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Cellular and biochemical parameters of exercise-induced oxidative stress: Relationship with training levels

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Abstract

To better clarify the relationship between physical activity and oxidative stress, we determined the effects of a maximal test in 18 young subjects with different training levels (six professional Athletes and 12 non-agonists (NA)). Redox homeostasis (total antioxidant activity (TAS), vitamin C and glutathione (GSH)), oxidative damage (diene conjugation and hemolysis), lymphocyte cell death and repair systems (apoptosis, micronuclei and Hsp70 expression) were evaluated. We found that agonistic training led to a chronic oxidative insult (high baseline values of oxidized glutathione (GSSG), micronuclei and hemolysis). On the contrary, NA with the lowest level of training frequency showed a well balanced profile at rest, but they were more susceptible to exercise-induced variations (GSSG/GSH and diene increased values), respect to the NA with an higher level of training. As almost all the parameters employed in this study showed inter-individual variations, the GSSG/GSH ratio remains the most sensitive and reliable marker of oxidative stress, accordingly with other data just reported in the literature.

Keywords: *Oxidative stress, redox balance, physical activity, DNA damage, apoptosis*

Introduction

Regularly performed, moderate exercise has many beneficial effects, whereas acute exercise can produce damage in skeletal muscle and other tissues [1,2]. During aerobic exercise, the O_2 consumption is incremented 10–20-folds in whole body, and 100–200-folds in skeletal muscle [3]. The increment of O_2 flux through the mitochondria (together with the inflammatory response at muscular level) leads to partial O_2 reduction, thereby triggering reactive oxygen species (ROS) production and antioxidant consumption [4,5]. Alterations of the redox state induce redox-sensitive signaling cascades, which, in turn, interfere with the expression of endogenous enzymatic (superoxide dismutase (SOD), catalase and glutathione peroxidase (GPx)) and not-enzymatic (glutathione (GSH)) antioxidants [6,7].

Despite the growing amount of data, the relationship between physical activity and oxidative stress is far from being linear, as well as widespread confirmed. Experimental evidence indicates that exhaustive exercise induces lipid peroxidation [8], DNA damage [9–11] and alteration of the antioxidant defence system [12]. Conversely, the protective effects of training are usually associated with the up-regulation of endogenous antioxidant defense and repair systems, thus explaining why trained individuals display less cell damage than untrained subjects [5,11,13–15].

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The different outcomes observed in literature appear to originate from different biochemical/cellular markers, as well as different exercise protocols adopted [16–18]. Indeed, variations in nature, intensity and duration of physical test performed can activate different patterns of oxidant–antioxidant balance leading to different responses in terms of cellular damage.

In our study, we investigated the effect of an exhaustive exercise on different hematic parameters in young subjects divided on the basis of their level of sport practice (being non-agonistic (NA) or professional). In particular, we evaluated redox homeostasis, by measuring the total antioxidant activity (TAS), as well as some hydrophilic circulating antioxidants (such as vitamin C and GSH levels) and oxidized/reduced GSH ratio (GSSG/GSH). Then, we studied how alterations in redox state can be translated in molecular and cellular damage. To this end, we measured serum diene conjugation (thus monitoring low-density lipoprotein oxidation) and *in vitro* erythrocyte sensitivity to oxidative damage induced by treatment with a free-radical initiator.

Finally, we used peripheral blood lymphocytes to investigate the effects of oxidative damage, by monitoring the rate of apoptotic cell death and defective DNA repair machinery (micronuclei), and the modulation of repair systems through expression of Hsp70, the most responsive heat shock protein to heat stress and exercise.

