Original Research

Antioxidant Supplementation and Tapering Exercise Improve Exercise-Induced Antioxidant Response

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Objective and Methods: The present controlled-training, double-blind study (supplemented, n = 7; placebo, n = 9) investigated whether taper training (TT) and antioxidant supplementation, i.e., 150 μg of selenium, 2000 IU of retinol, 120 mg of ascorbic acid and 30 IU of α-tocopherol, modulates antioxidant potential, redox status and oxidative damage occurrence both at rest and in response to exercise. Two weeks of TT followed four weeks of overloaded training. Dietary intakes were recorded. Before and after TT, triathletes did a duathlon consisting of 5-km run, 20-km bike and 5-km run. Biological studies were conducted at rest and after exercise.

Results: Whatever the nutritional status, TT induced a decrease in resting blood reduced glutathione (GSH) concentration ($p < 0.001$), erythrocyte superoxide dismutase (SOD) activity ($p < 0.0001$) and plasma total antioxidant status (TAS) ($p < 0.05$). Only in the supplemented group (Su) with TT, did plasma glutathione peroxidase (GSH-Px) activity decrease ($p < 0.05$) and $CD4^+$ cell concentration increase ($p < 0.05$). However, antioxidant supplementation increased plasma TAS increase in response to exercise and TT ($p < 0.05$). After exercise, TT also induced a lower decrease in blood reduced and oxidized (GSSG) glutathione ($p < 0.01$) in both groups, but TT had no effect on lipoperoxidation as estimated by plasma thiobarbituric reactive substances or on muscular damage occurrence estimated by plasma creatine kinase isoenzyme MB mass.

Conclusion: During TT, antioxidant supplementation at nutritional doses reinforces antioxidant status response to exercise, with an effect on exercise-induced oxidative stress, and no effect on oxidative damage.

INTRODUCTION

Reactive radical oxygen species (ROS) generation increases under aerobic endurance stress. The major source of ROS is thought to be the mitochondria of active muscle [1], but free radicals are also produced by red blood cells or during inflammatory response [2]. When the antioxidant system is not adapted to excessive production of ROS, oxidative stress is initiated. ROS are potent inducers of various cellular damage affecting lipids, proteins and nucleic acids. Imbalance between oxidants and antioxidants can affect the normal function of immune cells [3]. To prevent exercise-induced oxidative stress, the organism is well equipped with antioxidant defense systems including enzymes such as superoxide dismutase (SOD), catalase, and glutathione peroxidase (GSH-Px), and non-enzymatic substances such as reduced glutathione (GSH) and vitamins A, C, E, and selenium [1] that act in synergy. Vitamin E is considered the most important lipid-soluble exogenous antioxidant in humans. Vitamin C serves as an antioxidant directly by scavenging aqueous peroxyl radicals and indirectly by regenerating reduced vitamin E [4].

Relative to sedentary persons, athletes have lower vitamin C and GSH plasma concentration [5] and higher resting lipoperoxidation (LIPOX) indices [6]. Because of their well-known consumption of low-density nutrient food products, antioxidant supplementation might be appropriate for athletes requiring

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high-energy intakes [7]. Antioxidant supplementation has a favorable effect on LIPOX process in highly trained athletes [8] or in overloaded athletes [9] and prevents marginal status of vitamin C in professional basketball players [10]. Otherwise, although repetitive stimulations increase erythrocyte antioxidant enzyme activities [11–13] and reduced GSH concentration [12], their consequences on exercise-induced oxidative damage indices appear to be linked to subjects' adaptation state when engaged in studies. When subjects are initially not adapted to exercise, aerobic training reduces exercise-induced oxidative damage [2,14]. In adapted subjects, however, an overloaded training stimulus can provoke a transitory or prolonged lack of physiological and/or biochemical adaptations. To optimize performance, undesirable effects of overloading are commonly reduced by lowering training volume (tapering) before a major competition. Beneficial effects have been shown by decreased biochemical and physiological markers of training stress [15]. In this situation, the decrease in training volume could diminish oxidative stress and consequently oxidative damage through a decrease in prooxidant generation. Therefore, after an overloaded training (OT), we could expect taper training (TT) to reinforce antioxidant potential and decrease exercise-induced oxidative stress and damage.

We hypothesize that reinforcement of antioxidant potential by antioxidant supplementation with a complex of selenium, vitamin A, vitamin C and vitamin E would reduce oxidative stress and exercise-induced oxidative damage.

MATERIAL AND METHODS

Subjects and Study Design

Twenty volunteer French male triathletes were recruited to participate in a ten-week controlled training (four-week normal training, four-week overloaded training (OT) and two-week taper training (TT)) double-blind study. They were randomly assigned to two groups taking either an antioxidant complex supplement (Su group) or a placebo (Pla group). Triathletes were long-distance competitors in professional activities. All subjects were non-smokers, had no history of medical disorders, and had not taken antioxidant supplements for at least six months prior to the study. They were instructed to refrain from making any drastic changes in diet and to abstain from anti-inflammatory or analgesic drugs throughout the study. All the subjects gave written informed consent. The protocol was approved by the Committee for the Protection of Persons in Biochemical Research (No. 99002). One Pla group subject and three Su group subjects were excluded from the study because of injuries or for family reasons. After the four-week OT period, triathletes had significant signs of overtraining (increased performance time, increased epinephrine urinary concentration, and increased total profile of mood state score). Anthropometric and physiological characteristics are reported in Table 1.

