Data were tested for normal distribution using the Kolmogorov–Smirnov test. The main effect of time was obtained by using the repeated-measures ANOVA. Dependent on normal distribution of data, either paired *t*-tests (for normally distributed data) or Wilcoxon tests (for not normally distributed data) were then used to assess differences in the test variables, and all postrace values were compared with prerace (baseline) values. Pearson's or Spearman's correlations were used to examine any significant relationships. Subjects were divided into groups (percentiles) by exercise test variables including the relative IAT or the relative PO at $\dot{V}O_{2peak}$. One-factorial ANOVA and *post hoc* analyses with Bonferroni's test were then applied to assess whether differences in oxidative stress- and antioxidant-associated variables were associated with the percentile distribution. All statistical analyses were performed using SPSS 15.0 for Windows (SPSS Inc., Chicago, IL). Significance

TABLE 5. Significant associations with training and exercise test variables, performance, and oxidative stress markers.

	$\dot{V}O_{2peak}$	Rel. W_{peak}	IAT	Rel. IAT	PO La 2	Rel. PO La 2	PO La 3	Rel. PO La 3	Rel. PO La 3 %W_{peak}	WNET	Cycle Training	180-km Cycle Time
CD												
1 d POST					−0.33*	−0.37*	−0.36*	−0.46**				
Δ PRE to 1 d POST		−0.31*										
MDA												
PRE										−0.38*	−0.37*	
POST	−0.30*	−0.40**									−0.35*	
Δ PRE to POST		−0.34*										
1 d POST		−0.32*								−0.34*	−0.39*	
Δ PRE to 1 d POST		−0.32*										
oxLDL												
PRE		−0.30*		−0.29*	−0.35*	−0.37*	−0.34*	−0.36*				
POST		−0.37*		−0.30*								
1 d POST					−0.33*	−0.37*						
oxLDL:LDL												
PRE										0.42**	0.33*	
AOPP												
POST			0.31*	0.33*			0.31*		0.38**			−0.31*
Δ PRE to POST				0.34*	0.34*	0.39*	0.34*	0.37*	0.35*			−0.35*
1 d POST												−0.34*
Δ PRE to 1 d POST												−0.38*
AOPP:total protein												
POST		0.36*	0.31*	0.32*		0.38*		0.44**				
Δ PRE to POST				0.33*	0.33*	0.39**	0.33*	0.36*	0.32*			

CD, conjugated dienes; MDA, malondialdehyde; oxLDL, oxidized LDL; AOPP, advanced oxidation protein products; W_{peak}, peak PO; rel., relative; IAT, individual anaerobic threshold; PO La 2 (3), power output at 2 (3) mmol blood lactate·L^{-1}; WNET, weekly net endurance exercise time; PRE, 2 d prerace; POST, immediately postrace; 1 d POST, 1 d postrace; Δ PRE to (1 d) POST, change from prerace to immediately (1 d) postrace; *$P < 0.05$; **$P < 0.01$.

was set at a P value of <0.05 and is reported as $P < 0.05$, $P < 0.01$, and $P < 0.001$.

RESULTS

Race results. The average completion time was 10 h 52 min ± 61 min (mean ± SD; Table 1). The estimated average antioxidant intake during the race was 393 ± 219 mg vitamin C and 113 ± 59 mg alpha-tocopherol. There were no differences in the amount of the consumed antioxidants between the groups divided by exercise test variables. Out of 48, three study participants failed to complete the race due to self-reported fatigue. In addition, three subjects could not participate in one or more blood sample time points and thus were excluded from the analysis.

Plasma concentrations of lipoproteins and biochemical variables. Plasma concentrations of lipoproteins and UA can be found in Table 2. LDL decreased significantly ($P < 0.001$) immediately postrace (−15%) and 1 d postrace (−26%) and stabilized below prerace concentrations until 5 d postrace (−8%; $P < 0.01$). TC significantly decreased to below prerace values 1 d (−10%; $P < 0.001$) and 5 d after the race (−4%; $P < 0.01$), whereas HDL increased immediately (+9%; $P < 0.05$) and 1 d postrace (+12%; $P < 0.01$). VLDL and TG significantly ($P < 0.001$) increased postrace (+75 and +69%, respectively). Plasma levels of UA significantly ($P < 0.001$) increased immediately after the triathlon (+49%). Thereafter, UA concentrations gradually declined but remained significantly ($P < 0.001$) elevated at all time points ($P = 0.001$ for 19 d postrace) versus prerace values (Table 2).

