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5
6 **Prolonged depletion of antioxidant capacity following ultra-endurance exercise**
7
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Abstract

Purpose: The purpose of this study was to examine the short and long-term (up to 1-month) impact of an ultra-endurance running event on redox-homeostasis. **Methods:** Markers of oxidative stress and antioxidant capacity in peripheral blood were assessed following a single-stage 233 km (143 mile) running event. Samples were collected from nine men (mean age ± SD; 46.1 ± 5.3 y; body mass index 24.9 ± 2.3 $kg \cdot m^{-2}$, maximal-oxygen uptake 56.3 ± 3.3 $ml \cdot kg^{-1} \cdot min^{-1}$). Peripheral blood mononuclear cells (PBMC) were assayed for non-specific DNA damage (frank strand breaks) and damage to DNA caused specifically by oxidative stress (formamidopyrimidine DNA glycosylase (FPG)-dependent damage). Protein carbonylation (PC) and lipid peroxidation (LPO) were assessed in plasma. Reduced glutathione (GSH) was measured in whole blood. **Results:** PBMC frank strand breaks were elevated above baseline at 24 hours post-race ($p < .001$). FPG-dependent oxidative DNA damage was increased immediately post-race ($p < .05$). PC remained elevated for 7 days following the race ($p < .04$) whereas LPO was increased for 24 hours ($p < .05$) and fell below baseline 28 days later ($p < .05$). GSH, a measure of antioxidant capacity also showed a biphasic response, increasing by one third post-race ($p < .01$) and falling to two-thirds of baseline levels 24 hours later ($p < .001$). GSH remained depleted to approximately one third of pre-race values 28 days post-race ($p < .01$). **Conclusions:** Ultra-endurance exercise causes oxidative stress, which persists for 1 calendar month depending on the specific biomarker examined. These results suggest that ultra-endurance events are associated with a prolonged period of reduced protection against oxidative stress.

Keywords: Glutathione, comet assay, protein carbonylation, lipid peroxidation, ultra-endurance exercise.

51 **Introduction**

52 ***Paragraph number 1.*** Research consistently shows that ultra-endurance exercise results in the
53 formation of reactive oxygen species (ROS) (17, 27). The extent of this production has the
54 potential to overwhelm antioxidant defences, causing oxidative stress (13, 27). Oxidative stress is
55 associated with damage to proteins, lipids and DNA (27), which has been causally related to
56 ageing, and the development of cancer and cardiovascular disease (28, 39, 40). Consequently,
57 there has been substantial debate as to whether the health benefits of physical activity extend to
58 more extreme forms of endurance exercise (15, 17). For example, epidemiological studies show
59 that individuals taking part in an exceptionally large amount of physical activity have impaired
60 cardiovascular health (17).

61

62 ***Paragraph number 2.*** Exercise-induced oxidative stress is likely the result of superoxide ($O_2^{\bullet -}$)
63 production by the electron 'leak' in mitochondria, xanthine oxidase in endothelial cells, or from
64 the inflammatory response of activated leukocytes (13, 27). $O_2^{\bullet -}$ dismutates to hydrogen
65 peroxide (H_2O_2) and, in the presence of transition metals, forms highly reactive hydroxyl radicals
66 ($OH^{\bullet}$) which rapidly oxidise bodily molecules (13). Arguably, the most robust and accurate
67 methods for assessing oxidative stress are the measurement of ROS-induced modifications to
68 plasma proteins or damage to DNA in peripheral blood mononuclear cells (PBMC). (13). These
69 blood markers correlate well with similar measurements in tissues, and therefore provide an
70 indication of whole-body oxidative stress (37).