Table 1. Anthropometric and Physiological Data for Supplemented (Su, n = 7) and Placebo (Pla, n = 9) Triathletes

	Su Group	Pla Group
Age (years)	33.2 ± 9.8	32.6 ± 10.5
Body Mass (kg)	67.8 ± 3.6	68.9 ± 4.3
Height (cm)	175.7 ± 7.1	176.2 ± 7.1
Body Fat (%)	11.4 ± 3.7	11.5 ± 1.7
$\dot{V}O_{2max}$ (mL/minute/kg)	63.7 ± 1.5	64.1 ± 5.2

Values are expressed as mean ± standard deviation (SD).
HR = heart rate, $\dot{V}O_{2max}$ = maximal oxygen uptake.

Supplementation

The Su group consumed two oral tablets per day of an antioxidant complex preparation: Selenium A-C-E® (Richelet Laboratories, Paris, France; each tablet contained 75 μg of organic selenium, 1000 IU of retinyl acetate, 60 mg of ascorbic acid, and 15 IU of D-α-tocopheryl succinate). The Pla group received a placebo preparation, which was identical in amount, aspect and taste as for the Su group. Triathletes were told before the beginning of the study to comply carefully with the treatment and to take one tablet before breakfast and one tablet before lunch.

Training Program and Quantification

Referring to the competition program of long-distance triathletes, two-week TT followed a four-week OT phase. Training program before and during TT was set by a coach. Training loads were clearly defined for each subject, quantitatively by the collection of personal details about past training and qualitatively by functional assessments as described in Palazzetti *et al.* [16]. Individual training loads were quantified by a modified version of the method of Morton [16]. During the two-week TT, training volumes in swimming, cycling, and running were progressively and significantly reduced (Fig. 1, Table 2).

Dietary Record

Dietary records were kept each day of the study for each meal. All triathletes were instructed on proper nutritional recording, including estimating portion sizes. Data acquisition was made in grams, and daily nutritional consumption was quantified by the CIQUAL data base (Regal micro Windows 9x, NT Version 1.2, Max Feinberg, France).

Daily antioxidant micronutrient requirements for French sportsmen whose daily energy expenditure exceeds 2200 kcal (or 9.2 MJ) have recently been established by the "French Food Agency of Sanitary Security" [17]. A corrected coefficient is added for each antioxidant according to the daily energy expenditure over 2200 kcal. The Su and Pla groups did not differ in food antioxidant intakes, but they differed significantly ($p < 0.05$) in antioxidant supplement intake. Supplementation during TT permitted them to compensate their deficit in vitamin E (Fig. 2).

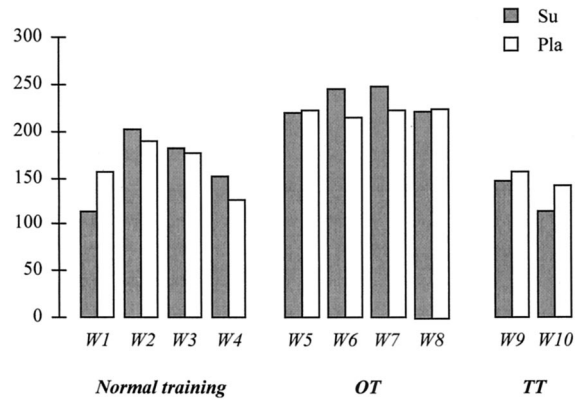

Fig. 1. Total weekly training load in supplemented (Su, n = 7) and placebo (Pla, n = 9) triathletes during normal training, overloaded training (OT) and taper training (TT). Total weekly training load is expressed in arbitrary units (AU). W1: week number 1 in training program.

Functional Assessments

Identical series of measurements were repeated by all triathletes after OT and after TT. Each test was performed at the same time of day.

Maximal Treadmill Test. Triathletes performed a continuous, incremental running test on a motorized treadmill (2500 ST, GYMROL, Andrezieux Boutheon, France). The test began with a warm-up at 10 km/hour (2% slope) for five minutes; running speed was then increased by 2 km/hour every two minutes up to 14 km/hour and by 1 km/hour to exhaustion.

During treadmill tests, ventilatory and gas exchange responses were measured on a breath-by-breath basis using an automatic spiroergometric system (Vmax 29, Sensor Medics, Rungis, France). Heart rate (HR) was monitored continuously and recorded using an electrocardiograph monitor (HELLIGE, SMS 182, Freibourg in Breisgau, Germany) and a telemetric system (Polar Accurex Plus, Polar Electro Oy, Kempele, Finland). The criteria used for determining $\dot{V}O_{2\,max}$ were a plateau in $\dot{V}O_2$ despite an increase in load or running speed, a respiratory exchange ratio (RER) over 1.1 and an HR over 90% of the predicted maximal HR.

Duathlon Test. All duathlon tests took place outdoors between March and April in Nice, France. Outside temperature ranged from 17°C to 22°C. Triathletes performed all testing in the same equipment conditions and drank the same energy beverage. Before each duathlon test, all triathletes warmed up during 30 minutes by alternating jogging and stretching. Running trials were performed alternating lawn and asphalt on a flat circuit. Cycling was performed on an exercise bike (Elite-Travel, Fontaniva, Italy) over which was positioned the personal bike of triathletes. Duathlon tests were performed at 84 ± 2% of $\dot{V}O_{2\,max}$.

