ULTRA-ENDURANCE EXERCISE: UNANSWERED QUESTIONS IN REDOX BIOLOGY AND IMMUNOLOGY

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ABSTRACT

Ultra-endurance races are extreme exercise events that can take place over large parts of a day, several consecutive days, or over weeks and months interspersed by periods of rest and recovery. Since the first ultra-endurance races in the late 1970s, around 1000 races are now held worldwide each year, and more than 100,000 people take part. While these athletes appear to be fit and healthy, there have been occasional reports of severe complications following ultra-endurance exercise. Thus, there is concern that repeated extreme exercise events could have deleterious effects on health which might be brought about by the high levels of reactive oxygen species (ROS) produced during exercise. Studies that have examined biomarkers of oxidative damage following ultra-endurance exercise have found measurements to be elevated for several days, which has usually been interpreted to reflect increased ROS production. Levels of the antioxidant molecule reduced glutathione (GSH) are depleted for one month or longer following ultra-endurance exercise, suggesting an impaired capacity to cope with ROS. This article summarises studies that have examined the oxidative footprint of ultra-endurance exercise in light of current thinking in redox biology and the possible health implications of such extreme exercise.

ULTRA-ENDURANCE EXERCISE

Traditional endurance exercise is usually defined as activity that is sustained for between thirty minutes and four hours [1]. The term "ultra-endurance" is used to describe a variety of extreme and prolonged exercise racing events, which can involve either single or multiple sporting modalities. These activities are usually undertaken with little or no rest, over large parts of a day or consecutive days. Other types of ultra-endurance races happen over several days or weeks, interspersed by periods of recovery, and can take place in a variety of environmental conditions (e.g., tropical, temperate or desert climates, sometimes at high altitude). Consequently, the physiological demands of ultra-endurance events differ considerably.

Various interpretations have been made as to what constitutes ultra-endurance exercise, some of which are sport-specific and defined by distances travelled, rather than the duration of exercise. For example, with foot races (i.e., walking or running), ultra-marathons involve competitors covering a distance greater than a traditional marathon (26.2 miles or 42.2 km; with typical marathon completion time ranging between 2 and 6 hours). With triathlon, ultra-distance exercise (also branded "ironman") involves swimming for 2.4 miles (3.8 km), cycling for 112.0 miles (180.2 km) and running for 26.2 miles (42.2 km). Typical ironman completion times range between ≈8 and 17 hours. While broader duration-based definitions of ultra-endurance exercise include activities undertaken for more than four hours [2], this review considers ultra-endurance exercise to be; performed for at least six hours [3]; running over a distance of ≥50 miles (80.4 km); and when triathlon events meet ultra-distance criteria. The majority of studies discussed examine continuous ultra-endurance exercise that it is not separated by periods of recovery (i.e., sleep). Although analysis of some oxidative stress biomarkers suggest an additive effect of repeated ultra-endurance exercise, analysis of other biomarkers suggests that clearance or repair processes can be initiated between sampling points and bouts of exercise [4]. Thus, studies investigating multi-day events including rest periods, or those that focus on nutritional interventions are beyond the scope of this review. The aim of this work is to discuss ultra-endurance exercise in the context of current thinking in redox biology, highlighting the possible implications of engagement in extreme exercise.

SCIENTIFIC INTEREST IN ULTRA-ENDURANCE EXERCISE

With a few exceptions (e.g., continental expeditions and the Tour de France cycling race), mass-participation in ultra-endurance exercise began in the late 1970s. It is now estimated that more than 1000 ultra-endurance events take place worldwide each year with more than 100,000 people competing [5]. Despite reports that prolonged exercise and large training loads may impair immunity and increase the incidence of upper respiratory tract infections [6], ultra-endurance athletes report fewer missed work or school days due to illness and injury compared with the normal population, and generally exhibit a low incidence of chronic disease [7]. However, health concerns have been raised about participation in ultra-endurance events, including long-term cardiac damage, potentially mediated by the high levels of reactive oxygen species (ROS) that are produced during exercise [2, 8]. Thus, a number of studies have examined whether redox homeostasis is altered after bouts of ultra-endurance exercise.

