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Reductive stress after exercise: The issue of redox individuality

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ABSTRACT

Exercise has been consistently used as an oxidant stimulus in redox biology studies. However, previous studies have focused on group differences and did not examine individual differences. As a result, it remains untested whether all individuals experience oxidative stress after acute exercise. Therefore, the main aim of the present study was to investigate whether some individuals exhibit unexpected responses after an acute eccentric (i.e., muscle-damaging) exercise session. Ninety eight ($N = 98$) young men performed an isokinetic eccentric exercise bout with the knee extensors. Plasma, erythrocytes and urine samples were collected immediately before and 2 days post-exercise. Three commonly used redox biomarkers (F_2-isoprostanes, protein carbonyls and glutathione) were assayed. As expected, the two oxidant biomarkers (F_2-isoprostanes and protein carbonyls) significantly increased 2 days after exercise (46% and 61%, respectively); whereas a significant decrease in glutathione levels (by −21%) was observed after exercise. A considerable number of the participants exhibited changes in the levels of biomarkers in the opposite, unexpected direction than the group average. More specifically, 13% of the participants exhibited a decrease in F_2-isoprostanes and protein carbonyls and 10% of the participants exhibited an increase in glutathione levels. Furthermore, more than 1 out of 3 individuals exhibited either unexpected or negligible (from 0% to ± 5%) responses to exercise in at least one redox biomarker. It was also observed that the initial values of redox biomarkers are important predictors of the responses to exercise. In conclusion, although exercise induces oxidative stress in the majority of individuals, it can induce reductive stress or negligible stress in a considerable number of people. The data presented herein emphasize that the mean response to a redox stimulus can be very misleading. We believe that the wide variability (including the cases of reductive stress) described is not limited to the oxidant stimulus used and the biomarkers selected.

Introduction

There is a consensus that a single session of exercise induces oxidative stress [1–3] and that the free radicals produced during exercise are important modulators of muscle and systemic adaptations to physical activity [4–6]. This is based on the results of exercise studies over the past three decades [7]. However, to the best of our knowledge, all exercise studies have focused on group differences and did not examine individuality in responses. As far as we know, the same holds true for all redox studies. Indeed, there is an indirect evidence that this is possibly the case considering that even well controlled studies have presented marked heterogeneity in responses in exercise-induced oxidative stress. For example, changes in F_2-isoprostanes (the reference biomarker of oxidative damage) varied greatly (from –27% to +181%) in response to acute exercise [8,9]. Likewise, significant variation exists in individual responsiveness of glutathione (the major antioxidant of erythrocytes) to acute exercise (from −63% to +40% [10,11]). It needs to be recognized that findings based on the level of a group may not fully apply to each member of that group [12]. As a result, it is untested whether all individuals experience oxidative stress after acute exercise.

Therefore, the main aim of the present study was to investigate whether some of the individuals will exhibit unexpected responses in the levels of three commonly used redox biomarkers (F_2-isoprostanes, protein carbonyls and glutathione) after an acute exercise session. The secondary aims of the study were (i) to quantify the inter-individual variability of the redox biomarkers in response to exercise, (ii) to investigate the potential dependence of the post-exercise changes on the initial values (iii) and to examine the relationships among the redox biomarkers both at rest and after the redox challenge. To

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Table 1
Physiological characteristics and analysis of daily energy intake of the study participants (mean ± SD).

	$N = 98$
Age (years)	23.5 ± 4.0
Height (cm)	175.5 ± 6.5
Body mass (kg)	76.3 ± 6.8
Body fat (%)	15.8 ± 4.6
Energy (kcal/day)	2407 ± 90
Carbohydrate (% energy)	55.0 ± 10
Fat (% energy)	27.6 ± 7.6
Protein (% energy)	17.4 ± 8.3
Vitamin C (mg)	121 ± 15
Vitamin E (mg, α-TE[a])	8.2 ± 1.1
Selenium (μg)	72.1 ± 24

[a] α-TE: alpha-tocopherol equivalents.

accomplish these aims, we used the eccentric exercise model as a physiological oxidant stimulus because it induces alterations in redox homeostasis that are characterized by long (lasting for up to four days after exercise) and large (even up to 40% compared to rest) increases in oxidant biomarkers [13–16]. Therefore, eccentric exercise may facilitate to unravel the potential individual differences on redox homeostasis responses. To our knowledge, this is the first study to have investigated the effect of exercise (or any other stimulus) on inter-individual variability of redox responses.

Materials and methods

Participants

Ninety eight ($N = 98$) young men (19–30 years old) participated in the present investigation (Table 1). All participants had stable body weight for at least a year. An individual was defined as having a stable body weight if his body weight did not change by more than ± 3 kg. Subjects were excluded from the study, if they had any history of musculoskeletal injury to the lower limbs that would limit the ability to perform the exercise session. The participants were asked to recall whether they had participated in regular resistance or aerobic training or in unaccustomed and/or heavy exercise (e.g., soccer game, competitive running, high-impact aerobics) in the 3 months before the study entry. Individuals who reported participation in such activities were precluded from the study. Smoking and consumption of nutritional supplementation the last three months before the study initiation were also exclusion criteria to participate in the present investigation. Volunteers were instructed to abstain from any strenuous exercise (except for the exercise session performed during the experimental procedure) during their participation in the study as well as for five days prior and 2 days following the exercise session (i.e., second sample collection). Subjects were also advised to refrain from taking anti-inflammatory or analgesic medications for the duration of the study. A written consent was obtained from all participants, after they were informed for the risks, discomforts and benefits involved in the study. The procedures were in accordance with the Helsinki declaration of 1975, as revised in 2000.

Study design

All participants performed an acute isokinetic eccentric exercise bout with the knee extensors of their preferred leg. Plasma, erythrocytes and urine were collected immediately before and 48 h post-exercise. The evaluation of muscle damage (isometric torque and creatine kinase), oxidant biomarkers (F_2-isoprostanes and protein carbonyls) and the non-enzymatic antioxidant (glutathione) was performed at the time of body fluid collection. Each volunteer was provided with a written set of instructions for monitoring dietary consumption and a record sheet for recording food intake.

Eccentric exercise protocol

The eccentric exercise session was performed on an isokinetic dynamometer (Cybex Norm, Ronkonkoma, NY). The exercise protocols were undertaken from the seated position (120° hip angle), after the participants were stabilized according to the manufacturer's instructions. Participants completed 5 sets of 8 eccentric maximal voluntary contractions [knee range, 0° (full extension) to 90° flexion] at an angular velocity of 60°/s. A 2-min rest interval was used between sets.

Muscle damage

The isokinetic dynamometer was used for the measurement of isometric knee extensor peak torque at 90° knee flexion. The average of the 3 maximal voluntary contractions with the preferred leg was recorded. To ensure that the subjects provided their maximal effort, the measurements were repeated if the difference between the lower and the higher torque values exceeded 10%. There was a 2-min rest between isometric efforts. Creatine kinase was assayed in plasma spectrophotometrically using a kit from Spinreact (Sant Esteve, Spain).

