



Citation for published version:

Turner, JE, Bennett, SJ, Bosch, JA, Griffiths, HR & Aldred, S 2014, 'Ultra-endurance exercise: Unanswered questions in redox biology and immunology', *Biochemical Society Transactions*, vol. 42, no. 4, pp. 989-95.
<https://doi.org/10.1042/BST20140120>

DOI:

[10.1042/BST20140120](https://doi.org/10.1042/BST20140120)

Publication date:

2014

Document Version

Peer reviewed version

[Link to publication](#)

Publisher Rights

Unspecified

The final version of record is available at: <http://dx.doi.org/10.1042/BST20140120>

University of Bath

General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

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

3
4 JAMES E. TURNER^{1*}, STUART J. BENNETT², JOS A. BOSCH^{3,4}, HELEN R.
5 GRIFFITHS² & SARAH ALDRED⁵

6
7 ¹ Department for Health, University of Bath, Claverton Down, Bath, BA2 7AY

8
9 ² Life and Health Sciences, Aston University, Birmingham, B4 7ET, UK

10
11 ³ School of Psychology, University of Amsterdam, Amsterdam, 1012WX, The
12 Netherlands

13
14 ⁴ Mannheim Institute of Public Health, Social and Preventive Medicine (MIPH),
15 Mannheim Medical Faculty, University of Heidelberg, Germany.

16
17 ⁵ School of Sport, Exercise and Rehabilitation Sciences, University of Birmingham,
18 B15 2TT, UK

19
20 *Corresponding author: James E. Turner, Department for Health, University of Bath,
21 Claverton Down, Bath, BA2 7AY, e-mail: j.e.turner@bath.ac.uk, tel: 01225 38 3566

22
23
24
25
26
27
28
29 **Keywords:** Oxidative stress, reactive oxygen species, antioxidants, peroxiredoxin,
30 protein oxidation, glutathione,
31

32

33 **ABSTRACT**

34 Ultra-endurance races are extreme exercise events that can take place over large parts
35 of a day, several consecutive days, or over weeks and months interspersed by periods
36 of rest and recovery. Since the first ultra-endurance races in the late 1970s, around
37 1000 races are now held worldwide each year, and more than 100,000 people take
38 part. While these athletes appear to be fit and healthy, there have been occasional
39 reports of severe complications following ultra-endurance exercise. Thus, there is
40 concern that repeated extreme exercise events could have deleterious effects on health
41 which might be brought about by the high levels of reactive oxygen species (ROS)
42 produced during exercise. Studies that have examined biomarkers of oxidative
43 damage following ultra-endurance exercise have found measurements to be elevated
44 for several days, which has usually been interpreted to reflect increased ROS
45 production. Levels of the antioxidant molecule reduced glutathione (GSH) are
46 depleted for one month or longer following ultra-endurance exercise, suggesting an
47 impaired capacity to cope with ROS. This article summarises studies that have
48 examined the oxidative footprint of ultra-endurance exercise in light of current
49 thinking in redox biology and the possible health implications of such extreme
50 exercise.