Materials and methods

Subjects and study procedure

The sample consisted of 18 young healthy male volunteers: 12 subjects (23.5 ± 2.1 years) practicing sport at NA (group) level and six professional road-racing cyclists (32.0 ± 4.9 years) (Athletes), regularly practicing training-race, the last one of which was performed two days before the test. NA group was subdivided into two subgroups according to the habitual frequency of training, on the basis of data collected by questionnaire; the differences found in training level was confirmed by differences in aerobic fitness levels, as resulting from the maximal oxygen uptake (VO_2max) observed during the test. Thus, three sub-samples were generated: sub-sample NA-Low, formed by six subjects with the lowest VO_2max (47.1 ± 3.6 ml/min/kg), corresponding to that with the lowest training frequency (3.6 ± 2.62 h/w); sub-sample NA-Int, constituted by six subjects with an intermediate VO_2max (56.7 ± 2.1 ml/min/kg), corresponding to the subgroup with the intermediate training frequency (8.1 ± 3.75 h/w); sub-sample Athletes formed by the six cyclists (VO_2max of 66.2 ± 4.2 ml/min/kg), that of course had the highest training frequency (12.5 ± 4.36 h/w) (Table I). All subjects were healthy, non-smokers, not taking any routine medication or dietary supplementation of vitamins and/or antioxidants. After informed consent, blood samples were collected in the presence of anticoagulants (EDTA or heparin) at rest (immediately before exercise), 30 min and 24 h after a cycle-ergometer exhaustive test. NA-Int as well as NA-Low subjects started the test with a load of 30 W, followed by further increments of 30 W every 2 min. Athletes started with a load of 100 W, with incremental steps of 25 W/min. Both for Athletes and NA groups test was carried out until volitional fatigue was attained or maximal heart rate was reached (Table I). During the test, cardiorespiratory parameters, such as VO_2, heart rate, systolic and diastolic blood pressure were measured (QUARKb2, COSMED, Italy). Capillary blood samples were also collected for lactate assay, before and during each test, to verify the overcoming of the aerobic threshold (6 mM) that indicates

Table I. Physiological measurements.

	NA-Low (mean ± SD)	NA-Int (mean ± SD)	Athletes (mean ± SD)
	Training data		
Training freq (d/w)	2.5 ± 1.38	4.0 ± 1.67	5.0 ± 1.63
Total hours of training (h/w)	3.6 ± 2.62⋆	8.1 ± 3.75	12.5 ± 4.36
	Physiological measurements		
Height (cm)	178.0 ± 8.34	175.8 ± 6.26	178.0 ± 0.07
Weight (kg)	71.2 ± 9.66	66.0 ± 9.70	70 ± 6.26
Body mass index (kg/m^2)	22.1 ± 1.83	21.6 ± 1.85	22.2 ± 1.78
	Exhaustive test		
Test duration (min)	17.2 ± 1.47	20.5 ± 3.62	16.0 ± 1.41
Heart rate max (bpm)	188.7 ± 12.91	192.5 ± 10.33	184.0 ± 4.94
VO_2max (ml/min/kg)	47.1 ± 3.79†	56.7 ± 1.84	66.2 ± 4.23
Lactate max (mM)	8.0 ± 0.43	9.2 ± 1.53	9.9 ± 1.41
Load max (watt)	246.0 ± 25.10‡	300.0 ± 36.74	412 ± 26.22

NA-Low vs. NA-Int.
⋆$p = 0.037$.†$p = 0.001$.‡$p = 0.027$.

the reaching of test exhaustiveness (Ebioplus, Eppendorf, Germany). Heart rate max and lactate max did not differ between groups, indicating that, despite an expected different max load, the intensity of the test was comparable (Table I).

Plasma and red blood cells were obtained by centrifugation at 800*g* for 20 min at 4°C. Purified lymphocytes were obtained by density-gradient centrifugation of whole blood on Ficoll-Paque (Amersham). After centrifugation at 1800 rpm for 30 min at room temperature, lymphocytes were collected from the interface, rinsed twice in phosphate buffered saline (PBS) and resuspended in RPMI 1640 medium (Gibco) supplemented with 10% foetal calf serum, penicillin and streptomycin (complete culture medium), at a density of 2.5×10^5 cells/ml.

Micronuclei

For each culture, whole blood (0.5 ml) was added to 9.5 ml of complete culture medium, supplemented with 2% phytohemagglutinin (PHA), and incubated for 48 h at 37°C. To block cytokinesis, 6 μg/ml cytochalasin B (Sigma) was added for the last 28 h of incubation. To obtain micronuclei samples, cultures were harvested according to the CBMN standard protocol [19]. Briefly, after centrifugation, the pellet was resuspended in an hypotonic solution (0.075 M KCl) for 8–10 min. Cells were re-centrifuged and fixed for 30 min in ice-cold methanol:glacial acetic acid (3:1). The fixation step was repeated twice, and finally cells were dropped on clean dry slides and air-dried. The slides were stained with 5% Giemsa in phosphate buffer pH 6.8, followed by a washing step in phosphate buffer for 2 min.