EXERCISE-INDUCED REACTIVE OXYGEN SPECIES PRODUCTION

Molecular species, including superoxide ($O_2\bullet^-$), hydrogen peroxide (H_2O_2,), nitric oxide (NO•) and peroxynitrite ($ONOO\bullet^-$), collectively referred to as ROS, are formed within and around most body cells through normal processes such as respiration and signalling [9]. A number of sources have been identified that increase ROS output in a variety of cells during exercise, including the mitochondrial electron transport chain, prostanoid metabolism, and the autoxidation of haemoglobin, myoglobin and catecholamines [9-11]. Another significant example includes the production of $O_2\bullet^-$ by nicotinamide adenine dinucleotide phosphate (NADPH)-oxidase for signalling purposes in contracting muscle and some types of immune cells (e.g., T-lymphocytes) or for destruction of pathogens by others (e.g., activated phagocytes such as neutrophils) [9, 10]. Finally, during ischemia reperfusion, the conversion of purines to uric acid, which normally proceeds via xanthine dehydrogenase, instead occurs via xanthine oxidase, producing $O_2\bullet^-$ [9, 10].

Within the intracellular and extracellular fluid, and embedded within cell or organelle membranes, various molecules with antioxidant properties exist to buffer ROS [9, 10]. Examples include vitamins (e.g., vitamin C; ascorbic acid, and vitamin E; tocopherol), enzymes (e.g., superoxide dismutase; SOD, catalase; CAT, glutathione peroxidase; GPx, peroxiredoxins; PRDXs and thioredoxins; TRXs) and various co-factors, such as the thiol, reduced glutathione (GSH). Sometimes these antioxidant molecules participate in signalling cascades themselves following interaction with ROS [9, 10]. However, it is thought that the production of ROS during exercise can be so large that antioxidant defences are overwhelmed, resulting in oxidative damage to proteins, lipids and DNA [9, 11]. Adducts on these molecules, referred to as biomarkers of oxidative stress, have been shown to be increased following ultra-endurance exercise, and speculated to indicate clinically relevant alterations to redox homeostasis [2, 9, 11].

ULTRA-ENDURANCE EXERCISE AND OXIDATIVE STRESS

Table 1 shows studies that have investigated non-stop ultra-marathons [12-17]. With the exception of one study [12] and the non-finishers included in another [15, 16], the duration of ultra-marathons (27 to 48 hours) was considerably longer than the ultra-distance triathlons [18-23] and multi-sport events [24, 25] shown in Table 2 (7.5 to 12.5 hours). It can also be seen that generally, the alterations in redox homeostasis, indicated by measuring biomarkers of oxidative stress, are more consistent between ultra-marathon athletes (and between different biomarkers in these studies), compared to investigations of ultra-distance triathletes (see Table 2). For example, biomarkers of lipid peroxidation, such as malondialdehyde (MDA), lipid hydroperoxides (LPO) and F_2-isoprostanes (F2iso) are consistently higher immediately after ultra-marathons, and remain elevated for 24 to 48 hours [12-15] (see Table 1). Thiobarbituric acid reactive substances (TBARS) appear to be a less robust measure of lipid peroxidation following exercise, often showing counter-intuitive decreases soon after ultra-marathons and ultra-distance triathlons [17, 19]. To assist with the interpretation the results above, the reader is directed towards a comprehensive review covering the strengths and weaknesses of commonly measured oxidative stress biomarkers [26].