71

72 ***Paragraph number 3.*** ROS production during exercise can also result in alterations to
73 antioxidant capacity. Measurement of blood antioxidant defences therefore provides another
74 informative method to assess oxidative stress (13). Conceivably, depletion of these defences

75 could result in decreased protection against ROS. In contrast, an up-regulation of antioxidant
76 defences is often observed following exercise (13, 27), and it is plausible that this process is one
77 of the mechanisms behind the beneficial adaptations to exercise (13, 27). One of the most
78 abundant antioxidant compounds in blood, as with most other body tissues, is reduced
79 glutathione (GSH) (36). GSH acts as an electron donor for the reductive detoxification of
80 hydrogen peroxide (H_2O_2) into water (H_2O) by the enzyme GSH peroxidase (GPx). Decreased
81 GSH is associated with cardiovascular disease and other non-communicable diseases, potentially
82 due to impaired protection leading to oxidative stress (29). GSH also has other important
83 functions, including regulation of immune function and acting as a co-factor for specific
84 enzymatic reactions (11, 16, 34). Thus, this molecule provides a useful measure of oxidative
85 stress.
86
87 ***Paragraph number 4.*** Many studies have oxidative stress in the context of ultra-endurance
88 exercise, but few have investigated a very prolonged and continuous bout of ultra-endurance
89 exercise consisting of just one sporting activity (e.g., 31). Several studies have examined ironman
90 triathlons, which involve swimming, cycling and running (e.g., 22). Investigations of this kind
91 therefore assume an equal contribution of effects from each sporting modality. Moreover, it is
92 currently unknown whether oxidative stress persists for more than 19 days following such
93 extreme exercise (22). In general, oxidative stress can last for up to 72 hours post-exercise,
94 depending on the biomarker investigated (19, 22, 31, 35). More prolonged effects are observed
95 when antioxidant capacity is the primary measurement. For example, decreased levels of
96 enzymatic antioxidants have been reported 19 days after an ironman competition (22). Although
97 it appears the oxidative footprint left by ultra-endurance exercise is relatively acute, some data is
98 clearly difficult to interpret due to complications in study design. Collecting samples before and

99 after multiple days of exercise takes into consideration periods of rest and recovery (e.g., 19).
100 Likewise, examining just one section of a multi-day event also includes effects caused by
101 previous days of exercise (e.g., 35). Further studies are therefore required to answer the question
102 of whether ultra-endurance exercise results in alterations to redox-homeostasis which are
103 detectable one month later.
104
105 ***Paragraph number 5.*** The present study examined the impact of a single-stage ultra-endurance
106 running event on redox-homeostasis over a period of 28 days. On the basis of prior research, it
107 was hypothesised that exercise would cause protein carbonylation, lipid peroxidation and damage
108 to DNA, which would be accompanied by a reduction in whole blood GSH concentration. It was
109 anticipated that all parameters would return to baseline levels within 28 days of the race.

Methods

Participants

Paragraph number 6. Nine healthy non-smoking middle-aged men (≥ 40 years) took part in this study (Table 1). Participants abstained from taking vitamin supplements for at least ten weeks prior to the ultra-endurance race. Participants provided informed consent, and the study was approved by the School of Sport and Exercise Sciences research ethics committee, of the University of Birmingham.

[INSERT TABLE 1 HERE]

Baseline measurements

Paragraph number 7. Participants visited the laboratory prior to the ultra-endurance race to have height and body mass recorded, and skin-fold measurements taken for the calculation of percentage body fat (9). Maximal oxygen consumption ($\dot{V}O_2$ max) was directly measured with a graded exercise test on a treadmill adapted from the Bruce protocol (38). Breath-by-breath measurements were recorded every 5 seconds throughout the test (Oxycon Pro, Jaegar, Germany). Heart rate and ratings of perceived exertion (RPE) were recorded during the final minute of each stage. In addition, participants had leisure time physical activity assessed using the international physical activity questionnaire (IPAQ) (5). IPAQ data was expressed as MET-minutes per week, where 1 MET (metabolic equivalent) is equal to resting metabolic rate.