Two independent cultures were settled for each experimental point and micronuclei count was evaluated by scoring 1000 binucleated cells/culture on coated slides.

Apoptosis

Purified lymphocytes were pelletted, rinsed twice with PBS and fixed with paraformaldehyde (4% in PBS) at 4°C for 1 h. After incubation, cells were resuspended in 500 μl PBS and gently dropped on a clean dry slide coated with poly-L-lysine. Cell apoptosis was evaluated by TdT-mediated dUTP-nick end labeling (TUNEL) assay, utilizing the "*In situ* cell detection Kit, AP" (Roche), according to the manufacturer's instructions. At least four independent cultures were settled for each experimental point. Apoptosis frequency was evaluated by scoring the number of TUNEL positive nuclei on at least 1000 cells analyzed.

Ascorbic acid (AA)

For AA measurements blood samples were treated with the reducing agent dithiothreitol (DTT) (1 mM) to prevent AA oxidation. Plasma was deproteinized by ice-cold 70% methanol (v/v), containing 1 mM EDTA and 1 mM DTT. After incubation for 5 min on ice, the samples were centrifuged at 10,000*g* for 10 min and the supernatant was immediately assayed by HPLC with UV detection [20] or frozen at −70°C for later analysis [21].

Total antioxidant status

Plasma TAS was determined spectrophotometrically (734 nm), accordingly to Miller et al. [22]. This method is based on the reactivity of plasmatic antioxidant compounds relative to a 1 mM Trolox (vitamin E analogue) standard.

Glutathione

Blood GSH content was quantified spectrofotometrically at 412 nm, by a 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB)-GSH reductase recycling assay, accordingly to the method of Anderson [23]. Briefly, 20 μl of blood samples were added to 100 μl of 10 mM HCl. Red blood cells were lysed by freezing and thawing three times; after centrifugation at 10,000*g* for 5 min at 4°C, the supernatant solution was deproteinized with 5% 5-sulfosalicylic acid. Oxidized glutathione (GSSG) was selectively measured in samples where reduced GSH was masked by pretreatment with 2-vinylpyridine (2%).

Diene conjugation

Serum diene conjugation was evaluated spectrophotometrically accordingly to the method of Vasankari et al. [24]. Absorbance at 300 nm was subtracted from that at 234 nm. Values were expressed as the Δ absorbance/ml of serum.

Hemolysis

Red blood cells sensitivity to hemolysis was evaluated spectrophotometrically at 540 nm, by treating erythrocytes for 3 h with 50 mM 2,2′-azo-bis(2-amidinopropane) dihydrochloride (AAPH) (a free-radical initiator) [25]. Hemolysis was expressed on the basis of the maximum absorbance (100%) in erythrocyte aliquots completely hemolyzed in distilled water.

Hsp70 expression

Purified lymphocytes were split in two fractions: one sample was incubated at 37°C and the other one was

in vitro stimulated with heat shock. Heat shock was triggered by incubating lymphocytes at 42°C for 45 min, then cells were allowed to recover at 37°C for 5–7 h; experimental conditions were chosen to induce an intensive stress response without cell damage. After incubation, cells were pelletted and lysed in RIPA buffer (50 mM Tris–HCl pH 7.4, 150 mM NaCl, 1 mM PMSF, 1 mM EDTA, 5 μg/ml aprotinin, 5 μg/ml leupeptin, 1% Triton x-100, 1% sodium deoxycholate and 0.1% SDS). After protein determination by Bradford Protein Assay (Bio-Rad Laboratories), samples were analysed for Hsp70 content by western blot. Briefly, 10 μg proteins were subjected to SDS-PAGE on a 10% polyacrylamide gel and electroblotted onto a PVDF membrane. Blots were blocked with 5% non-fat dry milk (Bio-Rad) and then incubated with anti-Hsp70 primary antibody (Santa Cruz Biotechnology). After washings and incubation with the horseradish peroxidase–conjugated secondary antibody, detection was carried out with ECL (Amersham).

