

# **Influence of vitamin C and vitamin E on redox signalling: implications for exercise adaptations**

J. N. Cobley, H. McHardy, J. P. Morton, M. G. Nikolaidis, G. L. Close

This is the accepted manuscript © 2015, Elsevier.  
Licensed under the Creative Commons Attribution-NonCommercial-  
NoDerivatives 4.0 International  
(<http://creativecommons.org/licenses/by-nc-nd/4.0/>)



The published article is available from doi:  
<http://dx.doi.org/10.1016/j.freeradbiomed.2015.03.018>

1 Influence of vitamin C and vitamin E on redox signalling: implications for exercise  
2 adaptations

3 Cobley JN<sup>1</sup>, McHardy H<sup>1</sup>, Morton JP<sup>2</sup>, Nikolaidis MG<sup>3</sup> & Close GL<sup>2</sup>

- 4
- 5 1. Division of Sport and Exercise Sciences, Abertay University, Dundee, UK, DD1 1HG
  - 6 2. Research Institute for Sport and Exercise Science, Liverpool John Moores University,  
7 Liverpool, UK, L3 3AF
  - 8 3. School of Physical Education and Sport Sciences at Serres, Aristotle University of  
9 Thessaloniki, Serres, Greece
- 10  
11  
12

13 **Address for correspondence:**

14 Dr James N Cobley  
15 Division of Sport and Exercise Sciences  
16 Abertay University  
17 Kydd Building  
18 Dundee  
19 DD1 1HG  
20 United Kingdom  
21 Email: [J.Cobley@abertay.ac.uk](mailto:J.Cobley@abertay.ac.uk)  
22 Tel: +44(0)1382 308178  
23

24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36

37 **Abstract**

38 The exogenous antioxidants vitamin C (ascorbate) and vitamin E ( $\alpha$ -tocopherol) often blunt  
39 favourable cell signalling responses to exercise, suggesting that redox signalling contributes  
40 to exercise adaptations. Current theories posit that this antioxidant paradigm interferes with  
41 redox signalling by attenuating exercise-induced reactive oxygen species (ROS) and reactive  
42 nitrogen species (RNS) generation. The well-documented *in vitro* antioxidant actions of  
43 ascorbate and  $\alpha$ -tocopherol and characterisation of the type and source of the ROS/RNS  
44 produced during exercise theoretically enables identification of the redox-dependent  
45 mechanism responsible for the blunting of favourable cell signalling responses to exercise.  
46 This review aimed to apply this reasoning to determine how the aforementioned antioxidants  
47 might attenuate exercise-induced ROS/RNS production. The principal outcomes of this  
48 analysis are (1) neither antioxidant is likely to attenuate nitric oxide signalling either directly  
49 (reaction with nitric oxide) or indirectly (reaction with derivatives, e.g. peroxynitrite) (2)  
50 neither antioxidant reacts appreciably with hydrogen peroxide, a key effector of redox  
51 signalling (3) ascorbate but not  $\alpha$ -tocopherol has the capacity to attenuate exercise-induced  
52 superoxide generation and (4) alternate mechanisms, namely pro-oxidant side reactions  
53 and/or reduction of bioactive oxidised macromolecule adducts, are unlikely to interfere with  
54 exercise-induced redox signalling. Out of all the possibilities considered, ascorbate mediated  
55 suppression of superoxide generation with attendant implications for hydrogen peroxide  
56 signalling is arguably the most cogent explanation for blunting of favourable cell signalling  
57 responses to exercise. However, this mechanism is dependent on ascorbate accumulating at  
58 sites rich in NADPH oxidases, principal contributors to contraction mediated superoxide  
59 generation, and outcompeting nitric oxide and superoxide dismutase isoforms. The major  
60 conclusions of this review are: (1) direct evidence for interference of ascorbate and  $\alpha$ -  
61 tocopherol with exercise-induced ROS/RNS production is lacking (2) theoretical analysis  
62 reveals that both antioxidants are unlikely to have a major impact on exercise-induced redox  
63 signalling and (3) it is worth considering alternate redox-independent mechanisms.

64 **Key words:** Vitamin C, Vitamin E, antioxidant, reactive oxygen species, reactive nitrogen  
65 species, exercise adaptations, oxidative stress

66 **Abbreviations:** 5LOX: 5-lipoxygenase; AP-1: Activating Protein 1; cGMP: Cyclic  
67 Guanosine Monophosphate; ERK: Extracellular Signal-Regulated Kinase; GSH: Glutathione  
68 (reduced); GSSG: Glutathione (oxidised); H<sub>2</sub>O<sub>2</sub>: Hydrogen Peroxide; HIF- $\alpha$ : Hypoxia  
69 Inducible Factor Alpha; HSF-1: Heat Shock Factor 1; HSP90: Heat Shock Protein 90;  
70 JNK:c-Jun N-terminal Kinase; KEAP-1: Kelch-like ECH-Associated Protein 1; NADPH  
71 oxidase: Nicotinamide Adenine Dinucleotide Phosphate-Oxidase; NF- $\kappa$ B: Nuclear Factor  
72 Kappa Beta; NO: Nitric Oxide; NOS: Nitric Oxide Synthase; Nrf2: Nuclear Factor  
73 (erythroid-derived 2)-like 2; p38 MAPK: p38 Mitogen Activated Protein Kinase; PGC-1 $\alpha$ :  
74 Peroxisome Proliferator-Activated Receptor Gamma Coactivator 1-alpha; PTEN:  
75 Phosphatase and Tensin Homolog; SHP-2: Src Homology Protein-2; SOD: Superoxide  
76 Dismutase; Src: STAT3: Signal Transducer and Activator of Transcription 3

78 **Introduction**

79 In the last year, many studies have observed that exogenous antioxidant supplementation,  
80 principally ascorbate and  $\alpha$ -tocopherol co-supplementation, blunts favourable molecular  
81 responses to exercise training [1-3]. These findings confirm some [4-7] but not others [8-14]  
82 in this area [reviewed in 15-18]. Irrespective of the outcome, all of the aforementioned  
83 studies share a common mechanistic rationale that depends on the antioxidant action of  
84 ascorbate and  $\alpha$ -tocopherol (see figure 1A). This redox dependent mechanism is often  
85 assumed, yet seldom confirmed by any biochemical measurements. That is, evidence to  
86 support the postulate that redox-dependent mechanisms are responsible for the observed  
87 results is rarely presented. A redox-dependent mechanism of action principally rests on the  
88 assumption that ascorbate and  $\alpha$ -tocopherol react appreciably with reactive oxygen species  
89 (ROS) and reactive nitrogen species (RNS) implicated in redox signalling (see box 1). In line  
90 with a recent commentary [19] the terms ROS/RNS are not used hereafter for two reasons (1)  
91 they convey limited mechanistic information and (2) the two electron oxidants that  
92 principally mediate redox signalling (e.g. peroxynitrite) are known. The well-documented *in*  
93 *vitro* antioxidant actions of ascorbate and  $\alpha$ -tocopherol and characterisation of the sources of  
94 superoxide and nitric oxide (NO) generation, precursors of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and  
95 peroxynitrite, during exercise in skeletal muscle enables the veracity of this assumption to be  
96 explored (see figure 1B). Possible redox-dependent mechanisms for these results are  
97 appraised herein.

98 **Redox signalling**

99 Cell signalling enables cells to integrate information provided by internal and external cues  
100 into an orchestrated biological response [20-22]. A fundamental aspect of cell signalling is  
101 the propagation, via regulated biochemical reactions, of specific and reversible  
102 compartmentalised signals [20-22]. There is an increasing realisation and indeed evidence  
103 base supporting the notion that redox-dependent mechanisms contribute to cell signalling  
104 processes [23-29]. The basic premise of redox signalling is that two electron oxidants,  
105 principally H<sub>2</sub>O<sub>2</sub>, regulate specific and reversible post-translational modifications to thiol  
106 (SH) moieties on target proteins implicated in cell signalling [27]. Salient modifications  
107 include *inter alia*: disulphide formation, sulfenic acid formation, S-nitrosylation and S-  
108 glutathionylation [23-31]. Of course, redox signalling is not limited to thiol modification with  
109 other processes contributing, notably oxidation of other amino acids (e.g. methionine) and  
110 oxidised macromolecule adducts (e.g. 4-hydroxynonenal [25, 32-33]). Whilst the biological  
111 importance of redox signalling is clear, the underpinning mechanisms are unresolved [23-25,  
112 34]. This is best evidenced by the chemical constraints that could limit the reaction of H<sub>2</sub>O<sub>2</sub>  
113 with thiol moieties on target proteins (see below [24-25]). It is, therefore, clear that redox  
114 signalling is important but that elucidating the underpinning mechanisms requires further  
115 research.