Ultra-endurance race

132 ***Paragraph number 8.*** Participants took part in The Grand Union Canal Race; a single-stage 233
133 km (145 mile) race from Birmingham to London in the UK. The route comprised mainly gravel
134 or paved tow-paths and bridleways adjacent to the canal. The race started at 06:00 on Saturday
135 the 23rd of May 2009, and participants who completed the race, crossed the finish line between
136 21:15 the next day, and 01:50 on Monday the 25th of May. Participants undertook the race at their
137 own pace. Diet was not controlled or recorded for logistical reasons and participants consumed
138 food and fluids *ad libitum*. Ambient temperatures during the race were 6-9°C at night and 20-
139 25°C during the day. Relative humidity was 57-64%.
140
141 *Experimental design*
142 ***Paragraph number 9.*** Blood samples were collected from a forearm vein less than 60 minutes
143 before the race and upon completion (mean 65 min ± 64 SD after). Additional blood draws were
144 taken at 24 hours (mean 26 hours ± 4 SD after), 7 days, and 28 days post-race. Participants were
145 seated for 5 min prior to all blood draws, and refrained from exercising for at least 7 days
146 following the race.
147
148 *Blood sample processing*
149 ***Paragraph number 10.*** Blood was collected into ethylene-diamine-tetra-acetic acid (EDTA)
150 vacutainer tubes (Becton-Dickinson, Oxford, UK) and immediately stored at 4°C. PBMC and
151 plasma were obtained within 60 min using a portable centrifuge (E8F Portafuge, LW scientific,
152 Georgia, USA). PBMC were isolated using density gradient centrifugation. Briefly, whole blood
153 was diluted 1:1 with Phosphate Buffered Saline (PBS; Invitrogen, Paisley, UK) and carefully
154 layered onto Ficoll-Paque PLUS (GE Healthcare, Uppsala, Sweden) before centrifuging at 1350
155 $\times g$ for 30 min. The buffy coat was aspirated, and cells washed in PBS at 1350 $\times g$ for 10 min.

156 The pellet was re-suspended 1:1 in foetal bovine serum (FBS) 10% dimethyl sulfoxide (DMSO).

157 Plasma was obtained by centrifuging whole blood at 1350 × *g* for 15 min. PBMC, plasma and

158 whole blood were immediately frozen in multiple aliquots using dry ice, and later transferred to

159 −80°C storage until analysis.

160

161 *General analytical procedures*

162 ***Paragraph number 11.*** Total plasma protein concentration was determined using the

163 bicinchoninic acid method as described by Smith *et al*. (32). Biochemical parameters were

164 expressed relative to protein concentration, cell number, or were corrected for changes in plasma

165 volume according to Dill and Costill (7) as appropriate. Haemoglobin and haematocrit was

166 assessed using a Coulter ACT^{diff} haematology analyser (Beckman-Coulter, High Wycombe, UK).

167 Chemicals were obtained from Sigma-Aldrich, UK, unless otherwise stated.

168

169 *Reduced glutathione*

170 ***Paragraph number 12.*** Reduced glutathione (GSH) was measured in whole blood frozen 1:1

171 with FBS (10% DMSO) using a commercially available fluorescence-based assay according to

172 manufacturer's instructions (Promega GSH-Glo, Wisconsin, USA).

173

174 *DNA damage*

175 ***Paragraph number 13.*** A non-specific measure of DNA damage, Frank strand breaks to PBMC

176 DNA, were measured using the alkaline single-cell gel electrophoresis (comet) assay (30). DNA

177 damage caused by oxidative stress was assessed using *Escherichia coli* Formamidopyrimidine

178 DNA glycosylase (FPG) digestion for the detection of oxidised purine lesions (FPG-dependent

179 oxidative DNA damage) (4). Briefly, samples and controls were re-suspended in 1 ml of