As shown in Tables 1 and 2, the effects of ultra-endurance exercise on antioxidant levels appear at first glance to be varied. Measures of antioxidant capacity have been shown to be increased [13, 14], decreased [17, 18] or exhibit no change [19, 25] in the hours and days after ultra-endurance exercise. The apparent inconsistency is likely due to one or both of the following factors. (A) The use of

different methodology to assess antioxidant capability (e.g., measuring the protein level of a single intracellular antioxidant enzyme *vs.* the reducing capacity of several extracellular antioxidants). For example, total plasma antioxidant capacity is typically elevated for one to two days after ultra-endurance exercise [14, 21]. While this antioxidant response is partly mediated by acute ascorbic acid flux from the adrenal glands [27], plasma antioxidant capacity is also influenced by acute dietary intake [28], which is often not controlled. Increased levels of intracellular antioxidants are perhaps less sensitive to acute fluctuations with diet [29], and reflect increased protein transcription or enzymatic re-synthesis in response to ROS [9, 10]. Thus, the levels of intracellular and extracellular antioxidants may change in response to different stimuli and may reflect different antioxidant mechanisms post-exercise. (B) The direction of change in a measurement of antioxidant capability is related to the biological properties of the antioxidant mechanism. For example, interaction between antioxidant molecules occurs across a series of reactions (e.g., detoxification of $O_2\bullet^-$ by SOD produces H_2O_2 that is converted to H_2O by a number of enzymes, including CAT, PRDXs and also GPx with GSH as a co-factor) [9, 10]. Thus, increased activity or levels of one antioxidant molecule influences the activity and levels of others, making it difficult to interpret values for single antioxidants unless all elements of this sequence are assessed in the same study, and in the same cell.

The overall picture is that ultra-endurance exercise results in a transient increase in antioxidant capability, and if the exercise-induced ROS production is severe or prolonged, then antioxidant molecules are depleted and may not be return to normal levels for at least one month [15]. Although the source of ROS during ultra-endurance exercise remains unclear, one study has shown that the capacity for mitochondria to produce ROS is increased immediately after 24 hours of running, cycling and kayaking, returning to normal within 28 hours [24].

THE GRAND UNION CANAL RACE: A 145-MILE ULTRA-MARATHON

Our group have contributed to understanding of how ultra-endurance exercise affects redox homeostasis by examining a single-stage, 145-mile ultra-marathon that took place over two days [15, 16]. Blood samples were collected for up to one month after the race, and multiple processes in redox biology were investigated in plasma, erythrocytes, and peripheral blood mononuclear cells [15, 16]. In the first report we showed that plasma lipid hydroperoxides were increased above pre-race values for 24 hours and plasma protein carbonyls were elevated for seven days [15] (see Table 1). Consistent with other reports [21] non-specific damage to lymphocyte DNA was detectable for 24 hours, some of which was oxidative-stress specific [15]. As has been shown previously by others, DNA damage is rapidly repaired by enzymes such as 8-oxoguanine DNA glycosylase the activity of which is up-regulated after exercise [30]. However, studies examining other measures of DNA damage, including chromosome breakages or abnormalities, and measures of mis-repaired DNA have shown little effect of exercise (see Table 2) [20].

Another notable finding from this report [15] was depletion of GSH measured in whole blood (i.e., GSH that is largely derived from erythrocytes) for one month, comparable to levels found in a number of pathologies [31]. This result suggests that ultra-endurance exercise either; results in an excessively large and sustained effect on ROS production beyond the end of the exercise period; affects the activity of enzymes that recycle or produce GSH (i.e., glutathione reductase, γ-glutamylcysteine, glutathione synthetase); or alternatively, depletes key precursors for GSH (i.e., L-glutamine and L-cysteine) as has been shown by some studies [6].

In our second report, we examined whether the depletion of GSH, the principal redox regulator in erythrocytes, generalised to similar molecules in lymphocytes, by examining the antioxidant enzyme peroxiredoxin-2 (PRDX2) [16]. This molecule is critical for lymphocyte function, including proliferation and activation [32], and if it is depleted following exercise, might partly explain the reports of dysregulated immunity following large volumes of exercise [6]. Confirming the generalisibility of persistent oxidative stress between cell types, lymphocyte PRDX2 showed comparable changes to GSH in erythrocytes [16]. Central to PRDX2 function is a redox active cysteine, which serves to reduce ROS, becoming oxidised to form different oligomeric or redox states of PRDX2, each with different fates. Mild oxidation results in the formation of sulphenic acid whereas severe oxidation (i.e., over-oxidation) produces sulphinic acid or sulphonic acid. While the first modification is reversible by antioxidants such as TRX, the latter two modifications are largely irreversible and subsequently cleared from the cell. Analysis in our second report [16] showed that PRDX2 was "over-oxidised" by ultra-endurance exercise suggesting that the mechanism for depleted PRDX2 might involve excessive production of ROS, subsequent change in oligomeric state and probable clearance by the proteasome [16]. The implications of these findings and others are presented in the next section.

