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Exercise-Induced Oxidative Stress
Myths, Realities and Physiological Relevance

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Abstract

Although assays for the most popular markers of exercise-induced oxidative stress may experience methodological flaws, there is sufficient credible evidence to suggest that exercise is accompanied by an increased generation of free radicals, resulting in a measurable degree of oxidative modifications to various molecules. However, the mechanisms responsible are unclear. A common assumption that increased mitochondrial oxygen consumption leads *per se* to increased reactive oxygen species (ROS) production is not supported by *in vitro* and *in vivo* data. The specific contributions of other systems (xanthine oxidase, inflammation, haem protein auto-oxidation) are poorly characterised. It has been demonstrated that ROS have the capacity to contribute to the development of muscle fatigue *in situ*, but there is still a lack of convincing direct evidence that ROS impair exercise performance *in vivo* in humans. It remains unclear whether exercise-induced oxidative modifications have little significance, induce harmful oxidative damage, or are an integral part of redox regulation. It *is* clear that ROS play important roles in numerous physiological processes at rest; however, the detailed physiological functions of ROS in exercise remain to be elucidated.

In the last two decades or so there has been an increasing awareness of the range of physiological processes involving free radicals. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) such as hydrogen peroxide (H_2O_2), superoxide ($O_2^{\bullet -}$), hypochlorous acid (HOCl) and nitric oxide ($NO^{\bullet}$) play important roles in, for example, the body's immune response,[1] redox regulation of gene transcription,[2] cell signalling,[3] and enzymology.[4] Thus, ROS and RNS are clearly essential for normal functioning. However, as with everything else in life, 'too much' is always a bad thing and the high reactivity of certain ROS makes it relatively easy for the intracellular redox status to slip into a state less compatible with optimal functioning. Oxidative stress is implicated in virtually every known disease[5] and there is an increasing body of evidence linking free radical production to the process of

aging.[6] These negative aspects of free radicals have given them a bad reputation that is hard to overcome. Consequently, after the emergence of evidence in the late 1970s indicating an increase in radical production with exercise, studies into exercise-induced oxidative stress predominantly seem to have focused on the perceived negative impact of ROS on exercise performance, and ways to counteract it. This article summarises the available evidence supporting the occurrence of exercise-induced oxidative stress, examines the proposed mechanisms for increased ROS generation with exercise, and discusses the relevance of exercise-induced ROS formation to health and performance.

1. Exercise-Induced Oxidative Stress: Experimental Evidence

In 1978, Dillard et al.[7] were the first to demonstrate that physical exercise can lead to increased lipid peroxidation by showing a 1.8-fold increase in exhaled pentane levels after 60 minutes of cycling at 25–75% of maximum oxygen consumption ($\dot{V}O_{2max}$). A few years later, Davies et al.[8] confirmed this finding of exercise-induced oxidative stress by demonstrating a 2- to 3-fold increase in free radical production during exhaustive exercise in rats, as detected by electron paramagnetic resonance (EPR) spectroscopy of intact or homogenised muscle and liver tissue. The short lifespan of free radicals prevents direct detection *in vivo* in humans, but EPR spectroscopy (particularly in combination with spin traps) seems a promising method to allow the detection of free radical species in biological samples *ex vivo*.[9,10] However, only few studies have used this method to establish the relationship between exercise and oxidative stress. The first exercise study to use EPR in combination with spin trapping examined the effect of exhaustive, incremental cycling on free radical levels in venous blood of untrained men.[9] A 350% increase in what were speculated to be lipid-derived oxygen-centered alkoxyl radicals was accompanied by a 42% rise in lipid peroxides and a 14% increase in thiobarbituric acid (TBARS; a measure of malondialdehyde [MDA], which is a by-product of the peroxidation of polyunsaturated fatty acids). Similar results were obtained in a further study in which it was also shown that pre-exercise ascorbic acid (vitamin C) supplementation attenuates the exercise-induced increase in free radicals.[10] By determining veno-arterial differences in spin adducts, Bailey et al.[11,12] were able to demonstrate that the active muscle bed is a source of increased free radical production during exercise. Furthermore, Groussard et al.[13] observed that free radical production peaked 20 minutes after a Wingate test for anaerobic power, which led them to suggest that the increase in post-exercise free radical production was mediated by xanthine oxidase.

Because of the difficulties in measuring free radical production directly, most human studies have used indirect markers to demonstrate exercise-induced oxidative stress. The majority of these have looked at markers of lipid peroxidation. A substantial amount of alleged support for the theory that exercise induces oxidative stress comes from data on the effects of exercise on TBARS. Although several studies have been unable to demonstrate an increase in TBARS levels,[14-31] and decreases have also been reported,[13,32,33] a large number of studies have found significant increases, some by as much as 220% (table I). However, there are some reservations about the validity of the TBARS assay in detecting lipid peroxidation *in vivo*. It is common knowledge, but largely ignored, that the TBARS assay is not specific to MDA, nor is lipid peroxidation the exclusive source of MDA.[34,35] Moreover, the various available assays experience methodological problems.[36] Although numerous authors have reported resting TBARS levels in plasma or serum of healthy human volunteers of up to several micromolar,[14,15,18,20,24,28,31,33,37-41] others have reported that TBARS levels are very low,[26,35,42] or out of the analytical range of the assay.[17,43] In our laboratory, we have submitted plasma of healthy human volunteers to a range of assay conditions similar to those described in the literature[44-46] without detecting measurable amounts of TBARS (unpublished data). Recovery of added MDA is excellent, however, suggesting that although the TBARS

Table I. Effects of exercise on thiobarbituric acid (TBARS)

Study[a]	Assay	Exercise protocol[b]	Subjects	Resting TBARS ± SD (μmol/L)	Δ Exercise (%)[c]
Viinikka et al.[14]	Fluoro	Max-test cycle	10 T M	2.0 ± 0.4	+10
Lovlin et al.[32]	Spec	5 min cycle 40%	6 UT M	2.26 ± 0.10 mmol/L	−12[d]
		5 min cycle 70%			−6
		Max-test cycle			+27[d]
Kanter et al.[37]	Spec	80km run	9 T M	3.6 ± 0.8	+59[d]
Maughan et al.[15]	Spec	45 min down-hill run	16 UT M	2.0 ± 0.1	NC
Duthie et al.[16]	Fluoro	21km run	7 T M	1.52 ± 0.23	+16
Kretzschmar et al.[33]	Fluoro	Max-test cycle	7 UT M	3.64 ± 0.74	−12[d]
			5 T M	3.29 ± 0.34	−33[d]
Sahlin et al.[17]	UV/HPLC	Cycle 98% to exh	8 MT M	<0.1	NC
Kanter and Eddy[38]	Spec	30 min 60%/5 min 90%	20 T/UT M	5 ± 1	~+30[d]
Ji[42]	Spec	Cycle 70% to exh	8 T F/M	0.12 ± 0.03	~+30
Maxwell et al.[49]	UV/HPLC	60 min box-stepping	8 UT F/M	0.89 ± 0.12	+38
Rokitzki et al.[39]	Spec	Max-test cycle	15 T F	7.6 ± 2.8	+31
Sen et al.[50]	Spec	Max-test	9 UT M	1.3 ± 0.2	~+30
		30 min cycle AT		1.2 ± 0.3	~+60[d]
		30 min cycle AnT		1.1 ± 0.1	~+90[d]
Hartmann et al.[18]	Fluoro	Max-test run	5 UT M	2.6 ± 0.6	+15
Vasankari et al.[51]	Spec	1km run	8 T M	1.05 ± 0.05	−7
		10km run	7 T M	0.98 ± 0.08	−11
Niess et al.[20]	Fluoro	Max-test run	5 UT M	2.58 ± 0.63	+12
Alessio et al.[52]	Fluoro	30 min run 80%	9 MT M	0.90 ± 0.12	+47[d]
Dufaux et al.[21]	Fluoro/HPLC	2.5h run	12 MT M	0.8 ± 0.1	NC
Margaritis et al.[22]	Fluoro/HPLC	Triathlon	12 T M	4.54 ± 0.70 mmol/L	−6
Ashton et al.[9]	Fluoro/HPLC	Max-test cycle	12 UT M	0.70 ± 0.05	+14[d]
Child et al.[53]	Fluoro/HPLC	21km run	17 T M	1.48 ± 0.39	+11[d]
Marzatico et al.[40]	Spec	Half marathon	6 T M	3.13 ± 0.4	+60[d]
		6 × 150m sprint	6 T M	2.85 ± 0.3	+220[d]
Szczesniak et al.[54]	Not given	Max-test run	13 T M	1.80 ± 0.03	+14[d]
Ashton et al.[10]	Fluoro/HPLC	Max-test cycle	10 UT M	0.70 ± 0.04	+14[d]
Borsheim et al.[55]	Spec	90 min cycle 58%	8 MT M	1.68 ± 0.08	+83[d]
Chung et al.[23]	Spec (TBARS)	30 min run 75%	11 UT F	0.65 ± 0.05	NC
	Spec (MDA)			1.55 ± 0.17	NC
Laaksonen et al.[56]	Spec	40 min cycle 60%	14 UT M	0.86 ± 0.37	+67[d]
Leaf et al.[57]	Spec	Max-test run	14 UT M	26.9 ± 25.7	−6
Surmen-Gur et al.[25]	Spec	Max-test cycle	9 UT F	5.2 ± 1.0	NC
Alessio et al.[26]	Spec, MPI	Max-test run	12 UT M/F	0.14 ± 0.01 μmol/g protein	+14
		Isometric 50% MVC to exh		0.14 ± 0.01 μmol/g protein	+7
Child et al.[58]	Fluoro/HPLC	21km run	14 T M	1.49 ± 0.16	~+30[d]
Hessel et al.[41]	Spec	Marathon	18 T M	11.46 ± 3.09	+11[d]
Sacheck et al.[27]	Fluoro/HPLC	45 min 10% downhill run	8 T F	1.5 ± 0.2	NC
Akova et al.[28]	Spec	Leg extension to exh	8 UT F	3.2 ± 0.3	NC
Groussard et al.[13]	Fluoro/HPLC	Wingate	8 UT M	?	−24[d]

Continued next page

Table I. Contd

Study[a]	Assay	Exercise protocol[b]	Subjects	Resting TBARS ± SD (μmol/L)	Δ Exercise (%)[c]
Quindry et al.[29]	Spec	Max-test run	9 MT M	1.9 ± 0.5	−5
		45 min 10% below LT		2.2 ± 0.4	+4
		45 min 10% above LT		2.3 ± 0.7	+4
Sacheck et al.[30]	Fluoro/HPLC	45 min 16% downhill run	16 MT M	0.40 ± 0.05	NC
Ramel et al.[31]	HPLC	Submax resistance exercise	7 T M	1.60 ± 0.38	+25
			10 UT M	2.09 ± 1.18	+19

a Selected studies are shown.

b The '%' symbol in the 'Exercise protocol' column indicates the percentage of maximum oxygen consumption.

c Δ Exercise is the change caused by exercise, and has been estimated from figures if not stated in the original article.

d Significant change.