180 Dulbecco's modified Eagle medium (DMEM). Control samples (approximately 4×10^5 cells)
181 were treated with 100 µM hydrogen peroxide or DMEM for 1 hour at 37°C. Samples and
182 controls were centrifuged at 200 × *g* for 5 min. Pellets were re-suspended and mixed in 25 µl
183 DMEM and 300 µl low-melting point agar. 150 µl of this cell suspension was added to two
184 separate glass slides (pre-coated with a thin layer of normal-melting point agar) and covered with
185 a glass cover-slip. Slides were left for 30 min at 4°C to solidify. Cover slips were removed, and
186 slides added to lysis buffer (2.5 M NaCl, 0.1 M Na_2EDTA, 10 mM Tris base, 1% Sodium *N*-
187 lauryl sarcosinate, 10% DMSO, and 1% Triton-X 100, pH 10.0) for 1 hour at 4°C. Slides were
188 washed (3 × 5 min) with FPG enzyme buffer (40 mM HEPES, 0.1 M KCl, 0.5 mM Na_2EDTA
189 and 0.2 mg/ml bovine serum albumin, pH 8.0). Parallel slides were treated with either 50 µl FPG
190 enzyme buffer containing 1 unit of FPG enzyme (Trevigen, Maryland, USA) or 50 µl FPG
191 enzyme buffer alone. Following treatment, slides were covered with cover-slips and incubated for
192 60 min at 37°C in a moist box. After incubation, cover-slips were removed, and slides were
193 transferred to a horizontal electrophoresis tank containing electrophoresis buffer (300 mM NaOH
194 and 1 mM Na_2EDTA, pH > 13.0) and left to stand for 20 min before electrophoresis at 32 V, 300
195 mA for 20 min ($1.8 V \cdot cm^{-1}$). Slides were neutralised by flooding (3 × 5 min) in neutralisation
196 buffer (0.4 M Tris, pH 7.5), and stained with 50 µl of 10 × Sybr Gold (Invitrogen Ltd, Paisley,
197 UK). Slides were stored at 4°C in a moist box, and examined within 24 hours using a
198 fluorescence microscope (20 × magnification, 515-560 nm excitation and 590 nm barrier filters
199 respectively; Zeis Axiovert 10, Germany). Median percentage of DNA in comet tails was
200 analysed in 100 comets using computer-based image analysis software (Comet Assay IV,
201 Perceptive Instruments, Suffolk, UK).
202

203 *Protein carbonylation*

204 ***Paragraph number 14.*** Plasma protein carbonylation was measured by enzyme-linked-
205 immunosorbent-assay (ELISA) described by(1). Samples and standards (0.5 mg/ml in sodium
206 carbonate buffer; 50 mM, pH 7.4, 50μl) were allowed to bind to 96-well multi-sorb plates for
207 60min at 37°C in triplicate (Nunc, Fisher Thermo Scientific, UK). 2, 4-dintrophenylhydrazine
208 (1mM, 50μl) in 2 M hydrochloric acid was added, and plates incubated at room temperature for
209 60 min. Plates were blocked overnight at 4°C with 0.1% tris-buffered saline-TWEEN-20. Mouse
210 anti-DNP antibody (1:1000, 50μl) was incubated with samples for 120 min at 37°C, followed by
211 a peroxidise-labelled rat anti-mouse antibody (1:5000, 50μl), incubated for 60 min at 37°C. A
212 citrate phosphate-based substrate (0.15 M, pH 5) was added, and plates left to develop in the dark
213 for 30 min at 37°C. The reaction was stopped with 2 M sulphuric acid and plates read at 490 nm.
214 Values were expressed as nmol/mg of protein.

215

216 *Lipid peroxidation*

217 ***Paragraph number 15.*** Lipid peroxides were assayed in plasma using a modification of the
218 method by el-Saadani *et al*. (10). Samples and positive and negative controls (1:1000 hydrogen
219 peroxide and distilled water respectively) were added to 96-well plates in triplicate. Working
220 reagent (0.2 M Potassium Phosphate, 0.12 M Potassium Iodide, 0.15 mM Sodium Azide, 2g/l
221 Triton-X, 0.1 g/l Alkylbenzyldimethylammonium, 10μM Ammonium Molybdate; 100 μl) was
222 added, and plates incubated at room temperature for 30 min. Plates were read at 340 nm. Lipid
223 peroxide concentration was calculated using the Beer-Lambert-Law with an extinction coefficient
224 of 24600. Values were expressed as nmol/ml plasma,

225

226 *Statistical analysis*

227 ***Paragraph number 16.*** Data were inspected for normal distribution using the Kolmogrov
228 Smirnov test. Non-normally distributed data were transformed logarithmically. For the comet
229 assay, measurements of percent (%) tail DNA were determined to assess the extent of DNA
230 damage as recommended by Duez *et al* (8). Responses to exercise were examined with repeated-
231 measures Analyses of Variance (ANOVAs). Differences compared to baseline were examined
232 using post-hoc paired samples *t*-tests. Statistical significance was accepted at the $p < .05$ level.
233 Effect sizes are presented as η^2. Data are presented as means ± standard error of the mean (SEM)
234 unless otherwise stated. Data were analysed using SPSS statistical package version 18.0 for
235 Windows (SPSS Inc, USA).