Profile of Mood States (POMS)

Every week, each triathlete completed the Profile of Mood States questionnaire (POMS) [18]. The relationship between physiological, biochemical disturbances and behavior and/or psychological indices is well documented. Originally the POMS was devised to evidence the occurrence of these disturbances in pathological situations. The POMS is now widely used as one of the tools for identifying overreaching or overtraining states. In our study, the POMS was administered to quantify the influence of training loads on Mood State and to verify whether the subjects were in an overreached state before the start of the TT period.

Blood Sampling Procedures

Venous blood samples were collected before and after two-week TT in resting and post-exercise conditions. Subjects reported to the laboratory after a day off and an overnight fast. The time of day for basal blood test was standardized to within 30 minutes for each subject, and all samples were taken between 6 a.m. and 8 a.m. Post-exercise venous blood samples were obtained immediately after the duathlon tests, which took

Table 2. Quantification of Training Loads in Swimming, Cycling, and Running in OT and TT for Supplemented (Su, n = 7) and Placebo (Pla, n = 9) Triathletes

		OT	TT	OT *vs.* TT Difference (%)
Swimming (km/week)	Su	7.6 ± 1.9	3.3 ± 1.5*	−57 ± 18
	Pla	7.5 ± 2.9	3.6 ± 1.5*	−45 ± 30
Cycling (km/week)	Su	280 ± 68	159 ± 38*	−40 ± 15
	Pla	252 ± 45	169 ± 46*	−31 ± 18
Running (km/week)	Su	39.9 ± 14.0	28.1 ± 7.8*	−37 ± 15
	Pla	42.9 ± 10.9	31.1 ± 10.3*	−26 ± 25
Total Training Load (AU)	Su	233 ± 83	129 ± 32*	−46 ± 12
	Pla	220 ± 70	149 ± 63*	−32 ± 11

Values are expressed as mean ± standard deviation (SD).

Total training loads are expressed in arbitrary units (AU).

* $p < 0.05$ relative to OT.

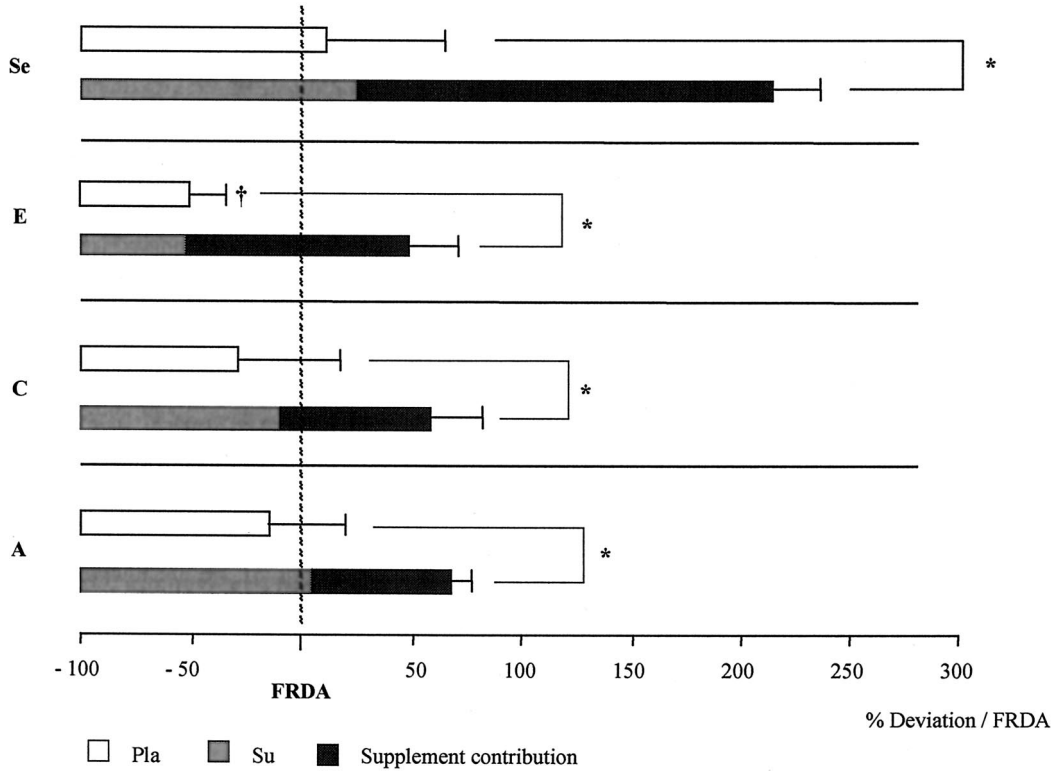

Fig. 2. Daily mean food and supplement vitamin A, C, and E and selenium (Se) intakes relative to the French Recommended Dietary Allowance (FRDA) in supplemented (Su, n = 7) and placebo (Pla, n = 9) triathletes during TT. *$p < 0.05$ relative to Pla group. $^{\dagger}p < 0.05$ relative to FRDA.

place the same day at the end of afternoon. Blood samples were collected by puncture from an antecubital vein. Whole blood (400 μL) for glutathione analysis was immediately treated as described later. The blood samples were centrifuged (4000 rpm, 4°C, 10 minutes), and plasma or serum was divided into aliquots and frozen in dry ice prior to storage at −80°C until assay.