Collection and handling of body fluids

A blood sample was drawn from a forearm vein and collected in EDTA tubes. The blood was centrifuged immediately at 1370*g* for 10 min at 4 °C and the plasma was collected. The packed erythrocytes were lysed with 1:1 (v/v) distilled water, inverted vigorously and centrifuged at 4000*g* for 15 min at 4 °C. For urine sampling, spot samples were collected in a container. For standardizing urine dilution, creatinine levels were measured using a kit (Fisher Diagnostics, Middletown, USA). Body fluid samples were stored at −80 °C and thawed only once before analysis.

Redox biomarkers

A competitive immunoassay was used for the determination of F_2-isoprostanes in urine (Cayman Chemical, Charlotte, USA). Urine was purified using the solid phase extraction cartridges. The purification and the subsequent ELISA assay were performed following the manufacturer's recommendations. Plasma protein carbonyls and erythrocyte glutathione were determined spectrophotometrically as described previously [17]. Oxidation of GSH was prevented using N-ethylmaleimide, which is widely considered the most appropriate blocking agent for preventing glutathione oxidation [18]. Positive controls have been used in both the ELISA and the spectrophotometric assays.

Statistical analysis

The distribution of all dependent variables was examined. The results showed that dependent variables were normally distributed (Kolmogorov–Smirnov test) and equal variance (Levene test) was not violated. *t*-Tests for paired samples were performed to compare the values of all depended variables between the two sample collections (pre–post). The coefficient of variation (CV) was calculated for post-exercise percent changes (i.e., based on absolute values) of all variables in order to quantify inter-individual variability. Non-linear correlation analysis was performed between the percent changes of all redox biomarkers and their initial values. Data are presented as mean ± standard deviation (SD) and the level of significance was set at $\alpha = .05$.

Results

Physical characteristics and dietary intake

The physiological characteristics and dietary intake of the participants are presented in Table 1. The reported fat intake is lower than those frequently reported in the literature [19–21]. We believe that there is an inconsistency between the reported and the consumed fat by the participants in our study. Indeed, a number of investigations have indicated that subjects are reporting lower fat intake compared to the actual consumed. For example, it has been shown that participants are reporting higher relative protein intake and lower relative fat intake indicating a tendency for subjects to underreport intake of foods that could be characterized as unhealthy [22,23].

Considering the strong effects of nutritional antioxidants on redox responses described in the literature [24,25], we performed a correlation analysis between antioxidant intake (i.e., vitamin C, vitamin E and selenium) through normal diet and redox biomarkers (both at rest and after exercise). Except for some spurious and low significant correlation coefficients antioxidant intake by food alone cannot predict the redox responses to eccentric exercise (Table 2). It is clear that the content of vitamin C, vitamin E and selenium would be more reliably estimated by determining their levels in blood plasma. However, in the present investigation, only protein carbonyls were measured in blood plasma. Therefore, it is rather improbable that plasma levels of vitamin C, vitamin E, selenium (or other nutritionally-derived antioxidant) could have seriously affected the large eccentric-exercise induced alterations in protein carbonyls.

Muscle damage

The isometric torque of the participants 2 days after exercise was significantly lower compared to the baseline value ($P < 0.001$), with an average reduction equal to 21% and with a CV of 49% among the participants. As expected, creatine kinase significantly increased 2 days following eccentric exercise with an average increase equal to 1251% and an inter-individual CV of 146% (Table 3).

Redox homeostasis

The two oxidant biomarkers (F_2-isoprostanes and protein carbonyls) increased significantly 48 hours after exercise ($P < 0.001$) with an average change equal to 46% and 61% and with an inter-individual CV of 71% and 80%, respectively. The non-enzymatic antioxidant glutathione significantly decreased after exercise. Average change of glutathione was −21% and the inter-individual CV was 79% (Table 3). Some individuals exhibited changes in the levels of biomarkers in the opposite to the expected direction (Table 4). More specifically, 13% of the participants exhibited a decrease in F_2-isoprostanes and protein carbonyls and 10% of the participants exhibited an increase in glutathione levels (Fig. 1). In addition, some participants exhibited negligible expected changes after exercise (from 0% to +5% in F_2-isoprostanes and protein carbonyls and from 0% to −5% in glutathione compared to the resting value): 7% in F_2-isoprostanes, 5% in protein carbonyls and 9% in glutathione. Of the 98 participants, unexpected or negligible responses were observed in 20% for F_2-isoprostanes, 18% for protein carbonyls and 19% for glutathione. Collectively, more than 1 out of 3 individuals exhibited either unexpected or negligible responses to exercise in at least one redox biomarker.

Correlation between percent changes and initial values

Non-linear correlation analysis (Fig. 2) between post-exercise percent change of each redox biomarker and its initial value revealed high correlations for protein carbonyls ($r = -.63$) and F_2-isoprostanes ($r = -.56$) and moderate correlation for glutathione ($r = -.37$).

Decision tree analysis

In order to shed more light on the potential effect of the antioxidant glutathione on the post-exercise induced responses, we constructed a decision tree to model the possible exercise-induced outcomes in F_2-isoprostanes and protein carbonyls based on glutathione values (Fig. 3). All participants ($N = 98$) were separated based on their initial glutathione levels and their post-exercise changes (%) in the levels of the two oxidant biomarkers (F_2-isoprostanes and protein carbonyls). The mean erythrocyte glutathione concentration of all participants was 3.06 μmol/g Hb. Dividing them based on the post-exercise changes in plasma protein carbonyls, we observed that some participants ($N = 35$) with much lower initial glutathione levels (2.71 μmol/g Hb) exhibited higher increases in protein carbonyls (i.e., a minimum of 87% increase; highlighted with a blue border) compared to the rest of the participants ($N = 63$), whose initial glutathione levels were 3.25 μmol/g Hb. However, looking at the post-exercise changes in the urinary F_2-isoprostanes of the 63 aforementioned participants, we observed that individuals with low initial glutathione levels exhibited either extremely large increases (higher than 83%) or large decreases (higher than 24%, indicative of reductive stress) in the levels of F_2-isoprostanes after exercise. In particular, the subgroup with the large increases in the levels of F_2-isoprostanes ($N = 7$) had initial levels of glutathione equal to 2.76 μmol/g Hb, while the subgroup with the large decreases and the potential reductive stress ($N = 8$) had initial levels of glutathione equal to 2.24 μmol/g Hb (highlighted with red borders). Based on these findings, it seems that the appearance of reductive stress (judging from changes in F_2-isoprostanes and protein carbonyls) is independent of the initial values of one of the most important antioxidants in vivo (glutathione). However, it remains surprising and noteworthy the fact that individuals with low initial glutathione levels exhibited changes toward opposite directions in the levels of F_2-isoprostanes. The exact nature of the mechanisms responsible for this observation is unclear.

Correlation between biomarkers

Correlation analysis among the resting values of redox biomarkers revealed a large positive correlation between F_2-isoprostanes and protein carbonyls ($r = .66$, $P < .001$) and moderate negative correlations between glutathione with both F_2-isoprostanes ($r = -.30$, $P = .002$) and protein carbonyls ($r = -.49$, $P < .001$) (Table 5). Likewise, concerning the correlations for post-exercise changes, a large positive correlation was found between F_2-isoprostanes and protein carbonyls ($r = .61$, $P < .001$) and small negative correlations between glutathione with F_2-isoprostanes ($r = -.203$, $P = .045$) and protein carbonyls ($r = -.281$, $P = .005$) (Table 6). Muscle torque was moderately correlated with protein carbonyls and only small correlation coefficients were revealed with F_2-isoprostanes and glutathione. Creatine kinase did not correlate significantly with any of the three redox biomarkers (Table 6).