IMPLICATIONS OF ULTRA-ENDURANCE EXERCISE: UNANSWERED QUESTIONS IN REDOX BIOLOGY AND IMMUNOLOGY

Should exercise-induced ROS production be prevented with antioxidant supplementation?

The long-term effects of excessive exercise-induced ROS production are unknown, but many athletes supplement their diets with antioxidants assuming protection from oxidative damage. In the context of ultra-endurance exercise, there is no consistent evidence that antioxidant supplements prevent elevated biomarkers of oxidative stress, and some studies have even shown an exacerbating effect of supplementation on biomarker frequency [33, 34]. Further, evidence shows that athletes are naturally equipped with a strong capacity to buffer exercise-induced ROS. In a detailed study of antioxidant capacity and ultra-endurance exercise, it was shown that plasma concentrations of most vitamins remained within a normal physiological range, were adequate compared to recommended values, remained at levels above those required to saturate cells, and provided protection against exercise-induced oxidative DNA damage [35]. However, significant decreases in carotenoids and γ-tocopherol below normal values were reported 24 hours after exercise [35]. In some studies deleterious effects of supplementation have been reported [33, 34], therefore, unless nutritionally deficient, dietary antioxidants are probably unnecessary for ultra-endurance athletes, except for perhaps during short periods of recovery [35].

It has been argued that the view of exercise in general causing "oxidative stress" needs revision [10, 11, 33]. Although ultra-endurance exercise probably causes a transient and manageable oxidative insult, regular exercise training results in adaptive processes [10, 33]. ROS-induced adaptation includes an increased capacity to buffer ROS (e.g., production of enzymatic antioxidants) but also changes associated with metabolism (e.g., mitochondrial biogenesis), improved exercise capacity (e.g., vasodilation) and other important health-related processes (e.g., insulin sensitivity, fatty acid storage, and glucose control) [10, 33]. Thus, the rationale for preventing or limiting exercise-induced ROS production has been questioned and

tested experimentally, with some studies showing that antioxidant supplementation negates the beneficial effects of exercise [36], and others showing that exercise adaptation occurs despite supplementation [37].

To understand whether exercise-induced ROS production should be limited or prevented, possible implications for cell function caused by protein oxidation could be examined in future research. Use of oxidative fluorescence difference gel electrophoresis (Oxi-DIGE), a novel gel-based proteomic technique, would allow for the redox proteome of blood samples collected at two different time-points (e.g., before and upon completion of ultra-endurance exercise) to be examined simultaneously, and might reveal proteins important for cell function that have been oxidatively modified [38].

Could decreased PRDX2 level and redox-state affect cell-mediated immunity after ultra-endurance exercise?

Changes in the level and redox-state of PRDX2 in lymphocytes, in particular T-lymphocytes, might in part explain inflammatory activity following ultra-endurance exercise [16]. Mild oxidation of PRDX2 (i.e., formation of sulphenic acid) is essential to control T-lymphocyte activation by buffering hydrogen peroxide levels [32]. However, depletion of PRDX2, due to excessive ROS production, over-oxidation (i.e., formation of sulphinic or sulphonic acid), and subsequent removal from the cell, could result in exacerbated T-lymphocyte activation and proliferation [16]. In support, mice lacking PRDX2 exhibit uncontrolled T-lymphocyte responses following viral challenge causing lethal inflammatory pathology [32]. Thus, PRDX2 over-oxidation might stimulate a T-lymphocyte derived inflammatory response, which is possible considering these cells are potent producers of cytokines such as interleukin-6 and tumour necrosis factor-α [39].