116

117

## 118 **Exogenous antioxidants, exercise and redox signalling**

119 One conceptual model of exercise adaptation posits that ‘exercise signals’ (e.g. altered  $\text{Ca}^{2+}$   
120 flux and energy status) during acute exercise bouts activate signalling pathways, that with  
121 repeated activation (multiple exercise bouts), yield exercise adaptations [35-37]. From a  
122 redox perspective, increased exercise-induced superoxide, NO, peroxynitrite and  $\text{H}_2\text{O}_2$   
123 generation is an ‘exercise signal’ implicated in the regulation of beneficial cyto-protective  
124 and mitochondrial exercise adaptations [38-41]. Cyto-protective adaptations confer increased  
125 resistance to oxidative stress owing to increased glutathione content, antioxidant enzyme  
126 activity and content coupled to up-regulation of cyto-protective proteins, notably heat shock  
127 proteins [42-45]. Mitochondrial adaptations are principally manifested by increased  
128 mitochondrial content and consequent metabolic adaptations post-training [46-49]. At the  
129 molecular level, increased contraction-mediated superoxide, NO, peroxynitrite and  $\text{H}_2\text{O}_2$   
130 generation is implicated in the regulation of several signalling proteins, including kinases  
131 (e.g. p38 MAPK [50]), transcriptional co-activators (e.g. PGC-1 $\alpha$  [51]) and transcription  
132 factors (e.g. NF- $\kappa$ B, HSF-1, AP-1 and Nrf2 [38-41; 52]). Akin to the parent discipline,  
133 knowledge of mechanisms underpinning exercise-induced redox signalling is fragmentary.  
134 That is, how contraction-mediated superoxide, NO, peroxynitrite and  $\text{H}_2\text{O}_2$  generation  
135 impacts the post-translational state of redox-sensitive signalling proteins remains to be fully  
136 resolved and demonstrated in an exercise setting. Exercise-induced redox signalling could  
137 involve free radical (e.g. superoxide) and non-radical mediated (e.g. peroxynitrite)  
138 mechanisms [26-28]. The aforementioned mechanisms will next be considered in turn but it  
139 is emphasised that the impact of ascorbate and  $\alpha$ -tocopherol cannot be fully appraised until  
140 the mechanistic nature of exercise-induced redox signalling is better understood. The need to  
141 advance knowledge of exercise-induced redox signalling constitutes a major theme of this  
142 review.

## 143 **Direct signalling**

144 Skeletal muscle contractions are associated with a transient increase in superoxide and NO  
145 generation, secondary to NADPH oxidase and nitric oxide synthase (NOS) isoform  
146 activation, respectively [53-56]. It is, therefore, necessary to consider whether (1) direct  
147 redox signalling by superoxide and NO is possible (2) ascorbate and  $\alpha$ -tocopherol react  
148 appreciably with either radical (3) this reaction out-competes other reactions and (4) any  
149 reaction interferes with compartmentalised redox signalling.

## 150 ***Superoxide***

151 There are several sources of superoxide in skeletal muscle, including: mitochondrial electron  
152 transport chain complex I and III, NADPH oxidases, dual oxidases, xanthine oxidase,  
153 uncoupled NOS isoforms, phospholipases and lipoxygenases [57-59]. Recent data suggest  
154 that NADPH oxidases are the principal contributors to contraction mediated superoxide  
155 generation [60-61]. NADPH oxidases are expressed at several locations in skeletal muscle,  
156 including: mitochondria, sarcolemma, transverse tubules and sarcoplasmic reticulum [60-64].  
157 From a signalling perspective, superoxide does not react appreciably with thiols ( $k \sim 10^3 \text{ M}^{-1}$

158 s<sup>-1</sup> [65]) and any reaction would have to outcompete the kinetically favourable ( $k \sim 10^9 \text{ M}^{-1} \text{ s}^{-1}$ )  
159 reaction of superoxide with superoxide dismutase (SOD) isoforms [66]. Hence, signalling  
160 via this mechanism is unlikely *in vivo* [23, 66]. It should be noted that the reaction of  
161 superoxide with thiols is complex and involves intermediate thiyl radicals that ultimately  
162 result in the regeneration of superoxide [29, 65]. It is also of note that superoxide is not that  
163 reactive with most biomolecules [66-67]. Indeed, superoxide is more of a reductant than an  
164 oxidant unless protonated [66-67]. Nevertheless, we do not exclude the possibility that  
165 elevated superoxide concentrations allied to target co-localisation might overcome this  
166 kinetic constraint [28, 68]. Whilst the reaction with thiols might be unlikely, superoxide can  
167 react with protein metal centres directly [69]. One example relevant to exercise is the  
168 involvement of superoxide in the regulation of HIF- $\alpha$ , a protein that regulates exercise-  
169 induced angiogenesis [70-71]. Superoxide can react with the metal centre of propyl  
170 hydroxylase, an inhibitor of HIF- $\alpha$ , converting Fe<sup>2+</sup> to Fe<sup>3+</sup> and inactivating the enzyme [72].  
171 Direct signalling by superoxide is, therefore, possible but comes with the caveat that this  
172 mechanism is not well characterised and thiol oxidation seems unlikely.

173 Although, under-characterised and indeed unlikely in some contexts (e.g. thiol oxidation)  
174 superoxide may contribute to exercise-induced redox signalling. Providing a potential  
175 mechanism for ascorbate and  $\alpha$ -tocopherol to blunt exercise-induced redox signalling  
176 provided either antioxidant reacts appreciably with superoxide.  $\alpha$ -tocopherol does not react  
177 appreciably with superoxide, partly owing to its poor solubility in aqueous solution and the  
178 negative charge of superoxide that restricts diffusion across biological membranes [69, 73]. It  
179 follows that  $\alpha$ -tocopherol is extremely unlikely to interfere with exercise-induced redox  
180 signalling in this fashion. A redox-independent mechanism is possible via inhibition of 5-  
181 lipoxygenase (5-LOX) activity [74-75] but this has not been demonstrated in skeletal muscle  
182 cell lines.

183 Ascorbate can directly react with superoxide ( $k \sim 10^5 \text{ M}^{-1} \text{ s}^{-1}$  [76]). From a kinetic  
184 perspective, therefore, ascorbate mediated scavenging of superoxide with attendant  
185 implications for redox signalling is possible. Human skeletal muscle is highly responsive to  
186 ascorbate supplementation [77-78]. Indeed, levels can be increased by ~3.5 fold post-  
187 supplementation [77]. Elevated ascorbate concentrations post-supplementation increase the  
188 likelihood of the ascorbate-superoxide reaction occurring. This could have signalling  
189 implications provided (1) ascorbate out-competes other reactants and (2) reacts in the relevant  
190 microdomain. Whether ascorbate out-competes other reactants, namely SOD isoforms and  
191 NO for superoxide [78], is not known. It is unlikely however, that ascorbate out-competes the  
192 diffusion-limited superoxide-NO reaction [78]. Redox signalling is compartmentalised and  
193 subject to intricate spatiotemporal regulation [80-86]. Spatiotemporal regulation of different  
194 redox-sensitive networks is controlled, in part, by various subcellular redox couples (e.g.  
195 GSH/GSSG) that are not in equilibrium [80-86]. That is, redox couples in different  
196 microdomains and organelles exhibit different redox potentials and are not necessarily  
197 interlinked [80-86]. For instance, a signalling event might involve oxidation of the  
198 cytoplasmic but not nuclear GSH pool [80-82]. It follows that, the reaction of ascorbate with  
199 superoxide requires spatial context for proper interpretation. For example, if it is assumed

200 that exercise-induced redox signalling occurred in the caveolae of the plasma membrane  
201 following NADPH oxidase activation and resultant superoxide generation. Then ascorbate  
202 would need to be present in this microdomain to effect a reduction in the amount of  
203 superoxide available for reaction with a target or dismutation to H<sub>2</sub>O<sub>2</sub>. In this scenario, the  
204 initial signalling event would be unperturbed by reaction of ascorbate with superoxide in  
205 other microdomains (e.g. cytoplasm). Signalling requires only a small proportion of the total  
206 target protein population to be modified hence it is noted that signalling could still proceed  
207 despite some reduction in superoxide and target protein modification levels. Whether  
208 ascorbate is present in the relevant microdomains remains an open question. Overall,  
209 ascorbate reacts with superoxide but the spatiotemporal nature of this reaction and its  
210 relevance to exercise-induced redox signalling requires further investigation.