236

237

238

239

240

241 **Results**

242 ***Paragraph number 17.*** Four men completed the ultra-endurance race (finishers) and five retired
243 prematurely due to exhaustion (non-finishers). There were no significant physiological
244 differences between finishers and non-finishers, nor were there differences in any of the
245 biochemical or immunological parameters investigated between the two groups (data not shown).
246 Running duration (hours), distance (km) or finishing status (i.e., finisher or non-finisher) did not
247 confound any of the results reported below, as determined by analysis of covariance (14). On this
248 basis, and the fact that all non-finishers had undertaken exercise for more than 12 hours (hh:mm;
249 mean 20:44 ± 07:15 SD), covering the distance of approximately three marathons (mean 126.7
250 km ± 29.7 SD), all participants were included in analyses.

251

252 *DNA damage in PBMC*

253 ***Paragraph number 18.*** Figure 1 shows frank strand breaks and FPG-dependent oxidative DNA
254 damage before and after ultra-endurance exercise. Frank strand breaks increased with exercise
255 (main effects of time; $F_{(4, 32)} = 11.7$, $p < .001$, $\eta^2 = .594$) and were elevated above baseline
256 immediately and 24 hours after the race (paired samples *t*-tests; $t_{(8)} < -5.2$, $p < .01$, see Figure 1).
257 An increase in FPG-dependent oxidative DNA damage was also observed (main effects of time;
258 $F_{(4, 32)} = 2.8$, $p < .05$, $\eta^2 = .257$) and this was elevated above baseline immediately post-race
259 (paired samples *t*-test; $t_{(8)} = -3.1$, $p < .05$, see Figure 1).

260

261 [INSERT FIGURE 1 HERE]

262

263 *Whole blood GSH*

264 ***Paragraph number 19.*** A biphasic antioxidant response was observed with ultra-endurance
265 exercise (main effects of time; $F_{(4, 32)} = 26.0$, $p < .001$, $\eta^2 = .764$, see Figure 2) which reflected
266 the elevated markers of oxidative stress. GSH concentration increased immediately post-race
267 (+32%; paired samples *t*-test; $t_{(8)} = -3.7$, $p < .01$) but was depleted to nearly two-thirds (−60%)
268 of baseline levels 24 hours later (paired samples *t*-test; $t_{(8)} = 6.1$, $p < .001$). At 7 days and 28 days
269 post-race, GSH remained at approximately half to one third (−45 to −31%) of baseline levels
270 respectively (paired samples *t*-tests; $t_{(8)} > 3.5$, $p < .01$, see Figure 2).
271
272 [INSERT FIGURE 2 HERE]
273
274 *Plasma protein carbonylation*
275 ***Paragraph number 20.*** Ultra-endurance exercise resulted in significant plasma protein
276 carbonylation (main effects of time; $F_{(4, 32)} = 7.6$, $p < .001$, $\eta^2 = .370$) (see Figure 3). Post-hoc
277 comparisons to baseline showed that plasma protein carbonyl concentration was elevated for 7
278 days after the race (paired samples *t*-tests; $t_{(8)} > -2.5$, $p < .04$, see Figure 3).
279
280 [INSERT FIGURE 3 HERE]
281
282 *Plasma lipid peroxidation*
283 ***Paragraph number 21.*** Ultra-endurance exercise resulted in plasma lipid peroxidation (main
284 effects of time; $F_{(4, 32)} = 14.0$, $p < .001$, $\eta^2 = .636$) (see Figure 4). Plasma lipid peroxide
285 concentration was elevated immediately post-race, and remained at this level for 24 hours, before
286 falling below baseline 28 days later (paired samples *t*-tests; $t_{(8)} < 3.3$, $p < .05$, see Figure 4).
287

288 [INSERT FIGURE 4 HERE]

289

290 **Discussion**

291 ***Paragraph number 22.*** Studies have shown that ultra-endurance exercise results in oxidative
292 stress, which is alleviated in a matter of hours or days depending on the biomarker assessed (19,
293 22, 31, 35). The present investigation expands upon previous investigations by employing a more
294 prolonged blood sampling regimen (up to 28 days post-race). This study measured several
295 oxidative stress biomarkers, including a comparison between non-specific DNA damage (i.e.,
296 frank strand breaks) and oxidative-specific DNA damage (FPG-dependent oxidative DNA
297 damage).