AnT = anaerobic threshold; **AT** = aerobic threshold; **exh** = exhaustion; **F** = females; **fluoro** = fluorometric; **HPLC** = high-performance liquid chromatography; **LT** = lactate threshold; **M** = males; **max-test** = exhaustive incremental test; **MDA** = malondialdehyde; **MPI** = *N*-methyl-2-phenylindoline; **MT** = moderately trained; **MVC** = maximal voluntary contraction; **NC** = no change; **spec** = spectrophotometric; **submax** = submaximal; **T** = trained; **UT** = untrained; **?** indicates information not provided in original article.

assay is clearly capable of detecting MDA, free plasma levels are very low. Some authors have suggested that protein-bound MDA should be released prior to analysis, and support this claim by showing that hot alkali digestion of samples prior to the assay produces significantly higher TBARS values.[47,48] However, we have found that deproteinising plasma samples prior to this treatment results in similarly high TBARS levels (unpublished data), which suggests that TBARS are produced during the assay, and do not accurately reflect the situation *in vivo*. How several authors have come to observe micromolar levels of TBARS remains unclear, as unfortunately information on exact procedures for the quantification of concentrations using standard curves is rarely provided. An example of a simple mistake could be to directly compare the absorbance of plasma samples with standard control samples consisting of known MDA concentrations in water, disregarding the substantial difference in baseline between the spectra.

Beside methodological problems, failure to correct for exercise-induced plasma volume changes may have been the cause for significant increases in several studies.[9,10,25,54] Furthermore, in the only study to include a no-exercise control condition, Borsheim et al.[55] observed an unexpected, and unexplained 69% increase in TBARS levels over a 3.5-hour period of bed-rest, no different from the 83% increase 2 hours after 90 minutes of submaximal cycling. It is our opinion that, considering the above, TBARS are unsuitable as markers of exercise-induced oxidative stress. More reliable support for the occurrence of exercise-induced oxidative stress is available from studies using alternative markers of lipid peroxidation (table II). Isoprostanes are stable, prostaglandin-like compounds formed *in vivo* through peroxidation of arachidonic acid, and can be detected in blood, urine and saliva. Their well documented mechanism of production,[59] combined with a potent oxidative activity,[60] makes these substances well suited as markers of oxidative stress *in vivo*. Several recent studies have provided indications for substantial increases in plasma isoprostane levels with strenuous cycling,[61] or prolonged running.[30,62-66] Controversy exists as to the timing of the maximum effect; Mastaloudis et al.[63] and Steensberg et al.[66] reported isoprostane levels to peak directly post-exercise, followed by a return to baseline within 1 hour or 1 day, respectively, whereas Sacheck et al.[30] did not observe a significant increase until 24 hours after cessation of exercise, with peak values at 72 hours post-exercise. Increases in plasma lipid peroxides,[9,10,26,29,41,62,65,67] and ethane and pentane in exhaled breath[7,24,68,69] also tend to indicate signs of exercise-induced lipid peroxidation, but measurement of conjugated dienes (CD) has produced less consistent results. A number

Table II. Effects of exercise on markers of lipid peroxidation

Study[a]	Exercise protocol[b]	Subjects	Δ Exercise (%)[c]
Isoprostanes			
McAnulty et al.[62]	3h run 70%	16 T M/F	+22[d]
Mastaloudis et al.[63]	50km run	11 T M/F	+75[d]
Nieman et al.[65]	80km run	13 T M/F	+40[d]
Sacheck et al.[30]	45 min 16% downhill run	16 MT M	+80[d]
Steensburg et al.[66]	2.5h run	11 T M	+56[d]
Waring et al.[61]	20 min cycle 80W	20 UT M/F	+60[d]
Mastaloudis et al.[64]	50km run	10 T M/F	+46
Lipid hydroperoxide			
Viguie et al.[73]	90 min cycle 65%	11 MT M	NC
Ashton et al.[9]	Max-test cycle	12 UT M	+42[d]
Ashton et al.[10]	Max-test cycle	10 UT M	+42[d]
Alessio et al.[26]	Max-test run	12 UT M/F	+25
	Isometric 50% MVC to exh		+36[d]
Hessel et al.[41]	Marathon	18 T M	+11[d]
Bailey et al.[67]	Max-test cycle	18 UT M	+22[d]
McAnulty et al.[62]	3h run 70%	16 T M/F	+230[d]
Nieman et al.[65]	80km run	13 T M/F	+8[d]
Quindry et al.[29]	Max-test run	9 MT M	+49
Conjugated dienes			
Duthie et al.[16]	21km run	7 T M	NC
Vasankari et al.[19]	1km run	8 T M	NC
	10km run	7 T M	+14[d]
	27km run	8 T M	+11[d]
Marzatico et al.[40]	Half marathon	6 T M	NC
	6 × 150m sprint	6 T M	+80[d]
Vasankari et al.[51]	31km run	8 T M	+9[d]
	Marathon	22 MT M	+7
Liu et al.[70]	Marathon	11 T M	NC
Ramel et al.[31]	Submax resistance exercise	7 T M	NC
		10 UT M	+27[d]
Pentane			
Dillard et al.[7]	60 min submax cycle	6 UT M/F	+80[d]
Pincemail et al.[68]	20 min submax cycle	5 UT M	>+300[d]
Kanter et al.[69]	Run 30 min 60%/5 min 90%	9 MT M	+250[d]
Leaf et al.[24]	Max-test run	7 UT M/F	>+10.000[d]
Ethane			
Leaf et al.[24]	Max-test run	7 UT M/F	>+900[d]

a Selected references are shown.

b The '%' symbol in the 'Exercise protocol' column indicates the percentage of maximum oxygen consumption.

c Δ Exercise is the change caused by exercise, and has been estimated from figures if not stated in the original article.

d Significant change.

exh = exhaustion; **F** = females; **M** = males; **max-test** = exhaustive incremental test; **MT** = moderately trained; **MVC** = maximal voluntary contraction; **NC** = no change; **submax** = submaximal; **T** = trained; **UT** = untrained.

of studies have demonstrated an increase in levels of CD after exercise,[19,31,40,51] whereas others did not observe such an effect.[16,70] The lag-time of CD formation has been shown to be reduced after exercise suggesting increased oxidative stress,[70] but remained unchanged,[51] or even increased in other studies.[71,72]

Markers of oxidative modifications to DNA have also shown inconsistent results. While urinary levels of 8-oxo-7,8-dihydroxy-2′-deoxyguanosine (8-oxodG; a degradation product of the DNA base guanine) mostly remain stable after exercise,[30,74-76] studies using single cell gel electrophoresis (SCG) have indicated signs of oxidative stress starting during exercise[77] and lasting for up to several days.[18,20,78] Few data are available on the effects of exercise on protein oxidation in human subjects. Alessio et al.[26] observed a significant increase in plasma protein carbonyls after exhaustive, incremental running, but not after isometric hand-gripping exercise to exhaustion. Prolonged running resulted in increased carbonyl levels in a study by Radak et al.,[79] but Chevion et al.[80] reported a decrease in levels after 50km and 80km marches.

Beside changes in levels of indirect markers of oxidative stress, the effects of exercise on antioxidant levels have been studied. The most frequently used antioxidant marker is the glutathione redox status, the ratio between the fractions of reduced (GSH) and oxidised glutathione (GSSG). Although controversy exists about what fractions of total glutathione (TGSH) consist in the reduced and oxidised forms, considering the activity of enzymes such as glutathione reductase and the physiological importance of glutathione being kept in the reduced form, it is highly unlikely that the GSH/GSSG ratio *in vivo* approaches parity as regularly reported in the exercise science literature.[16,21,23,42,50,56,73,81-84] Thus, although the majority of studies investigating the effects of exercise on glutathione redox-status seems to support the occurrence of exercise-induced oxidative stress (table III), it appears that similar to the TBARS-assay, methodology for the measurement of glutathione redox status influences reported values to a considerable extent. We have observed substantial oxidation of glutathione in blood samples subjected to freezing/thawing (unpublished data), which can be taken as an example of the importance of adequate sample preparation. Several other pitfalls in sample preparation have been identified and discussed by Rossi et al.[85]

Plasma levels of tocopherol (vitamin E), the major lipid soluble antioxidant, have been reported to increase following cycling at 100% $\dot{V}O_{2max}$ to exhaustion,[88] and after a triathlon.[89] In human erythrocytes, tocopherol levels increased progressively up to 48 hours after a half-marathon.[16] It has been suggested that an exercise-induced increase in tocopherol may be caused by co-release with free fatty acids during lipolysis in adipose tissue.[90] However, in a study by Camus et al.[91] tocopherol mobilisation during exercise was not affected by β-adrenergic blockade, rendering this theory unlikely. In two other studies, prolonged submaximal exercise did not appear to lead to changes in plasma tocopherol levels.[16,73] More recently, Kawai et al.[92] reported that whereas serum tocopherol levels were unaffected by exercise, erythrocyte tocopherol levels actually decreased. Schneider et al.[93] observed a tendency for serum tocopherol to decrease between 3 and 24 hours after an exhaustive treadmill run.

Plasma ascorbic acid levels increase as a response to exercise,[16,63,73,94,95] but values rapidly return to baseline levels after cessation of exercise.[63,94] Levels drop below pre-exercise values 1 day after strenuous exercise, and may remain low for at least 3 days.[63,93,95] It is unclear what causes the exercise-induced changes in concentrations of tocopherol and ascorbic acid, or to what extent these changes influence oxidative stress. A rise in plasma antioxidant levels might enhance the antioxidant defences in the blood, but may reduce defences at the sites they are mobilised from.