51
52
53
54
55
56
57
58

59 **ULTRA-ENDURANCE EXERCISE**

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

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

91
92 **SCIENTIFIC INTEREST IN ULTRA-ENDURANCE EXERCISE**

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

106
107 **EXERCISE-INDUCED REACTIVE OXYGEN SPECIES PRODUCTION**

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

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

134 135 **ULTRA-ENDURANCE EXERCISE AND OXIDATIVE STRESS**

136 Table 1 shows studies that have investigated non-stop ultra-marathons [12-
137 17]. With the exception of one study [12] and the non-finishers included in another
138 [15, 16], the duration of ultra-marathons (27 to 48 hours) was considerably longer
139 than the ultra-distance triathlons [18-23] and multi-sport events [24, 25] shown in
140 Table 2 (7.5 to 12.5 hours). It can also be seen that generally, the alterations in redox
141 homeostasis, indicated by measuring biomarkers of oxidative stress, are more
142 consistent between ultra-marathon athletes (and between different biomarkers in these
143 studies), compared to investigations of ultra-distance triathletes (see Table 2). For
144 example, biomarkers of lipid peroxidation, such as malondialdehyde (MDA), lipid
145 hydroperoxides (LPO) and F_2 -isoprostanes (F_2 iso) are consistently higher
146 immediately after ultra-marathons, and remain elevated for 24 to 48 hours [12-15]
147 (see Table 1). Thiobarbituric acid reactive substances (TBARS) appear to be a less
148 robust measure of lipid peroxidation following exercise, often showing counter-
149 intuitive decreases soon after ultra-marathons and ultra-distance triathlons [17, 19].
150 To assist with the interpretation the results above, the reader is directed towards a
151 comprehensive review covering the strengths and weaknesses of commonly measured
152 oxidative stress biomarkers [26].

153 As shown in Tables 1 and 2, the effects of ultra-endurance exercise on
154 antioxidant levels appear at first glance to be varied. Measures of antioxidant capacity
155 have been shown to be increased [13, 14], decreased [17, 18] or exhibit no change
156 [19, 25] in the hours and days after ultra-endurance exercise. The apparent
157 inconsistency is likely due to one or both of the following factors. (A) The use of

158 different methodology to assess antioxidant capability (e.g., measuring the protein
159 level of a single intracellular antioxidant enzyme vs. the reducing capacity of several
160 extracellular antioxidants). For example, total plasma antioxidant capacity is typically
161 elevated for one to two days after ultra-endurance exercise [14, 21]. While this
162 antioxidant response is partly mediated by acute ascorbic acid flux from the adrenal
163 glands [27], plasma antioxidant capacity is also influenced by acute dietary intake
164 [28], which is often not controlled. Increased levels of intracellular antioxidants are
165 perhaps less sensitive to acute fluctuations with diet [29], and reflect increased protein
166 transcription or enzymatic re-synthesis in response to ROS [9, 10]. Thus, the levels of
167 intracellular and extracellular antioxidants may change in response to different stimuli
168 and may reflect different antioxidant mechanisms post-exercise. (B) The direction of
169 change in a measurement of antioxidant capability is related to the biological
170 properties of the antioxidant mechanism. For example, interaction between
171 antioxidant molecules occurs across a series of reactions (e.g., detoxification of $O_2^{\bullet-}$
172 by SOD produces H_2O_2 that is converted to H_2O by a number of enzymes, including
173 CAT, PRDXs and also GPx with GSH as a co-factor) [9, 10]. Thus, increased activity
174 or levels of one antioxidant molecule influences the activity and levels of others,
175 making it difficult to interpret values for single antioxidants unless all elements of this
176 sequence are assessed in the same study, and in the same cell.

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

184

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

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

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

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

227

228 **IMPLICATIONS OF ULTRA-ENDURANCE EXERCISE: UNANSWERED** 229 **QUESTIONS IN REDOX BIOLOGY AND IMMUNOLOGY**

230

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

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

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

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

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

269

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

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

284 It is not known whether the depletion in lymphocyte PRDX2 seven days after
285 ultra-endurance exercise [16] might also be evident in other cells of the immune
286 system, and this merits further investigation. For example, dendritic cells are
287 important tissue sentinels that detect and ingest invading pathogens and parts of dying
288 or infected body cells in order to initiate immune responses. PRDX2 allows dendritic
289 cell differentiation by regulating hydrogen peroxide levels via its mild oxidation,
290 providing protection from ROS-induced cell death [40]. However, if PRDX2 is
291 depleted in dendritic cells due to over-oxidation and clearance, the ensuing non-
292 reducing intracellular environment, associated with depletion of other antioxidants,
293 such as thiols, could impair anti-viral immunity. For example, when dendritic cells
294 activate T-lymphocytes, cysteine is provided to increase lymphocyte surface thiols
295 [41]. Further, other dendritic cell processes could be impaired, considering that
296 oxidative stress has been shown to prevent antigen processing [42].

