

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 (H₂O₂), superoxide (O₂^{•-}), hypochlorous acid (HOCl) and nitric oxide (NO[•]) 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 ($\dot{V}O_{2\max}$). 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 1. 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
Rokitzi 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
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 <i>in vivo</i>. 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
Jj ^[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 produc-

tion 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

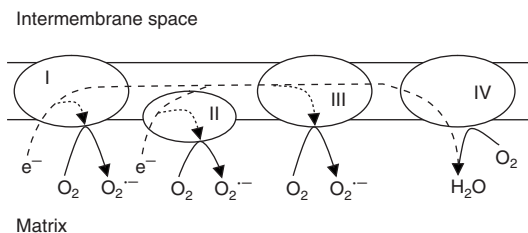


Fig. 1. In the respiratory chain, electrons are transferred from complexes I, II and III to complex IV (dashed line), after which oxygen acts as the final electron acceptor. However, in state 4 respiration, inadequate coupling of electron transfer at complexes I, II and III can cause 'leakage' of electrons 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 quinone 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 Ca²⁺ 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.

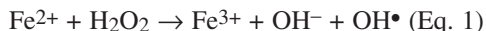
An alternative mechanism for the increase in free radical production with exercise could be one similar to that seen in ischaemia-reperfusion injury. Intense exercise is associated with transient tissue hypoxia in several organs, as blood flow is redistributed to cover the increased blood supply in active 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. Plas-

ma 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.

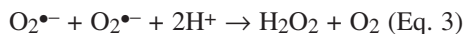
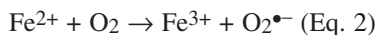
Free radicals may mediate cell damage, but conversely, exercise-induced cell damage could lead to an increased production of ROS itself. Exercise can result in microvascular dysfunction, oedema and cell damage through mechanical shear forces or through a disturbance of normal cellular metabolism.^[123] 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):



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 ($\text{Fe}^{4+} = \text{O}^{2-}$), with concomitant formation of a free radical ($\text{R}\cdot$) 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]



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

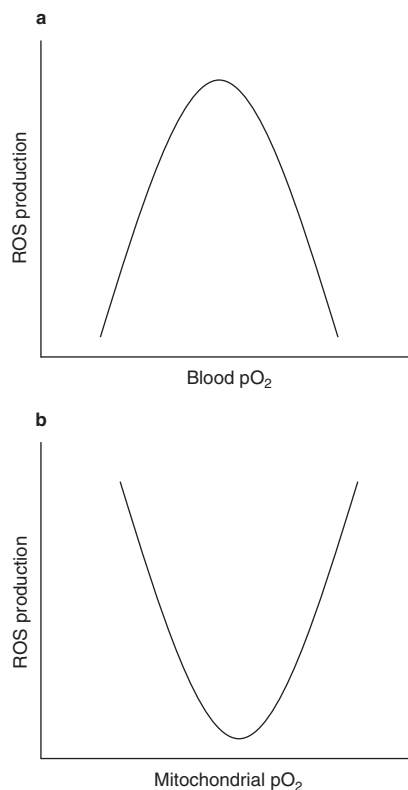


Fig. 2. Model depicting the differential effects of partial oxygen pressure (pO_2) on radical production by: (a) oxyhaemoglobin auto-oxidation, and (b) intracellular mechanisms that are not yet fully elucidated. Whereas superoxide-production by haem proteins has a bell-shaped dependence on pO_2 ,^[137] 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 sup-

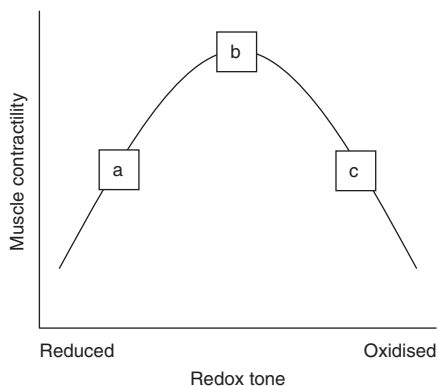


Fig. 3. The optimal-redox-tone model proposed by Reid and co-workers state that the basal, presumably homeostatically regulated, intracellular redox tone (a) is more reduced than the redox tone optimal for muscle contraction (b). Exercise-induced reactive oxygen species production slightly alters the 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).

plementation *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- α -phenyl-nitron, α -(4-pyridyl-1-oxide) *N*-tert-butyl nitron 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]

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 than 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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