There may be several reasons why some investigators[75,76,96] have failed to observe signs of exercise-induced oxidative stress. First, the use of different test subjects might influence the findings of different studies. Factors such as training status, age and sex have scarcely been looked into, but could potentially play a role. Furthermore, research has

Table III. Effects of exercise on blood glutathione redox status

Study[a]	GSH trap	Exercise	Subjects	Resting GSH (mmol/L)[b]	GSH/GSSG pre-ex[b]	GSH/GSSG post-ex[b]
Kretzschmar et al.[33]	NEM	Max-test cycle	7 UT M	0.008[c]	8	7
			5 T M	0.015[c]	18	11
Sastre et al.[86]	NEM	Max-test run	? T M	0.8	14	8[d]
Vina et al.[87]	NEM	Max-test run	?	0.88	23	13[d]
Margaritis et al.[22]		Triathlon	12 T M	0.63	22	22
Hessel et al.[41]	NEM	Marathon	18 T M	1.91	25	24[d]
(The reported GSH/GSSG ratios listed below are unlikely to occur *in vivo*. Thus, conclusions based on the results of these studies should be taken with caution.)						
Gohil et al.[81]		Max-test cycle	8 MT M	0.24	1	1
		90 min cycle 65%		0.42	3	0.2[d]
Duthie et al.[16]		21km run	7 T M	0.77[e]	3	2
Camus et al.[82]		Uphill walk	8 MT M	0.55	2.2	2.3
		Downhill run		0.55	1.8	1.8
Ji[42]		Cycle 70% to exh	8 T M	0.55	2.6	3.7[d]
Laires et al.[83]		40 min run submax	8 UT M	3.2[e]	5	5
Viguie et al.[73]		90 min cycle 65%	11 MT M	0.55	1	0.4[d]
Sen et al.[50]	2-VP	Max-test	9 UT M	0.6	3	2[d]
		30 min cycle AT		0.6	3	2[d]
		30 min cycle AnT		0.6	3	2[d]
Dufaux et al.[21]		2.5h run	12 MT M	0.7	6	1[d]
Chung et al.[23]	2-VP	30 min run 75%	11 UT F	0.86	2.3	1.7[d]
Laaksonen et al.[56]	2-VP	40 min cycle 60%	14 UT M	0.77	5	3[d]
Lee et al.[84]	2-VP	60 EE 150% MIC	8 UT M	0.8	3	3

a Selected references are shown.

b Resting GSH and the ratio GSH/GSSG have been estimated from figures if not stated in the original article.

c In plasma.

d Significant change.

e mg/g haemoglobin.

2-VP = 2-vinylpyridine; **AnT** = anaerobic threshold; **AT** = aerobic threshold; **EE** = eccentric exercise; **ex** = exercise; **exh** = exhaustion; **F** = females; **GSH** = reduced glutathione; **GSSG** = oxidised glutathione; **M** = males; **max-test** = exhaustive incremental test; **MIC** = maximal isokinetic contraction; **MT** = moderately trained; **NEM** = *N*-ethylmaleimide; **submax** = submaximal; **T** = trained; **UT** = untrained; **?** indicates information not provided in original article.

incorporated a large range of exercise intensities. Only exercise of sufficient intensity or duration appears to lead to a large enough increase in free radical production to overwhelm the antioxidant defences.[20,29,97] Beside this, levels of some products of oxidative reactions may not be elevated directly after exercise, and can reach their maximal levels hours,[15,98] or even days[18,20,30,74,78] after the end of exercise. Additionally, several authors who used multiple markers have found indications of increased oxidative stress after exercise with some of these, whereas others did not change.[20,99] Finally, Liu et al.[99] demonstrated that the response to oxidative stress can be quite different between various organs. It was suggested that differences between organs are dependent on several factors, including oxygen consumption ($\dot{V}O_2$), antioxidant levels, and presence of adequate repair systems. It can be concluded that the absence of signs of oxidative stress after exercise does not necessarily imply that oxidative damage did not occur.

2. Mechanisms of Increased Free Radical Production with Exercise

The available evidence seems to support the occurrence of exercise-induced oxidative stress, but it

remains unclear what causes the increase in free radical production. Various mechanisms have been identified, but specific contributions from each of these to total ROS production are poorly characterised. Several mechanisms may act synergistically, and it is possible that different types of exercise involve different mechanisms of free radical production. However, it seems to be commonly assumed amongst exercise scientists that the most important source of free radicals, both at rest and during exercise, is a 'leak' in the respiratory chain in the mitochondrial inner-membrane. Many of the redox centres in the four enzyme complexes making up the respiratory chain can be oxidised by molecular oxygen, resulting in the formation of the superoxide radical. The organisation of the respiratory chain is such, however, that oxygen is consumed almost exclusively by cytochrome c oxidase (complex IV). The structure and redox chemistry within complex IV does not allow any superoxide leakage (the ferrous oxy complex being immediately reduced to a stable ferryl intermediate preventing any single electron reduction of the oxygen). However, inadequate coupling of electron transfer between the electron acceptors of complex II and III has been shown to cause ~2% of total $\dot{V}O_2$ to be converted to superoxide radicals under resting conditions.[100,101]

This finding has led to the widespread interpretation that a substantial increase in ROS generation is to be expected during exercise, as oxygen flux through active muscle may increase by as much as ~100 times the resting value to meet increased energy demands. However, *in vitro* studies by Boveris and Chance[101] showed that the radical leak only occurs during state 4 respiration (low $\dot{V}O_2$; high membrane potential; low adenosine triphosphate [ATP] production), and not during state 3 respiration (high $\dot{V}O_2$; lower membrane potential; high ATP production); this would imply a decrease in radical production with exercise rather than an increase.

Later studies by Herrero and Barja[102] extended and confirmed these original findings of a decrease in radical production as $\dot{V}O_2$ and ATP synthesis increased. Indeed, the increased superoxide production under 'resting conditions' appears to be a feedback trigger activating mitochondrial uncoupling proteins,[103] dropping the membrane potential, increasing $\dot{V}O_2$, and hence preventing radical damage to mitochondrial proteins (e.g. aconitase). 'Exercise' at least in the test tube, does not increase mitochondrial free radical production.

It is also worth noting that Boveris and Chance[101] used supraphysiological partial oxygen pressure (pO_2) values, whereas during exercise cytosolic pO_2 has been shown to remain constant at all intensities, and mitochondrial pO_2 is expected to decrease.[104,105] Consequently, it has been suggested that it is the decrease in mitochondrial pO_2 rather than increased oxygen flux that is the cause of the exercise-induced increase in ROS production.[106] Support for this theory comes from studies demonstrating increased oxidative stress under hypoxic conditions[67] and with isometric exercise, which does not require a large increase in $\dot{V}O_2$, but may nonetheless reduce mitochondrial pO_2.[26] Recently, Bailey et al.[12] demonstrated a relationship between the veno-arterial difference in spin-adduct concentration and conditions previously shown to induce a decrease in mitochondrial pO_2,[105] rather than with conditions of increased oxygen flux. Although direct evidence for a mechanism by which an exercise-induced decrease in mitochondrial pO_2 can cause an increase in ROS formation is lacking, it can be concluded that the widespread notion that an increase in mitochondrial oxygen flux *per se* is the cause for this increased radical production is unlikely to be correct (figure 1).

Apart from the enzyme complexes of the respiratory chain, other mitochondrial enzymes may contribute to the exercise-induced increase in free radical production. Recent animal experiments suggest that the mitochondrial membrane-bound glycerol 3-phosphate dehydrogenase (mGPDH) is a more important source of free radicals than the complexes of the electron transport chain.[107,108] Together with the cytosolic cGPDH this enzyme is part of the glycerol-3-phosphate shuttle which, similarly to the malate-aspartate shuttle, acts to transport the reduced form of nicotinamide-adenine dinucleotide

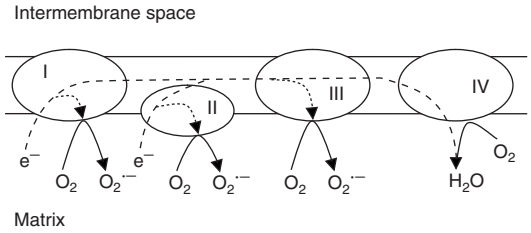

Fig. 1. In the respiratory chain, electrons are transferred from complexes I, II and III to complex IV (dashed line), after which oxygen acts as the final electron acceptor. However, in state 4 respiration, inadequate coupling of electron transfer at complexes I, II and III can cause 'leakage' of electrons to oxygen (dotted lines). Conversely, an increase in oxygen flux at state 3 respiration (as occurs during exercise) would be expected to 'pull' electrons through the respiratory chain at complex IV at an increased rate, decreasing superoxide generation at complexes I–III. In biochemical terms, the steady-state concentrations of the flavin or quinoine radicals that reduce oxygen to superoxide decrease as the mitochondrial membrane potential is lowered during state 3 respiration.

(NADH) into the mitochondria. The activity of mGPDH is regulated by Ca^{2+} levels,[109] and increases with physical activity.[110] Increased mGPDH activity has been shown to correlate with increased glycerol-3-phosphate-dependent hydrogen peroxide production.[111] However, as of yet no information on the importance of this enzyme to exercise-induced ROS production in humans is available.

An alternative mechanism for the increase in free radical production with exercise could be one similar to that seen in ischaemia-reperfusion injury. Intense exercise is associated with transient tissue hypoxia in several organs, as blood flow is redistributed to cover the increased blood supply in active skeletal muscles and the skin. Additionally, during exercise performed at intensities at or above $\dot{V}O_{2max}$, muscle fibres may undergo relative hypoxia as oxygen supply cannot match energy demand.[112] The ischaemic conditions trigger conversion of xanthine dehydrogenase (XDH) to xanthine oxidase (XOD), probably through a disturbance of calcium homeostasis activating calcium-dependent proteases that catalyse cleavage of a portion of XDH.[113] Upon reoxygenation of the hypoxic tissues after exercise, XOD produces superoxide as a by-product of the degradation of hypoxanthine (HX) into xanthine (X), and subsequently into urate. Plasma XOD levels have indeed been demonstrated to increase following exercise,[87,114-116] but there are indications that the XOD-mediated increase in free radicals is substrate-limited rather than enzyme-limited.[117] However, exercise has also been shown to increase HX, X, and urate levels in the blood.[17,114,118-120] Studies using allopurinol to inhibit XOD activity[121,122] have provided further evidence to indicate that XOD is a source of free radicals following exercise. Production of ROS by XOD may lead to oxidative stress for several hours following exercise, and is not restricted to skeletal muscle. However, as the increase in XOD expression seems to occur predominantly after exercise, it seems unlikely that this mechanism is responsible for the enhanced free radical generation *during* exercise.