### 211 *Nitric oxide*

212 NOS isoforms utilise L-arginine to catalyse NO production [87]. The principal NOSs in  
213 skeletal muscle are nNOS (localised to the sarcolemma), eNOS (localised to the  
214 mitochondria) and iNOS the inducible isoform [88-89]. Skeletal muscle contractions increase  
215 intra and extracellular NO generation [55-56]. NO activates guanylate cyclases, via reversible  
216 heme group binding, to generate the signalling biomolecule cGMP [87]. This signalling  
217 mechanism is associated with several physiological outcomes, notably vasodilation following  
218 NO generation by vascular endothelial cells [90], but is not generally considered to be redox  
219 signalling *per se* [25]. Rather, NO based redox signalling is typically indirect in nature,  
220 proceeding through reaction of NO with other radicals [28]. Any reaction of exogenous  
221 antioxidants with NO directly would, therefore, be of consequence for indirect signalling. In  
222 this regard, NO reacts rapidly with other ROS/RNS, notably superoxide, but reacts slowly  
223 with other cellular biomolecules [91]. Hence, ascorbate and  $\alpha$ -tocopherol have limited ability  
224 to suppress NO directly [69]. It is, however, recognised that ascorbate could influence NO  
225 bioavailability with possible implications for indirect signalling [92-93]. NOS mediated NO  
226 generation is contingent upon several co-factors, notably tetrahydrobiopterin (BH<sub>4</sub> [94]). Low  
227 levels of BH<sub>4</sub> and/or ablated BH<sub>4</sub> binding uncouple NOS isoforms resulting in the production  
228 of superoxide [93]. NOS uncoupling is implicated in the pathophysiology of cardiovascular  
229 disease [95]. Ascorbate is suggested to prevent NOS isoform uncoupling and thus enhance  
230 NO bioavailability [92]. The underpinning mechanisms remain to be fully resolved but might  
231 involve superoxide suppression [92], reduction in BH<sub>4</sub> oxidation and/or reduction of oxidised  
232 intermediaries (e.g. BH<sub>3</sub> [93]). The implication of this is unclear from a signalling perspective  
233 and may not be relevant in non-pathological settings. Overall, neither antioxidant can  
234 interfere with NO signalling by direct reaction but ascorbate might influence NO  
235 bioavailability, the outcome of this being unclear in an exercise setting.

### 236 **Indirect signalling**

#### 237 *Peroxynitrite*

238 Peroxynitrite, a term encompassing peroxynitrite anion and its protonated form peroxynitrous  
239 acid, is an extremely labile reactive species generated by the diffusion controlled reaction

240 between NO and superoxide ( $k \sim 4-16 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$  [97-100]). The aforementioned reaction  
241 proceeds at a significantly faster rate than the reaction of superoxide with SOD isoforms ( $k \sim$   
242  $1-2 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$  [88, 101]), rendering peroxynitrite generation a likely fate of NO and  
243 superoxide produced during muscle contractions [102]. From a signalling perspective, direct  
244 signalling by peroxynitrite is unlikely owing to rapid reaction with peroxiredoxins ( $k \sim 10^6-$   
245  $10^7 \text{ M}^{-1} \text{ s}^{-1}$  [99, 103-106]) and  $\text{CO}_2$  ( $k \sim 5.8 \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$  [99, 104, 107-108]). The rather slow  
246 reaction ( $k \sim 10^2 \text{ M}^{-1} \text{ s}^{-1}$  for ascorbate [69]) of both ascorbate and  $\alpha$ -tocopherol with  
247 peroxynitrite is unlikely to outcompete the aforementioned rapid reactants. It is improbable  
248 that this reaction out-competes the moderate reaction of peroxynitrite with glutathione ( $k =$   
249  $1.35 \times 10^3 \text{ M}^{-1} \text{ s}^{-1}$  [77]), given the abundance, present at millimolar concentrations in most  
250 cells, of glutathione. Further, diffusion of peroxynitrite across biological membranes is  
251 limited, rendering reaction with  $\alpha$ -tocopherol unlikely [77]. It is necessary, therefore, to  
252 consider whether ascorbate or  $\alpha$ -tocopherol can modulate indirect peroxynitrite signalling.

253 Indirect peroxynitrite signalling could proceed via (1) coupled sensing and metabolism  
254 mechanism, wherein peroxiredoxins function as sensor proteins that transmit the signal (2)  
255 reaction with glutathione and generation of thiyl radicals and/or (3) radical derivatives of the  
256 reaction of peroxynitrite with  $\text{CO}_2$  [25, 28]. Ascorbate and  $\alpha$ -tocopherol are unlikely to  
257 interfere with any peroxiredoxin associated sensing-metabolism signalling. This would  
258 necessitate outcompeting two highly abundant and efficient reactants,  $\text{CO}_2$  and  
259 peroxiredoxins, for peroxynitrite and hence will not be further considered herein.  
260 Analogously, neither antioxidant will likely out-compete glutathione to blunt any thiyl radical  
261 associated signalling. In any case, the principal biological fate of peroxynitrite is rapid  
262 reaction with  $\text{CO}_2$  to generate short-lived intermediaries (e.g. nitrosoperoxocarbonate) that  
263 can form radical products following homolysis, notably carbonate radical and nitrogen  
264 dioxide [99, 104, 107-108]. It is possible that signalling proceeds through carbonate radical  
265 and nitrogen dioxide, as both are one electron oxidants [109] that could be implicated in thiol  
266 based signalling [28]. The capacity of these radicals to be second messengers in redox  
267 signalling might be limited by their non-selective reaction with protein thiols. Both radicals  
268 can initiate protein nitration with attendant implications for redox signalling [110]. For  
269 instance, nitration of HSP90 at specific residues (Tyr 33 & 56) induces neuronal apoptosis via  
270 the Fas pathway [110]. It can also inactivate antioxidant enzymes (e.g. SOD2 and GPx1 [111-  
271 113]), which could facilitate transient transmission of a redox signal [114-115]. As a  
272 signalling paradigm, protein nitration could be limited by its random nature and lack of  
273 reversibility. Nevertheless, ascorbate or  $\alpha$ -tocopherol mediated scavenging of carbonate  
274 radical and nitrogen dioxide could blunt subsequent thiol and/or protein nitration based  
275 signalling.

276 Ascorbate reacts with both carbonate radical and nitrogen dioxide [109]. In particular, the  
277 reaction of ascorbate with nitrogen dioxide ( $k \sim 3.5 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$ ) is similar to glutathione ( $k \sim$   
278  $2 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$ ) and the reaction of nitrogen dioxide with tyrosine radical ( $k \sim 3.2 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$ ),  
279 an intermediate in the formation of nitrated proteins [77, 116]. Increased ascorbate  
280 concentrations post-supplementation could facilitate scavenging to attenuate nitrogen dioxide  
281 mediated protein nitration or thiol oxidation. The relevance of this for redox signalling is ill



282 defined and this represents a considerable caveat. Further, ascorbate would have to attenuate  
283 nitrogen dioxide formation proximal to the signalling reaction (nitrogen dioxide-protein  
284 tyrosine residue) as blunting signalling depends on interfering with spatially regulated  
285 cascades [80-83]. Distal reactions would be likely to just attenuate macromolecule damage  
286 without impinging redox signalling [80-83]. Any reaction of  $\alpha$ -tocopherol with carbonate  
287 radical is likely biologically irrelevant, since the charge state of carbonate radical restricts  
288 diffusion through lipid bilayers [109, 117]. In contrast, nitrogen dioxide is uncharged and can  
289 react with  $\alpha$ -tocopherol ( $k \leq 10^6 \text{ M}^{-1} \text{ s}^{-1}$  [116]). However,  $\alpha$ -tocopherol is not considered an  
290 efficient nitrogen dioxide scavenger [116] and is likely out-competed by other reactants (e.g.  
291 glutathione), despite any increases in  $\alpha$ -tocopherol membrane content post-supplementation.  
292 Overall, it is clear that (1) neither antioxidant is likely to interfere with indirect signalling  
293 associated with peroxiredoxins or glutathione (2)  $\alpha$ -tocopherol is unlikely to interfere with  
294 any carbonate and nitrogen dioxide signalling but this is theoretically possible for ascorbate  
295 and (3) the importance of carbonate radical and nitrogen dioxide for redox signalling is  
296 unclear, questioning the biological relevance of any interference.