It is not known whether the depletion in lymphocyte PRDX2 seven days after ultra-endurance exercise [16] might also be evident in other cells of the immune system, and this merits further investigation. For example, dendritic cells are important tissue sentinels that detect and ingest invading pathogens and parts of dying or infected body cells in order to initiate immune responses. PRDX2 allows dendritic cell differentiation by regulating hydrogen peroxide levels via its mild oxidation, providing protection from ROS-induced cell death [40]. However, if PRDX2 is depleted in dendritic cells due to over-oxidation and clearance, the ensuing non-reducing intracellular environment, associated with depletion of other antioxidants, such as thiols, could impair anti-viral immunity. For example, when dendritic cells activate T-lymphocytes, cysteine is provided to increase lymphocyte surface thiols [41]. Further, other dendritic cell processes could be impaired, considering that oxidative stress has been shown to prevent antigen processing [42].

Could ultra-endurance exercise result in latent viral reactivation?

Herpes viruses are ubiquitous in the population and are never eliminated by the immune system, remaining dormant ('latent') for prolonged periods in infected host cells, interrupted by periods of viral replication and disease ('reactivation'). Examples include varicella zoster virus; the cause of chicken pox and shingles, Epstein-Barr virus; the cause of infectious mononucleosis, and cytomegalovirus; implicated in ageing of the immune system. Viral reactivation has been shown in response to a variety of physiological and psychological stressors, such as very strenuous exercise training, spaceflight, depression, anxiety, and other forms of acute psychological stress [43-45]. Moreover, conditions associated with oxidative stress

and inflammation (e.g., systemic lupus erythematosus) are associated with viral reactivation [46]. We hypothesise that ultra-endurance exercise may also result in viral reactivation, which might be brought about by an indirect or direct effect of ROS. First, ultra-endurance exercise might impair the control of latent viruses due to suppression of cell-mediated immunity by oxidative stress. Second, exercise-induced ROS production might stimulate viral replication directly considering that another persistent virus, human immunodeficiency virus (HIV), has been shown to reactivate via redox-mediated transcription of NFκB in virus-harboring cells [47].

Could decreased GSH levels potentiate acetaminophen (paracetamol) toxicity?

Ultra-endurance exercise depletes erythrocyte GSH levels by ≈66% for 24 hours, and levels remain ≈33% lower than normal one month later [15]. Animal studies have shown that exercise-induced changes in the levels of GSH measured in blood also reflect changes in a variety of body tissues, including skeletal and cardiac muscle, and organs such as the spleen, brain, thymus and liver [48]. If ultra-endurance exercise depletes liver GSH to the same extent as erythrocyte GSH [15] then these effects are comparable to acute acetaminophen overdose, which can lower liver GSH by ≈80-90% [48]. Other animal studies have shown that very strenuous exercise impairs liver detoxification of acetaminophen and potentiates hepatotoxicity [49]. Considering the likelihood of ultra-endurance competitors requiring analgesic medication, the possibility of an impaired capacity to detoxify acetaminophen is of particular relevance. Moreover, due to recent papers showing that acetaminophen can improve sports performance by increasing power output during exercise and reducing thermal strain [50], there is a possibility that use of this medication will become widespread in athletes. Future research is therefore warranted to examine whether other pain relief might be more appropriate for ultra-endurance athletes (e.g., non-steroidal anti-inflammatory drugs, that are cleared by other pathways).

CONCLUSION

As the first wave of ultra-endurance athletes, now aged between 60-70 years, are examined in studies and receive routine healthcare, data providing insight into the long term health benefits or risks of ultra-endurance exercise will soon become available. In anticipation, studies continue to examine the effects of ultra-endurance exercise on redox homeostasis. The present review highlighted some of the main findings in this area, discussed possible consequences of exercise induced ROS production, and suggested several avenues for further research that may help to advance the field.

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Table 1. Oxidative stress and ultra-marathons.