Biological Analyses

Oxidized and Reduced Glutathione. Immediately after venipunction, 400 μL of whole blood was transferred into a tube containing 3600 μL of 6% (v/v) metaphosphoric acid in water. The solution was mixed and centrifuged for ten minutes at 4°C. Acidic protein-free supernatants were stored at −80°C until analysis. Glutathione level was determined using enzymatic cycling of reduced glutathione (GSH) by means of NADPH and glutathione reductase (GR) coupled with DTNB. We estimated oxidized glutathione (GSSG) according to the method of Akerboom and Sies, slightly modified [19]. For this we masked GSH by adding 10 μL of 2-vinyl-pyridine to 500 μL of deproteinized extract adjusted to pH 6 with triethanolamine. The mixture was allowed to stand for 60 minutes. The fraction of GSH was calculated as GSH = total glutathione −2 GSSG.

Index of Lipid Peroxidation. Thiobarbituric acid reactants were evaluated in plasma by a Perkin Elmer Model LS 50 fluorometer (Perkin-Elmer Ltd, Bucks, UK) with a malondialdehyde-kit (Sobioda, Grenoble, France) as previously described [20].

CK-MB Mass. The mass level of creatine kinase isoenzyme MB (CK-MB mass) was determined in plasma by immunoassay using the ELISA sandwich principle with fluorogenic marker.

Metalloenzymes. Plasma and erythrocyte selenium-dependent GSH-Px activities were evaluated using terbutyl hydroperoxide (Sigma Chemical Co, Via Coger, Paris, France) as substrate instead of hydrogen peroxide. This technique was adapted on a Hitachi 904 analyzer. Results are expressed as μmoles of NADPH (Boehringer-Mannheim, Germany) oxidized per minute per gram of hemoglobin for erythrocyte GSH-Px and as unit per liter for plasma GSH-Px.

Erythrocyte Cu-Zn SOD activity was measured after hemoglobin precipitation by monitoring the autoxidation of pyrogallol according to [21]. This technique was adapted on a Hitachi 904 analyzer.

Total Antioxidant Status (TAS). TAS was measured in plasma by chemiluminescent technique using a Hitachi 904 analyzer with a Total Antioxidant Status Randox-kit (Randox Laboratories Ltd, Roissy, France).

Selenium Determination. Serum selenium concentrations were determined with a Perkin Elmer 5100 (Norwalk, CT) equipped with an HGA 600 furnace, an electron discharge lamp and a Zeeman background correction [22].

Table 3. Physiological, Biological and Psychological Parameters before and after TT for Supplemented (Su, n = 7) and Placebo (Pla, n = 9) Triathletes

	Before TT		After TT	
	Su	Pla	Su	Pla
$\dot{V}O_{2\,max}$ (mL/minute/kg)	63.7 ± 1.5	64.1 ± 5.2	65.8 ± 2.8*	65.9 ± 5.4*
Duathlon Time (minutes)	68.8 ± 8.5	69.7 ± 9.5	67.7 ± 8.3*	67.2 ± 9.6*
Hematocrit (L/L)	0.44 ± 0.04	0.45 ± 0.02	0.45 ± 0.04	0.45 ± 0.03
Hemoglobin (mmol/L)	9.24 ± 0.71	9.24 ± 0.50	9.34 ± 0.60	9.33 ± 0.58
Total POMS-Score	93 ± 15	122 ± 22	84 ± 11*	107 ± 18*

Values are expressed as mean ± standard deviation (SD).
* $p < 0.05$ relative to before TT.

Vitamin Determination. Vitamin C concentration was evaluated by fluorimetry using an automated method in serum after stabilization and extraction with a 5% metaphosphoric acid solution according to Speek *et al.* [23]. Retinol and α-tocopherol concentrations were determined by HPLC as described by [24].

Hematological Parameters. Hematocrit and hemoglobin, leukocyte, neutrophil, lymphocyte, $CD4^+$ and $CD8^+$ concentrations were determined by an automated cytometer (Coulter counter VCS).

Statistical Analysis

All data are expressed as means and standard deviations (SD). To determine the main effect of training, one-way ANOVA (Treatment) with repeated measures (OT, TT) was used to analyze estimated daily energy intake, energy expenditure, macronutrient intake, body mass and body fat. Student's *t* test for paired values was used to compare estimated daily micronutrient intakes and French Recommended Dietary Allowances (FRDA) in OT and TT. Physiological and psychological data were analyzed by one-way ANOVA with repeated measures to determine interaction effect between treatments (Su group, Pla group) and training (Before-TT, After-TT). Biochemical data were analyzed by two-way ANOVA (Treatment, TT) with repeated measures (Pre-exercise, Post-exercise) to determine firstly interaction effects between treatment, training, and duathlon (Treatment × TT × Exercise), treatment and training (Treatment × TT), and treatment and duathlon (Treatment × Exercise), and secondly to determine the main effects (Treatment, TT or Exercise). When significant changes were observed in ANOVA tests, Fisher's PLSD *post hoc* test was applied to locate the source of significant differences. Statistical significance was set at $p < 0.05$.