### 297 *Hydrogen peroxide*

298 Several aspects of redox signalling have been attributed to  $\text{H}_2\text{O}_2$ , a relatively stable and  
299 membrane permeable reactive oxygen species [23-29, 118-121]. The basic mechanism of  
300  $\text{H}_2\text{O}_2$  mediated signalling involves changes in target protein function following oxidation of  
301 cysteine residues to form sulfenic acid and disulphide bonds [26-27]. The reaction of  $\text{H}_2\text{O}_2$   
302 with highly abundant enzymes, notably glutathione peroxidase ( $k \sim 10^8 \text{ M}^{-1} \text{ s}^{-1}$  [122]), catalase  
303 ( $k \sim 2.0 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$  [123]) and peroxiredoxins ( $10^5$ - $10^8 \text{ M}^{-1} \text{ s}^{-1}$  [106, 124]), proceeds at a  
304 significantly faster rate than its reaction with reactive cysteine residues on low abundant  
305 signalling proteins (e.g. KEAP1 estimated  $k \sim 140 \text{ M}^{-1} \text{ s}^{-1}$  [125]). It would, at first glance,  
306 seem that  $\text{H}_2\text{O}_2$  signalling would be precluded, owing to the  $\text{H}_2\text{O}_2$  signal being metabolised  
307 before reaction with target proteins [23, 25]. There are several explanations for redox  
308 signalling proceeding despite this chemical bottleneck (see 28, 125), however three are  
309 particularly cogent. First, the  $\text{H}_2\text{O}_2$  metabolising enzymes could act as sensors themselves, as  
310 has been suggested for peroxiredoxin isoforms [25; 126]. Indeed, peroxiredoxin 2 acts as a  
311 signal receptor and transmitter in STAT3 signalling [127]. Second, post-translational  
312 modifications (e.g. phosphorylation) could alter the catalytic efficiency of  $\text{H}_2\text{O}_2$  metabolising  
313 enzymes, permitting transient transmission of a redox signal [25, 114-115]. Third, co-  
314 localisation of target and source allied to a favourable target protein microenvironment,  
315 principally manifested by an exposed thiol with low  $\text{p}K_a$  [23-29; 128-129]. It is apparent that  
316 the mechanistic details of  $\text{H}_2\text{O}_2$  mediated signalling require further investigation [23].

317 Despite the aforementioned mechanistic considerations,  $\text{H}_2\text{O}_2$  mediated signalling is  
318 implicated in the regulation of kinases, phosphatases, transcriptional co-activators and  
319 transcription factors in various subcellular compartments [23-29; 125]. For instance, kinases  
320 and phosphatases modulate cell signalling via catalysing phosphorylation and  
321 dephosphorylation of protein residues, respectively [129-130]. Oxidation of cysteine residues  
322 in the catalytic domain of these enzymes, results in reversible activation of tyrosine kinases  
323 (e.g. Src [130]) and inactivation of phosphatases (e.g. PTEN and SHP-2 [131]). This redox

324 signalling paradigm is important for the propagation of growth factor signalling (e.g.  
325 epidermal growth factor), as demonstrated by genetic over-expression of H<sub>2</sub>O<sub>2</sub> metabolising  
326 enzymes [132]. Indeed, growth factor activation stimulates localised H<sub>2</sub>O<sub>2</sub> generation in  
327 several cell types, probably owing to NADPH oxidase mediated superoxide production and  
328 subsequent dismutation to H<sub>2</sub>O<sub>2</sub> [130]. In an exercise setting, H<sub>2</sub>O<sub>2</sub> mediated inactivation of  
329 mitogen activated protein kinase phosphatase could promote p38 MAPK, JNK and ERK  
330 activation, proteins implicated in exercise-induced cell signalling [36]. Although, the precise  
331 events have yet to be defined, H<sub>2</sub>O<sub>2</sub> is likely a key effector of exercise-induced redox  
332 signalling.

333 It is noteworthy that neither ascorbate nor  $\alpha$ -tocopherol react appreciably with H<sub>2</sub>O<sub>2</sub> [133]  
334 and hence, *prima facie*, have limited capacity to directly impact this important redox  
335 signalling mechanism. Even if they could react with H<sub>2</sub>O<sub>2</sub>, both ascorbate and  $\alpha$ -tocopherol  
336 would be unlikely to out-compete endogenous H<sub>2</sub>O<sub>2</sub> reactants, such as peroxiredoxins [24].  
337 There are, however, two indirect mechanisms that warrant consideration. First, SOD isoforms  
338 catalyse the dismutation of superoxide to H<sub>2</sub>O<sub>2</sub> [134]. Ascorbate could indirectly attenuate  
339 the H<sub>2</sub>O<sub>2</sub> signal via reaction with superoxide, provided spatiotemporal concerns are satisfied,  
340 localised reaction with superoxide in the relevant microdomain (see superoxide section), and  
341 other reactants are outcompeted (e.g. NO). Any attenuation of the H<sub>2</sub>O<sub>2</sub> signal could have  
342 ramifications for superoxide generation since NADPH oxidases are, in part, activated by  
343 H<sub>2</sub>O<sub>2</sub> [135]. However, Nox4 is a NADPH oxidase expressed in skeletal muscle that can  
344 generate H<sub>2</sub>O<sub>2</sub> directly [63; 136]. It is extremely unlikely that ascorbate diminishes Nox4  
345 mediated H<sub>2</sub>O<sub>2</sub> generation. Any indirect inhibition is not possible for  $\alpha$ -tocopherol owing to  
346 lack of appreciable reaction with superoxide [69]. Second, the reaction of hydrogen peroxide  
347 with transition metal centres can yield superoxide and/or hydroxyl radical [69]. It is possible  
348 that these radicals could then transmit a local signal that could be scavenged. However, there  
349 are two major problems with this hypothesis (1) the random nature precludes specific  
350 signalling and (2) the reaction of either antioxidant with hydroxyl radical is biologically  
351 meaningless, since hydroxyl radical reacts with the first biomolecule it encounters [137-138].  
352 Overall, we do not exclude indirect interference with H<sub>2</sub>O<sub>2</sub> signalling, probably via reaction  
353 of ascorbate with superoxide, but emphasise that experimental support in an exercise setting  
354 is required.