Study	Subjects[1]	Mode	Distance	Duration	Samples[2]	Summary of selected results
Kanter [12] (1998)	9 m	Running	50 miles	≈ 8.5 h	Post	**Serum:** ↑MDA
Nieman [13] (2003)	22/9 m/f	Running	99 miles	≈ 27 h	Post	**Plasma:** ↑F2iso, ↑LPO, ↑FRAP
Skenderi [14] (2008)	16/2 m/f	Running	153 miles	≈ 33 h	Post	**Plasma:** ↑F2iso, =MDA, ↑TAC, **Rbc:** $\downarrow^{ns}$ GSH
					48 h	**Plasma:** ↑F2iso,↓MDA, ↑TAC, **Rbc:** $\downarrow^{ns}$ GSH
Turner [15, 16] (2011 & 2013)	9 m	Running	145 miles	≈12 - 40 h	Post	**Plasma:** ↑PC, ↑LPO, **Rbc:** ↑GSH, **PBMC:** ↑DNA, ↑FPG, $\uparrow^{ns}$ PRDX2
					24 h	**Plasma:** ↑PC, ↑LPO, **Rbc:** ↓GSH, **PBMC:** ↑DNA, = FPG, = PRDX2
					7 days	**Plasma:** ↑PC, = LPO, **Rbc:** ↓GSH, **PBMC:** = DNA, = FPG, ↓ PRDX2
					28 days	**Plasma:** = PC, ↓LPO, **Rbc:** ↓GSH, **PBMC:** = DNA, = FPG, = PRDX2
Klapcinska [17] (2013)	7 m	Running	≈174 miles	48 h	Post	**Plasma:** $\uparrow^{ns}$ TBARS, **Rbc:** $\downarrow^{ns}$ SOD, $\uparrow^{ns}$ GSH
					24 h	**Plasma:** $\downarrow^{ns}$ TBARS, **Rbc:** $\downarrow^{ns}$ SOD, ↑ GSH
					48 h	**Plasma:** = TBARS, **Rbc**: $\downarrow^{ns}$ SOD, $\uparrow^{ns}$ GSH

Legend for Table 1: [1] m/f is males/females. [2] all post-exercise samples compared to a pre-race sample. ↑ statistically significant increase, ↓ statistically significant decrease, $\uparrow^{ns}$ non-significant increase, $\downarrow^{ns}$ non-significant decrease, = no change, **Serum:** cell free component of clotted blood, **Plasma:** cell free component of anticoagulated blood, **Rbc:** erythrocytes, **PBMC:** peripheral blood mononuclear cells, **PC:** plasma protein carbonylation, **TBARS**: Thiobarbituric acid reactive substances, **MDA:** malondialdehyde, **F2iso:** F2isoprostanes, **LPO:** lipid hydroperoxides, **FRAP:** ferric reducing ability of plasma, **TAC**: total antioxidant capacity of plasma, **CAT:** catalase, **SOD**: superoxide dismutase, **GPx:** glutathione peroxidase, **GSH:** reduced glutathione, **DNA:** non-specific DNA damage, **FPG:** formamidopyrimidine glycosylase sensitive DNA damage, **PRDX2:** Peroxiredoxin-2.

Table 2. Oxidative stress and ultra-distance triathlon or multi-sport events.