RESULTS

TT Effects

The two-week TT program led to a significant decrease ($p < 0.01$) in total POMS-score, duathlon time (−3%) (Table 3) and daily total energy expenditure (−26%) (Table 4) and to a significant increase in $\dot{V}O_{2\,max}$ (+3%) (Table 3) and body fat

Table 4. Estimation of Daily Energy Intake, Energy Expenditure, Macronutrient and Micronutrient Intakes, Body Mass and Body Fat during OT and TT for Supplemented (Su, n = 7) and Placebo (Pla, n = 9) Triathletes

	OT		TT	
	Su	Pla	Su	Pla
Energy Intake (MJ/day)	15.0 ± 3.2	13.8 ± 3.0	15.0 ± 3.3	13.2 ± 2.9
Energy Expenditure (MJ/day)	17.0 ± 1.9	16.8 ± 2.4	12.1 ± 1.2*	12.7 ± 1.7*
Carbohydrates (g/day)	486 ± 92	469 ± 99	404 ± 92	385 ± 94
Lipids (g/day)	122 ± 40	127 ± 40	124 ± 41	125 ± 36
Proteins (g/day)	130 ± 31	122 ± 27	133 ± 38	117 ± 29
Selenium (μg/day)#	98 ± 17	83 ± 21	102 ± 19	90 ± 33
Vitamin A (μg RE/day)#	1164 ± 331	1086 ± 507	984 ± 171	835 ± 369
Vitamin C (mg/day)#	187 ± 76	138 ± 83	164 ± 52	134 ± 75
Vitamin E (mg TE/day)#	11 ± 4	11 ± 5	10 ± 2	11 ± 3
Body Mass (kg)	67.9 ± 3.6	68.9 ± 4.3	67.8 ± 3.2	69.5 ± 4.3
Body Fat (%)	11.4 ± 3.7	11.5 ± 1.7	11.8 ± 3.7*	12.1 ± 1.8*

Values are expressed as mean ± standard deviation (SD).
# Data do not include antioxidant supplementation (Selenium A-C-E®).
RE = retinol equivalent, TE = tocopherol equivalent.
* $p < 0.05$ relative to OT.

Table 5. Exogenous Cofactor, Antioxidant Vitamins and Retinol Levels in Supplemented (Su, n = 7) and Placebo (Pla, n = 9) Triathletes before and after TT in Pre- and Post-Exercise Conditions

		Before TT			After TT			ANOVA		
		Pre-Exercise	Post-Exercise	Δ (Post- *vs.* Pre-)	Pre-Exercise	Post-Exercise	Δ (Post- *vs.* Pre-)	(TT Effect)	(Ex. Effect)	(TT × Ex. Effect)
Selenium										
(μmol/L)	Su	1.35 ± 0.16#	1.48 ± 0.12#*	0.13 ± 0.13	1.33 ± 0.13#	1.45 ± 0.12#*	0.11 ± 0.07	NS	$p < 0.001$	NS
	Pla	1.08 ± 0.12	1.08 ± 0.15*	0.02 ± 0.09	1.06 ± 0.09	1.12 ± 0.16*	0.06 ± 0.10			
Ascorbic acid										
(μmol/L)	Su	69.6 ± 8.6	108.1 ± 16.6*	38.5 ± 14.3	60.1 ± 9.4	103.1 ± 25.8*	43.0 ± 20.1	NS	$p < 0.0001$	NS
	Pla	60.9 ± 13.8	84.6 ± 33.5*	29.9 ± 23.9	69.3 ± 18.3	97.8 ± 18.7*	33.1 ± 4.9			
α-tocopherol										
(μmol/L)	Su	28.1 ± 4.8	31.9 ± 5.6*	3.9 ± 1.8	29.6 ± 7.4	31.4 ± 5.7*	1.8 ± 2.7‡	NS	$p < 0.001$	$p < 0.05$
	Pla	23.1 ± 5.1	26.3 ± 6.4*	2.8 ± 2.5	24.5 ± 6.1	25.9 ± 7.2*	1.9 ± 1.6‡			
Retinol										
(μmol/L)	Su	1.89 ± 0.33	2.09 ± 0.39*	0.20 ± 0.13	2.01 ± 0.35†	2.24 ± 0.38†*	0.23 ± 0.11	$p < 0.05$	$p < 0.0001$	NS
	Pla	1.87 ± 0.23	2.05 ± 0.39*	0.25 ± 0.22	2.03 ± 0.28†	2.13 ± 0.29†*	0.11 ± 0.12			

Values are expressed as mean ± standard deviation (SD).

# $p < 0.001$ relative to Pla group.

† $p < 0.05$ relative to Before TT.

* $p < 0.05$ relative to Pre-exercise.

‡ $p < 0.05$ relative to interaction effect between TT and exercise.

(+4%) (Table 4). However, TT had no effect on hematocrit and hemoglobin concentration, body mass (Table 3), daily total energy intakes and daily macronutrient intakes (Table 4). Also during TT, we observed no variation in antioxidant micronutrient intakes (Table 4) and circulating antioxidant concentrations, except for serum retinol, whose concentrations increased with TT (+6%) (Table 5). In contrast, plasma TAS concentration was lower after TT than before (−2%, $p < 0.05$) (Fig. 3). Moreover, for endogenous antioxidant, both SOD activity (Table 6) and blood GSH concentrations (Fig. 4) were lower after TT (−5% and −7%, respectively). Blood GSSG concentrations were decreased in response to TT (−9%, $p < 0.05$) (Fig. 4). However, no significant change was found in lipoperoxidation and muscle damage markers (Table 7). Furthermore, polymorphonuclear eosinophil, lymphocyte and $CD8^+$ cell concentrations were significantly increased with TT (+23%, +8% and +20%, respectively). $CD4^+$ to $CD8^+$ cell ratio was decreased with TT (−12%) (Table 8).