Data analysis

Collected data have been organized in a database and analyzed with the statistic software SPSS (SPSS 10 for Windows). The results are expressed as mean ± standard deviation (SD) and range. Statistical significance was set to $p < 0.05$.

All the data were tested for their normal distribution with the one-sample Kolmogorof-Smirnov test. To analyze the significance of the mean differences between the two groups for the physiological data we utilize the unpaired *t*-test.

A univariate GLM for repeated measures was used to analyze the differences between and within groups in relation to the test performance. For fixed between-subjects factors, Bonferroni test was used as *post hoc* multiple comparison test.

Results

Redox homeostasis

The baseline value of antioxidant status parameters appeared to be unrelated to training level; indeed AA, TAS and total GSH levels were similar in all groups (Table II). All parameters were not affected by exercise. In agreement with the literature reporting an AA efflux by the adrenal gland [26], only a transient increase was found 30 min after exercise in all samples and it returned to baseline levels after 24 h (within-subjects: $F = 6.218$, $p = 0.006$) (Table II).

The GSSG/GSH ratio is often used as a marker of oxidative stress: an increase indicates that ROS production exceeds total reducing capacity [27]. Athletes, although showing significant higher basal values (0.22 ± 0.07, range 0.19) with respect to NA-Low (0.04 ± 0.02, range 0.07) and NA-Int (0.07 ± 0.03, range 0.1) sub-samples (between-subjects: $F = 139.3$, $p = 0.001$) confirmed by the Bonferroni *post hoc* test (Athletes vs. NA-Int: $p = 0.003$, 95% CL = −0.17; −0.04; Athletes vs. NA-Low: $p = 0.001$, 95% CL = −0.18; −0.04), did not show any modification after the physical test (Figure 1). On the other hand, NA-Low subjects showed a significant increasing trend both after 30 min (0.08 ± 0.04, range 0.13) and 24 h (0.12 ± 0.06, range 0.17) (within-subjects: $F = 8.627$, $p = 0.003$), while NA-Int subjects showed unchanged values over time (Figure1).

Oxidative damage

Serum diene conjugates are generated during early steps of lipid peroxidation [24]. Values were similar in all samples; 24 h after exercise, this marker showed a tendency to increase (within-subjects: $F = 3.68$, $p = 0.038$), more pronounced in NA-Low individuals (Table II).

Although exhaustive exercise did not alter the percentage of hemolysis induced by AAPH, differences were found in relation to the training level (between-subjects: $F = 5.9$, $p = 0.013$). In fact, from Bonferroni *post hoc* test Athletes resulted significantly higher than NA-Low subjects ($p = 0.014$, 95% CL = −39.35; −4.18) (Table II).

Cell death and repair systems

The spontaneous apoptosis frequency in peripheral lymphocytes was very low, ranging between 3.0 ± 2.2 (NA-Low group) and 4.1 ± 1.2 (NA-Int group) apoptotic cells/1000 lymphocytes, in agreement with published data [28], without differences among the three groups (Figure 2A). The mean values increased 30 min after the exhaustive exercise in all three groups (NA-Low: 4.5 ± 1.2, range 4.2; NA-Int: 6.0 ± 3.8, range 7.1; Athletes: 7.4 ± 4.00, range 9.1 vs. 3.4 ± 2.4, range 6), although a statistically significant difference could be determined only in the Athletes (within-subjects: $F = 12.23$, $p = 0.004$). All mean values returned to pre-exercise levels or lower 24 h after the test (Figure 2A).

A relevant individual variability in micronuclei induction was observed in all sub-samples. Compared to NA-Low and NA-Int sub-samples, Athletes had higher basal micronuclei frequency, this difference becomes statistically significant at unpaired *t*-test, when we compare Athletes value with the NA-Low and NA-Int sub-samples taken together (10.73 ± 5.4, range 13.1 vs. 5.34 ± 3.4, range 12.8; $t = -2.45$, 95% CL = −10.07; −0.67, $p = 0.028$). Nevertheless, the Athletes basal value appeared to be unchanged after exercise (Figure 2B).