297

298 *Could ultra-endurance exercise result in latent viral reactivation?*

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

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

316

317 *Could decreased GSH levels potentiate acetaminophen (paracetamol) toxicity?*

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

335

336 **CONCLUSION**

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

345

346

347 **ACKNOWLEDGEMENTS**

348 The authors are thankful to all of the volunteers who took part in the Grand Union
349 Canal Race 2009 and Nicholas Hurren, Sarah Jackman, and Natalie Riddell who
350 assisted with data collection. Funding for this study was provided by the University of
351 Birmingham UK. Analytical methods for peroxiredoxin-2 were developed with
352 financial support from COST CM1001 and MARK-AGE (EU FP7 Large-scale
353 integrating project HEALTH-F4-2008-200880).

354

355

356

357
358
359
360
361
362
363
364
365
366
367
368
369
370
371
372
373
374
375
376
377
378
379
380
381
382
383
384
385
386
387
388
389
390
391
392
393
394
395
396
397
398
399
400
401
402
403
404
405
406
407
408
409
410
411
412
413
414
415

REFERENCES

1. Coyle, E.F., *Physiological determinants of endurance exercise performance*. J Sci Med Sport, 1999. **2**(3): p. 181-9.
2. Knez, W.L., J.S. Coombes, and D.G. Jenkins, *Ultra-endurance exercise and oxidative damage : implications for cardiovascular health*. Sports Medicine, 2006. **36**(5): p. 429-41.
3. Zaryski, C. and D.J. Smith, *Training principles and issues for ultra-endurance athletes*. Curr Sports Med Rep, 2005. **4**(3): p. 165-70.
4. Radák, Z., et al., *Super-marathon race increases serum and urinary nitrotyrosine and carbonyl levels*. Eur J Clin Invest, 2003. **33**: p. 726-730.
5. *International Association of Ultrarunners*. 27/03/14]; Available from: <http://www.iau-ultramarathon.org>.
6. Walsh, N.P., et al., *Position statement. Part two: Maintaining immune health*. Exerc Immunol Rev, 2011. **17**: p. 64-103.
7. Hoffman, M.D. and E. Krishnan, *Health and exercise-related medical issues among 1,212 ultramarathon runners: baseline findings from the Ultrarunners Longitudinal TRacking (ULTRA) Study*. PLoS One, 2014. **9**(1): p. e83867.
8. Wortley, G. and A.A. Islas, *The problem with ultra-endurance athletes*. Br J Sports Med, 2011. **45**(14): p. 1085.
9. Powers, S.K. and M.J. Jackson, *Exercise-induced oxidative stress: cellular mechanisms and impact on muscle force production*. Physiol Rev, 2008. **88**(4): p. 1243-76.
10. Gomes, E.C., A.N. Silva, and M.R. de Oliveira, *Oxidants, antioxidants, and the beneficial roles of exercise-induced production of reactive species*. Oxid Med Cell Longev, 2012. **2012**: p. 756132.
11. Nikolaidis, M.G., et al., *Redox biology of exercise: an integrative and comparative consideration of some overlooked issues*. J Exp Biol, 2012. **215**(Pt 10): p. 1615-25.
12. Kanter, M.M., et al., *Serum creatine kinase and lactate dehydrogenase changes following an eighty kilometer race. Relationship to lipid peroxidation*. European Journal of Applied Physiology and Occupational Physiology, 1988. **57**(1): p. 60-3.
13. Nieman, D.C., et al., *Immune and oxidative changes during and following the Western States endurance run*. International Journal of Sports Medicine, 2003. **24**: p. 541-547.
14. Skenderi, K.P., et al., *Changes in free radical generation and antioxidant capacity during ultramarathon foot race*. Eur J Clin Invest, 2008. **38**(3): p. 159-65.
15. Turner, J.E., et al., *Prolonged depletion of antioxidant capacity after ultraendurance exercise*. Med Sci Sports Exerc, 2011. **43**(9): p. 1770-6.
16. Turner, J.E., et al., *The antioxidant enzyme peroxiredoxin-2 is depleted in lymphocytes seven days after ultra-endurance exercise*. Free Radic Res, 2013. **47**(10): p. 821-8.
17. Klapinska, B., et al., *Metabolic responses to a 48-h ultra-marathon run in middle-aged male amateur runners*. Eur J Appl Physiol, 2013. **113**(11): p. 2781-93.
18. Ginsburg, G.S., 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): p. 221-5.
19. Margaritis, I., et al., *No evidence of oxidative stress after a triathlon race in highly trained competitors*. Int J Sports Med, 1997. **18**(3): p. 186-90.
20. Reichhold, S., et al., *No acute and persistent DNA damage after an Ironman triathlon*. Cancer Epidemiol Biomarkers Prev, 2008. **17**(8): p. 1913-9.
21. Neubauer, O., et al., *No indications of persistent oxidative stress in response to an ironman triathlon*. Med Sci Sports Exerc, 2008. **40**(12): p. 2119-28.
22. Wagner, K.H., et al., *Well-trained, healthy triathletes experience no adverse health risks regarding oxidative stress and DNA damage by participating in an ultra-endurance event*. Toxicology, 2009.
23. Pinho, R.A., et al., *Oxidative stress and inflammatory parameters after an ironman race*. Clin J Sport Med, 2010. **20**(4): p. 306-11.
24. Sahlin, K., et al., *Ultraendurance exercise increases the production of reactive oxygen species in isolated mitochondria from human skeletal muscle*. J Appl Physiol, 2010. **108**(4): p. 780-7.