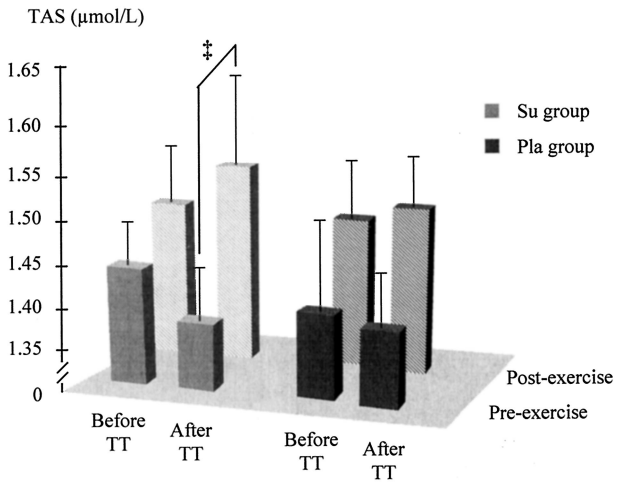

Fig. 3. Variations in plasma total antioxidant status (TAS) before and after TT in pre- and post-exercise conditions in supplemented (Su, n = 7) and placebo (Pla, n = 9) triathletes. ‡$p < 0.05$ relative to interaction effect between Treatment, TT and Exercise.

Treatment Effects

Compliance with the treatment was 97%. For exogenous circulating antioxidants, treatment induced a positive effect on selenium concentrations only (Table 5). For the endogenous antioxidant system, only plasma GSH-Px activity was significantly increased (Table 6). No effect of treatment was found on oxidative stress, lipoperoxidation and muscular damage markers (Table 7) or on concentrations of leukocytes, neutrophils, lymphocytes, $CD4^+$ and $CD4^+$:$CD8^+$ ratio (Table 8).

Interaction Effect between Treatment and TT

For endogenous antioxidant, ANOVA showed a significant interaction effect between Treatment and TT on resting plasma GSH-Px activity (Table 6). As shown by *post hoc* analysis, plasma GSH-Px activity was significantly decreased in Su group (−5%, $p < 0.05$) in response to TT; no change in activity was shown for Pla group. The decrease in blood GSH concentrations in response to TT tended to be higher with supplementation ($p = 0.07$) (Fig. 4). No significant interaction effect between Treatment and TT was observed on GSH:GSSG ratio, and lipoperoxidation and muscular damage markers (Table 7). In contrast, a positive Treatment effect during TT was found on $CD4^+$ cell concentrations (Table 8).

Table 6. Parameters of Endogenous Antioxidant Potential in Supplemented (Su, n = 7) and Placebo (Pla, n = 9) Triathletes before and after TT in Pre- and Post-Exercise Conditions

		Before TT			After TT			ANOVA		
		Pre-Exercise	Post-Exercise	Δ (Post- *vs.* Pre-)	Pre-Exercise	Post-Exercise	Δ (Post- *vs.* Pre-)	(Treat. × TT Effect)	(Treat. × Ex. Effect)	(TT × Ex. Effect)
SOD (U/mg Hb)	Su	1.34 ± 0.09	1.31 ± 0.06	−0.03 ± 0.04§	1.25 ± 0.08†	1.25 ± 0.09†	0.004 ± 0.04§	NS	$p < 0.01$	NS
	Pla	1.33 ± 0.15	1.34 ± 0.12	0.01 ± 0.04	1.28 ± 0.13†	1.32 ± 0.14†	0.03 ± 0.03			
e GSH-Px (U/g Hb)	Su	43.7 ± 9.5	45.2 ± 10.8*	1.5 ± 2.1	44.1 ± 10.2	44.9 ± 10.6*	0.8 ± 0.7	NS	NS	NS
	Pla	39.6 ± 9.9	40.5 ± 10.7*	1.0 ± 1.0	40.0 ± 10.8	40.1 ± 10.4*	0.1 ± 1.1			
p GSH-Px (U/L)	Su	472.9 ± 62.2#	492.0 ± 66.5#*	19.1 ± 21.1	452.4 ± 64.5#	498.1 ± 53.9#*	45.7 ± 19.6¥‡	$p < 0.05$	NS	$p < 0.05$
	Pla	397.7 ± 46.3	415.0 ± 62.6*	17.3 ± 26.0	401.3 ± 50.4	429.4 ± 59.0*	28.1 ± 10.0‡			

Values are expressed as mean ± standard deviation (SD).

e GSH-Px = erythrocyte glutathione peroxidase activity, p GSH-Px = plasma glutathione peroxidase activity.

# $p < 0.05$ relative to Pla group.

† $p < 0.001$ relative to Before TT.

* $p < 0.01$ relative to Pre-Exercise.

¥ $p < 0.05$ relative to interaction effect between Treatment and TT.

§ $p < 0.05$ relative to interaction effect between Treatment and Exercise.

‡ $p < 0.05$ relative to interaction effect between TT and exercise.