Also Hsp70 expression showed relevant inter-individual variations not related to fitness levels;

Table II. Redox markers.

Time	NA-Low (mean ± SD) (range)			NA-Int (mean ± SD) (range)			Athletes (mean ± SD) (range)		
	0	30 min	24 h	0	30 min	24 h	0	30 min	24 h
TAS (mM Trolox equivalents)	0.9 ± 0.2 0.5	1.0 ± 0.2 0.5	0.9 ± 0.2 0.7	0.8 ± 0.2 0.5	0.9 ± 0.2 0.5	0.8 ± 0.3 0.8	0.9 ± 0.3 0.9	0.9 ± 0.2 0.6	0.8 ± 0.1 0.3
AA (10^{-5} M)	1.7 ± 0.9⋆ 2.2	2.4 ± 1.2⋆ 2.9	2.1 ± 1.3⋆ 3.2	2.4 ± 1.4⋆ 3.4	2.8 ± 1.8⋆ 4.2	2.0 ± 1.3⋆ 3.3	3.0 ± 1.5⋆ 4.5	3.7 ± 1.1⋆ 3.1	2.5 ± 1.2⋆ 2.9
Tot Glutathione (10^{-4} M)	11.7 ± 1.9 5.8	13.7 ± 2.3 6.0	13.2 ± 3.3 8.7	11.8 ± 3.1 3.9	13.1 ± 3.2 4.7	10.6 ± 2.4 7.2	13.5 ± 3.3 8.9	14.7 ± 4.0 9.8	12.5 ± 1.0 2.6
GSSG (10^{-4} M)	0.5 ± 0.1 0.7	1 ± 0.2 0.8	1.4 ± 0.2 1.5	0.8 ± 0.2 1.0	1.2 ± 0.3 1.1	1.0 ± 0.2 1.3	2.4 ± 0.6 1.7	2.8 ± 0.7 2.6	1.5 ± 0.1 1.7
GSH (10^{-4} M)	11.2 ± 1.8 5.6	12.7 ± 2.0 6.8	11.8 ± 2.9 8.9	11.0 ± 2.7 3.5	11.9 ± 2.8 4.0	9.6 ± 2.2 6.9	11.1 ± 2.7 8.1	11.9 ± 3.2 7.6	11.0 ± 0.9 4.3
Diene conjugation (ΔOD/ml)	9.5 ± 3.3† 8.6	9.8 ± 2.4† 6.7	10.1 ± 2.9† 8.3	8.6 ± 1.4† 3.6	8.9 ± 1.1† 3.2	9.1 ± 1.1† 3.2	8.8 ± 1.0† 2.3	9.5 ± 1.0† 2.7	8.9 ± 0.8† 1.9
Hemolysis (%)	27.7 ± 10.5‡ 25	28.1 ± 7.0‡ 21.1	26.5 ± 8.1‡ 19	34.4 ± 9.4 28	33.3 ± 10.1 26.5	33.1 ± 7.7 21.8	53.3 ± 15.0‡ 38	43.7 ± 11.7‡ 31.7	50.4 ± 24.7‡ 57

The letters in superscript indicates a significant ANOVA for repeated measures with the following p values.
⋆$p = 0.006$.†$p = 0.038$.‡$p = 0.013$.

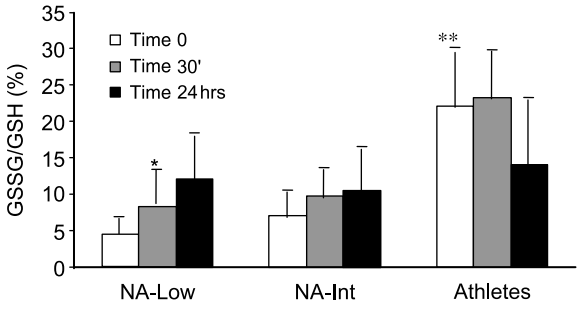

Figure 1. Effect of fitness level and of a single bout of exhaustive exercise on GSSG content. The GSSG/GSH ratio was measured in NA-Low, NA-Int subjects and Athletes, at rest (white bars), 30'min (grey bars) and 24 h (black bars) after exhaustive exercise. Data are presented as mean ± SD of experiments performed in triplicate. Significant statistical changes: ⋆NA-Low by time $p = 0.003$; ⋆⋆Athletes vs. NA-Low and NA-Int subjects at rest ($p = 0.001$).

indeed, in all groups, some individuals showed an increase in Hsp70 levels, others showed no change, and others showed a decrease in protein levels after exercise (data not shown). However, *in vitro* heat shock of lymphocytes showed increased Hsp70 baseline expression, especially in NA-Int (indicating ability to counteract stress) (Figure 2C).