- 416 25. Dantas de Lucas, R., et al., *Increased platelet oxidative metabolism, blood oxidative*
417 *stress and neopterin levels after ultra-endurance exercise*. J Sports Sci, 2014. **32**(1):
418 p. 22-30.
- 419 26. Griffiths, H.R., et al., *Biomarkers*. Mol Aspects Med, 2002. **23**(1-3): p. 101-208.
- 420 27. Gleeson, M., J.D. Robertson, and R.J. Maughan, *Influence of exercise on ascorbic*
421 *acid status in man*. Clin Sci (Lond), 1987. **73**(5): p. 501-5.
- 422 28. Cao, G. and R.L. Prior, *Postprandial increases in serum antioxidant capacity in older*
423 *women*. J Appl Physiol, 2000. **89**(3): p. 877-83.
- 424 29. Shin, J.W., et al., *Erythrocyte malondialdehyde and glutathione levels in vitiligo*
425 *patients*. Ann Dermatol, 2010. **22**(3): p. 279-83.
- 426 30. Radak, Z., et al., *Marathon running alters the DNA base excision repair in human*
427 *skeletal muscle*. Life Sci, 2003. **72**(14): p. 1627-33.
- 428 31. Townsend, D.M., K.D. Tew, and H. Tapiero, *The importance of glutathione in human*
429 *disease*. Biomed Pharmacother, 2003. **57**(3-4): p. 145-55.
- 430 32. Michalek, R.D., et al., *Peroxiredoxin II regulates effector and secondary memory*
431 *CD8+ T cell responses*. J Virol, 2012. **86**(24): p. 13629-41.
- 432 33. Peternej, T.T. and J.S. Coombes, *Antioxidant supplementation during exercise*
433 *training: beneficial or detrimental?* Sports Med, 2011. **41**(12): p. 1043-69.
- 434 34. Nieman, D.C., et al., *Vitamin E and immunity after the Kona Triathlon World*
435 *Championship*. Med Sci Sports Exerc, 2004. **36**(8): p. 1328-35.
- 436 35. Neubauer, O., et al., *Antioxidant responses to an acute ultra-endurance exercise:*
437 *impact on DNA stability and indications for an increased need for nutritive*
438 *antioxidants in the early recovery phase*. Br J Nutr, 2010. **104**(8): p. 1129-38.
- 439 36. Ristow, M., et al., *Antioxidants prevent health-promoting effects of physical exercise*
440 *in humans*. Proc Natl Acad Sci U S A, 2009. **106**(21): p. 8665-70.
- 441 37. Yfanti, C., et al., *Effect of antioxidant supplementation on insulin sensitivity in*
442 *response to endurance exercise training*. Am J Physiol Endocrinol Metab, 2011.
443 **300**(5): p. E761-70.
- 444 38. Baraibar, M.A., R. Ladouce, and B. Friguet, *Proteomic quantification and*
445 *identification of carbonylated proteins upon oxidative stress and during cellular aging*.
446 J Proteomics, 2013. **92**: p. 63-70.
- 447 39. Lorre, K., et al., *Interleukin-1 and B7/CD28 interaction regulate interleukin-6*
448 *production by human T cells*. Clin Immunol Immunopathol, 1994. **70**(1): p. 81-90.
- 449 40. Van Brussel, I., et al., *Transcript and protein analysis reveals better survival skills of*
450 *monocyte-derived dendritic cells compared to monocytes during oxidative stress*.
451 PLoS One, 2012. **7**(8): p. e43357.
- 452 41. Martner, A., et al., *Redox remodeling by dendritic cells protects antigen-specific T*
453 *cells against oxidative stress*. J Immunol, 2011. **187**(12): p. 6243-8.
- 454 42. Preynat-Seauve, O., et al., *Oxidative stress impairs intracellular events involved in*
455 *antigen processing and presentation to T cells*. Cell Stress Chaperones, 2003. **8**(2):
456 p. 162-71.
- 457 43. Gleeson, M., et al., *Epstein-Barr virus reactivation and upper-respiratory illness in*
458 *elite swimmers*. Med Sci Sports Exerc, 2002. **34**(3): p. 411-7.
- 459 44. Mehta, S.K., et al., *Reactivation of latent viruses is associated with increased plasma*
460 *cytokines in astronauts*. Cytokine, 2013. **61**(1): p. 205-9.
- 461 45. Rector, J.L., et al., *Consistent associations between measures of psychological*
462 *stress and CMV antibody levels in a large occupational sample*. Brain Behav Immun,
463 2014.
- 464 46. Su, B.Y., et al., *Incidental discovery of high systemic lupus erythematosus disease*
465 *activity associated with cytomegalovirus viral activity*. Med Microbiol Immunol, 2007.
466 **196**(3): p. 165-70.
- 467 47. Baruchel, S. and M.A. Wainberg, *The role of oxidative stress in disease progression*
468 *in individuals infected by the human immunodeficiency virus*. J Leukoc Biol, 1992.
469 **52**(1): p. 111-4.
- 470 48. Mitchell, J.R., et al., *Acetaminophen-induced hepatic necrosis. IV. Protective role of*
471 *glutathione*. J Pharmacol Exp Ther, 1973. **187**(1): p. 211-7.
- 472 49. Lew, H. and A. Quintanilha, *Effects of endurance training and exercise on tissue*
473 *antioxidative capacity and acetaminophen detoxification*. Eur J Drug Metab
474 Pharmacokinet, 1991. **16**(1): p. 59-68.