Plasma concentrations of markers of oxidative stress. Plasma concentrations of oxidative stress markers are shown in Table 3. A considerable (+91%) and significant increase in CD occurred immediately after the ironman triathlon ($P < 0.001$) and remained significantly elevated 1 d postrace (+13%; $P < 0.01$) when compared with prerace values. MDA trended to increase immediately after the race (+7%; $P = 0.06$), then increased further and reached statistical significance 1 d postrace (+9%; $P < 0.01$). There was a significant decrease in oxidized low-density lipoprotein (oxLDL) below prerace values immediately postrace (−13%; $P < 0.05$) and 1 d postrace (−24%; $P < 0.01$), whereas a tendency toward an increase in the oxLDL:LDL ratio 1 d postrace (+8%; $P = 0.07$) occurred. Plasma AOPP concentrations significantly ($P < 0.001$) increased by 25% immediately postrace and remained significantly ($P = 0.01$) elevated 1 d after the competition (+21%). Similarly, the AOPP:TP ratio peaked by 20% higher than prerace immediately postrace ($P < 0.01$) and remained significantly ($P < 0.05$) elevated by 16% higher than prerace values 1 d after the race. All markers of oxidative stress had returned to prerace values 5 d after the ironman triathlon, and 19 d postrace, all parameters were still similar to prerace concentrations (Table 3).

TABLE 6. Significant associations with plasma antioxidant capacity.

	$\dot{V}O_{2peak}$	W_{peak}	Rel. W_{peak}	IAT	Rel. IAT	PO La 2	Rel. PO La 2	PO La 3	Rel. PO La 3	Rel. PO La 3 %W_{peak}	Total Race Time	180-km Cycle Time	42.2-km Run Time	UA	GSH-Px
TEAC															
PRE															0.41**
POST	0.33*	0.30*	0.39**	0.34*	0.36*	0.33*	0.36*	0.36*		0.42**	−0.31*				0.37**
Δ PRE to POST	0.38*	0.41**	0.47***	0.45**	0.49***	0.46***	0.49***	0.48***	0.44**	0.56***	−0.44**	−0.40**	−0.35**	0.54***	
1 d POST															0.40**
Δ PRE to 1 d POST				0.34*	0.31*			0.31*		0.33*					
5 d POST															0.37*
19 d POST															0.47**

TEAC, Trolox equivalent antioxidant capacity; W_{peak}, peak PO; rel., relative; IAT, individual anaerobic threshold; PO La 2 (3), power output at 2 (3) mmol blood lactate·L^{-1}; PRE, 2 d prerace; POST, immediately postrace; 1 (5) [19] d POST, 1 d (5) [19] postrace; Δ PRE to POST, change from pre- to immediately postrace; Δ PRE to 1 d POST, change from prerace to 1 d postrace; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

Antioxidant capacity of plasma and activities of antioxidant erythrocyte enzymes. The time course of TEAC is shown in Figure 1, and antioxidant enzyme activities can be found in Table 4. A sharp elevation of TEAC was observed in response to the ironman triathlon (+48%; $P < 0.001$), and values remained significantly ($P < 0.001$) higher than prerace until 1 d after the race (+25%). Five and 19 d postrace, TEAC values were similar to prerace. There was a significant decrease in the activities of erythrocyte SOD (−6%; $P < 0.001$) and CAT (−4%; $P < 0.05$) immediately postrace. There was a trend toward decreased GSH-Px activity 1 d postrace (−4%; $P = 0.08$) but no significant changes during the monitoring period. SOD and CAT both followed a biphasic pattern during the recovery period and 19 d postrace, and athletes had moderate but significant decreases in the activities of SOD and CAT compared with prerace values (−5%; $P < 0.001$ and −6%; $P < 0.01$, respectively; Table 4).