Discussion

A major difficulty in evaluating the relationship between oxidative damage and physical activity is the lack of unambiguous parameters of oxidative stress *in vivo*. Furthermore, equivocal results mainly arise from a plethora of factors, including nature and timing of exercise, age and fitness of subjects, and

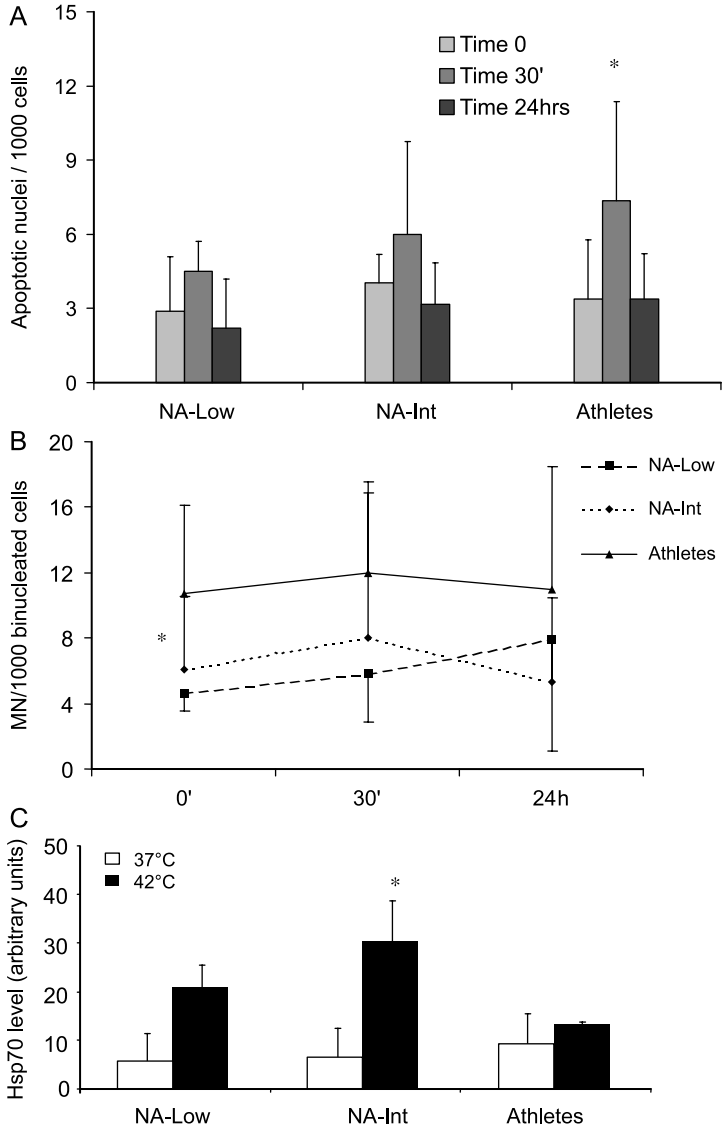

Figure 2. Effect of fitness level and of a single bout of exhaustive exercise on apoptosis and DNA-repair system efficiency. Apoptotic (A) and micronuclei (MN) (B) frequency in NA-Low, NA-Int and Athletes, at rest (white bars), 30 min (grey bars) and 24 h (black bars) after exhaustive exercise (C). Hsp70 expression in NA-Low, NA-Int subjects and Athletes, in normal conditions (white bars) and after thermal shock (black bars). All values are expressed as mean ± SD. See Materials and methods section for experimental details. Significant statistical changes: (A) ⋆Athletes by time $p = 0.003$. (B) ⋆Athletes vs. NA-Low and NA-Int subjects basal values: *t*-test for independent samples $t = -2.475$, $p = 0.028$). (C) ⋆NA-Int 37 vs. 42°C $p = 0.03$.

methodology assessing oxidative stress. In the present study, we evaluated multiple markers to give a broader point of view concerning redox balance and fitness level.