- 475 50. Mauger, A.R., et al., *Acute acetaminophen (paracetamol) ingestion improves time to*
476 *exhaustion during exercise in the heat.* Exp Physiol, 2014. **99**(1): p. 164-71.
477
478
479
480

481
482

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 48 h	Plasma: ↑F2iso, =MDA, ↑TAC, Rbc: ↓ ^{ns} GSH Plasma: ↑F2iso, ↓MDA, ↑TAC, Rbc: ↓ ^{ns} GSH
Turner [15, 16] (2011 & 2013)	9 m	Running	145 miles	≈12 - 40 h	Post 24 h 7 days 28 days	Plasma: ↑PC, ↑LPO, Rbc: ↑GSH, PBMC: ↑DNA, ↑FPG, ↑ ^{ns} PRDX2 Plasma: ↑PC, ↑LPO, Rbc: ↓GSH, PBMC: ↑DNA, = FPG, = PRDX2 Plasma: ↑PC, = LPO, Rbc: ↓GSH, PBMC: = DNA, = FPG, ↓ PRDX2 Plasma: = PC, ↓LPO, Rbc: ↓GSH, PBMC: = DNA, = FPG, = PRDX2
Klapcinska [17] (2013)	7 m	Running	≈174 miles	48 h	Post 24 h 48 h	Plasma: ↑ ^{ns} TBARS, Rbc: ↓ ^{ns} SOD, ↑ ^{ns} GSH Plasma: ↓ ^{ns} TBARS, Rbc: ↓ ^{ns} SOD, ↑ GSH Plasma: = TBARS, Rbc: ↓ ^{ns} SOD, ↑ ^{ns} GSH