Table 7. Markers of Free Radical Production and Muscle Damage in Supplemented (Su, n = 7) and Placebo (Pla, n = 9) Triathletes before and after TT in Pre- and Post-Exercise Conditions

		Before TT			After TT			ANOVA
		Pre-Exercise	Post-Exercise	Δ (Post- *vs.* Pre-)	Pre-Exercise	Post-Exercise	Δ (Post- *vs.* Pre-)	(TT × Ex. Effect)
GSH:GSSG ratio	Su	65.9 ± 19.7	30.2 ± 4.6*	−35.6 ± 20.7	55.0 ± 23.1	38.5 ± 7.6*	−16.5 ± 21.0	
	Pla	76.0 ± 28.7	31.0 ± 4.3*	−39.3 ± 29.2	105.2 ± 79.7	37.4 ± 5.7*	−74.0 ± 82.6	NS
TBARS (μmol/L)	Su	2.20 ± 0.31	2.35 ± 0.36*	0.15 ± 0.20	2.15 ± 0.34	2.24 ± 0.43*	0.10 ± 0.09	
	Pla	1.92 ± 0.26	2.05 ± 0.32*	0.09 ± 0.18	1.98 ± 0.39	2.02 ± 0.47*	0.10 ± 0.17	NS
CK-MB mass (ng/L)	Su	3.86 ± 1.67	5.86 ± 5.01*	2.00 ± 3.56	4.86 ± 3.62	6.29 ± 4.11*	1.43 ± 1.81	
	Pla	3.78 ± 4.99	5.78 ± 4.69*	1.89 ± 1.17	3.67 ± 1.58	5.00 ± 1.41*	1.12 ± 2.47	NS

Values are expressed as mean ± standard deviation (SD).

* $p < 0.05$ relative to Pre-Exercise.

Interaction Effect between Treatment and Exercise

ANOVA showed a significant interaction effect between Treatment and Exercise on erythrocyte SOD activity (Table 6). The decrease in blood GSH:GSSG ratio in response to exercise tended to be lower with supplementation ($p = 0.10$) (Table 7).

Interaction Effects between TT and Exercise

Whatever the nutritional status, TT significantly decreased the exercise-induced increase in α-tocopherol serum concentration (−39%, $p < 0.05$), but did not induce an effect on other exogenous circulating antioxidant concentrations (Table 5). TT, however, further increased the exercise-induced increase in plasma TAS (+63%) (Fig. 3) and in GSH-Px activity (+115%) (Table 6). Moreover, TT caused a significant decrease in exercise-induced oxidative stress, as shown by the lower (−52%) decrease in blood GSH and lower (−29%) increase in blood GSSG (Fig. 4). Yet the decrease in oxidative stress was not accompanied by significant changes in lipoperoxidation and muscular damage markers (Table 7).

Interaction Effects among Treatment, TT and Exercise

ANOVA showed a significant interaction effect among Treatment, TT and Exercise on plasma TAS (Fig. 3). This effect was mainly due to a significantly higher exercise-induced TAS increase after TT in Su group (+235%, $p < 0.05$), evidenced by post-hoc analysis. For oxidative stress, the exercise-induced blood GSSG increase after TT tended to be lower in Su group ($p = 0.08$) (Fig. 4). No significant interaction effect was found on exogenous circulating antioxidant concentrations (Table 5), on the endogenous antioxidant system (Table 6, Fig. 4) or on lipoperoxidation and muscular damage markers (Table 7).

DISCUSSION

Antioxidant supplementation is effective against oxidative damage in highly trained subjects [8,10]. We asked whether

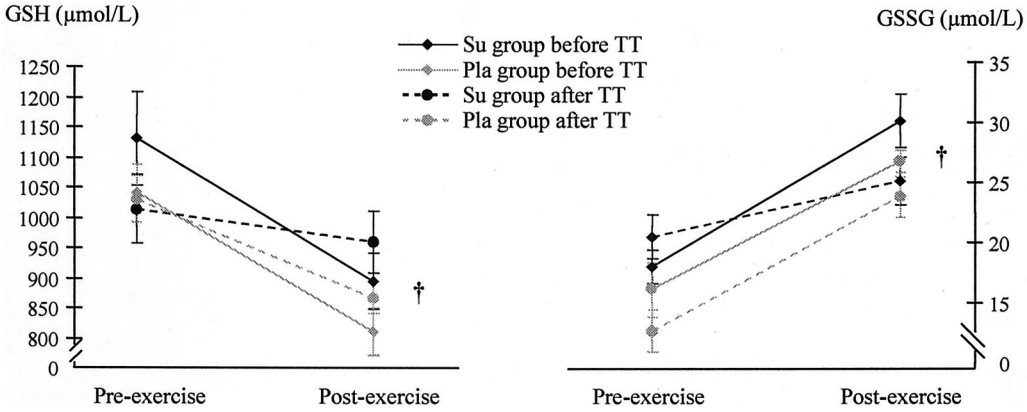

Fig. 4. Variations in blood reduced (GSH) and oxidized (GSSG) glutathione concentration before and after TT in pre- and post-exercise conditions in supplemented (Su, n = 7) and placebo (Pla, n = 9) triathletes. $^{\dagger}p < 0.05$ relative to interaction effect between TT and Exercise.

antioxidant supplementation could protect against exercise-induced oxidative damage during a period of reduced training stress.

Although neither LIPOX nor exercise-induced increase in muscle damage varied with TT and/or supplementation, TT reduced the magnitude of oxidative stress enhancement, as evidenced by the lower GSH oxidation. The decrease, with TT, in resting blood GSH and TAS concentrations and SOD activity had no impact on the GSH:GSSG ratio or on cellular indices of oxidative damage. The very low magnitude of changes in these antioxidant levels can explain the absence of biological effects. This absence may reflect the antioxidant system's adaptability in maintaining redox homeostasis. As oxidized forms of vitamins E and C require GSH to be reduced, the decrease in vitamin E and C oxidation, due to reduced training loads, could thus decrease the need for GSH in the cell and consequently the synthesis rates. This notion is consistent with the increase in glutathione synthesis after oxidative stress [25].