Associations with training and exercise test variables, performance, and oxidative stress markers. Various significant negative correlations were obtained between parameters of lipid peroxidation and training and exercise test variables, which are shown in Table 5. In contrast, the prerace oxLDL:LDL ratio correlated positively with the weekly net endurance exercise time ($r = 0.42$; $P < 0.01$). Significant positive correlations were observed between postrace indices of protein oxidation and some exercise test variables, whereas triathlon-induced changes in AOPP were inversely related with the cycle split time ($P < 0.05$; Table 5) and the total race time ($P = 0.053$).

Associations with training and exercise test variables, performance, and plasma antioxidant capacity and antioxidant enzyme activities. There were multiple positive correlations with changes in TEAC and prerace training and exercise test variables that are summarized in Table 6. Exemplary, the change of TEAC from pre- to immediately postrace correlated with the percentage of maximum PO at 3 mmol·L^{-1} blood lactate ($r = 0.56$; $P < 0.001$). In addition, positive correlations were noted between the exercise-induced changes in TEAC and UA ($r = 0.54$; $P < 0.001$; Table 6), and both changes in TEAC and UA correlated negatively with the total race time ($r = -0.44$ and $r = -0.48$, respectively; both $P < 0.01$). Significant positive correlations were observed between activities of erythrocyte GSH-Px with TEAC (Table 6). Furthermore, GSH-Px activities correlated positively with the percentage of maximum PO at 3 mmol·L^{-1} blood lactate at prerace ($r = 0.35$; $P < 0.05$), 1 d postrace ($r = 0.39$; $P < 0.01$), and 19 d postrace ($r = 0.36$; $P < 0.05$).

Groups divided by the relative PO at $\dot{V}O_{2peak}$ and the relative IAT: effects on LDL oxidation and plasma antioxidant capacity. On the basis of the group distribution into percentiles by the relative PO at $\dot{V}O_{2peak}$, a trend was observed insofar as lower oxLDL concentrations immediately postrace were associated with higher levels in relative PO at $\dot{V}O_{2peak}$ (differences between all groups: $P = 0.056$). Furthermore, athletes in the group with the highest relative PO at $\dot{V}O_{2peak}$ (top percentile) had significantly ($P < 0.05$) lower oxLDL concentrations immediately postrace than those athletes in the group with the lowest relative PO at $\dot{V}O_{2peak}$ (lowest percentile), and an according trend was noted with prerace oxLDL concentrations ($P = 0.059$). The association of pre- to postrace changes in TEAC with the percentile distribution by the relative IAT is shown in Figure 2. TEAC increased with the relative IAT across the percentiles, and the differences between all groups were $P = 0.18$. Moreover, the TEAC response was significantly ($P < 0.05$) higher in the subject group with the highest relative IAT (top percentile) compared with the group with the lowest IAT (lowest percentile; Fig. 2).

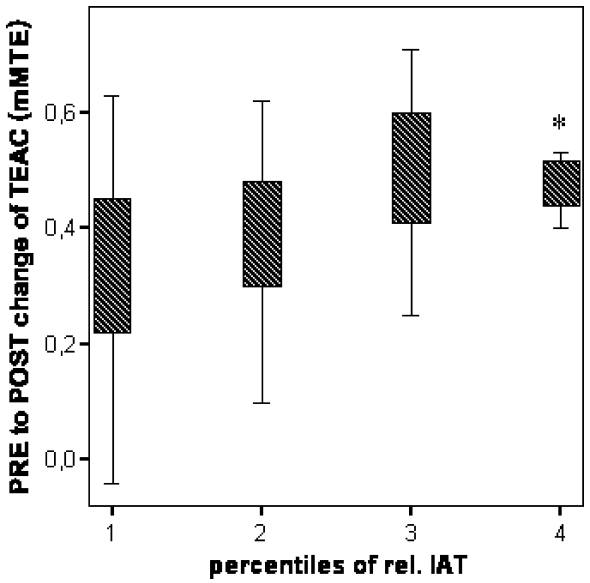

FIGURE 2—Association of the prerace (PRE) to postrace (POST) change of Trolox equivalent antioxidant capacity (TEAC) with the percentiles of the relative IAT (rel. IAT). TE indicates Trolox equivalents; percentile 1, ≤2.64 W·kg^{-1}; percentile 2, 2.65–2.89 W·kg^{-1}; percentile 3, 2.90–3.24 W·kg^{-1}; percentile 4, ≥3.25 W·kg^{-1}. *Significant different from percentile 1 ($P < 0.05$); differences between all percentiles: $P = 0.18$.