### 355 **Removal of the cysteine modification once formed: S-Nitrosylation as an exemplar** 356 **paradigm**

357 Ascorbate and  $\alpha$ -tocopherol might remove redox modifications once formed and this could  
358 interfere with exercise-induced redox signalling. S-Nitrosylation (S-NO) is considered as an  
359 exemplar paradigm. S-NO defines the attachment of NO to cysteine [139]. NO is a weak  
360 nitrating agent and cannot generate S-NO directly [140]. Indeed, the precise reactions  
361 involved in S-NO formation *in vivo* are ill-defined [141]. It is suggested that transition metal  
362 catalysed pathways, formation of dinitrogen trioxide and thiyl radical species contribute to S-  
363 NO generation [142-143]. Knowledge of exercise-induced S-NO events are limited but the  
364 following observations support a role (1) protein kinases and phosphatases are S-nitrosylated  
365 [139] (2) transcription factors implicated in exercise adaptations are S-nitrosylated, including

366 HIF- $\alpha$  [144], p53 [145] and NF- $\kappa$ B [52] and (3) the ryanodine receptor type I is S-  
367 nitrosylated with attendant implications for Ca<sup>2+</sup> signalling and muscle function [146].  
368 Ascorbate can denitrosylate proteins indeed this property forms the basis of the biotin-switch  
369 assay, a S-NO analytical tool [147-148]. Denitrosylation can proceed in a copper dependent  
370 or independent manner [149]. The former is unlikely *in vivo* given the chelation of transition  
371 metals whilst the latter is associated with high ascorbate concentrations (5-50 mM), and even  
372 then only partial denitrosylation of a sample occurs [27]. Whether ascorbate dependent  
373 denitrosylation occurs at physiological concentrations and in the relevant cellular  
374 microdomains is debatable but should not be discounted at this stage. The literature  
375 appertaining to denitrosylation reactions involving  $\alpha$ -tocopherol is limited and hence its  
376 feasibility and relevance *in vivo* is an open question. Nevertheless, similar concentration,  
377 localisation and specificity concerns apply. Further, it is unlikely that exogenous antioxidants  
378 exert an effect greater than the existing endogenous denitrosylation system [139]. This  
379 system includes the S-nitrosoglutathione and thioredoxin pathway and enzymes such as:  
380 protein disulphide isomerase, SOD isoforms and xanthine oxidase [150]. Taken together, two  
381 observations are apparent (1) S-NO modifications relevant to the adaptive exercise response  
382 require investigation (2) the effect of ascorbate and  $\alpha$ -tocopherol on the skeletal muscle S-NO  
383 proteome is not known. Ascorbate and  $\alpha$ -tocopherol are unlikely to interfere with other  
384 modifications (e.g. S-glutathionylation) once formed as there is limited chemical basis for  
385 any direct interference.

## 386 **Alternate mechanisms**

### 387 ***Reduction of potentially bioactive oxidised macromolecule adducts***

388 Direct signalling by indiscriminately reactive one electron oxidants, notably hydroxyl radical,  
389 is limited by lack of specificity, precluding signalling via conventional mechanisms (e.g.  
390 protein post-translational modifications [26-27]). Indirect signalling might be afforded by the  
391 generation of oxidised lipid, DNA and protein adducts [151-152]. In particular, pre-treatment  
392 of cells with low-doses of lipid peroxidation products (e.g. 4-hydroxynonenal) induces  
393 favourable responses, notably activation of the Nrf-2-KEAP1 pathway, that protect against  
394 the stress imposed by a subsequent oxidative challenge [153-154]. Nrf-2-KEAP1 pathway  
395 activation is likely to proceed via S-alkylation of KEAP1 and subsequent inactivation, an  
396 event that promotes the nuclear translocation of Nrf-2 [66, 155]. Interestingly, S-alkylation  
397 also regulates NADPH oxidase activity [156], facilitating a putative negative feedback loop.  
398 The sensing of damaged proteins and DNA adducts by chaperones and repair enzymes,  
399 respectively, could provoke an adaptive response. Cell signalling processes are subject to  
400 intricate spatiotemporal regulation [20-22, 80-85]. Macromolecule oxidation, secondary to  
401 hydroxyl radical attack, fails to satisfy this fundamental signalling requirement, being  
402 inherently random and non-specific [137-138, 157]. Whether levels of oxidised  
403 macromolecules serve as a general non-specific redox rheostat that informs signalling  
404 responses is an open question. Nevertheless, this is unlikely on a global level owing to the  
405 compartmentalised and specific nature of cell signalling [20-22].

406 Acute exercise bouts are usually, but not always [see 158], associated with an increase in  
407 oxidised macromolecule adducts [159]. If these products were acting in a signalling fashion,  
408 this postulate requires investigation in an exercise setting, then an ascorbate and  $\alpha$ -tocopherol  
409 mediated reduction in oxidised macromolecule adducts might blunt this potentially  
410 favourable response (see figure 2). Although, both antioxidants scavenge radicals implicated  
411 in the initiation of macromolecule oxidation the effects of antioxidant supplementation on  
412 oxidised adduct levels are variable [137-138]. This is best exemplified in pathological  
413 contexts wherein global levels of oxidised macromolecule adducts are constitutively elevated  
414 [160], possibly reflecting deregulated redox signalling. In these settings, ascorbate and  $\alpha$ -  
415 tocopherol supplementation does not decrease disease incidence and generally only  
416 marginally decreases macromolecule oxidation [137-138, 161-164]. This might reflect a  
417 failure of ascorbate and  $\alpha$ -tocopherol to accumulate in redox signalling compartments and  
418 effect a reduction in the levels of a reactive species or indeed a failure to react appreciably  
419 with the relevant species [161-164]. Further, positive effects are generally evident in  
420 individuals presenting with ascorbate and  $\alpha$ -tocopherol deficiency at baseline [165]. Of  
421 course, the nature of macromolecule oxidation at rest compared to exercise are likely  
422 different. In an exercise setting, ascorbate and  $\alpha$ -tocopherol afford limited protection against  
423 exercise-induced macromolecule damage [166]. Indeed, a recent meta-analysis concluded  
424 that  $\alpha$ -tocopherol does not reduce exercise-induced lipid peroxidation [166]. Overall, a  
425 signalling role of oxidised macromolecules is speculative in an exercise setting and neither  
426 antioxidant consistently protects against exercise-induced macromolecule oxidation.  
427 Reduction of potentially bioactive oxidised macromolecule adducts does not likely explain  
428 the attenuation of favourable cell signalling responses to exercise training following  
429 ascorbate and  $\alpha$ -tocopherol supplementation.

### 430 ***Pro-oxidant potential***

431 The oxidation of ascorbate results in the formation of an ascorbyl radical [93]. Ascorbyl  
432 radical is unlikely to exert pro-oxidant effects *in vivo* owing to its poor reactivity and  
433 existence of glutathione and NADPH dependent recycling systems [167]. Ascorbate has well-  
434 documented pro-oxidant properties *in vitro* when free transition metal are present [76].  
435 Ascorbate can reduce  $\text{Fe}^{3+}$  to  $\text{Fe}^{2+}$ , and  $\text{Fe}^{2+}$  can then in turn react with  $\text{O}_2$  to generate  
436 superoxide [176]. Ascorbate can also generate hydroxyl radical and  $\text{H}_2\text{O}_2$  via classical Fenton  
437 chemistry [177]. Indeed, this is the basis for the use of pharmacological intravenous ascorbate  
438 administration as a cancer treatment owing to the toxicity of  $\text{H}_2\text{O}_2$  to certain cancer cells  
439 [177-178]. This treatment paradigm bypasses gut metabolism removing the absorption  
440 constraints that restrict peak plasma ascorbate concentrations to  $\sim 200 \mu\text{M}$  following even  
441 high-dose oral supplementation [178]. The relevance of these pro-oxidant effects *in vivo* is  
442 highly debated, and indeed controversial, especially in non-pathological contexts [178]. Any  
443 pro-oxidant action is likely dependent on the availability of transition metals. It is emphasised  
444 that these are largely sequestered by the metallothionein family, transferrin and ferritin [170].  
445 Despite the intracellular sequestration of certain transition metals, cells still contain small  
446 ( $\sim 20 \mu\text{M}$ ) un-sequestered pools of free iron that could participate in pro-oxidation reactions  
447 [171]. Interestingly, microarray analysis has revealed that metallothionein mRNA abundance

448 is significantly enriched following acute endurance exercise [172]. This could reflect a stress  
449 response to exercise-induced perturbations in intracellular transition metal handling. Such  
450 perturbations are likely to be greater following exercise that evokes muscle damage, given  
451 that muscle injury increases labile iron levels in skeletal muscle [173] possibly owing to  
452 increased hemolysis [174]. The aforementioned scenarios would permit increased free  
453 transition metal availability and pro-oxidant ascorbate potential. Any pro-oxidant actions  
454 could elevate the 'redox' signal from an adaptive to maladaptive threshold. This supposition  
455 is, however, speculative at present. Some species (e.g. mice and rodents) retain the capacity  
456 to endogenously manufacture ascorbate from glucose owing to expression of gulonolactone  
457 oxidase [175]. Humans harbour a defunct gulonolactone oxidase gene and hence need to  
458 acquire ascorbate exogenously, via dietary sources. Disruption of ascorbate homeostasis in  
459 lower order species with large dose supplementation could favour pro-oxidant and cytotoxic  
460 effects that contribute to blunted training adaptations.