483 **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
484 significant decrease, ↑^{ns} non-significant increase, ↓^{ns} non-significant decrease, = no change, **Serum:** cell free component of clotted blood, **Plasma:** cell free
485 component of anticoagulated blood, **Rbc:** erythrocytes, **PBMC:** peripheral blood mononuclear cells, **PC:** plasma protein carbonylation, **TBARS:** Thiobarbituric
486 acid reactive substances, **MDA:** malondialdehyde, **F2iso:** F2isoprostanes, **LPO:** lipid hydroperoxides, **FRAP:** ferric reducing ability of plasma, **TAC:** total
487 antioxidant capacity of plasma, **CAT:** catalase, **SOD:** superoxide dismutase, **GPx:** glutathione peroxidase, **GSH:** reduced glutathione, **DNA:** non-specific DNA
488 damage, **FPG:** formamidopyrimidine glycosylase sensitive DNA damage, **PRDX2:** Peroxiredoxin-2.
489

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 6 h 24 h 48 h 96 h	Plasma: =TBARS, Rbc: =GSH, =GSSG, =SOD, =GPx Plasma: ↓TBARS, Rbc: =GSH, =GSSG, =SOD, =GPx Plasma: =TBARS, Rbc: =GSH, =GSSG, =SOD, =GPx Plasma: =TBARS, Rbc: =GSH, =GSSG, =SOD, =GPx Plasma: =TBARS, Rbc: =GSH, =GSSG, =SOD, =GPx
Reichold [20] (2008)	20 m	Tri	≈ 10.5 h	Post 5 days 19 days	PBMC: ↓Micronuclei, =Nucleoplasmic bridges, =Nuclear buds PBMC: ↓Micronuclei, =Nucleoplasmic bridges, ↑Nuclear buds PBMC: ↓Micronuclei, ↓Nucleoplasmic bridges, =Nuclear buds
Neubauer [21] (2008)	42 m	Tri	≈ 11 h	Post 24 h	Plasma: ↑TAC, ↑MDA, Rbc: ↓SOD, ↓ CAT, =GPx, PBMC: ↑DNA, ↓ ^{ns} ENDO, ↓ ^{ns} FPG Plasma: ↑TAC, =MDA, Rbc: =SOD, ↓ CAT, =GPx, PBMC: ↑DNA, ↓ ^{ns} ENDO, ↓ ^{ns} FPG
Wagner [22] (2009)				5 days 19 days	Plasma: =TAC, =MDA, Rbc: ↓SOD, ↓ ^{ns} CAT, =GPx, PBMC: =DNA, ↑ ^{ns} ENDO, ↓ ^{ns} FPG Plasma: =TAC, =MDA, Rbc: ↓SOD, ↓ CAT, =GPx, PBMC: =DNA, ↓ ^{ns} ENDO, ↓ ^{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 28h	Mitochon: ↑ ^{ns} HNE, ↑ ROS, Muscle: ↑ ^{ns} GPx, =SOD Mitochon: ↑ HNE, = ROS, Muscle: ↑ GPx, =SOD
Dantas de Lucas [25] (2014)	11 m	Multi	≈ 10 h	Post	Plasma: ↑PC, Rbc: ↑TBARS, = CAT

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

498 **Nucleoplasmic bridges** and **Nuclear buds** both originate from mis-repaired DNA. **DNA:** non-specific DNA damage, **ENDO:** endonuclease III sensitive DNA
499 damage, **FPG:** formamidopyrimidine glycosylase sensitive DNA damage,
500