Free radicals may mediate cell damage, but conversely, exercise-induced cell damage could lead to an increased production of ROS itself. Exercise can result in microvascular dysfunction, oedema and cell damage through mechanical shear forces or through a disturbance of normal cellular metabolism.[123] As a result, exercise triggers an inflammatory response, characterised by infiltration of the affected areas by neutrophils and other phagocytic cells, followed by a respiratory burst involving production of superoxide, hydrogen peroxide and other ROS. Myeloperoxidase (MPO), an iron-containing enzyme found in neutrophils, subsequently catalyses the conversion of hydrogen peroxide into hypochlorous acid, a highly potent oxidant. Neutrophil levels and activation,[29,124-127] and MPO levels[68,82,124,126,128-131] have been convincingly demonstrated to increase following exercise, and may remain elevated for hours[29,125] to days.[132] Nonetheless, the understanding of the relevance of exercise-induced neutrophilia and elevated MPO levels on oxidative stress remains incomplete.

Recently, another theory on the mechanism of increased free radical production with exercise has been put forward, which involves haem proteins.[133] Haem proteins like haemoglobin (Hb) and myoglobin (Mb) contain iron, a redox active transition metal, which is able to generate or remove ROS.

Traditionally, free radical production by transition metals has been thought to involve Fenton chemistry: the oxidation of ferrous iron by hydrogen peroxide, generating the highly reactive hydroxyl radical (equation 1):

$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH^\bullet$ (Eq. 1)

However, free iron levels in the body are generally maintained at sub-micromolar levels,[134] rendering it unlikely that Fenton chemistry is the sole mechanism of free radical production under normal conditions *in vivo*. This is especially likely to be true for the plasma assays used in most sports science studies, given the excess of the redox-inactive iron chelator, transferrin, in the healthy population. An alternative pathway for the production of free radicals by haem proteins involves auto-oxidation of haem proteins, e.g. oxyhaemoglobin (oxyHb) and oxymyoglobin (oxyMb). This is a constant source of superoxide radicals, which are subsequently converted to hydrogen peroxide (equations 2 and 3). Already in the 1950s it was shown that reaction of hydrogen peroxide with methaemoglobin (metHb) or metmyoglobin (metMb) leads to the production of free radicals;[135] ferric haem is oxidised to the ferryl form ($Fe^{4+} = O^{2-}$), with concomitant formation of a free radical ($R^\bullet$) on the globin protein (equation 4). Both are thought to be potentially harmful oxidants with reactivity similar to the hydroxyl radical.[133] The globin-based free radicals have been observed in human blood, and were shown to be the same as those that are formed in the reaction of purified metHb with hydrogen peroxide.[136]

$Fe^{2+} + O_2 \rightarrow Fe^{3+} + O_2^{\bullet-}$ (Eq. 2)

$O_2^{\bullet-} + O_2^{\bullet-} + 2H^+ \rightarrow H_2O_2 + O_2$ (Eq. 3)

$R\text{-}Fe^{3+} + H_2O_2 \rightarrow R^{\bullet+}\text{-}Fe^{4+}{=}O^{2-} + H_2O$ (Eq. 4)

In contrast to the relationship between free radical production and mitochondrial pO_2, the auto-oxidation rate of Hb has an unusual bell-shaped dependence on blood pO_2 (figure 2). Balagopalakrishna et al.[137] demonstrated that metHb and superoxide production are enhanced at intermediate oxygenation (~40–80%), whereas these rates are reduced at low and high pO_2 values. Arterial Hb oxygenation is close to 100%, both at rest and during exercise, which implies that Hb auto-oxidation in arterial blood will invariably be low. However, Hb oxygenation drops steadily as blood flows through capillaries and supplies oxygen to active aerobic tissues. Hb oxygenation is lowest in venous blood and averages about 70% at rest. During exercise, the oxygen supply to tissues increases, with a concomitant drop in oxygenation of venous blood. Nearly all oxygen can be released from Hb with sufficiently high exercise intensities. Thus, superoxide production in capillary and venous blood at rest

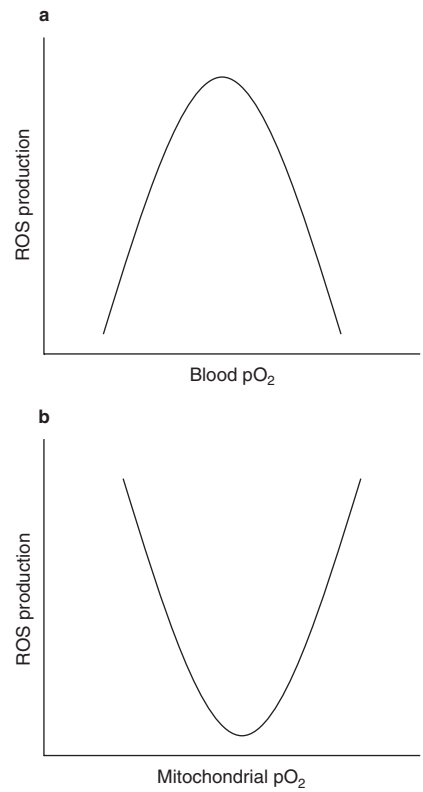

Fig. 2. Model depicting the differential effects of partial oxygen pressure (pO_2) on radical production by: (**a**) oxyhaemoglobin auto-oxidation, and (**b**) intracellular mechanisms that are not yet fully elucidated. Whereas superoxide-production by haem proteins has a bell-shaped dependence on pO_2,[137] data by Bailey et al.[67] and Boveris and Chance[101] indicates the relationship between mitochondrial pO_2 and radical formation is U-shaped. This example serves to illustrate the ambiguity of different processes involved in altering redox tone, and suggests that assessment of oxidative stress in blood samples may provide quite different information compared with oxidative stress status in muscle tissue. **ROS** = reactive oxygen species.

will be relatively high, but may not be maximal. With an increase in tissue oxygen demand superoxide production might increase, but will return to a lower rate in venous blood at high exercise intensities. Therefore, it is difficult to predict the effect of exercise on haem-related production of free radicals. However, even if metHb-related superoxide production is reduced with exercise, hydrogen peroxide generated through other mechanisms may still react with metHb to produce ferryl iron and protein-bound free radicals as shown in equation 4, thus providing a pathway for oxidative stress. Little data are available to determine the relevance of haem-related mechanisms to exercise-induced oxidative stress, but preliminary findings from our laboratory have indicated a short-lived, but consistent exercise-induced increase in levels of an iron-chlorin species that is produced solely through interaction of Hb with peroxides.[138]

3. Exercise-Induced Oxidative Stress: Health and Performance

Some authors[57,139] have looked on the exercise-induced increase in free radical production as a paradox: an apparently healthy act (exercise) leading to detrimental effects through damage to various molecules and tissues. This is somewhat of a misunderstanding as exercise in itself is not a healthy act (causing dehydration, substrate depletion, muscle damage, inflammation); it is the recovery after exercise that is healthy. Exercise training has been shown to enhance antioxidant defences, for example by increasing superoxide dismutase and glutathione peroxidase activities,[140-148] and in this respect the exercise-induced increase in free radical production can be seen as no different from other responses to exercise: a certain load disturbs homeostasis, resulting in adaptations in the body to be able to cope with a similar load in the future.

Human antioxidant defences are adequate to prevent oxidative stress at 'normal' levels of free radical production, but there is by no means an excess of antioxidants present. The antioxidant defence system seems to control levels of free radicals, rather than eliminating them completely.[149] This seems logical, as free radicals have numerous physiological functions, and their complete elimination would affect normal functioning in a negative way. Exercise may have the capacity to increase the production of free radicals compared with resting levels, but it seems that if exercise would produce free radicals to an extent that would cause adverse effects to health, antioxidant defences would have developed to be able to cope with this additional stress (in this respect, it is interesting to note that cardiac and skeletal muscles have a considerably lower antioxidant capacity than organs like liver and kidney[150-152]). However, it is possible that just like other acute effects of exercise, increased production of free radicals impairs performance during, or in subsequent exercise.

There are several possible mechanisms for a detrimental effect of ROS on exercise performance. Oxidative damage to adenosine triphosphatase (ATPase) pumps can significantly reduce calcium uptake by the sarcoplasmic reticulum,[153-156] interfering with muscle excitation-contraction coupling and reducing muscle contractility. Similarly, ROS may affect the ability to develop action potentials required for muscle contraction through damaging ATPase pumps required for potassium influx back into skeletal muscle cells.[157,158] Furthermore, muscle contractile proteins (fast and slow myosin heavy chains), and mitochondrial enzymes required for energy provision (succinate dehydrogenase, cytochrome oxidase) appear to be susceptible to oxidative damage.[159]

Experimental evidence supporting a negative effect of free radicals on performance comes mainly from several *in situ* studies demonstrating altered contractile function, decreased maximal force, and enhanced fatigue rates in animal skeletal muscle fibres treated with various oxidants, effects that could usually be prevented with antioxidants, or reversed by reducing agents.[160-164] An additional finding of several of these studies has been that low levels of ROS are essential for optimal muscle contraction,[160-162,164] and in view of this information Reid and co-workers[164,165] proposed a model in which the basal redox tone of muscle cells is more

reduced than the redox tone optimal for muscle contraction. A moderate contraction-induced increase in ROS production was suggested to move the intracellular redox tone towards a more oxidised, and therefore more optimal state for muscle contraction (figure 3). Prolonged, or high-intensity exercise, however, would have the potential to 'overshoot' this optimal redox tone, resulting in ROS-induced fatigue. An implication of this model would be that for optimal performance it may be beneficial for the basal cellular redox tone to be reduced to such an extent that the exercise-induced increase in ROS production would bring the redox tone as close to optimal as possible. Intuitively, this appears feasible with acute antioxidant supplementation, whereas long-term supplementation may not alter the homeostatically regulated intracellular redox tone (note that the optimal basal redox tone for functioning at rest may be more oxidised than for high-intensity exercise). Analogous to administration of substances like sodium bicarbonate prior to exercise in order to influence pH and enhance performance through increased buffer capacity, pre-exercise antioxidant supplementation may provide a buffer for changes to the intracellular redox tone. To date, a performance-enhancing effect with antioxidant supplementation *in vivo* has only been demonstrated in studies using pre-exercise intravenous injection as the means of administration. Swimming time to exhaustion has been improved in mice after administration of the spin-traps *N*-tert-butyl-alpha-phenylnitrone, alpha-(4-pyridyl-1-oxide) *N*-tert-butyl nitrone and 5,5-dimethylpyrroline-*N*-oxide,[166] tocopherol,[166] GSH,[167,168] and GSH ethyl esters,[168] whereas injection of *N*-acetylcysteine (NAC) has been shown to attenuate muscle fatigue in humans.[169,170] Conversely, intravenously administered NAC did not enhance time to exhaustion during intense, intermittent cycling[171] or submaximal cycling.[172] The majority of studies examining the effects of antioxidants on exercise performance in humans have employed long-term supplementation, and to date no performance-enhancing effects have been observed with this strategy, despite the fact that the increase in levels of markers of oxidative stress has often been found to be attenuated.[173-183]

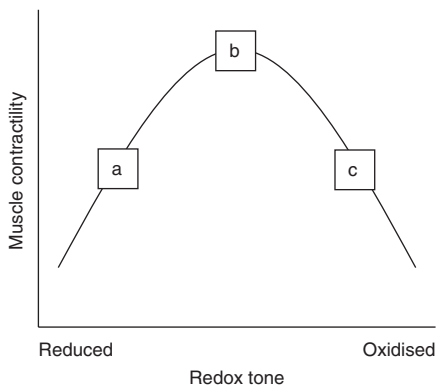

Fig. 3. The optimal-redox-tone model proposed by Reid and coworkers state that the basal, presumably homeostatically regulated, intracellular redox tone (**a**) is more reduced than the redox tone optimal for muscle contraction (**b**). Exercise-induced reactive oxygen species production slightly alters the redox tone providing more favourable conditions required for exercise, but has the potential to 'overshoot' and result in a redox tone that is too oxidised (**c**), contributing to fatigue (reproduced from Reid,[165] with permission).