461 Similar to ascorbate, any pro-oxidant effect of  $\alpha$ -tocopherol could elevate the 'redox' signal  
462 from an adaptive to maladaptive threshold. The oxidation of  $\alpha$ -tocopherol yields  $\alpha$ -tocopherol  
463 radical [75]. Although,  $\alpha$ -tocopherol radical is capable of inducing lipid peroxidation *in vitro*,  
464 this has not been consistently documented *in vivo* [93,176]. Toxicity of  $\alpha$ -tocopherol  
465 radical is thought to be limited by ascorbate mediated recycling of  $\alpha$ -tocopherol radical to  $\alpha$ -  
466 tocopherol [93]. Indeed, this reason is often cited as a justification for  $\alpha$ -tocopherol and  
467 ascorbate co-supplementation [16]. Ascorbate mediated recycling of  $\alpha$ -tocopherol radical is  
468 well documented *in vitro* but evidence for this interaction *in vivo*, particularly in humans, is  
469 often inconsistent [69]. Recycling can also be achieved by glutathione [177], which could be  
470 an important contributor *in vivo*. Analogous to ascorbate, tocopherol isoforms can exert  
471 transition metal dependent pro-oxidation effects *in vitro* but their sequestration and  
472 localisation is likely to limit this possibility *in vivo* [75]. Overall, it is unlikely that  $\alpha$ -  
473 tocopherol is acting in a pro-oxidant fashion to diminish exercise-induced redox signalling.

## 474 **Perspectives**

475 Beyond theory and speculation there is a paucity of evidence supporting the notion that  
476 ascorbate and  $\alpha$ -tocopherol supplementation interferes with exercise-induced redox signalling  
477 via a redox-dependent 'scavenging' mechanism. Unfortunately, obtaining supporting  
478 evidence is hampered by several analytical limitations. Electron spin resonance and  
479 fluorescent based probe technology are not readily applicable to the *in vivo* human situation  
480 and many fluorescent probes are prone to experimental artefact, that is, spurious side-  
481 reactions that artificially amplify the signal [178-180]. Interpretation of these techniques in  
482 animal and cell culture models is complicated by interspecies differences (e.g. rodents can  
483 manufacture ascorbate) and the oxidative stress that cell culture can impose [181-182]. This  
484 has fostered a reliance on biochemical footprints, such as lipid peroxidation biomarkers (e.g.  
485 malondialdehyde [44, 157]). A change in a biochemical footprint does not necessarily reflect a  
486 redox-dependent scavenging effect of exogenous antioxidants it could simply reflect  
487 differential repair or dietary changes [69, 133]. Redox signalling occurs in specific cellular  
488 compartments hence altered macromolecule oxidation levels do not necessarily reflect the  
489 incidence of redox signalling [80-86]. That is, redox signalling does not require global

490 changes in oxidised macromolecule adducts to occur [80-82]. Instead, specific, reversible and  
491 compartmentalised signals define redox signalling [80-86]. Whether assaying global levels of  
492 oxidised macromolecule adducts provides any useful information on the interference of  
493 ascorbate and  $\alpha$ -tocopherol supplementation with exercise-induced redox signalling is  
494 therefore debatable.

495 In considering possible technical solutions, redox proteomics enables quantitative and  
496 unbiased analysis of redox-regulated post-translational modifications implicated in cell  
497 signalling [183-187]. However, signalling proteins might be masked by the abundance of  
498 metabolic and contractile proteins in skeletal muscle [183-187]. Further, determining the  
499 functionality of novel modifications would require further experimentation [188]. Application  
500 of redox proteomics to the study of exercise-induced redox signalling is strongly encouraged.  
501 Another way might be to analyse redox regulated end-points, such as activity and abundance  
502 of antioxidant enzymes and heat shock proteins [46]. Ascorbate and  $\alpha$ -tocopherol  
503 supplementation did not interfere with antioxidant enzyme and heat shock protein abundance  
504 when this approach was recently applied [8]. This might suggest a lack of a redox dependent  
505 mode of action since these outcome markers are one principal end-point of exercise-induced  
506 redox signalling. However, this approach provides limited mechanistic information being  
507 unable to identify the nature of any possible interference [189]. Overall, it is clear that further  
508 mechanistic research is required and that redox proteomics represents an admiral starting  
509 point.

510 Ascorbate and  $\alpha$ -tocopherol could act in a redox independent manner to attenuate favourable  
511 cell signalling responses to exercise training. Ascorbate is a co-factor for  $\alpha$ -ketoglutarate  
512 dependent dioxygenases (e.g. prolyl 4-hydroxylase [93,169,175]) and also promotes HIF- $\alpha$   
513 repression via proline hydroxylation [190-191]. This is particularly relevant to exercise given  
514 the role of HIF- $\alpha$  in the regulation of angiogenesis, growth, apoptosis and metabolism [192-  
515 193]. Of interest, ascorbate can regulate the activity of enzymes implicated in the regulation  
516 of histone methylation [194-195], an epigenetic process that regulates exercise adaptations  
517 [196]. Similarly,  $\alpha$ -tocopherol can inhibit 5-LOX, protein kinase C isoforms and  
518 phospholipase A<sub>2</sub> which could influence exercise-induced cell signalling [197-199].  
519 Inhibition of these enzymes is suggested to be redox independent and appears to be related to  
520 the interaction of  $\alpha$ -tocopherol with signalling proteins [197-199]. This could explain the  
521 observation that several genes (e.g. tropomyosin) are regulated by  $\alpha$ -tocopherol [197].  
522 Altogether, it is possible that redox-independent actions contribute and this is worthy of  
523 further investigation.

524 Irrespective of the mechanism, redox dependent or independent, blunted cell signalling  
525 responses following ascorbate and  $\alpha$ -tocopherol supplementation have seldom translated to  
526 impaired whole-body exercise adaptations (e.g. diminished increases in aerobic capacity [1]).  
527 There are several possible explanations for this however, two are particularly cogent. First,  
528 changes at the whole-body level are a product of peripheral and central adaptations hence any  
529 peripheral impairment can be compensated for [15]. Second, the molecular processes  
530 measured are often stress responses and have rarely been shown to be either essential to  
531 adaptation and/or predict the magnitude of adaptation [200]. Further, signalling processes

532 have an in built reserve capacity, therefore, suppression of an upstream signal does not  
533 always translate to blunted downstream responses [20-22]. When it is considered that a  
534 whole-body response is the reflection of highly regulated processes across several cell types  
535 it is unsurprising that blunted activation of one or two regulatory proteins fails to impact  
536 adaptation. The physiological relevance of an impaired molecular response to functional end-  
537 points is, therefore, debatable.

## 538 **Conclusion**

539 Current paradigms posit that ascorbate and  $\alpha$ -tocopherol supplementation act as antioxidants  
540 to diminish global superoxide, NO, peroxynitrite and H<sub>2</sub>O<sub>2</sub> levels and thus affect an  
541 attenuation of exercise-induced redox signalling. For this to be possible, it is contended here  
542 that the criteria outlined in box 1 must be satisfied. Our largely theoretical analysis reveals  
543 that all of assumptions implicit in a redox dependent mechanism of action are not met for any  
544 of the aforementioned species. The best candidate for a scavenging effect represents the  
545 reaction of ascorbate with superoxide, with attendant implications for H<sub>2</sub>O<sub>2</sub> signalling. Even  
546 in this case, it is unclear whether the requisite chemical (out-competing other reactants) and  
547 spatiotemporal (co-localisation with relevant targets) concerns are satisfied. It is readily  
548 acknowledged that the present analysis is limited by knowledge of the mechanisms  
549 underpinning exercise-induced redox signalling being fragmentary. It is also emphasised that  
550 a nuanced view of kinetics in space, time and context is warranted. That is, kinetic  
551 information is usually derived from *in vitro* experiments that do not faithfully mimic the *in*  
552 *vivo* situation. A situation characterised by compartment specific redox potentials and pH  
553 characteristics, all of which could influence the reaction of ascorbate and  $\alpha$ -tocopherol with a  
554 given species and thus our conclusions. Despite the aforementioned caveats, a clear challenge  
555 to the current interpretational framework is presented. It cannot be assumed that just because  
556 a molecule has 'antioxidant properties' that it is acting as an antioxidant to attenuate exercise-  
557 induced redox signalling *in vivo*. Further, in the current context altered global levels of  
558 oxidised macromolecules should not be used to evidence an attenuation of exercise-induced  
559 redox signalling. Indeed, it is our view that redox signalling networks that are insulated from  
560 nutritional antioxidants have evolved. Whilst ascorbate and  $\alpha$ -tocopherol could scavenge  
561 reactive species that diffuse out of signalling microdomains the insulation could protect  
562 against any major interference. This observation may be novel in an exercise setting but is  
563 consistent with the failure of nutritional antioxidant therapy to modify diseases associated  
564 with oxidative stress and pathological disruption of redox signalling. It is hoped that the  
565 present dialogue stimulates investigations into the molecular mechanisms underpinning the  
566 blunting of exercise-induced redox signalling following ascorbate and  $\alpha$ -tocopherol  
567 supplementation. It is emphasised that this discourse applies only to the antioxidants  
568 discussed and should not be extrapolated to other antioxidants, since antioxidants are not  
569 biochemically and functionally homogenous [133]. In this regard, it might be worthwhile  
570 exploring alternate antioxidant paradigms, such as N-acetyl-cysteine [201].