Discussion

The current knowledge on redox biology is based on the average effects observed in groups of individuals. To our knowledge, this is the first attempt to investigate the individual variation of redox biomarkers. More specifically, we examined whether some individuals respond unexpectedly to acute exercise (an accepted oxidant stimulus) on the level of three commonly used redox biomarkers (F_2-isoprostanes, protein carbonyls and glutathione). Eccentric exercise was used as an oxidant stimulus to produce extensive and long-lasting changes in redox biomarkers and unravel the potential individual differences on redox homeostasis responses. The measurements were performed before and 48 h post-exercise in three different biological matrices (i.e., plasma, erythrocytes and urine) in order to provide a

Table 2
Correlation coefficients between resting values of biomarkers and antioxidant intake.

	F_2-isoprostanes	Protein carbonyls	Glutathione
Vitamin C			
Pre-exercise	$r = -.143$, $P = .161$	$r = .075$, $P = .461$	$r = .077$, $P = .451$
Post-exercise	$r = .119$, $P = .241$	$r = .080$, $P = .434$	$r = .010$, $P = .926$
% Change	$r = .263$, $P = .009$	$r = .035$, $P = .732$	$r = -.075$, $P = .462$
Vitamin E			
Pre-exercise	$r = -.212$, $P = .036$	$r = .031$, $P = .764$	$r = -.037$, $P = .721$
Post-exercise	$r = -.085$, $P = .405$	$r = -.006$, $P = .954$	$r = .040$, $P = .698$
% Change	$r = .181$, $P = .074$	$r = .012$, $P = .909$	$r = -.004$, $P = .966$
Selenium			
Pre-exercise	$r = -.131$, $P = .198$	$r = -.201$, $P = .047$	$r = -.103$, $P = .312$
Post-exercise	$r = -.080$, $P = .432$	$r = -.120$, $P = .237$	$r = .104$, $P = .310$
% Change	$r = .056$, $P = .582$	$r = .106$, $P = .299$	$r = .173$, $P = .088$

Table 3
Initial and 48 h post-exercise values of biomarkers (mean ± SD).

	Pre-exercise	48 h Post-exercise	% Change	CV[#]
F_2-isoprostanes (pg/mg creatinine)	690 ± 220	950 ± 320*	46 ± 48	71
	(220–1050)	(320–1590)	(−62–196)	
Protein carbonyls (nmol/mg pr.)	.50 ± .22	.72 ± .25*	61 ± 62	80
	(.19–1.01)	(.21–1.20)	(−61–253)	
Glutathione (μmol/g Hb)	3.06 ± 1.05	2.31 ± .98*	−21 ± 30	79
	(.90–5.30)	(.70–4.50)	(−76–110)	
Muscle torque (Nm)	195 ± 39	153 ± 37*	−21 ± 16	49
	(127–318)	(78–255)	(−56–25)	
Creatine kinase (U/L)	120 ± 30	1518 ± 1901*	1251 ± 1826	146
	(72–221)	(129–10,829)	(39–11,299)	

The numbers in brackets show the minimum and maximum values.
*Significantly different with respect to the initial value ($P < .001$).
[#]The coefficient of variation (CV) was calculated based on absolute values.

Table 4
Participants with 1, 2 or 3 unexpected responses.

Participants ($N = 98$)	1 Unexpected response ($N = 8$)		2 Unexpected responses ($N = 11$)		3 Unexpected responses ($N = 2$)	
	GSH:	4	F_2-IsoPs-PC:	7	F_2-IsoPs-PC-GSH:	2
	F_2-IsoPs:	3	PC-GSH:	3		
	PC:	1	F_2-IsoPs-GSH:	1		

F_2-IsoPs: F_2-isoprostanes; GSH: glutathione; and PC: protein carbonyls.

Fig. 1. Percent change in redox biomarker levels for each individual. Individuals exhibited unexpected responses are highlighted with red color. This kind of data presentation may offer a more fair representation of redox biological reality.

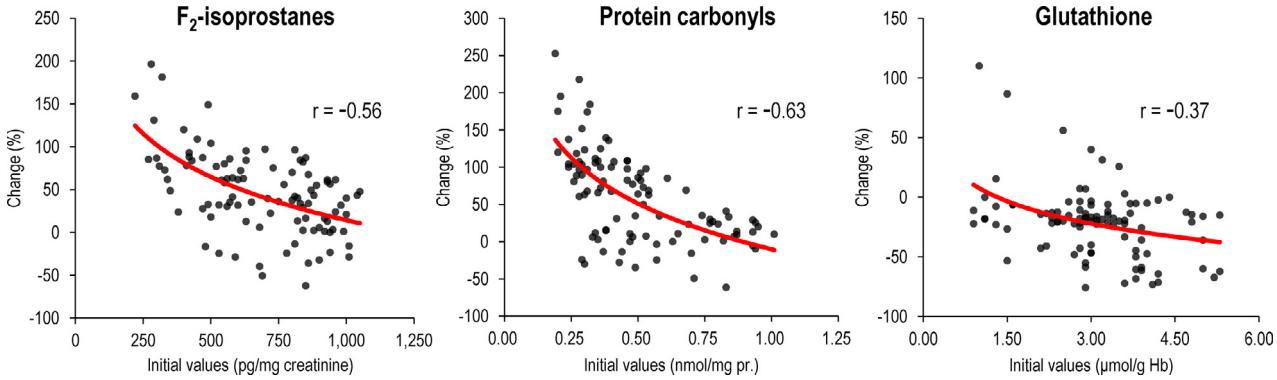

Fig. 2. Non-linear correlation analysis between post-exercise percent change of each redox biomarker and its initial values.