DISCUSSION

The major finding of the present study was that there are no indications of persistent oxidative damage during a single bout of ultraendurance exercise. Although most (but not all) oxidative stress markers temporarily increased after the ironman triathlon, the current results indicate the importance of the acute exercise-induced alterations in antioxidant capacity of the athletes, which were associated with a variety of physiological training-related determinants. Our data suggest that these training- and/or exercise-induced biochemical and physiological responses in the antioxidant defense system are able to counteract severe or persistent oxidative damage to cell compounds and blood lipids after extremely demanding exercise. Considering the current concerns about health consequences for ultraendurance

athletes, these findings provide important and novel information because oxidative stress and recovery responses after ultraendurance exercise, up to date, have not been followed for such a long time course.

Most but not all previous studies have shown increased oxidative stress as immediate responses to acute bouts of ultraendurance exercise. Various indices of lipid peroxidation such as MDA (11), lipid hydroperoxides (22), or F_2-isoprostanes (19,22,23) were found to be elevated after long-distance triathlons (11,23), an 80-km race (22), and another 50-km ultramarathon (19). Contrary, after other long-distance triathlon races, authors reported either no evidence of oxidative stress (17) or even a decrease in the susceptibility of plasma lipids to peroxidation (7). Some of these methods such as the TBARS assay have been criticized for insufficient accuracy, specificity, and validity (9,10,13). Therefore, we measured CD, as this method is considered as a specific marker for the initial phase of lipid peroxidation (6,36,37), and detected MDA by high-performance liquid chromatography (HPLC). Moreover, the study is the first in which attention is drawn on the effects of ultraendurance exercise on oxLDL and AOPP. Both parameters are seen as novel and reliable biomarkers that indicate long-term effects of oxidative stress (4,24) such as after a high volume training period.

In the current investigation, different amplitudes and kinetics were observed in the examined oxidative stress markers. Although there was an immediate and marked rise in CD, followed by a rapid decline, MDA increased only slightly immediately after race completion but rose to significant levels 1 d after the ironman triathlon. One explanation for the differences in the changes of these indices is that CD are primary oxidation products formed during initial reactions of lipid peroxidation, whereas MDA is produced at a latter stage of the lipid peroxidation chain reactions (6,36). Furthermore, our data suggest that enhanced postrace antioxidant defenses (described later in detail) might have played a role in preventing a more pronounced rise in MDA. AOPP concentrations and AOPP:TP ratio peaked immediately postrace. Importantly, despite a decrease (from immediately postrace to 1 d postrace) in CD, AOPP, and AOPP:TP ratio, all these markers in addition to MDA remained significantly above prerace values 1 d postrace. This apparently indicates that peroxidation of membranes or/and blood lipids as well as oxidative modification of plasma proteins are sustained for at least 1 d after prolonged strenuous exercise. Although a delayed removal of oxidized products cannot be excluded, our findings that concentrations of nutritive antioxidants dropped below prerace values 1 d postrace (21) (probably reflecting increased antioxidant consumption associated with the counteracting of increased ROS formation) further support the concept of continued oxidative stress responses. Although augmented susceptibility of LDL particles to oxidation (16) and increased lipid peroxides levels (8) persisted over 4 to 8 d after a marathon run, Mastaloudis et al. (19) reported that F_2-isoprostanes (together with IL-6) had returned to prerace values 1 d postrace in ultramarathon runners. Observed correlations between protein oxidation markers and markers of muscle damage and inflammation (unpublished results) might point to muscular inflammatory processes as a source of this low-grade oxidative stress response 1 d postrace (6,13,38). Crucially, oxLDL even significantly decreased below prerace values after the race. This change is most likely a consequence of an enhanced lipoprotein metabolism and the decline of LDL cholesterol itself as demonstrated after another ironman race (7). However, the oxLDL:LDL ratio showed a modest and only temporary trend to increase 1 d after the competition. In line with observations of Ginsburg et al. (7), who reported a reduced susceptibility of plasma lipids to peroxidation in male ironman competitors, this finding suggests that a single bout of ultraendurance exercise might not contribute to the development or progression of atherosclerosis lesions based upon the oxidative modification of LDL hypothesis (34). Moreover, as all oxidative stress markers had returned to prerace values 5 d postrace and remained at prerace levels 19 d postrace, there are no indications of persistent oxidative stress during an ironman triathlon.