## 571 **Conflict of interest**

572 No conflicts of interest, financial or otherwise, are declared by the authors.

573 **Acknowledgements**

574 Prof Barry Halliwell (National University of Singapore), Prof Christine Winterbourn (University of Otago), Dr  
575 Chris McGlory (McMaster University), Mr Dom Partridge (Aberdeen University) and Dr George Sakellariou  
576 (Liverpool University) are all thanked for critically reading and providing insightful comments on formative  
577 drafts of the manuscript. J.N.C is supported by the Carnegie Trust (R70103).

578 **References**

- 579 1. Paulsen, G.; Cumming, K.T.; Holden, G.; Hallen, J.; Ronnestad, B.R.; Sveen, O.; et  
580 al. Vitamin C and E supplementation hampers cellular adaptation to endurance in  
581 humans: a double-blind, randomised, controlled trial. *J Physiol.* 592:1887-1901; 2014.
- 582 2. Paulsen, G.; Hamarsland, H.; Cumming, K.T.; Johansen, R.E.; Hulmi, J.J.; Borsheim,  
583 E.; et al. Vitamin C and E supplementation alters protein signalling after a strength  
584 training session, but not muscle growth during 10 weeks of training. *J Physiol.*  
585 592:5391-5408; 2014.
- 586 3. Venditti, P.; Napolitano, D.; Barone, D.; Di Meo, S. Vitamin E supplementation  
587 modifies adaptive responses to training in rat skeletal muscle. *Free Radic Res.* 48:  
588 1179-1189; 2014.
- 589 4. Gomez-Cabrera, M.C.; Borrás, C.; Pallardo, V.F.; Sastre, J.; Ji, L.L; Vina, J.  
590 Decreasing xanthine oxidase-mediated oxidative stress prevents useful cellular  
591 adaptations to exercise in rats. *J Physiol.* 567:113-120; 2005.
- 592 5. Gomez-Cabrera, M.C.; Domenech, E.; Romagnoli, M.; Arduini, A.; Borrás C.;  
593 Pallardo, V.F.; et al. Oral administration of vitamin C decreases muscle mitochondrial  
594 biogenesis and hampers training-induced adaptations in endurance performance. *Am J*  
595 *Clin Nutr.* 87:142-149; 2008.
- 596 6. Khassaf, M.; McArdle, A.; Esanu, C.; Vasilaki, A.; McArdle, F.; Griffiths, R.D.; et al.  
597 Effect of vitamin C supplementation on antioxidant defence and stress proteins in  
598 human lymphocytes and skeletal muscle. *J Physiol.* 549:645-652; 2003.
- 599 7. Ristow, M.; Zarse, K.; Oberbach, A.; Kloting, N.; Birringer, M.; Kiehnopf, M.; et al.  
600 Antioxidants prevent health-promoting effects of physical exercise in humans. *Proc*  
601 *Natl Acad Sci USA.* 106:8665-8670; 2009.
- 602 8. Cumming, K.T.; Raastad, T.; Holden, G.; Bastani, N.E.; Schneeberger, D.; Paronetto,  
603 M.P.; et al. Effects of vitamin C and E supplementation on endogenous antioxidant  
604 systems and heat shock proteins in response to endurance training. *Physiol Rep.* 2:  
605 e12142; 2014.
- 606 9. Higashida, K.; Kim, S.H.; Higuchi, M.; Holloszy, J.O.; Han, D.H. Normal adaptations  
607 to exercise despite protection against oxidative stress. *Am J Physiol Endocrinol*  
608 *Metab.* 301:E779-E784; 2011.
- 609 10. Theodorou, A.A.; Nikolaidis, M.G.; Paschalis, S.; Koutsias, S.; Panayiotou, G.;  
610 Fatouros, I.G.; et al. No effect of antioxidant supplementation on muscle performance  
611 and blood redox status adaptations to eccentric training. *Am J Clin Nutr.* 93:1373-  
612 1383; 2011.
- 613 11. Roberts, L.A.; Beattie, K.; Close, G.L.; Morton, J.P. Vitamin C consumption does not  
614 impair training-induced improvements in exercise performance. *Int J Sports Physiol*  
615 *Perform.* 6:58-69.; 2011.



- 616 12. Wadley, G.D.; McConell, G.K. High-dose antioxidant vitamin C supplementation  
617 does not prevent acute exercise-induced increases in markers of skeletal muscle  
618 mitochondrial biogenesis in rats. *J Appl Physiol.* 108:1719-1726; 2010.
- 619 13. Yfanti, C.; Akerstrom, T.; Neilsen, A.R.; Mounier, R.; Mortensen, O.H.; Lykkesfeldt,  
620 J.; et al. Antioxidant supplementation does not alter endurance training adaptation.  
621 *Med Sci Sports Exerc.* 42:1388-1395; 2010.
- 622 14. Yfanti, C.; Neilsen, A.R.; Akerstrom, T.; Neilsen, S.; Rose, A.J.; Richter, E.A.; et al.  
623 Effect of antioxidant supplementation on insulin sensitivity in response to endurance  
624 exercise training. *Am J Physiol Endocrinol Metab.* 300:E761-E770; 2011.
- 625 15. Close, G.L.; Jackson, M.J. Antioxidants and exercise: a tale of the complexities of  
626 relating signalling processes to physiological function? *J Physiol.* 592:1721-1722;  
627 2014.
- 628 16. Nikolaidis, M.G.; Kerksick, C.M.; Lamprecht, M.; McAunlty, S.R. Does vitamin C  
629 and E supplementation impair favourable adaptations of regular exercise? *Oxid Med*  
630 *Cell Longev.* 2012:707941; 2012.
- 631 17. Slattery, K.; Bentley, D.; Coutts, A.J. The role of oxidative, inflammatory and  
632 neuroendocrinological systems during exercise stress in athletes: Implications of  
633 antioxidant supplementation on physiological adaptation during intensified physical  
634 training. *Sports Med.* DOI: 10.1007/s40279-014-0282-7; 2014.
- 635 18. Mankowski, R.T.; Anton, S.D.; Buford, T.W.; Leewenburgh, C. Dietary antioxidants  
636 as modifiers of physiologic adaptations to exercise. *Med Sci Sports Exerc.* DOI:  
637 10.1249/MSS.0000000000000620; 2015.
- 638 19. Forman, H.J.; Augusto, O.; Brigelius-Flohe, R.; Dennery, P.A.; Kalyanaraman, B.;  
639 Ishiropoulos, H.; et al. A suggested guide to free radical research terminology and  
640 methodology. *Free Radic Biol Med.* 78:233-235; 2015.
- 641 20. Kholodenko, B.N. Cell-signalling dynamics in space and time. *Nat Rev Mol Cell*  
642 *Biol.* 7:165-176; 2006.
- 643 21. Kholodenko, B.; Hancock, J.F.; Kolch, W. Cell signalling ballet in space and time.  
644 *Nat Rev Mol Cell Biol.* 11:414-426; 2010.
- 645 22. Smock, R.G.; Gierasch, L.M. Sending signals dynamically. *Science.* 324:198-203;  
646 2009.
- 647 23. Forman, H.J.; Maiorino, M.; Ursini, F. Signaling functions of reactive oxygen species.  
648 *49:835-842; 2010.*
- 649 24. Forman, H.J.; Ursini, F.; Maiorino, M. An overview of mechanisms of redox  
650 signaling. *J Mol Cell Cardiol.* 73:2-9; 2014.
- 651 25. Brigelius-Flohe, R; Flohe, L. Basic principles and emerging concepts in the redox  
652 control of transcription factors. *Antioxid Redox Signal.* 15:2335-2381; 2011.
- 653 26. Holmstrom, K.M.; Finkel, T. Cellular mechanisms and physiological consequences of  
654 redox-dependent signalling. *Nat Rev Mol Cell Biol.* 15:411-421; 2014.
- 655 27. Janssen-Heininger, Y.M.; Mossman, B.T.; Heintz, N.H.; Forman, H.J.;  
656 Kalyanaraman, B.; Finkel, T.; et al. Redox-based regulation of signal transduction:  
657 principles: pitfalls, and promises. *Free Radic Biol Med.* 45:1-17; 2008.
- 658 28. Winterbourn, C.C. Are free radicals involved in thiol-based redox signalling? *Free*  
659 *Radic Biol Med.* <http://dx.doi.org/10.1016/j.freeradbiolmed.2014.08.017i>; 2014.