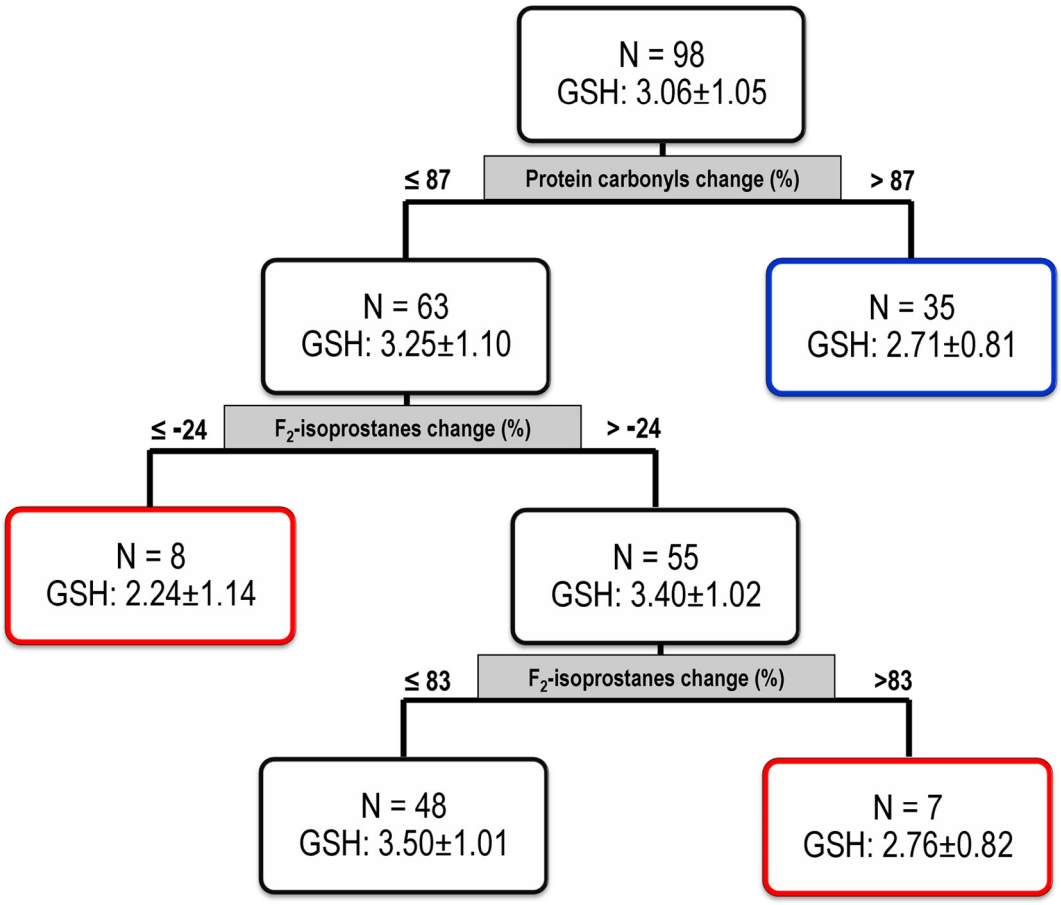

Fig. 3. Decision tree analysis based on initial glutathione (GSH) levels and post-exercise changes (%) in the levels of F_2-isoprostanes and protein carbonyls.

Table 5
Correlation coefficients between the resting values of biomarkers.

	F_2-isoprostanes	Protein carbonyls	Glutathione	Muscle torque
F_2-isoprostanes	–			
Protein carbonyls	$r = .663$, $P < .001$	–		
Glutathione	$r = -.306$, $P = .002$	$r = -.490$, $P < .001$	–	
Muscle torque	$r = -.101$, $P = .321$	$r = -.055$, $P = .591$	$r = .001$, $P = .990$	–
Creatine kinase	$r = -.038$, $P = .708$	$r = .116$, $P = .254$	$r = -.149$, $P = .143$	$r = -.021$, $P = .834$

Table 6
Correlation coefficients between the percent post-exercise changes of biomarkers.

	F_2-isoprostanes	Protein carbonyls	Glutathione	Muscle torque
F_2-isoprostanes	–			
Protein carbonyls	$r = .610$, $P < .001$	–		
Glutathione	$r = -.203$, $P = .045$	$r = -.281$, $P = .005$	–	
Muscle torque	$r = -.238$, $P = .018$	$r = -.456$, $P < .001$	$r = .332$, $P = .001$	–
Creatine kinase	$r = -.036$, $P = .727$	$r = .013$, $P = .899$	$r = -.094$, $P = .359$	$r = -.240$, $P = .017$

more integrative picture of redox alterations. The main finding of this study is the wide heterogeneity of the changes in redox biomarkers in response to exercise highlighted by the fact that exercise induced reductive stress or no stress in a considerable number of participants instead of the expected oxidative stress. Taking into account that free radicals are currently considered important signaling molecules for exercise adaptations [6] individuals who responded with reductive stress after exercise may not be benefited from exercise by the same extent with the individuals who responded with oxidative stress.

Exercise induced reductive stress in some individuals and no stress in other individuals

Although the role of exercise as an oxidant stimulus is uncontested [2,3,26], this stimulus seems not to be perceived similarly by all individuals. More specifically, some of the participants exhibited changes in the level of the redox biomarkers in an unexpected and opposite to the intuitive average direction. In fact, 13% of the participants exhibited a decrease in F_2-isoprostanes and protein carbonyls and 10% of the participants exhibited an increase in glutathione levels after exercise. Furthermore, 21% of the participants showed unexpected responses in one to three redox biomarkers. These results indicate that contrary to the common belief, an exercise session did not induce oxidative stress (in at least one biomarker) in 1 out of 5 participants. Instead, reductive stress was observed in this cohort. Furthermore, more than 1 out of 3 individuals exhibited either unexpected or negligible (from 0% to $\pm$ 5%) responses to exercise in at least one redox biomarker. Experimental treatments that affect the dependent variables in both directions can reduce the ability to detect statistical significance. Variability can dramatically reduce statistical power (i.e., the probability that a test will detect an effect that actually exists). Based on our results, we believe that part of the ongoing controversies presented in redox biology (e.g., antioxidant supplementation on mortality) are due to the large variability of redox biomarkers and that effects on redox biomarkers occur in both directions.

Several studies that chose to show individual values, instead of using mean value bars or tables, have indirectly indicated that some individuals experience reductive stress after exercise, despite the fact that the average group responses indicated oxidative stress. For example, in the work of Vollaard et al. [27] some participants exhibited decreased levels of oxidized hemoglobin and increased reduced to oxidized glutathione ratio (indicative of reductive stress) after exercise. In Dufaux et al. [28] the ratio of reduced to oxidized glutathione increased after exercise in some participants. In Rodriquez et al. [29] a small number of healthy (control) and unhealthy (chronic obstructive pulmonary disease) individuals exhibited decreased levels of protein carbonylation after exercise both in blood and muscle. Finally in Dantas de Lucas et al. [30] a number of individuals exhibited decreased plasma protein oxidation and erythrocyte lipid peroxidation levels after ultra-endurance exercise. Consequently, presenting individual differences along with group means is worthwhile for revealing "unanticipated" effects.

The initial values of redox biomarkers are important predictors of the responses to exercise

One fundamental question to consider is the nature of the mechanisms responsible for the heterogeneity in redox responses to exercise. This question was addressed in the present study by performing non-linear correlation analysis between the initial values of each biomarker and the percent changes after exercise. The results of these analyses showed a moderate to high correlation between the initial values of glutathione ($r = -.37$), F_2-isoprostanes ($r = -.56$) and protein carbonyls ($r = -.63$) with their respective percent change after the strong oxidant stimulus (i.e., eccentric exercise). That is, individuals with higher initial values in the oxidant biomarkers (i.e., F_2-isoprostanes and protein carbonyls) tended to exhibit smaller percent increases after exercise and vice versa. On the other hand, individuals with higher initial values in glutathione tended to exhibit higher percent decreases after exercise and vice versa. To the best of our knowledge, only one other study has addressed the issue of the initial values on redox responses [31]. This carefully executed study indicated that the concentration of oxidant biomarkers are likely to decrease after the supplementation of vitamin C and E only if they are already high. Based on our results, the finding of Block et al. can be extended to include the oxidant stimuli as well.