A wide range of correlations with training volume, training status, and oxidative stress markers were present (Table 5). Consistent with a previous study of Knez et al. (11), who reported a dose–response relationship of resting MDA concentrations with time spent training, we observed a positive correlation between the prerace oxLDL:LDL ratio and the weekly net endurance exercise. In contrast to the demonstrated oxLDL responses to acute ultraendurance exercise, this might reflect cumulative oxidative stress that had been attributed to high training volumes or overload training (6,18). On the other hand, our data revealed that those athletes with the highest PO at $\dot{V}O_{2peak}$ had significantly lower plasma oxLDL (but not LDL) concentrations after the triathlon compared with the subjects with the lowest PO at $\dot{V}O_{2peak}$ (percentile distribution). Additionally, MDA before, after, and 1 d postrace concentrations were also lower with higher training status and with increasing weekly training loads. Furthermore, we found many negative associations with markers of lipid peroxidation and variables associated with training status, both prerace as well as in response to the race (Table 5), indicating that better training levels might confer enhanced protection against oxidative stress and consequent damage of lipids and/or result in a decrease in free radical formation. Thus, the results from the present study support the idea that endurance training reduces postexercise oxidative stress (6,13).

Interestingly, opposite to the triathlon-induced effects on markers of lipid peroxidation, AOPP and AOPP:TP ratio were positively related to some training-associated variables. In addition with the finding that AOPP rose with the performance in the cycle split time in the ironman race, these data might imply that there is an intensity-related response in protein oxidation because better trained athletes were capable of competing the ironman at higher

intensities and therefore had more pronounced changes in protein oxidation. On the basis of previous observations in exercised animals (27), these results possibly suggest that proteins are more prone to free-radical–induced oxidation during strenuous endurance exercise than lipids. Taken together, our results reveal a complex picture of oxidative stress during exhaustive endurance exercise that emphasizes the importance to use multiple markers and to monitor them in a longer time course for several reasons. First, recent research has shown that there are optimal time points for the detection of maximum concentrations of oxidative stress markers (20,38). Second, our results support findings (27,28) that lipids and proteins might be affected differently by exercise-induced oxidative stress. Since, up to now, there is limited data regarding the effects of exercise on protein oxidation (38), which has striking consequences for cell function (28), it seems crucial not to focus exclusively on indices of lipid peroxidation. Finally, little information is available on the complete resumption of recovery in these indices especially after ultraendurance exercise (17,19), which might be important for assessing possible deleterious health effects.