298

299 ***Paragraph number 23.*** The results confirmed that ultra-endurance exercise is associated with
300 increases in markers of oxidative stress. FPG-dependent oxidative DNA damage was increased
301 immediately post-race, whereas frank strand breaks and lipid peroxidation were elevated for 24
302 hours. Augmented protein oxidation was detected 7 days after the race. In addition, we showed
303 for the first time, that whole blood GSH exhibits a large biphasic response; increasing by one-
304 third post-race, and falling almost two-thirds below normal levels 24 hours later. Strikingly, the
305 antioxidant capacity of blood remained at one-third of initial values 28 days after the race - a
306 similar level of GSH depletion as seen in Alzheimers disease patients (3).

307

308 ***Paragraph number 24.*** The present study showed that lipids were susceptible to peroxidation for
309 24 hours after ultra-endurance exercise, which is consistent with previous reports (22, 31). This
310 increase in lipid peroxidation was accompanied by a fall in lipid peroxide concentration 28 days
311 post-race. Increased lipid peroxidation is associated with cardiovascular disease, and some

312 markers of oxidised lipids (e.g., malondialdehyde) are mutagenic to DNA increasing the risk of
313 cancer (23, 28). Conversely, decreased lipid peroxidation has previously been observed following
314 exercise (12, 18) and it has been suggested that this may be one of the mechanisms behind the
315 atheroprotective effect of physical activity (12, 15). Further, increased protein oxidation was
316 detected for up to7 days post-race which could partly be attributed to the chemical stability of
317 protein carbonyl groups (6). Protein carbonylation has been associated with altered protein
318 function and receptor interaction; for example carbonylation of the protein moiety of low density
319 lipoprotein is known to increase uptake into blood monocytes (2).
320
321 ***Paragraph number 25.*** In the present study, ultra-endurance exercise caused an increase in frank
322 strand breaks, which remained elevated for 24 hours. Damage to DNA is linked to increased
323 cancer and cardiovascular disease risk (39), and elevated levels of DNA damage have been found
324 in atherosclerotic plaques (20). Following ultra-endurance exercise, some of this damage was
325 attributable to exercise-induced ROS production, since FPG-dependent oxidative DNA damage
326 was increased post-race, returning to baseline within 24 hours. The transient appearance of FPG-
327 dependent oxidative DNA damage, and the subsequent return to baseline, likely reflects up-
328 regulation of repair mechanisms in which oxidised purine bases (e.g., 8-oxoguanine) are repaired
329 or removed by 8-oxoguanine DNA glycosylase (OGG1) (26). Indeed, OGG1 activity is increased
330 approximately 16-18 hours after a marathon (25) which coincides with the return of FPG-
331 dependent oxidative DNA damage to baseline in this study. It is possible that frank strand breaks
332 did not follow the same pattern of repair, because this includes oxidised pyrimidines which are
333 not repaired by OGG1. Instead, pyrimidine lesions are repaired by endonuclease III homolog
334 (NTH1), the activity of which, is not up-regulated following exercise (25). In addition, these
335 purine lesions would not be detected (and therefore not included in our measure of oxidative