Other factors may also affect the wide inter-individual variability of redox responses presented in this study. Several physiological, habitual and technical factors have been proposed as potential sources of the inter-individual variability [32–35]. However, the complex nature of biological systems and networks seems to be the major contributor to this variability [36]. Redox integration is a property of oxidant/antioxidant systems that refers to the extent to which their components are correlated through functional, structural, developmental or evolutionary interdependency [37]. Exercise-induced oxidative stress can reduce the integration among redox components of the blood [38]. Different levels of reduced biochemical integration after exercise among individuals can partly explain the high variability in the responses among the participants observed in the present study. Rankinen and Bouchard [39], beyond other factors, support the genetic hypothesis (i.e., alleles at key genes) of the heterogeneous responses. It is also important to consider that redox biomarkers are chemical substances, whose biological effects, multiple functional roles, actions and production pathways are largely still unknown [40–43]. Moreover, their levels greatly vary in the general population even at rest [44–48] and most of them exhibit a complex synergistic interdependency [38,49]. It becomes therefore reasonable to propose that a considerable part of the observed redox individuality results from the inherent biological complexity.

Relationships among redox biomarkers

At resting state and after exercise, a high correlation was found between F_2-isoprostanes and protein carbonyls ($r = .66$ and $r = .61$, respectively) and only moderate or small correlations between the two aforementioned biomarkers and glutathione. The fact that F_2-isoprostanes and protein carbonyls were highly correlated is not unexpected, since both redox biomarkers are oxidative modified products of free radicals. Accordingly, glutathione produced much lower

correlation coefficients with the oxidant biomarkers. In general, it is evident that the oxidant biomarkers (i.e., F_2-isoprostanes and protein carbonyls) are considerably affected by the same mechanism (both are end-products of free radical mediated oxidation) and as a result provide a similar description of the perturbation in redox homeostasis. On the other hand, this is not valid for glutathione which is a molecule dynamically transforming from a reduced to an oxidized form. Concerning muscle damage biomarkers, the only statistically significant correlations appeared after exercise and only between muscle torque and the redox biomarkers. Muscle torque was negatively and moderately correlated with protein carbonyls, while small negative correlations were observed with F_2-isoprostanes and glutathione. The fact that creatine kinase did not correlate with any redox biomarker may be due to the extremely large variability in the percent increase of creatine kinase among individuals (from 39% up to 11,299%). The low or no significant correlations appeared between redox biomarkers and muscle damage biomarkers indicate that the oxidative stress measured in blood after exercise derives, at least in part, from the blood and not just from the muscle [50].

What are the origins of blood and urine oxidative stress after eccentric exercise?

The delay in the appearance of oxidative stress in body fluids and the concurrent alterations in muscle damage biomarkers after exercise indicate that much of the oxidative stress is not a direct consequence of mechanical or metabolic stress developed during exercise, but instead, oxidative stress is largely attributable to muscle damage. Major immunological responses (both at systemic and muscle level) occur during the post-eccentric exercise period which can affect redox homeostasis of blood and urine. After eccentric actions, neutrophils and other phagocytic cells are activated and recruited to the site of the initial damage [51]. These immunity cells produce superoxide anion ($O_2^{\bullet-}$) with the catalytic action of the reduced nicotinamide adenine dinucleotide phosphate (NADPH)-oxidase system [52]. Other cells can also activate NADPH oxidase after exercise (and contribute to body fluids oxidative stress) such as skeletal muscle [53] and endothelium [5] while upregulation of NADPH oxidase in smooth muscle cells has been reported after mechanical stimuli [54]. $O_2^{\bullet-}$ can be converted to hydrogen peroxide (H_2O_2) by superoxide dismutase. H_2O_2 is not a free radical, but it is considered a reactive species because of its toxicity and capacity to cause reactive species formation. In leukocytes, myeloperoxidase transforms H_2O_2 in hypochlorous acid (HOCl), one of the strongest physiological oxidants. These reactants can also destroy adjacent healthy muscle tissue. As many researchers have reported, this oxidative process has been directly shown to occur 2–4 days after eccentric actions in animals and humans [55]. Consequently, the major part of the delayed oxidative stress detected in blood and urine after muscle-damaging exercise probably comes from neutrophils and macrophages that are recruited to the site of the initial damage. This delayed onset of free radical production after eccentric exercise can activate several redox-sensitive transcription factors in cells and tissues. For example, nuclear factor kappa B (NF-κB) has been found upregulated after eccentric exercise in skeletal muscle [56] and leukocytes [57]. NF-kB has also been found upregulated after other oxidant stimuli in epithelium [58] and smooth muscle [59], tissues that are in contact or in the vicinity of blood. This upregulation of redox-sensitive transcription factors by free radicals can, in turn, lead to release of inflammatory cytokines [60,61]. In fact, either muscle-damaging or non-muscle-damaging exercise have been shown to induce the production of cytokines in leukocytes [62], skeletal muscle [63], endothelium [64] and smooth muscle [65] among others. All these tissues can release their cytokines in the circulation leading to oxidative stress in blood and urine. It was noted that free radicals and cytokines both stimulate the stress response and are re-activated by the same pathways, thereby giving rise to a vicious cycle, further contributing to oxidative stress [66,67]. That explains, it is conceivable that the reductive stress appeared in some individuals after exercise and may occur by downregulating some or all of these complex steps orchestrating the redox responses to eccentric exercise. It should be underlined that we consider eccentric exercise as just the original stimulus to initiate the cascade of redox-related biochemical events presented above. Certainly, any type of exercise (or any other environmental stimulus) cannot directly cause redox changes; these are always mediated by certain biochemical triggers.

In the present study no muscle biopsies were collected therefore limiting our view to blood and urine. However, the issue of blood vs. muscle measurements is a controversial topic in the literature. At present, the prevailing idea in the field is that blood is largely considered an inert body fluid, a kind of "sink" that passively accepts reactive species and oxidative products produced mainly from tissues (in our case contracting skeletal muscles). In our opinion, however, this frequently adopted tissue- or muscle-centric point of view has not been put into a rigorous test [17,50,68–72]. In fact, we believe that at least some of the effects of exercise on muscle redox homeostasis are partially due to changes in the redox composition of blood [73,74]. Additionally, several recent and high-prolific studies highlight the role of blood-borne factors in reversing aging in heart and brain [75,76]. As a result, we believe that investigating the effects of exercise (or any other environmental factor) on blood redox homeostasis has a scientific value in itself.

Conclusions

We believe that the evidence presented in this paper for the presence of individual differences in redox biology responses is strong. What is less clear is the exact nature of the mechanisms responsible for the heterogeneity in redox responses. We found that initial levels are a major determinant but certainly many others are awaited to be revealed in future investigations (e.g., genetics, age, loss of biochemical integration). The main finding of the study is that exercise induces reductive stress in some humans and no stress in other humans. We believe that the wide inter-individual variability described in this paper is not limited to the oxidant stimulus used and the three redox biomarkers selected. The data presented herein emphasize that the mean response to a redox stimulus can be misleading. Consequently, future research should present results for each individual separately along with the average responses.

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References

[1] M.G. Nikolaidis, A. Kyparos, I.S. Vrabas, F_2-isoprostane formation, measurement and interpretation: the role of exercise, Progress in Lipid Research. 50 (2011) 89–103. http://dx.doi.org/10.1016/j.plipres.2010.10.002, 20951733.

[2] S.K. Powers, W.B. Nelson, M.B. Hudson, Exercise-induced oxidative stress in humans: cause and consequences, Free Radical Biology and Medicine. 51 (2011) 942–50. http://dx.doi.org/10.1016/j.freeradbiomed.2010.12.009, 21167935.