4. Conclusions and Future Perspectives – Think Positive!

Two widespread dogmas in the exercise science literature are that: (i) exercise increases free radical production; and (ii) this is a negative side effect that needs to be prevented. As most of the evidence supporting the occurrence of exercise-induced oxidative stress is derived from the methodologically dubious marker TBARS, or from the potentially flawed measurement of glutathione-redox status, this link is not as strong as generally assumed. However, on the whole, there seems to be sufficient credible evidence to support an increase in ROS production as a result of exercise. As to the relevance of this phenomenon, there are no indications that exercise-induced oxidative stress has any negative impact on health, whereas evidence for the theory that increased ROS production may impair exercise performance *in vivo* is limited. One should bear in mind that oxidative modification is not necessarily the same as oxidative damage. It is interesting that antioxidant defences in skeletal muscle are relatively low compared with other tissues, and that although exercise training improves antioxidant

defences, this does not attenuate exercise-induced oxidative stress in highly trained athletes. This strongly suggests that there is little need for the body to completely prevent oxidative modifications in muscle. Thus, rather than unambiguously pointing towards a negative side effect of exercise, various observations seem to indicate that increased ROS production is a desired, or even required consequence of exercise, which is controlled to such an extent that it occurs whenever the relative exercise intensity is sufficiently high, regardless of training status.

Controlled production of specific ROS could serve distinct physiological functions depending on mode and intensity of exercise, and site of production. These functions could include, for example, redox-regulation of vascular tone, substrate metabolism and muscle contractility, but it is also tempting to speculate that ROS produced during exercise are involved in the molecular mechanisms by which physiological adaptations to exercise training are brought about. This possibility was first raised by Davies et al.[8] more then 20 years ago, and has since been raised by a number of others.[184,185] ROS make ideal signalling molecules (high, often selective reactivity; short lifespan; several distinct production mechanisms; present in all cells), and their production during and after exercise could provide a link between the disturbance of homeostasis by exercise and the resulting adaptations brought about by alterations in gene transcription. Recently, some of the molecular mechanisms have been elucidated by which ROS activate genes responsible for regulating processes such as iron metabolism[186,187] and the response to hypoxia,[188] and it would not be inconceivable that the mechanisms by which exercise induces gene transcription responsible for training adaptations proceed in very similar ways. If the type of exercise were to influence the mechanism or site of production of ROS this could also account for the occurrence of differential training adaptations in response to strength-, sprint- and endurance-type exercise. It can be concluded that although the question whether increased ROS production may impair exercise performance deserves further attention, it should not be overlooked that ROS may serve hitherto unidentified physiological functions. It may be time to shift the emphasis of research in the field of exercise-induced oxidative stress from being predominantly negative, to a more positive perspective.

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References

1. Knight JA. Review: free radicals, antioxidants, and the immune system. Ann Clin Lab Sci 2000; 30 (2): 145-58
2. Turpaev KT. Reactive oxygen species and regulation of gene expression. Biochemistry (Mosc) 2002; 67 (3): 281-92
3. Thannickal VJ, Fanburg BL. Reactive oxygen species in cell signaling. Am J Physiol Lung Cell Mol Physiol 2000; 279 (6): L1005-28
4. Cooper CE. Ferryl states and protein free radicals. In: Rice-Evans CA, Burdon RH, editors. Free radical damage and its control. Amsterdam: Elsevier, 1993: 67-111
5. Halliwell B, Chirico S. Lipid peroxidation: its mechanism, measurement, and significance. Am J Clin Nutr 1993; 57 (5 Suppl.): 715S-24S
6. Golden TR, Hinerfeld DA, Melov S. Oxidative stress and aging: beyond correlation. Aging Cell 2002; 1 (2): 117-23
7. Dillard CJ, Litov RE, Savin WM, et al. Effects of exercise, vitamin E, and ozone on pulmonary function and lipid peroxidation. J Appl Physiol 1978; 45 (6): 927-32
8. Davies KJ, Quintanilha AT, Brooks GA, et al. Free radicals and tissue damage produced by exercise. Biochem Biophys Res Commun 1982; 107 (4): 1198-205
9. Ashton T, Rowlands CC, Jones E, et al. Electron spin resonance spectroscopic detection of oxygen-centred radicals in human serum following exhaustive exercise. Eur J Appl Physiol Occup Physiol 1998; 77 (6): 498-502
10. Ashton T, Young IS, Peters JR, et al. Electron spin resonance spectroscopy, exercise, and oxidative stress: an ascorbic acid intervention study. J Appl Physiol 1999; 87 (6): 2032-6
11. Bailey DM, Davies B, Young IS, et al. EPR spectroscopic detection of free radical outflow from an isolated muscle bed in exercising humans. J Appl Physiol 2003; 94 (5): 1714-8
12. Bailey DM, Young IS, McEneny J, et al. Regulation of free radical outflow from an isolated muscle bed in exercising humans. Am J Physiol Heart Circ Physiol 2004; 287 (4): H1689-99
13. Groussard C, Rannou-Bekono F, Machefer G, et al. Changes in blood lipid peroxidation markers and antioxidants after a single sprint anaerobic exercise. Eur J Appl Physiol 2003; 89 (1): 14-20
14. Viinikka L, Vuori J, Ylikorkala O. Lipid peroxides, prostacyclin, and thromboxane A2 in runners during acute exercise. Med Sci Sports Exerc 1984; 16 (3): 275-7