It is not clear how exogenous antioxidants affect the efficiency of the endogenous antioxidant system. The higher activity in the seleno-dependent enzyme GSH-Px with supplementation is obviously related to the selenium contained in the mixture. This is consistent with data on sedentary [26] and on trained but previously sedentary subjects [27]. Resting plasma GSH-Px activity was decreased in the supplemented group only during TT, even though plasma selenium concentration remained unchanged. Thus, it could result from repletion of critical pools of selenium, particularly the high priority selenoprotein P, given the wide function of selenium. The decrease in training load and the resulting decrease in free radical production can explain the lack of antioxidant supplement efficacy on antioxidant systems measured at rest. The major effects of antioxidant supplementation in our exercise conditions can be accounted for by the fact that antioxidant synergic action can be evidenced when oxidative stress is increased [28].

$CD4^+$ cell concentration, known to decrease with heavy training [29], increased during TT in our study with antioxidant supplementation. Lymphocyte proliferation is suppressed by ROS [4], and most nutritional deficits affect T-cell functions [30]. Yet the mechanisms remain unclear. Several mechanisms can be involved, and they appear tightly linked with the ability of selenium to increase the regulation of interleukin-2 (Il-2) receptor expression on activated lymphocyte surface and on NK cells, thus facilitating interaction with Il-2, which stimulates clonal expansion [31]. Moreover, selenium—contained in GSH-Px selenoproteins—could limit ROS damaging effects on those cells [32].

Table 8. Concentrations of Leukocytes, Neutrophils, Lymphocytes, $CD4^+$ and $CD4^+$:$CD8^+$ Ratio in Supplemented (Su, n = 7) and Placebo (Pla, n = 9) Yriathletes before and after TT

	Su Group			Pla Group			ANOVA	
	Before TT	After TT	Δ (After *vs.* Before)	Before TT	After TT	Δ (After *vs.* Before)	(TT Effect)	(Treat. × TT Effect)
Leukocytes (10^9/L)	5.59 ± 1.10	5.83 ± 0.64	0.24 ± 0.99	6.43 ± 1.23	6.22 ± 0.60	−0.21 ± 1.29	NS	NS
Neutrophils (10^9/L)	2.84 ± 1.16	2.80 ± 0.52	−0.04 ± 0.97	3.64 ± 1.20	3.42 ± 0.60	−0.22 ± 1.08	NS	NS
Lymphocytes (10^9/L)	1.91 ± 0.54	2.16 ± 0.60*	0.24 ± 0.17	1.96 ± 0.32	2.00 ± 0.31*	0.04 ± 0.27	$p < 0.05$	NS
$CD4^+$ (10^9/L)	0.73 ± 0.22	0.80 ± 0.26	0.07 ± 0.09	0.77 ± 0.16	0.74 ± 0.15	−0.02 ± 0.07‡	NS	$p < 0.05$
$CD4^+$:$CD8^+$	1.55 ± 0.57	1.35 ± 0.51*	−0.20 ± 0.29	1.75 ± 0.54	1.51 ± 0.46*	−0.24 ± 0.23	$p < 0.01$	NS

Values are expressed as mean ± standard deviation (SD).

* $p < 0.05$ relative to Before TT.

‡ $p < 0.05$ relative to interaction effect between Treatment and TT.

In trained athletes, some parameters of endogenous antioxidant defense are correlated with $\dot{V}O_{2max}$ [13] or training load [12]. Conservation of training intensity during TT allows $\dot{V}O_{2\,max}$ to increase and avoids the decrease in oxidative capacity that occurs in the detraining process. To our knowledge, no studies relative to detraining have been performed on those biochemical parameters. The two-week TT led to decreased resting erythrocyte SOD activity, but no effect was found on erythrocyte GSH-Px activity. As TT is characterized by a decrease in total physical activity, the consecutive decrease in anion superoxide production could induce the decrease in resting SOD activity. Thus SOD activity, which seems to require a higher training load to be up-regulated [2,11], would be more sensitive than erythrocyte GSH-Px to a reduced training load. Nevertheless, the mechanisms regulating enzymatic antioxidant activities in erythrocytes remain unclear.

Ascorbate, α-tocopherol, β-carotene, but also GSH, urate and albumin provide antioxidant protection in human extra-cellular fluids. Their levels can be measured through TAS concentration. The decrease in resting plasma TAS level with TT could be explained by a lowered urate synthesis consecutive to a lowered activation of xanthine oxidase, involved in free radical generation during exhaustive exercise [32]. TT induced a statistically significant decrease in the resting antioxidant system, yet the low magnitude of changes suggests there are few biological effects on oxidative damage at rest. On the other hand, TT combined with supplementation greatly up-regulated the exercise-induced response of antioxidant systems. These large variations may be related to the lower exercise-induced glutathione oxidation. Antioxidant responses in exercise and in resting conditions thus have to be considered separately. Antioxidant complex supplementation may have increased the ability of free radicals to be scavenged in serum during physical activity.

CONCLUSION

The main finding of the study is the upregulation of antioxidant response to exercise with TT and antioxidant supplementation including a complex of selenium and vitamins A, C and E. Upregulation could promote the decrease in oxidative stress specifically in exercise conditions, without reducing exercise-induced oxidative damage. Supplementation at the dose used compensated for the deficit in vitamin E intake. Note that the effects of the antioxidant mixture in this study were observed for nutritional doses, meaning they can be provided by food. The upholding of normal nutritional status, with respect to antioxidant intakes, plays a key role in antioxidant adaptive effects during training, mostly in response to exercise-induced stress.

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