[3] Z. Radak, Z. Zhao, E. Koltai, H. Ohno, M. Atalay, Oxygen consumption and usage during physical exercise: the balance between oxidative stress and ROS-dependent adaptive signaling, Antioxidants Redox Signaling. 18 (2013) 1208–46. http://dx.doi.org/10.1089/ars.2011.4498, 22978553.

[4] S. Fittipaldi, I. Dimauro, N. Mercatelli, D. Caporossi, Role of exercise-induced reactive oxygen species in the modulation of heat shock protein response, Free Radical Research. 48(1) (2014) 52–70, .

[5] L. Gliemann, M. Nyberg, Y. Hellsten, Nitric oxide and reactive oxygen species in limb vascular function: what is the effect of physical activity? Free Radical Research. 48(1) (2014) 71–83, .

[6] S.K. Powers, J. Duarte, A.N. Kavazis, E.E. Talbert, Reactive oxygen species are signalling molecules for skeletal muscle adaptation, Experimental Physiology. 95 (2010) 1–9. http://dx.doi.org/10.1113/expphysiol.2009.050526, 19880534.

[7] K.J. Davies, L. Packer, G.A. Brooks, Biochemical adaptation of mitochondria, muscle, and whole-animal respiration to endurance training, Archives of Biochemistry and Biophysics. 209 (1981) 539–54. http://dx.doi.org/10.1016/0003-9861(81)90312-X, 7294809.

[8] U. Dreissigacker, M. Wendt, T. Wittke, D. Tsikas, N. Maassen, Positive correlation between plasma nitrite and performance during high-intensive exercise but not oxidative stress in healthy men, Nitric Oxide : Biology and Chemistry / Official Journal of the Nitric Oxide Society. 23 (2010) 128–35. http://dx.doi.org/10.1016/j.niox.2010.05.003, 20451646.

[9] S.R. McAnulty, L.S. McAnulty, D.C. Nieman, J.D. Morrow, L.A. Shooter, S. Holmes, et al, Effect of alpha-tocopherol supplementation on plasma homocysteine and oxidative stress in highly trained athletes before and after exhaustive exercise, Journal of Nutritional Biochemistry. 16 (2005) 530–7, .

[10] L.L. Ji, A. Katz, R. Fu, M. Griffiths, M. Spencer, Blood glutathione status during exercise: effect of carbohydrate supplementation, Journal of Applied Physiology (Bethesda, Md. : 1985). 74 (1993) 788–92, .

[11] Y. Michailidis, A.Z. Jamurtas, M.G. Nikolaidis, I.G. Fatouros, Y. Koutedakis, I. Papassotiriou, et al, Sampling time is crucial for measurement of aerobic exercise-induced oxidative stress, Medicine and Science in Sports and Exercise. 39 (2007) 1107–13, .

[12] C. Bouchard, T. Rankinen, Individual differences in response to regular physical activity, Medicine and Science in Sports and Exercise. 33(6 Suppl.) (2001) S446–S451, .

[13] M.G. Nikolaidis, V. Paschalis, G. Giakas, I.G. Fatouros, Y. Koutedakis, D. Kouretas, et al, Decreased blood oxidative stress after repeated muscle-damaging exercise, Medicine and Science in Sports and Exercise. 39 (2007) 1080–9, .

[14] M.G. Nikolaidis, A.Z. Jamurtas, V. Paschalis, I.G. Fatouros, Y. Koutedakis, D. Kouretas, The effect of muscle-damaging exercise on blood and skeletal muscle oxidative stress: magnitude and time-course considerations, Sports Medicine (Auckland, N.Z.). 38 (2008) 579–606, .

[15] M.G. Nikolaidis, A. Kyparos, C. Spanou, V. Paschalis, A.A. Theodorou, G. Panayiotou, et al, Aging is not a barrier to muscle and redox adaptations: applying the repeated eccentric exercise model, Experimental Gerontology. 48 (2013) 734–43. http://dx.doi.org/10.1016/j.exger.2013.04.009, 23628501.

[16] AA Theodorou, MG Nikolaidis, V Paschalis, S Koutsias, G Panayiotou, IG Fatouros, et al, No effect of antioxidant supplementation on muscle performance and blood redox status adaptations to eccentric training, American Journal of Clinical Nutrition. 93 (2011) 1373–83, .

[17] M.G. Nikolaidis, A. Kyparos, K. Dipla, A. Zafeiridis, M. Sambanis, G.V. Grivas, et al, Exercise as a model to study redox homeostasis in blood: the effect of protocol and sampling point, Biomarkers : Biochemical indicators of Exposure, Response, and Susceptibility to Chemicals. 17 (2012) 28–35. http://dx.doi.org/10.3109/1354750X.2011.635805, 22288504.

[18] D. Giustarini, I. Dalle-Donne, A. Milzani, P. Fanti, R. Rossi, Analysis of GSH and GSSG after derivatization with N-ethylmaleimide, Nature Protocols. 8 (2013) 1660–9. http://dx.doi.org/10.1038/nprot.2013.095, 23928499.

[19] A.H. Goris, M.S. Westerterp-Plantenga, K.R. Westerterp, Undereating and underrecording of habitual food intake in obese men: selective underreporting of fat intake, American Journal of Clinical Nutrition. 71(1) (2000) 130–4, .

[20] M.B. Livingstone, A.E. Black, Markers of the validity of reported energy intake, Journal of Nutrition. 133 (2003) S895–S920, .

[21] S. Voss, A. Kroke, K. Klipstein-Grobusch, H. Boeing, Is macronutrient composition of dietary intake data affected by underreporting? Results from the EPIC-Potsdam Study. European Prospective Investigation into Cancer and Nutrition, European Journal of Clinical Nutrition. 52(2) (1998) 119–26, .

[22] B.L. Heitmann, L. Lissner, Dietary underreporting by obese individuals: is it specific or non-specific? British Medical Jounal (Clinical Research Ed.). 311 (1995) 986–9. http://dx.doi.org/10.1136/bmj.311.7011.986, 7580640.

[23] J.A. Tooze, A.F. Subar, F.E. Thompson, R. Troiano, A. Schatzkin, V. Kipnis, Psychosocial predictors of energy underreporting in a large doubly labeled water study, American Journal of Clinical Nutrition. 79(5) (2004) 795–804, .

[24] M.C. Gomez-Cabrera, E. Domenech, M. Romagnoli, A. Arduini, C. Borras, F.V. Pallardo, et al, Oral administration of vitamin C decreases muscle mitochondrial biogenesis and hampers training-induced adaptations in endurance performance, American Journal of Clinical Nutrition. 87(1) (2008) 142–9, .

[25] M. Ristow, K. Zarse, A. Oberbach, N. Klöting, M. Birringer, M. Kiehntopf, et al, Antioxidants prevent health-promoting effects of physical exercise in humans, Proceedings of the National Academy of Sciences of the United States of America. 106(21) (2009) 8665–70, .

[26] M.G. Nikolaidis, A. Kyparos, C. Spanou, V. Paschalis, A.A. Theodorou, I.S. Vrabas, Redox biology of exercise: an integrative and comparative consideration of some overlooked issues, Journal of Experimental Biology. 215 (2012) 1615–25. http://dx.doi.org/10.1242/jeb.067470, 22539728.