15. Maughan RJ, Donnelly AE, Gleeson M, et al. Delayed-onset muscle damage and lipid peroxidation in man after a downhill run. Muscle Nerve 1989; 12 (4): 332-6
16. Duthie GG, Robertson JD, Maughan RJ, et al. Blood antioxidant status and erythrocyte lipid peroxidation following distance running. Arch Biochem Biophys 1990; 282 (1): 78-83
17. Sahlin K, Ekberg K, Cizinsky S. Changes in plasma hypoxanthine and free radical markers during exercise in man. Acta Physiol Scand 1991; 142 (2): 275-81
18. Hartmann A, Niess AM, Grunert-Fuchs M, et al. Vitamin E prevents exercise-induced DNA damage. Mutat Res 1995; 346 (4): 195-202
19. Vasankari T, Kujala U, Heinonen O, et al. Measurement of serum lipid peroxidation during exercise using three different methods: diene conjugation, thiobarbituric acid reactive material and fluorescent chromolipids. Clin Chim Acta 1995; 234 (1-2): 63-9
20. Niess AM, Hartmann A, Grunert-Fuchs M, et al. DNA damage after exhaustive treadmill running in trained and untrained men. Int J Sports Med 1996; 17 (6): 397-403
21. Dufaux B, Heine O, Kothe A, et al. Blood glutathione status following distance running. Int J Sports Med 1997; 18 (2): 89-93
22. Margaritis I, Tessier F, Richard MJ, et al. No evidence of oxidative stress after a triathlon race in highly trained competitors. Int J Sports Med 1997; 18 (3): 186-90
23. Chung SC, Goldfarb AH, Jamurtas AZ, et al. Effect of exercise during the follicular and luteal phases on indices of oxidative stress in healthy women. Med Sci Sports Exerc 1999; 31 (3): 409-13
24. Leaf DA, Kleinman MT, Hamilton M, et al. The effect of exercise intensity on lipid peroxidation. Med Sci Sports Exerc 1997; 29 (8): 1036-9
25. Surmen-Gur E, Ozturk E, Gur H, et al. Effect of vitamin E supplementation on post-exercise plasma lipid peroxidation and blood antioxidant status in smokers: with special reference to haemoconcentration effect. Eur J Appl Physiol Occup Physiol 1999; 79 (6): 472-8
26. Alessio HM, Hagerman AE, Fulkerson BK, et al. Generation of reactive oxygen species after exhaustive aerobic and isometric exercise. Med Sci Sports Exerc 2000; 32 (9): 1576-81
27. Sacheck JM, Decker EA, Clarkson PM. The effect of diet on vitamin E intake and oxidative stress in response to acute exercise in female athletes. Eur J Appl Physiol 2000; 83 (1): 40-6
28. Akova B, Surmen-Gur E, Gur H, et al. Exercise-induced oxidative stress and muscle performance in healthy women: role of vitamin E supplementation and endogenous oestradiol. Eur J Appl Physiol 2001; 84 (1-2): 141-7
29. Quindry JC, Stone WL, King J, et al. The effects of acute exercise on neutrophils and plasma oxidative stress. Med Sci Sports Exerc 2003; 35 (7): 1139-45
30. Sacheck JM, Milbury PE, Cannon JG, et al. Effect of vitamin E and eccentric exercise on selected biomarkers of oxidative stress in young and elderly men. Free Radic Biol Med 2003; 34 (12): 1575-88
31. Ramel A, Wagner KH, Elmadfa I. Plasma antioxidants and lipid oxidation after submaximal resistance exercise in men. Eur J Nutr 2004; 43 (1): 2-6
32. Lovlin R, Cottle W, Pyke I, et al. Are indices of free radical damage related to exercise intensity? Eur J Appl Physiol Occup Physiol 1987; 56 (3): 313-6
33. Kretzschmar M, Muller D, Hubscher J, et al. Influence of aging, training and acute physical exercise on plasma glutathione and lipid peroxides in man. Int J Sports Med 1991; 12 (2): 218-22
34. Bird RP, Draper HH. Comparative studies on different methods of malonaldehyde determination. Methods Enzymol 1984; 105: 299-305
35. Lepage G, Munoz G, Champagne J, et al. Preparative steps necessary for the accurate measurement of malondialdehyde by high-performance liquid chromatography. Anal Biochem 1991; 197 (2): 277-83
36. Halliwell B, Gutteridge JMC. Free radicals in biology and medicine. Oxford: University Press, 1998
37. Kanter MM, Lesmes GR, Kaminsky LA, et al. Serum creatine kinase and lactate dehydrogenase changes following an eighty kilometer race: relationship to lipid peroxidation. Eur J Appl Physiol Occup Physiol 1988; 57 (1): 60-3
38. Kanter MM, Eddy DE. Effects of antioxidants supplementation on serum markers of lipid peroxidation and skeletal muscle damage following eccentric exercise [abstract]. Med Sci Sports Exerc 1992; 24 (5): S17
39. Rokitzki L, Logemann E, Sagredos AN, et al. Lipid peroxidation and antioxidative vitamins under extreme endurance stress. Acta Physiol Scand 1994; 151 (2): 149-58
40. Marzatico F, Pansarasa O, Bertorelli L, et al. Blood free radical antioxidant enzymes and lipid peroxides following long-distance and lactacidemic performances in highly trained aerobic and sprint athletes. J Sports Med Phys Fitness 1997; 37 (4): 235-9
41. Hessel E, Haberland A, Muller M, et al. Oxygen radical generation of neutrophils: a reason for oxidative stress during marathon running? Clin Chim Acta 2000; 298 (1-2): 145-56
42. Ji LL. Antioxidant enzyme response to exercise and aging. Med Sci Sports Exerc 1993; 25 (2): 225-31
43. Largilliere C, Melancon SB. Free malondialdehyde determination in human plasma by high-performance liquid chromatography. Anal Biochem 1988; 170 (1): 123-6
44. Buege JA, Aust SD. Microsomal lipid peroxidation. Methods Enzymol 1978; 52: 302-10
45. Esterbauer H, Cheeseman KH. Determination of aldehydic lipid peroxidation products: malonaldehyde and 4-hydroxynonenal. Methods Enzymol 1990; 186: 407-21
46. Mihara M, Uchiyama M. Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. Anal Biochem 1978; 86 (1): 271-8
47. Carbonneau MA, Peuchant E, Sess D, et al. Free and bound malondialdehyde measured as thiobarbituric acid adduct by HPLC in serum and plasma. Clin Chem 1991; 37 (8): 1423-9
48. Hong YL, Yeh SL, Chang CY, et al. Total plasma malondialdehyde levels in 16 Taiwanese college students determined by various thiobarbituric acid tests and an improved high-performance liquid chromatography-based method. Clin Biochem 2000; 33 (8): 619-25
49. Maxwell SR, Jakeman P, Thomason H, et al. Changes in plasma antioxidant status during eccentric exercise and the effect of vitamin supplementation. Free Radic Res Commun 1993; 19 (3): 191-202
50. Sen CK, Rankinen T, Vaisanen S, et al. Oxidative stress after human exercise: effect of N-acetylcysteine supplementation. J Appl Physiol 1994; 76 (6): 2570-7
51. Vasankari TJ, Kujala UM, Vasankari TM, et al. Effects of acute prolonged exercise on-serum and LDL oxidation and antioxidant defences. Free Radic Biol Med 1997; 22 (3): 509-13

52. Alessio HM, Goldfarb AH, Cao G. Exercise-induced oxidative stress before and after vitamin C supplementation. Int J Sport Nutr 1997; 7 (1): 1-9
53. Child RB, Wilkinson DM, Fallowfield JL, et al. Elevated serum antioxidant capacity and plasma malondialdehyde concentration in response to a simulated half-marathon run. Med Sci Sports Exerc 1998; 30 (11): 1603-7
54. Szczesniak L, Karolkiewicz J, Deskur E, et al. The influence of exercise-induced oxidative stress on binding and degradation of 125I-insulin by the receptors on erythrocytes. J Physiol Pharmacol 1998; 49 (3): 421-32
55. Borsheim E, Knardahl S, Hostmark AT. Short-term effects of exercise on plasma very low density lipoproteins (VLDL) and fatty acids. Med Sci Sports Exerc 1999; 31 (4): 522-30
56. Laaksonen DE, Atalay M, Niskanen L, et al. Blood glutathione homeostasis as a determinant of resting and exercise-induced oxidative stress in young men. Redox Rep 1999; 4 (1-2): 53-9
57. Leaf DA, Kleinman MT, Hamilton M, et al. The exercise-induced oxidative stress paradox: the effects of physical exercise training. Am J Med Sci 1999; 317 (5): 295-300
58. Child RB, Wilkinson DM, Fallowfield JL. Effects of a training taper on tissue damage indices, serum antioxidant capacity and half-marathon running performance. Int J Sports Med 2000; 21 (5): 325-31
59. Roberts LJ, Morrow JD. Products of the isoprostane pathway: unique bioactive compounds and markers of lipid peroxidation. Cell Mol Life Sci 2002; 59 (5): 808-20
60. Fam SS, Morrow JD. The isoprostanes: unique products of arachidonic acid oxidation – a review. Curr Med Chem 2003; 10 (17): 1723-40
61. Waring WS, Convery A, Mishra V, et al. Uric acid reduces exercise-induced oxidative stress in healthy adults. Clin Sci (Lond) 2003; 105 (4): 425-30
62. McAnulty SR, McAnulty LS, Nieman DC, et al. Influence of carbohydrate ingestion on oxidative stress and plasma antioxidant potential following a 3h run. Free Radic Res 2003; 37 (8): 835-40
63. Mastaloudis A, Leonard SW, Traber MG. Oxidative stress in athletes during extreme endurance exercise. Free Radic Biol Med 2001; 31 (7): 911-22
64. Mastaloudis A, Morrow JD, Hopkins DW, et al. Antioxidant supplementation prevents exercise-induced lipid peroxidation, but not inflammation, in ultramarathon runners. Free Radic Biol Med 2004; 36 (10): 1329-41
65. Nieman DC, Henson DA, McAnulty SR, et al. Influence of vitamin C supplementation on oxidative and immune changes after an ultramarathon. J Appl Physiol 2002; 92 (5): 1970-7
66. Steensberg A, Morrow J, Toft AD, et al. Prolonged exercise, lymphocyte apoptosis and F2-isoprostanes. Eur J Appl Physiol 2002; 87 (1): 38-42
67. Bailey DM, Davies B, Young IS. Intermittent hypoxic training: Implications for lipid peroxidation induced by acute normoxic exercise in active men. Clin Sci (Lond) 2001; 101 (5): 465-75
68. Pincemail J, Camus G, Roesgen A, et al. Exercise induces pentane production and neutrophil activation in humans: effect of propranolol. Eur J Appl Physiol Occup Physiol 1990; 61 (3-4): 319-22
69. Kanter MM, Nolte LA, Holloszy JO. Effects of an antioxidant vitamin mixture on lipid peroxidation at rest and postexercise. J Appl Physiol 1993; 74 (2): 965-9
70. Liu ML, Bergholm R, Bergholm R, et al. A marathon run increases the susceptibility of LDL to oxidation in vitro and modifies plasma antioxidants. Am J Physiol 1999; 276: E1083-91
71. Case D, Baer JT, Subbiah MT. The effect of prolonged exercise on lipid peroxidation in eumenorrheic female runners. Med Sci Sports Exerc 2004; 31 (10): 1390-3
72. Ginsburg GS, Agil A, O'Toole M, et al. Effects of a single bout of ultraendurance exercise on lipid levels and susceptibility of lipids to peroxidation in triathletes. JAMA 1996; 276 (3): 221-5
73. Viguie CA, Frei B, Shigenaga MK, et al. Antioxidant status and indexes of oxidative stress during consecutive days of exercise. J Appl Physiol 1993; 75 (2): 566-72
74. Hartmann A, Pfuhler S, Dennog C, et al. Exercise-induced DNA effects in human leukocytes are not accompanied by increased formation of 8-hydroxy-2'-deoxyguanosine or induction of micronuclei. Free Radic Biol Med 1998; 24 (2): 245-51
75. Nielsen HB, Hanel B, Loft S, et al. Restricted pulmonary diffusion capacity after exercise is not an ARDS-like injury. J Sports Sci 1995; 13 (2): 109-13
76. Witt EH, Reznick AZ, Viguie CA, et al. Exercise, oxidative damage and effects of antioxidant manipulation. J Nutr 1992; 122 (3 Suppl.): 766-73
77. Mastaloudis A, Yu TW, O'Donnell RP, et al. Endurance exercise results in DNA damage as detected by the comet assay. Free Radic Biol Med 2004; 36 (8): 966-75
78. Hartmann A, Plappert U, Raddatz K, et al. Does physical activity induce DNA damage? Mutagenesis 1994; 9 (3): 269-72
79. Radak Z, Ogonovszky H, Dubecz J, et al. Super-marathon race increases serum and urinary nitrotyrosine and carbonyl levels. Eur J Clin Invest 2003; 33 (8): 726-30
80. Chevion S, Moran DS, Heled Y, et al. Plasma antioxidant status and cell injury after severe physical exercise. Proc Natl Acad Sci U S A 2003; 100 (9): 5119-23
81. Gohil K, Viguie C, Stanley WC, et al. Blood glutathione oxidation during human exercise. J Appl Physiol 1988; 64 (1): 115-9
82. Camus G, Felekidis A, Pincemail J, et al. Blood levels of reduced/oxidized glutathione and plasma concentration of ascorbic acid during eccentric and concentric exercises of similar energy cost. Arch Int Physiol Biochim Biophys 1994; 102 (1): 67-70
83. Laires MJ, Madeira F, Sergio J, et al. Preliminary study of the relationship between plasma and erythrocyte magnesium variations and some circulating pro-oxidant and antioxidant indices in a standardized physical effort. Magnes Res 1993; 6 (3): 233-8
84. Lee J, Goldfarb AH, Rescino MH, et al. Eccentric exercise effect on blood oxidative-stress markers and delayed onset of muscle soreness. Med Sci Sports Exerc 2002; 34 (3): 443-8
85. Rossi R, Milzani A, Dalle-Donne I, et al. Blood glutathione disulfide: in vivo factor or in vitro artifact? Clin Chem 2002; 48 (5): 742-53
86. Sastre J, Asensi M, Gasco E, et al. Exhaustive physical exercise causes oxidation of glutathione status in blood: prevention by antioxidant administration. Am J Physiol 1992; 263 (5 Pt 2): R992-5
87. Vina J, Sastre J, Asensi M, et al. Assay of blood glutathione oxidation during physical exercise. Methods Enzymol 1995; 251: 237-43
88. Pincemail J, Deby C, Camus G, et al. Tocopherol mobilization during intensive exercise. Eur J Appl Physiol Occup Physiol 1988; 57 (2): 189-91