336 DNA damage) by FPG in our assay. As well as the risks that DNA damage might confer, damage
337 to lymphocytes might inhibit cell-mediated immunity post-exercise, which is in line with reports
338 of increased infection risk following endurance exercise events (24).
339
340 ***Paragraph number 26.*** Perhaps the most striking finding of this study is that GSH was depleted
341 to one third of baseline levels at 28 days post-race. The baseline GSH concentrations observed in
342 this study were not elevated when compared to other reports in healthy individuals (e.g., 31).
343 GSH is a molecule that is key in cellular redox status regulation (11, 16, 34, 36) and
344 consequences of prolonged GSH depletion may include a compromise in immunity. For example,
345 lower GSH is associated with decreased lymphocyte proliferation and increased viral
346 reactivation. (16, 33, 34)
347
348 ***Paragraph number 27.*** A limitation of this investigation is that a control group was not included.
349 However, prior studies employing a non-exercise control did not observe differences in oxidative
350 stress during the resting control trial (e.g., 21). A further potential limitation is that only 4 out 5
351 subjects completed the race. All subjects were included in analyses because even non-finishers
352 engaged in more than 12 hours of continuous exercise, and covered the distance of more than
353 three marathons. Importantly, there were no physiological, biochemical, or immunological
354 differences between finishers and non-finishers. Thus, as we expected, controlling statistically for
355 finishing status (i.e., finisher *versus.* non-finisher), duration of exercise, or running distance had
356 no effects on the results reported.
357
358 ***Paragraph number 28.***

This study confirms that ultra-endurance exercise causes oxidative stress, which persists for 1 calendar month post-exercise, depending on the specific biomarker examined. For the first time, we show that blood antioxidant capacity, as measured by GSH, is significantly depleted below baseline for 28 days post-race. These results suggest that ultra-endurance events are associated with a period of reduced protection against oxidative stress.

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Conflict of interest

None.

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Figure captions

Figure 1. Frank strand breaks to PBMC DNA (closed bars) and FPG-dependent oxidative DNA damage (open bars). Data are Means ± SEM, expressed as percent (%) DNA in the comet tail. ** $p < .01$, * $p < .05$ compared to baseline (paired samples t-tests).

Figure 2. Whole blood reduced glutathione (GSH) concentration. Data are Means ± SEM. *** $p < .001$, ** $p < .01$ compared to baseline (paired samples t-tests).

Figure 3. Plasma protein carbonyl concentration. Data are Means ± SEM. *** $p < .001$, ** $p < .01$, * $p < .05$ compared to baseline (paired samples t-tests).

Figure 4. Plasma lipid peroxide concentration. Data are Means ± SEM. * $p < .05$ compared to baseline (paired samples t-tests).

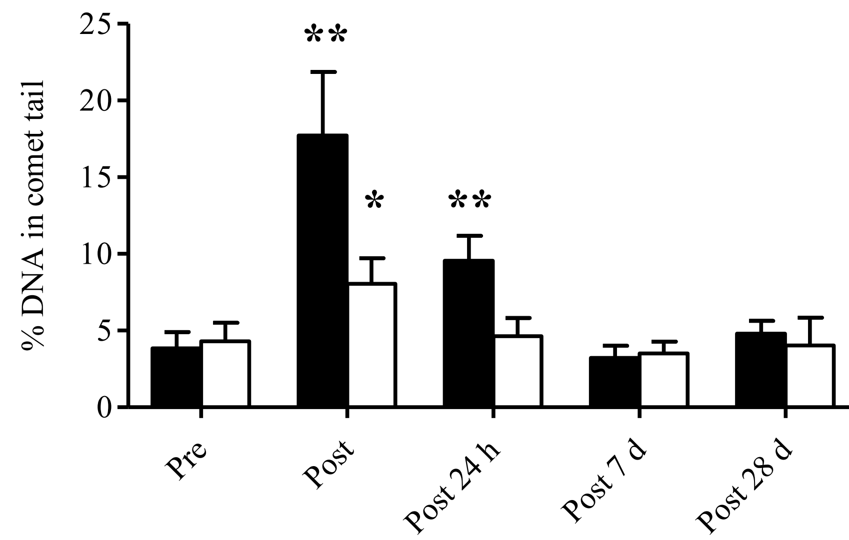
Table 1. Characteristics of participants and results from the ultra-endurance race (mean ± SD).