[27] N.B. Vollaard, B.J. Reeder, J.P. Shearman, P. Menu, M.T. Wilson, C.E. Cooper, A new sensitive assay reveals that hemoglobin is oxidatively modified in vivo, Free Radical Biology & Medicine. 39(9) (2005) 1216–28. http://dx.doi.org/10.1016/j.freeradbiomed.2005.06.012, 16214037.

[28] B. Dufaux, O. Heine, A. Kothe, U. Prinz, R. Rost, Blood glutathione status following distance running, International Journal of Sports Medicine. 18(2) (1997) 89–93, .

[29] D.A. Rodriguez, S. Kalko, E. Puig-Vilanova, M. Perez-Olabarría, F. Falciani, J. Gea, et al, Muscle and blood redox status after exercise training in severe COPD patients, Free Radical Biology & Medicine. 52(1) (2012) 88–94. http://dx.doi.org/10.1016/j.freeradbiomed.2011.09.022, 22064359.

[30] de Lucas R. Dantas, F. Caputo, de Souza K. Mendes, A.R. Sigwalt, K. Ghisoni, L. Silveira, et al, Increased platelet oxidative metabolism, blood oxidative stress and neopterin levels after ultra-endurance exercise, Journal of Sports Sciences. 32 (2014) 22–30, .

[31] G. Block, C.D. Jensen, J.D. Morrow, N. Holland, E.P. Norkus, G.L. Milne, et al, The effect of vitamins C and E on biomarkers of oxidative stress depends on baseline level, Free Radical Biology & Medicine. 45 (2008) 377–84. http://dx.doi.org/10.1016/j.freeradbiomed.2008.04.005, 18455517.

[32] R.J. Bloomer, K.H. Fisher-Wellman, Blood oxidative stress biomarkers: influence of sex, exercise training status, and dietary intake, Gender Medicine. 5 (2008) 218–28. http://dx.doi.org/10.1016/j.genm.2008.07.002, 18727988.

[33] O. García, T. Mandina, A.I. Lamadrid, A. Diaz, A. Remigio, Y. Gonzalez, et al, Sensitivity and variability of visual scoring in the comet assay. Results of an inter-laboratory scoring exercise with the use of silver staining, Mutation Research. 556 (2004) 25–34. http://dx.doi.org/10.1016/j.mrfmmm.2004.06.035, 15491629.

[34] A.K. Kant, B.I. Graubard, Ethnic and socioeconomic differences in variability in nutritional biomarkers, American Journal of Clinical Nutrition. 87 (2008) 1464–71, .

[35] J.A. Simoneau, C. Bouchard, Human variation in skeletal muscle fiber-type proportion and enzyme activities, American Journal of Physiology. 257 (1989) E567–E572, .

[36] J.A. Papin, J.L. Reed, B.O. Palsson, Hierarchical thinking in network biology: the unbiased modularization of biochemical networks, Trends in Biochemical Sciences. 29 (2004) 641–7. http://dx.doi.org/10.1016/j.tibs.2004.10.001, 15544950.

[37] D. Costantini, P. Monaghan, N.B. Metcalfe, Biochemical integration of blood redox state in captive zebra finches (*Taeniopygia guttata*), Journal of Experimental Biology. 214(7) (2011) 1148–52. http://dx.doi.org/10.1242/jeb.053496, 21389200.

[38] D. Costantini, P. Monaghan, N.B. Metcalfe, Loss of integration is associated with reduced resistance to oxidative stress, Journal of Experimental Biology. 216 (2013) 2213–20. http://dx.doi.org/10.1242/jeb.083154, 23470664.

[39] T. Rankinen, C. Bouchard, Gene-physical activity interactions: overview of human studies, Obesity (Silver Spring, Md.). 16 (2008) S47–S50, .

[40] M. Bruschi, G. Candiano, L. Santucci, G.M. Ghiggeri, Oxidized albumin. The long way of a protein of uncertain function, Biochimica et Biophysica Acta. 1830(12) (2013) 5473–9, .

[41] M. Carocho, I.C. Ferreira, A review on antioxidants, prooxidants and related controversy: natural and synthetic compounds, screening and analysis methodologies and future perspectives, Food and Chemical Toxicology : An international Journal Published For the British Industrial Biological Research Association. 51 (2013) 15–25. http://dx.doi.org/10.1016/j.fct.2012.09.021, 23017782.

[42] S. Kar, M. Kavdia, Endothelial NO and $O(_2)(-)$ production rates differentially regulate oxidative, nitroxidative, and nitrosative stress in the microcirculation, Free Radical Biology and Medicine. 63C (2013) 161–74.

[43] Y. Samuni, S. Goldstein, O.M. Dean, M. Berk, The chemistry and biological activities of N-acetylcysteine, Biochimica et Biophysica Acta. 1830 (2013) 4117–29. http://dx.doi.org/10.1016/j.bbagen.2013.04.016, 23618697.

[44] S. Argüelles, A. Gómez, A. Machado, A. Ayala, A preliminary analysis of within-subject variation in human serum oxidative stress parameters as a function of time, Rejuvenation Research. 10 (2007) 621–36. http://dx.doi.org/10.1089/rej.2006.0528, 18047415.

[45] J. Helmersson, S.F. Basu, (2)-Isoprostane and prostaglandin F(2 alpha)metabolite excretion rate and day to day variation in healthy humans, Prostaglandins, Leukotrienes, and Essential Fatty Acids. 65 (2001) 99–102. http://dx.doi.org/10.1054/plef.2001.0295, 11545626.

[46] A. Pilger, D. Germadnik, K. Riedel, I. Meger-Kossien, G. Scherer, H.W. Rüdiger, Longitudinal study of urinary 8-hydroxy-2′-deoxyguanosine excretion in healthy adults, Free Radical Research. 35 (2001) 273–80. http://dx.doi.org/10.1080/10715760100300811, 11697126.

[47] H. Su, M. Gornitsky, G. Geng, A.M. Velly, H. Chertkow, H.M. Schipper, Diurnal variations in salivary protein carbonyls levels in normal and cognitively impaired human subjects, Age. 30 (2008) 1–9. http://dx.doi.org/10.1007/s11357-007-9042-z, 19424868.

[48] E. Valencia, A. Marin, G. Hardy, Circadian rhythmicity of whole-blood glutathione in healthy subjects, Nutrition (Burbank, Los Angeles County, California). 17 (2001) 731–3. http://dx.doi.org/10.1016/S0899-9007(01)00653-0, 11527661.

[49] B. Halliwell, J.M.C. Gutteridge, Free Radicals in Biology and Medicine, New York, Oxford University Press, 2007.

[50] M.G. Nikolaidis, A.Z. Jamurtas, Blood as a reactive species generator and redox status regulator during exercise, Archives of Biochemistry and Biophysics. 490 (2009) 77–84, .

[51] J.G. Tidball, S.A. Villalta, Regulatory interactions between muscle and the immune system during muscle regeneration, American Journal of Physiology. Regulatory, Integrative and Comparative Physiology. 298(5) (2010) R1173–R1187. http://dx.doi.org/10.1152/ajpregu.00735.2009, 20219869.

[52] B. Halliwell, Phagocyte-derived reactive species: salvation or suicide? Trends in Biochemical Sciences. 31(9) (2006) 509–15. http://dx.doi.org/10.1016/j.tibs.2006.07.005, 16890439.