89. Kawai Y, Iwane H, Takanami Y. Vitamin E is mobilized in relation to lipolysis after strenuous endurance exercise [abstract]. Med Sci Sports Exerc 1994; 26 (5): S7
90. Kawai Y, Iwane H, Takanami Y. Mechanisms of the increase in serum vitamin E level through strenuous endurance exercise [abstract]. Med Sci Sports Exerc 1995; 27 (5): S9
91. Camus G, Pincemail J, Roesgen A, et al. Tocopherol mobilization during dynamic exercise after beta-adrenergic blockade. Arch Int Physiol Biochim 1990; 98 (1): 121-6
92. Kawai Y, Shimomitsu T, Takanami Y, et al. Vitamin E level changes in serum and red blood cells due to acute exhaustive exercise in collegiate women. J Nutr Sci Vitaminol (Tokyo) 2000; 46 (3): 119-24
93. Schneider M, Niess AM, Rozario F, et al. Vitamin E supplementation does not increase the vitamin C radical concentration at rest and after exhaustive exercise in healthy male subjects. Eur J Nutr 2003; 42 (4): 195-200
94. Garry PJ, Appenzeller O. Vitamins A and C and endurance races. Ann Sports Med 1983; 1: 82-4
95. Gleeson M, Robertson JD, Maughan RJ. Influence of exercise on ascorbic acid status in man. Clin Sci (Colch) 1987; 73 (5): 501-5
96. Inoue T, Mu Z, Sumikawa K, et al. Effect of physical exercise on the content of 8-hydroxydeoxyguanosine in nuclear DNA prepared from human lymphocytes. Jpn J Cancer Res 1993; 84 (7): 720-5
97. Poulsen HE, Loft S, Vistisen K. Extreme exercise and oxidative DNA modification. J Sports Sci 1996; 14 (4): 343-6
98. Koyama K, Kaya M, Ishigaki T, et al. Role of xanthine oxidase in delayed lipid peroxidation in rat liver induced by acute exhausting exercise. Eur J Appl Physiol Occup Physiol 1999; 80 (1): 28-33
99. Liu J, Yeo HC, Overvik-Douki E, et al. Chronically and acutely exercised rats: biomarkers of oxidative stress and endogenous antioxidants. J Appl Physiol 2000; 89 (1): 21-8
100. Boveris A, Oshino N, Chance B. The cellular production of hydrogen peroxide. Biochem J 1972; 128 (3): 617-30
101. Boveris A, Chance B. The mitochondrial generation of hydrogen peroxide: general properties and effect of hyperbaric oxygen. Biochem J 1973; 134 (3): 707-16
102. Herrero A, Barja G. ADP-regulation of mitochondrial free radical production is different with complex I- or complex II-linked substrates: implications for the exercise paradox and brain hypermetabolism. J Bioenerg Biomembr 1997; 29 (3): 241-9
103. Murphy MP, Echtay KS, Blaikie FH, et al. Superoxide activates uncoupling proteins by generating carbon-centered radicals and initiating lipid peroxidation: studies using a mitochondria-targeted spin trap derived from alpha-phenyl-N-tert-butylnitrone. J Biol Chem 2003; 278 (49): 48534-45
104. Richardson RS, Leigh JS, Wagner PD, et al. Cellular PO2 as a determinant of maximal mitochondrial O(2) consumption in trained human skeletal muscle. J Appl Physiol 1999; 87 (1): 325-31
105. Richardson RS, Newcomer SC, Noyszewski EA. Skeletal muscle intracellular PO(2) assessed by myoglobin desaturation: response to graded exercise. J Appl Physiol 2001; 91 (6): 2679-85
106. Bailey DM. What regulates exercise-induced reactive oxidant generation: mitochondrial O(2) flux or PO(2)? Med Sci Sports Exerc 2001; 33 (4): 681-2
107. Drahota Z, Chowdhury SK, Floryk D, et al. Glycerophosphate-dependent hydrogen peroxide production by brown adipose tissue mitochondria and its activation by ferricyanide. J Bioenerg Biomembr 2002; 34: 105-13
108. Miwa S, St-Pierre J, Partridge L, et al. Superoxide and hydrogen peroxide production by *Drosophila* mitochondria. Free Rad Biol Med 2003; 35 (8): 938-48
109. MacDonald MJ, Brown LJ. Calcium activation of mitochondrial glycerol phosphate dehydrogenase restudied. Arch Biochem Biophys 1996; 326 (1): 79-84
110. Rasmussen UF, Krustrup P, Bangsbo J, et al. The effect of high-intensity exhaustive exercise studied in isolated mitochondria from human skeletal muscle. Pflugers Arch 2001; 443 (2): 180-7
111. Jesina P, Kholova D, Bolehovska R, et al. Glycerophosphate-dependent hydrogen peroxide production by rat liver mitochondria. Physiol Res 2004; 53 (3): 305-10
112. Packer L. Oxidants, antioxidant nutrients and the athlete. J Sports Sci 1997; 15 (3): 353-63
113. Roy RS, McCord JM. Superoxide and ischemia: conversion of xanthine dehydrogenase to xanthine oxidase. In: Greenvald R, Cohen G, editors. Oxy radicals and their scavenger systems. New York: Elsevier Science Publishing, 1983: 145-53
114. Volek JS, Kraemer WJ, Rubin MR, et al. L-carnitine L-tartrate supplementation favorably affects markers of recovery from exercise stress. Am J Physiol Endocrinol Metab 2002; 282 (2): E474-82
115. Hellsten Y, Hansson HA, Johnson L, et al. Increased expression of xanthine oxidase and insulin-like growth factor I (IGF-I) immunoreactivity in skeletal muscle after strenuous exercise in humans. Acta Physiol Scand 1996; 157 (2): 191-7
116. Hellsten Y, Frandsen U, Orthenblad N, et al. Xanthine oxidase in human skeletal muscle following eccentric exercise: a role in inflammation. J Physiol 1997; 498 (Pt 1): 239-48
117. Xia Y, Zweier JL. Substrate control of free radical generation from xanthine oxidase in the postischemic heart. J Biol Chem 1995; 270 (32): 18797-803
118. Bangsbo J, Sjodin B, Hellsten-Westing Y. Exchange of hypoxanthine in muscle during intense exercise in man. Acta Physiol Scand 1992; 146 (4): 549-50
119. Hellsten-Westing Y, Kaijser L, Ekblom B, et al. Exchange of purines in human liver and skeletal muscle with short-term exhaustive exercise. Am J Physiol 1994; 266 (1 Pt 2): R81-6
120. Hellsten Y, Ahlborg G, Jensen-Urstad M, et al. Indication of in vivo xanthine oxidase activity in human skeletal muscle during exercise. Acta Physiol Scand 1988; 134 (1): 159-60
121. Vina J, Gimeno A, Sastre J, et al. Mechanism of free radical production in exhaustive exercise in humans and rats; role of xanthine oxidase and protection by allopurinol. IUBMB Life 2000; 49 (6): 539-44
122. Heunks LM, Vina J, van Herwaarden CL, et al. Xanthine oxidase is involved in exercise-induced oxidative stress in chronic obstructive pulmonary disease. Am J Physiol 1999; 277 (6 Pt 2): R1697-704
123. Pyne DB. Exercise-induced muscle damage and inflammation: a review. Aust J Sci Med Sport 1994; 26 (3-4): 49-58
124. Morozov VI, Pryatkin SA, Kalinski MI, et al. Effect of exercise to exhaustion on myeloperoxidase and lysozyme release from blood neutrophils. Eur J Appl Physiol 2003; 89 (3-4): 257-62
125. Yamada M, Suzuki K, Kudo S, et al. Effect of exhaustive exercise on human neutrophils in athletes. Luminescence 2000; 15 (1): 15-20
126. Suzuki K, Nakaji S, Yamada M, et al. Impact of a competitive marathon race on systemic cytokine and neutrophil responses. Med Sci Sports Exerc 2003; 35 (2): 348-55