In agreement with previous studies in marathon runners (16,37), but probably investigated for the first time in ultraendurance athletes, plasma antioxidant capacity rose markedly after the ironman race. The alteration in the total antioxidant capacity of plasma can be seen as an early adaptive response to oxidative stress (26), which might have prevented initiation of lipid peroxidation to a certain degree. One day after the race, TEAC declined but still remained elevated above prerace values. Although TEAC was still found to be increased 4 d after a marathon (16), it had returned to prerace levels 5 d postrace along with oxidative stress markers in the present study. Our data suggest several mechanisms for the observed postrace increase in the antioxidant capacity of plasma. On the one hand, the increase in TEAC might be a result of the elevation of vitamin C (which change correlated with that of TEAC) and alpha-tocopherol (21), attributed to the intake of these antioxidants during the race as well as tissue mobilization (19). On the other hand, concomitant with previous findings (16,19), the current results imply that UA is responsible for the rise in TEAC to a considerable extent (Table 4). Plasma concentrations of the potent hydrophilic antioxidant UA are known to rise during intense exercise being produced from increased purine metabolism (6,13,38) and possibly also due to impaired renal clearance (19). We found that both TEAC and UA increased with performance in the ironman triathlon. Consequently, these results suggest that those athletes with a higher training and performance status could push themselves harder, which in turn resulted in higher concentrations of UA after the race. This phenomenon cannot be considered as a specific training adaptation, but it contributes to the performance-linked increase of TEAC. Of further interest, we observed that the ironman-induced change of TEAC was associated with the relative IAT (percentile distribution), that is, TEAC increased in athletes with greater performance ability (Fig. 2). Moreover, several other training physiological determinants (associated with training and performance capacity at different exercise intensities) seem to play important roles in promoting such a protective response in antioxidant defenses of plasma (Table 6). Interestingly, TEAC was positively related to GSH-Px activities throughout the monitoring period (Table 6), which may imply a synergistic interaction between erythrocytes and plasma antioxidant capacity. However, although we noted that GSH-Px was linked with training status, it is unclear whether or to which extent training-induced adaptations of endogenous antioxidant defenses (in particular antioxidant enzymes) might have contributed to the rise of TEAC after the ironman race. In previous studies, high-dosed antioxidant supplementation in competitors of ultraendurance races had either beneficial (19), adverse (i.e., pro-oxidant) (23,11), or no effects (22) on oxidative stress changes. With the exception of the reported relationship between the changes in vitamin C and TEAC, there was no association found between plasma levels of nutritive antioxidant and oxidative stress or antioxidant responses in the present study. Despite individual differences in the plasma concentrations of nutritive antioxidants, this observation may be because antioxidant status of all subjects was in a normal physiological range (21).

Several studies showed increased antioxidant erythrocyte enzyme activities after relatively short bouts of aerobic exercise (6), whereas different patterns or opposite effects (decreases) were seen after a 171-km cycling mountain stage (2) or a marathon (8). Only a small number of studies have examined adaptations to or acute effects of ultraendurance exercise on the antioxidant system (11,17). Recently, Knez et al. (11) reported that activities of all key antioxidant enzymes in erythrocytes declined immediately after an ironman race. Except for GSH-Px (which only trended to decrease), our data further confirm these somewhat unexpected results as we also observed a significant decrease in the activities of SOD and CAT after the competition. In general, modifications of antioxidant enzyme activities after exercise characterize either adaptation (an increase in the activity at first) or utilization (a decrease if oxidative stress is overwhelming) (6). These decreases had, hypothetically, been attributed to a modification of the catalytic centers and subsequent inactivation of enzymes due to a disturbed redox balance induced by augmented oxidative stress (2,11,38). Contrary to the acute effects of the ironman triathlon, the attenuation of SOD and CAT activities 19 d after the competition to below prerace values likely can be explained by a down-regulation during the recovery period (6). Moreover, we noted that activities of GSH-Px were positively associated with the percentage PO at a blood lactate concentration of 3 $mmol \cdot L^{-1}$. This finding supports data that this enzyme may be highly responsive to endurance training in general (11,17) and to training at higher-intensities in particular. This conclusion is

supported by evidence in the skeletal muscle response to exercise (25).

CONCLUSION

The present data indicate that a single bout of ultra-endurance exercise is associated with a systemic acute and elevated oxidative stress response. Although the disturbance in the oxidant/antioxidant balance was sustained for at least 1 d after the ironman triathlon, there are no indications of persistent detrimental health effects due to oxidative stress. Moreover, our results provide further evidence that there are chronic training-induced biochemical adaptations (resulting either in a decrease in free radical production and/or in an enhancement of the antioxidant defenses) and that manifold training-associated determinants might be responsible for these protective responses to a certain extent. Weekly training loads as well as training at *different* intensities seem to be factors in the improvement of antioxidant defense mechanisms after prolonged intense exercise. Finally, the present investigation illustrates that even minor differences in the training status among well-trained athletes can result in significantly different outcomes in the training- and exercise-induced responses of oxidative stress and antioxidant-related parameters. Generally, these data imply that acute ultraendurance exercise does not cause longer lasting alterations in systemic oxidative stress markers, probably due to improved antioxidant responses to strenuous exercise in well-trained athletes.

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