[53] G.K. Sakellariou, M.J. Jackson, A. Vasilaki, Redefining the major contributors to superoxide production in contracting skeletal muscle. The role of NAD(P)H oxidases, Free Radical Research. 48(1) (2014) 12–29, .

[54] H. Hitomi, T. Fukui, K. Moriwaki, K. Matsubara, G.P. Sun, M. Rahman, et al, Synergistic effect of mechanical stretch and angiotensin II on superoxide production via NADPH oxidase in vascular smooth muscle cells, Journal of Hypertension. 24(6) (2006) 1089–95. http://dx.doi.org/10.1097/01.hjh.0000226199.51805.88, 16685209.

[55] G.L. Close, A. Kayani, A. Vasilaki, A. McArdle, Skeletal muscle damage with exercise and aging, Sports Medicine (Auckland, N.Z.). 35(5) (2005) 413–27. http://dx.doi.org/10.2165/00007256-200535050-00004, 15896090.

[56] R.D. Hyldahl, L. Xin, M.J. Hubal, S. Moeckel-Cole, S. Chipkin, P.M. Clarkson, Activation of nuclear factor-κB following muscle eccentric contractions in humans is localized primarily to skeletal muscle-residing pericytes, FASEB Journal : Official Publication of the Federation of American Societies For Experimental Biology. 25(9) (2011) 2956–66, .

[57] R. Jiménez-Jiménez, M.J. Cuevas, M. Almar, E. Lima, D. García-López, Paz J.A. De, et al, Eccentric training impairs NF-kappaB activation and over-expression of inflammation-related genes induced by acute eccentric exercise in the elderly, Mechanisms of Ageing and Development. 129(6) (2008) 313–21. http://dx.doi.org/10.1016/j.mad.2008.02.007, 18377953.

[58] A. Barchowsky, E.J. Dudek, M.D. Treadwell, K.E. Wetterhahn, Arsenic induces oxidant stress and NF-kappa B activation in cultured aortic endothelial cells, Free Radical Biology & Medicine. 21(6) (1996) 783–90. http://dx.doi.org/10.1016/0891-5849(96)00174-8, 8902524.

[59] L. Wu, The pro-oxidant role of methylglyoxal in mesenteric artery smooth muscle cells, Canadian Journal of Physiology and Pharmacology. 83(1) (2005) 63–8. http://dx.doi.org/10.1139/y04-112, 15759051.

[60] K. Esposito, F. Nappo, R. Marfella, G. Giugliano, F. Giugliano, M. Ciotola, et al, Inflammatory cytokine concentrations are acutely increased by hyperglycemia in humans: role of oxidative stress, Circulation. 106(16) (2002) 2067–72. http://dx.doi.org/10.1161/01.CIR.0000034509.14906.AE, 12379575.

[61] M. Guha, W. Bai, J.L. Nadler, R. Natarajan, Molecular mechanisms of tumor necrosis factor alpha gene expression in monocytic cells via hyperglycemia-induced oxidant stress-dependent and -independent pathways, Journal of Biological Chemistry. 275(23) (2000) 17728–39. http://dx.doi.org/10.1074/jbc.275.23.17728, 10837498.

[62] F.E. Lund, Cytokine-producing B lymphocytes – key regulators of immunity, Current Opinion in Immunology. 20(3) (2008) 332–8. http://dx.doi.org/10.1016/j.coi.2008.03.003, 18417336.

[63] K. Hamada, E. Vannier, J.M. Sacheck, A.L. Witsell, R. Roubenoff, Senescence of human skeletal muscle impairs the local inflammatory cytokine response to acute eccentric exercise, FASEB Journal : Official Publication of the Federation of American Societies For Experimental Biology. 19(2) (2005) 264–6, .

[64] J.R. Teijaro, K.B. Walsh, S. Cahalan, D.M. Fremgen, E. Roberts, F. Scott, et al, Endothelial cells are central orchestrators of cytokine amplification during influenza virus infection, Cell. 146(6) (2011) 980–91. http://dx.doi.org/10.1016/j.cell.2011.08.015, 21925319.

[65] W.T. Gerthoffer, C.A. Singer, Secretory functions of smooth muscle: cytokines and growth factors, Molecular Interventions. 2(7) (2002) 447–56. http://dx.doi.org/10.1124/mi.2.7.447, 14993407.

[66] N. Khaper, S. Bryan, S. Dhingra, R. Singal, A. Bajaj, C.M. Pathak, et al, Targeting the vicious inflammation-oxidative stress cycle for the management of heart failure, Antioxidants & Redox Signaling. 13(7) (2010) 1033–49, .

[67] A. Voigt, A. Rahnefeld, P.M. Kloetzel, E. Krüger, Cytokine-induced oxidative stress in cardiac inflammation and heart failure – how the ubiquitin proteasome system targets this vicious cycle, Frontiers in Physiology. 4 (2013) 42.

[68] P.W. Buehler, A.I. Alayash, Redox biology of blood revisited: the role of red blood cells in maintaining circulatory reductive capacity, Antioxidants & Redox Signaling. 7(11–12) (2005) 1755–60. http://dx.doi.org/10.1089/ars.2005.7.1755, 16356136.

[69] U. Kerkweg, K. Pamp, J. Fieker, F. Petrat, R.C. Hider, H. de Groot, Release of redox-active iron by muscle crush trauma: no liberation into the circulation, Shock (Augusta, GA). 33(5) (2010) 513–18, .

[70] P.R. Kvietys, D.N. Granger, Role of reactive oxygen and nitrogen species in the vascular responses to inflammation, Free Radical Biology & Medicine. 52(3) (2012) 556–92. http://dx.doi.org/10.1016/j.freeradbiomed.2011.11.002, 22154653.

[71] M.G. Nikolaidis, A. Kyparos, I.S. Vrabas, Cell redox homeostasis: reading Conti et al. data from a blood-centric perspective, Medicine and Science in Sports and Exercise. 44(1) (2012) 190, .

[72] Z. Radak, H.Y. Chung, S. Goto, Systemic adaptation to oxidative challenge induced by regular exercise, Free Radical Biology & Medicine. 44(2) (2008) 153–9. http://dx.doi.org/10.1016/j.freeradbiomed.2007.01.029, 18191751.

[73] V. Conti, G. Corbi, G. Russomanno, V. Simeon, N. Ferrara, W. Filippelli, et al, Oxidative stress effects on endothelial cells treated with different athletes' sera, Medicine and Science in Sports and Exercise. 44(1) (2012) 39–49, .

[74] V. Conti, G. Russomanno, G. Corbi, G. Guerra, C. Grasso, W. Filippelli, et al, Aerobic training workload affects human endothelial cells redox homeostasis, Medicine and Science in Sports and Exercise. 45(4) (2013) 644–53, .

[75] F.S. Loffredo, M.L. Steinhauser, S.M. Jay, J. Gannon, J.R. Pancoast, P. Yalamanchi, et al, Growth differentiation factor 11 is a circulating factor that reverses age-related cardiac hypertrophy, Cell. 153(4) (2013) 828–39, .

[76] S.A. Villeda, J. Luo, K.I. Mosher, B. Zou, M. Britschgi, G. Bieri, et al, The ageing systemic milieu negatively regulates neurogenesis and cognitive function, Nature. 477(7362) (2011) 90–4. http://dx.doi.org/10.1038/nature10357, 21886162.