Study	Subjects[1]	Mode	Duration	Samples[2]	Summary of selected results
Ginsburg [18] (1996)	26/13 m/f	Tri	≈ 12.5 h	Post	**Plasma:** ↓Vit A, =Vit C, = Vit E, ↓LPO
Margaritis [19] (1997)	12 m	Tri	≈ 7.5 h	Post	**Plasma:** =TBARS, **Rbc:** =GSH, =GSSG, =SOD, =GPx
				6 h	**Plasma:** ↓TBARS, **Rbc:** =GSH, =GSSG, =SOD, =GPx
				24 h	**Plasma:** =TBARS, **Rbc:** =GSH, =GSSG, =SOD, =GPx
				48 h	**Plasma:** =TBARS, **Rbc:** =GSH, =GSSG, =SOD, =GPx
				96 h	**Plasma:** =TBARS, **Rbc:** =GSH, =GSSG, =SOD, =GPx
Reichold [20] (2008)	20 m	Tri	≈ 10.5 h	Post	**PBMC:** ↓Micronuclei, =Nucleoplasmic bridges, =Nuclear buds
				5 days	**PBMC:** ↓Micronuclei, =Nucleoplasmic bridges, ↑Nuclear buds
				19 days	**PBMC:** ↓Micronuclei, ↓Nucleoplasmic bridges, =Nuclear buds
Neubauer [21] (2008)	42 m	Tri	≈ 11 h	Post	**Plasma:** ↑TAC, ↑MDA, **Rbc:** ↓SOD, ↓ CAT, =GPx, **PBMC:** ↑DNA, $\downarrow^{ns}$ ENDO, $\downarrow^{ns}$ FPG
				24 h	**Plasma:** ↑TAC, =MDA, **Rbc:** =SOD, ↓ CAT, =GPx, **PBMC:** ↑DNA, $\downarrow^{ns}$ ENDO, $\downarrow^{ns}$ FPG
Wagner [22] (2009)				5 days	**Plasma:** =TAC, =MDA, **Rbc:** ↓SOD, $\downarrow^{ns}$CAT, =GPx, **PBMC:** =DNA, $\uparrow^{ns}$ ENDO, $\downarrow^{ns}$ FPG
				19 days	**Plasma:** =TAC, =MDA, **Rbc:** ↓SOD, ↓ CAT, =GPx, **PBMC:** =DNA, $\downarrow^{ns}$ ENDO, $\downarrow^{ns}$ FPG
Pinho [23] (2010)	18 m	Tri	NR	Post	**Plasma:** ↑TBARS, ↑LPO, ↑PC, **Rbc:** ↑SOD, ↑CAT
Sahlin [24] (2010)	8 m	Multi	24 h	Post	**Mitochon:** $\uparrow^{ns}$ HNE, ↑ ROS, **Muscle**: $\uparrow^{ns}$ GPx, =SOD
				28h	**Mitochon:** ↑ HNE, = ROS, **Muscle:** ↑ GPx, =SOD
Dantas de Lucas [25] (2014)	11 m	Multi	≈ 10 h	Post	**Plasma:** ↑PC, **Rbc:** ↑TBARS, = CAT

Legend for Table 2: [1] m/f is males/females. [2] all post-exercise samples compared to a pre-race sample. **NR:** Not reported. **Tri:** Ultra-distance triathlon, **Multi:** running, cycling, kayaking, ↑ statistically significant increase, ↓ statistically significant decrease, $\uparrow^{ns}$ non-significant increase, $\downarrow^{ns}$ non-significant decrease, = no change, **Serum:** cell free component of clotted blood, **Plasma:** cell free component of anticoaggulated blood, **Rbc:** erythrocytes, **PBMC:** peripheral blood mononuclear cells, **Mitochon:** muscle mitochondria, **Muscle:** homogenised muscle, **PC:** plasma protein carbonylation, **TBARS**: Thiobarbituric acid reactive substances, **HNE:** 4-hydroxynonenal, **MDA:** malondialdehyde, **F2iso:** F2isoprostanes, **LPO:** lipid hydroperoxides, **FRAP:** ferric reducing ability of plasma, **TAC**: total antioxidant capacity of plasma, **CAT:** catalase, **SOD**: superoxide dismutase, **GPx:** glutathione peroxidase, **GSH:** reduced glutathione, **GSSG:** oxidized glutathione **ROS:** reactive oxygen species, **Micronuclei:** result from chromosome breakages or chromosomes lagging behind at anaphase during cell division,

Nucleoplasmic bridges and **Nuclear buds** both originate from mis-repaired DNA. **DNA:** non-specific DNA damage, **ENDO:** endonuclease III sensitive DNA damage, **FPG:** formamidopyrimidine glycosylase sensitive DNA damage,