Characteristics of participants	*n* = 9
Age (years)	46.1 ± 5.3
BMI ($kg \cdot m^{-2}$)	24.9 ± 2.3
Body fat (%)	14.1 ± 2.4
LTPA[a] (Median $MET \cdot min \cdot week^{-1}$)[b]	3393 (2040-4850)
Measured $\dot{V}O_2$ max ($ml \cdot kg^{-1} \cdot min^{-1}$)	56.3 ± 3.3
Maximum heart rate at $\dot{V}O_2$ max (bpm)	175 ± 5
Ultra-endurance race results	
Running distance (km)	174.1 ± 60.0
Running duration (hh:mm)	30:02 ± 12:14

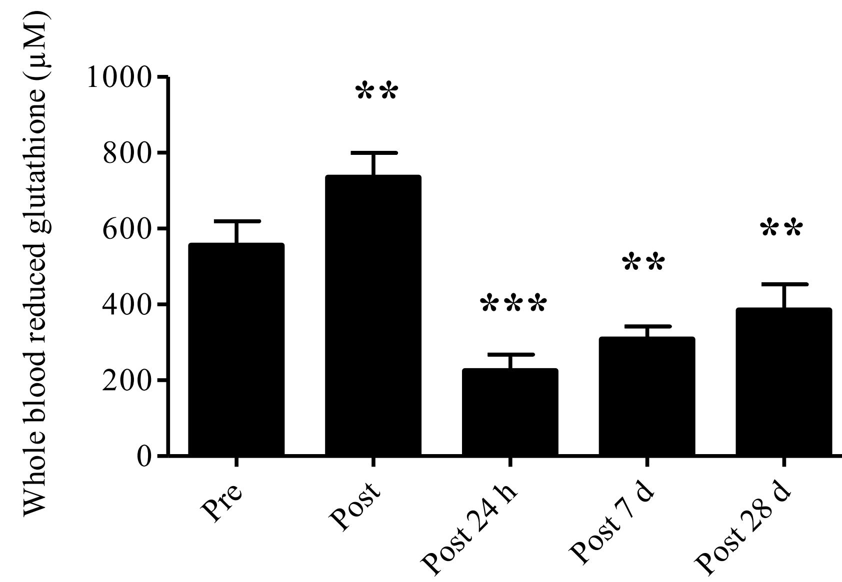
[a] Leisure Time Physical Activity, as assessed by the international physical activity questionnaire (IPAQ).

[b] Data are medians (min-max).

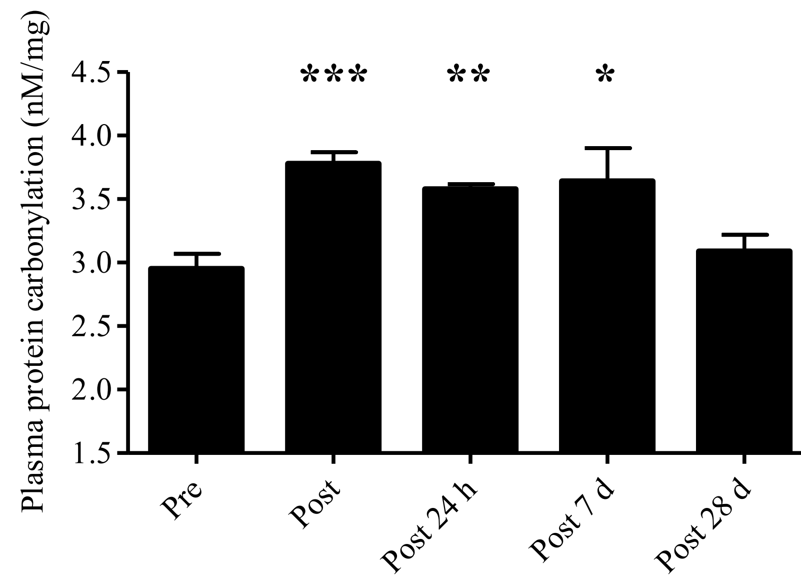
Turner. Ultra-running and glutathione depletion. Figure 1.



Turner. Ultra-running and glutathione depletion. Figure 2.



Turner. Ultra-running and glutathione depletion. Figure 3.



Turner. Ultra-running and glutathione depletion. Figure 4.