Regarding the biochemical markers, we studied redox balance, through the analysis of TAS, vitamin C, total GSH and GSSG/GSH ratio. TAS is an indicator of overall plasma antioxidant defences (uric acid, protein thiols, AA, α-tocopherol and trappers of transition metals); vitamin C is involved in biochemical pathways correlated to exercise metabolism and protection against exercise-triggered ROS generation [26]; GSH is a primary component of the cellular antioxidant network. In our subjects, exercise did not exert any significant alteration in these parameters, with the exception of the GSSG/GSH ratio that was directly correlated to training and exercise: Athletes showed 5-fold levels with respect to NA-Low subjects which, on the contrary, appeared to be more susceptible to exercise-induced variations (3-fold after 24 h). Our finding confirmed that this marker is a powerful redox sensor able to reveal early changes induced by physical stress [29,30]. In agreement with the hypothesis that cellular markers are more sensitive than plasma sensors of oxidative stress [31], no changes in serum diene conjugation were found between the groups, while erythrocyte sensitivity to hemolysis was higher in Athletes.

The effect on DNA-damage susceptibility was studied by measuring micronuclei [19,32], generated in mitotic cells by chromatide-type, chromosome-type and/or mitotic apparatus aberrations [32]. Reflecting a DNA damage escaped from the DNA repair machinery, data obtained by this assay add a different endpoint of genotoxicity to those given by the 8-OHdG and COMET assays, usually employed to detect exercise-induced DNA damage [11,14,33–36]. Also in this case, differences among the groups were seen, and Athletes showed the highest levels at rest.

NA-Int subjects showed the best thermotolerance, as indicated by the highest levels of Hsp70 protein (an inducible molecular chaperone involved in the early stages of the recovery process [37]) after temperature shifting. The lack of response in Athletes maybe was due to metabolic adaptation derived from thermoregulatory responses other than Hsp70 (different heat shock proteins and/or mitochondrial uncoupling proteins).

Unlike DNA damage and Hsp70 expression, the influence of exhaustive exercise on the frequency of lymphocyte apoptosis was similar in all three groups, with a slight increase immediately after exercise, and a complete recover within the 24 h.

Actually, the changes in cellular redox state during physical activity may be an important trigger of apoptosis [9], but other mechanisms, including metabolic and hormonal changes, or changes in CD95-receptor expression, have been reported to be related to exercise-induced apoptosis in lymphocytes [28].

Cellular, molecular and biochemical markers showed a large inter-individual variability that indicates a strong genetic effect detected elsewhere, especially regarding the clastogenic susceptibility [38,39]. Although our difficulty to predict an unambiguous response to strenuous exercise, nonetheless some useful considerations should be made. First of all, our data support the hypothesis of chronic stress in the athlete group, caused by their habitual intense weekly-schedule of training. Inasmuch, the stress induced by last training-race, that was performed two days before the test, is not worsened by a single bout of our test-exercise. These findings could be explained considering that, when the exercise is enough strenuous/prolonged, muscle injury can occur [9,34,35], with the subsequent release of inflammatory cytokines, which, in turn, lead to enhanced ROS production and DNA damage in lymphocytes [4]. The second observation emerging from our study is that NA-Low subjects are more susceptible to alterations of redox homeostasis. These individuals show the maximum variation after exercise; this should indicate that low/inconstant physical training is associated with lack of adaptive responses, as already reported in Ref. [40]. Finally, NA-Int subjects showed an intermediate situation, with low basal levels of redox markers that remained unchanged after the exhaustive test. In conclusion, we observed biochemical and cellular parameters confirming that very intense physical training could be associated with chronic oxidative insult, while mild stress derived by moderate training induces stimulation and maintenance of repair pathways. In addition, the most sensitive marker remains the GSSG/GSH ratio, which could be routinely used as an early index of altered redox homeostasis and oxidative damage risk.

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