127. Suzuki K, Sato H, Kikuchi T, et al. Capacity of circulating neutrophils to produce reactive oxygen species after exhaustive exercise. J Appl Physiol 1996; 81 (3): 1213-22
128. Bury TB, Pirnay F. Effect of prolonged exercise on neutrophil myeloperoxidase secretion. Int J Sports Med 1995; 16 (6): 410-2
129. Camus G, Pincemail J, Ledent M, et al. Plasma levels of polymorphonuclear elastase and myeloperoxidase after uphill walking and downhill running at similar energy cost. Int J Sports Med 1992; 13 (6): 443-6
130. Wetzstein CJ, Shern-Brewer RA, Santanam N, et al. Does acute exercise affect the susceptibility of low density lipoprotein to oxidation? Free Radic Biol Med 1998; 24 (4): 679-82
131. Niess AM, Sommer M, Schlotz E, et al. Expression of the inducible nitric oxide synthase (iNOS) in human leukocytes: responses to running exercise. Med Sci Sports Exerc 2000; 32 (7): 1220-5
132. Childs A, Jacobs C, Kaminski T, et al. Supplementation with vitamin C and N-acetyl-cysteine increases oxidative stress in humans after an acute muscle injury induced by eccentric exercise. Free Radic Biol Med 2001; 31 (6): 745-53
133. Cooper CE, Vollaard NB, Choueiri T, et al. Exercise, free radicals and oxidative stress. Biochem Soc Trans 2002; 30 (2): 280-4
134. Emerit J, Beaumont C, Trivin F. Iron metabolism, free radicals, and oxidative injury. Biomed Pharmacother 2001; 55 (6): 333-9
135. Gibson JF, Ingram DJ, Perutz MF. Orientation of the four haem groups in haemoglobin. Nature 1956; 178 (4539): 906-8
136. Svistunenko DA, Patel RP, Voloshchenko SV, et al. The globin-based free radical of ferryl hemoglobin is detected in normal human blood. J Biol Chem 1997; 272 (11): 7114-21
137. Balagopalakrishna C, Manoharan PT, Abugo OO, et al. Production of superoxide from hemoglobin-bound oxygen under hypoxic conditions. Biochemistry 1996; 35 (20): 6393-8
138. Vollaard NBJ, Reeder BJ, Shearman JP, et al. A new sensitive assay reveals that hemoglobin is oxidatively modified in vivo. Free Radic Biol Med 2005; 39 (9): 1216-28
139. Kanter M. Free radicals, exercise and antioxidant supplementation. Proc Nutr Soc 1998; 57 (1): 9-13
140. Elosua R, Molina L, Fito M, et al. Response of oxidative stress biomarkers to a 16-week aerobic physical activity program, and to acute physical activity, in healthy young men and women. Atherosclerosis 2003; 167 (2): 327-34
141. Criswell D, Powers S, Dodd S, et al. High intensity training-induced changes in skeletal muscle antioxidant enzyme activity. Med Sci Sports Exerc 1993; 25 (10): 1135-40
142. Higuchi M, Cartier LJ, Chen M, et al. Superoxide dismutase and catalase in skeletal muscle: adaptive response to exercise. J Gerontol 1985; 40 (3): 281-6
143. Leeuwenburgh C, Fiebig R, Chandwaney R, et al. Aging and exercise training in skeletal muscle: responses of glutathione and antioxidant enzyme systems. Am J Physiol 1994; 267 (2 Pt 2): R439-45
144. Powers SK, Criswell D, Lawler J, et al. Influence of exercise and fiber type on antioxidant enzyme activity in rat skeletal muscle. Am J Physiol 1994; 266 (2 Pt 2): R375-80
145. Powers SK, Lawler J, Criswell D, et al. Aging and respiratory muscle metabolic plasticity: effects of endurance training. J Appl Physiol 1992; 72 (3): 1068-73
146. Oh-ishi S, Kizaki T, Nagasawa J, et al. Effects of endurance training on superoxide dismutase activity, content and mRNA expression in rat muscle. Clin Exp Pharmacol Physiol 1997; 24 (5): 326-32
147. Oh-ishi S, Kizaki T, Ookawara T, et al. Endurance training improves the resistance of rat diaphragm to exercise-induced oxidative stress. Am J Respir Crit Care Med 1997; 156 (5): 1579-85
148. Somani SM, Husain K. Exercise training alters kinetics of antioxidant enzymes in rat tissues. Biochem Mol Biol Int 1996; 38 (3): 587-95
149. Halliwell B. Free radicals, proteins and DNA: oxidative damage versus redox regulation. Biochem Soc Trans 1996; 24 (4): 1023-7
150. Evelson P, Travacio M, Repetto M, et al. Evaluation of total reactive antioxidant potential (TRAP) of tissue homogenates and their cytosols. Arch Biochem Biophys 2001; 388 (2): 261-6
151. Di Meo S, Venditti P, De Leo T. Tissue protection against oxidative stress. Experientia 1996; 52 (8): 786-94
152. Cao G, Giovanoni M, Prior RL. Antioxidant capacity in different tissues of young and old rats. Proc Soc Exp Biol Med 1996; 211 (4): 359-65
153. Xu KY, Zweier JL, Becker LC. Hydroxyl radical inhibits sarcoplasmic reticulum Ca(2+)-ATPase function by direct attack on the ATP binding site. Circ Res 1997; 80 (1): 76-81
154. Morris TE, Sulakhe PV. Sarcoplasmic reticulum Ca(2+)-pump dysfunction in rat cardiomyocytes briefly exposed to hydroxyl radicals. Free Radic Biol Med 1997; 22 (1-2): 37-47
155. Scherer NM, Deamer DW. Oxidation of thiols in the Ca2+-ATPase of sarcoplasmic reticulum microsomes. Biochim Biophys Acta 1986; 862 (2): 309-17
156. Scherer NM, Deamer DW. Oxidative stress impairs the function of sarcoplasmic reticulum by oxidation of sulfhydryl groups in the Ca2+-ATPase. Arch Biochem Biophys 1986; 246 (2): 589-601
157. Lawler JM, Hu Z, Barnes WS. Effect of reactive oxygen species on K+ contractures in the rat diaphragm. J Appl Physiol 1998; 84 (3): 948-53
158. Sen CK, Kolosova I, Hanninen O, et al. Inward potassium transport systems in skeletal muscle derived cells are highly sensitive to oxidant exposure. Free Radic Biol Med 1995; 18 (4): 795-800
159. Haycock JW, Jones P, Harris JB, et al. Differential susceptibility of human skeletal muscle proteins to free radical induced oxidative damage: a histochemical, immunocytochemical and electron microscopical study in vitro. Acta Neuropathol (Berl) 1996; 92 (4): 331-40
160. Lamb GD, Posterino GS. Effects of oxidation and reduction on contractile function in skeletal muscle fibres of the rat. J Physiol 2003; 546 (Pt 1): 149-63
161. Oba T, Koshita M, Yamaguchi M. H2O2 modulates twitch tension and increases Po of Ca2+ release channel in frog skeletal muscle. Am J Physiol 1996; 271 (3 Pt 1): C810-8
162. Reid MB, Khawli FA, Moody MR. Reactive oxygen in skeletal muscle III: contractility of unfatigued muscle. J Appl Physiol 1993; 75 (3): 1081-7
163. Barclay JK, Hansel M. Free radicals may contribute to oxidative skeletal muscle fatigue. Can J Physiol Pharmacol 1991; 69 (2): 279-84
164. Andrade FH, Reid MB, Allen DG, et al. Effect of hydrogen peroxide and dithiothreitol on contractile function of single skeletal muscle fibres from the mouse. J Physiol 1998; 509: 565-75

165. Reid MB. Invited review: redox modulation of skeletal muscle contraction: what we know and what we don't. J Appl Physiol 2001; 90 (2): 724-31
166. Novelli GP, Bracciotti G, Falsini S. Spin-trappers and vitamin E prolong endurance to muscle fatigue in mice. Free Radic Biol Med 1990; 8 (1): 9-13
167. Novelli GP, Falsini S, Bracciotti G. Exogenous glutathione increases endurance to muscle effort in mice. Pharmacol Res 1991; 23 (2): 149-55
168. Leeuwenburgh C, Ji LL. Glutathione and glutathione ethyl ester supplementation of mice alter glutathione homeostasis during exercise. J Nutr 1998; 128 (12): 2420-6
169. Travaline MJ, Sudarshan S, Roy BG, et al. Effect of N-acetylcysteine on human diaphragm strength and fatigability. Am J Respir Crit Care Med 1997; 156 (5): 1567-71
170. Reid MB, Stokic DS, Koch SM, et al. N-acetylcysteine inhibits muscle fatigue in humans. J Clin Invest 1994; 94 (6): 2468-74
171. Medved I, Brown MJ, Bjorksten AR, et al. N-acetylcysteine infusion alters blood redox status but not time to fatigue during intense exercise in humans. J Appl Physiol 2003; 94 (4): 1572-82
172. Medved I, Brown MJ, Bjorksten AR, et al. Effects of intravenous N-acetylcysteine infusion on time to fatigue and potassium regulation during prolonged exercise. J Appl Physiol 2003; 96 (1): 211-7
173. Shephard RJ, Campbell R, Pimm P, et al. Vitamin E, exercise, and the recovery from physical activity. Eur J Appl Physiol Occup Physiol 1974; 33 (2): 119-26
174. Watt T, Romet TT, McFarlane I, et al. Vitamin E and oxygen consumption [letter]. Lancet 1974; II (7876): 354-5
175. Lawrence JD, Bower RC, Riehl WP, et al. Effects of alpha-tocopherol acetate on the swimming endurance of trained swimmers. Am J Clin Nutr 1975; 28 (3): 205-8
176. Sharman IM, Down MG, Norgan NG. The effects of vitamin E on physiological function and athletic performance of trained swimmers. J Sports Med Phys Fitness 1976; 16 (3): 215-25
177. Braun B, Clarkson PM, Freedson PS, et al. Effects of coenzyme Q10 supplementation on exercise performance, $\dot{V}O_{2max}$, and lipid peroxidation in trained cyclists. Int J Sport Nutr 1991; 1 (4): 353-65
178. Snider IP, Bazzarre TL, Murdoch SD, et al. Effects of coenzyme athletic performance system as an ergogenic aid on endurance performance to exhaustion. Int J Sport Nutr 1992; 2 (3): 272-86
179. Rokitzki L, Logemann E, Huber G, et al. Alpha-tocopherol supplementation in racing cyclists during extreme endurance training. Int J Sport Nutr 1994; 4 (3): 253-64
180. Laaksonen R, Fogelholm M, Himberg JJ, et al. Ubiquinone supplementation and exercise capacity in trained young and older men. Eur J Appl Physiol Occup Physiol 1995; 72 (1-2): 95-100
181. Malm C, Svensson M, Ekblom B, et al. Effects of ubiquinone-10 supplementation and high intensity training on physical performance in humans. Acta Physiol Scand 1997; 161 (3): 379-84
182. Weston SB, Zhou S, Weatherby RP, et al. Does exogenous coenzyme Q10 affect aerobic capacity in endurance athletes? Int J Sport Nutr 1997; 7 (3): 197-206
183. Itoh H, Ohkuwa T, Yamazaki Y, et al. Vitamin E supplementation attenuates leakage of enzymes following 6 successive days of running training. Int J Sports Med 2000; 21 (5): 369-74
184. Patwell DM, Jackson MJ. Contraction-induced oxidants as mediators of adaptation and damage in skeletal muscle. Exerc Sport Sci Rev 2004; 32 (1): 14-8
185. Jackson MJ. Free radicals in skin and muscle: damaging agents or signals for adaptation? Proc Nutr Soc 1999; 58 (3): 673-6
186. Iwai K, Drake SK, Wehr NB, et al. Iron-dependent oxidation, ubiquitination, and degradation of iron regulatory protein 2: implications for degradation of oxidized proteins. Proc Natl Acad Sci U S A 1998; 95 (9): 4924-8
187. Kang DK, Jeong J, Drake SK, et al. Iron regulatory protein 2 as iron sensor: iron-dependent oxidative modification of cysteine. J Biol Chem 2003; 278 (17): 14857-64
188. Zhu H, Bunn HF. Signal transduction: how do cells sense oxygen? Science 2001; 292 (5516): 449-51

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