- 660 29. Winterbourn, C.C. & Hampton, M.B. Thiol chemistry and specificity in redox  
661 signalling. *Free Radic Biol Med.* 45:549-561; 2008.
- 662 30. Lo Conte, M.; Carroll, S.K. The redox biochemistry of protein sulfenylation and  
663 sulfinylation. *J Bio Chem.* 288:26480-26488; 2013.
- 664 31. Gallogy, M.M.; Mieyal, J.J. Mechanisms of reversible protein glutathionylation in  
665 redox signaling and oxidative stress. *Curr Opin Pharmacol.* 7:381–391; 2007.
- 666 32. Drazic, A.; Winter, J. The physiological roles of methionine oxidation. *Biochim*  
667 *Biophys Acta.* 1844:1367-82; 2014.
- 668 33. Erickson, J.R.; Joiner, M.L.; Guan, X.; Kutschke, W.; Yang, J.; Oddis, C.V.; et al. A  
669 dynamic pathway for calcium-independent activation of CaMKII by methionine  
670 oxidation. *Cell.* 133: 462-474; 2008.
- 671 34. Winterbourn, C.C. Reconciling the chemistry and biology of reactive oxygen species.  
672 *Nat Chem Biol.* 4:278-286; 2008.
- 673 35. Coffey, V.G.; Hawley, J.A. The molecular basis of training adaptation. *Sports Med.*  
674 37:737-763; 2007.
- 675 36. Egan, B.; Zierath, J.R. Exercise metabolism and the molecular regulation of skeletal  
676 muscle adaptation. *Cell Metab.* 17:162-184; 2013.
- 677 37. Perry, C.G.R.; Lally, J.; Holloway, G.P.; Heigenhauser, G.J.F.; Bonen, A.; Spriet, L.I.  
678 Repeated transient mRNA bursts precede increases in transcriptional and  
679 mitochondrial proteins during training in human skeletal muscle. *J Physiol.* 588:4795-  
680 4810; 2010.
- 681 38. Gomez-Cabrera, M.C.; Domenech, E.; Vina, J. Moderate exercise is an antioxidant.  
682 *Free Radic Biol Med.* 44:126-131; 2008.
- 683 39. Powers, S.K.; Durate, J.; Kavazis A.N.; Talbert, E.E. Reactive oxygen species are  
684 signalling molecules for muscle adaptation. *Exp Physiol.* 95: 1-9; 2010.
- 685 40. Radak, Z.; Zhao, Z.; Kolati, E.; Ohno, H.; Atalay, M. Oxygen consumption and usage  
686 during physical exercise: The balance between oxidative stress and ROS-dependent  
687 adaptive signalling. *Antiox Redox Signal.* 18:1208-1246; 2012.
- 688 41. Powers, S.K.; Jackson, M.J. Exercise-induced oxidative stress: Cellular mechanisms  
689 and impact on muscle force production. *Physiol Rev.* 88:1243-1276; 2008.
- 690 42. Cogley, J.N.; Sakellariou, G.K.; Owens, D.J.; Murray, S.; Waldron, S.; Gregson, W.;  
691 et al. Lifelong training preserves some redox-regulated adaptive responses following  
692 an acute exercise stimulus in aged human skeletal muscle. *Free Radic Biol Med.*  
693 70:23-32; 2014.
- 694 43. Ji, L.L. Modulation of skeletal muscle antioxidant defence by exercise: Role of redox  
695 signalling. *Free Radic Biol Med.* 44:142-152; 2008.
- 696 44. Nikolaidis, M.G.; Margaritelis, N.V.; Paschalis, V.; Theodorou, A.A.; Kyparos, A.;  
697 Vrabas I.S. (Common questions and tentative answers on how to assess oxidative  
698 stress after antioxidant supplementation and exercise. In Lamprecht M (Ed)  
699 *Antioxidants in Sport Nutrition.* CRC Press, New York, pp. 221-246; 2014.
- 700 45. Morton, J.P.; Kayani, A.C.; McArdle, A.; Drust, B. The exercise-induced stress  
701 response of skeletal muscle with specific emphasis on humans. *Sports Med.* 39:643-  
702 662; 2009.

- 703 46. Cobley, J.N.; Bartlett, J.D.; Kayani, A.C.; Murray, S.W.; Louhelainen, J.; Donovan,  
704 T. et al. PGC-1 $\alpha$  transcriptional response and mitochondrial adaptation to acute  
705 exercise is maintained in skeletal muscle of sedentary elderly males. *Biogerontology*.  
706 13: 621-631; 2012.
- 707 47. Egan, B.; Carson, B.P.; Garcia-Roves, P.M.; Chibalin, A.V.; Sarsfeild, F.M.; Barron,  
708 N.; et al. Exercise intensity-dependent regulation of peroxisome proliferator-activated  
709 receptor coactivator-1 mRNA abundance is associated with differential activation of  
710 upstream signalling kinases in human skeletal muscle. *J Physiol*. 588:1779-1790;  
711 2010.
- 712 48. Holloszy, J.O.; Coyle, E.F. Adaptations of skeletal muscle to endurance exercise and  
713 their metabolic consequences. *J Appl Physiol*. 56:831-838; 1984.
- 714 49. Safdar, A.; Little, J.P.; Stokl, A.J.; Hettinga, B.P.; Akhtar, M.; Tarnopolsky, M.A.  
715 Exercise increases mitochondrial PGC-1 $\alpha$  content and promotes nuclear-  
716 mitochondrial cross-talk to coordinate mitochondrial biogenesis. *J Biol Chem*.  
717 286:10605-10617; 2011.
- 718 50. Wadley, G.D.; Nicloa, M.A.; Hiam, D.S.; McConell, G.K. Xanthine oxidase  
719 inhibition attenuates skeletal muscle signalling following acute exercise but does not  
720 impair mitochondrial adaptations to endurance training. *Am J Physiol Endocrinol*  
721 *Metab*. 304:E853-E862; 2013.
- 722 51. Kang, C.; O'Moore, K.; Dickman, J.R.; Ji, L.L. Exercise activation of muscle  
723 peroxisome proliferator activated receptor- $\gamma$  co-activator-1 $\alpha$  is redox sensitive. *Free*  
724 *Radic Biol Med*. 47:1394-1400; 2009.
- 725 52. Reynaert, N. L.; Ckless, K.; Korn, S.H.; Vos, N.; Guala, A.S.; Wouters, E.F.; et al.  
726 Nitric oxide represses inhibitory  $\kappa$ B kinase through S-nitrosylation. *Proc Natl Acad*  
727 *Sci USA*. 101:8945-8950; 2004.
- 728 53. Close, G.L.; Ashton, T.; McArdle, A.; Jackson, M.J. Microdialysis studies of  
729 extracellular reactive oxygen species production in skeletal muscle: factors  
730 influencing the reduction of cytochrome c and hydroxylation of salicylate. *Free Radic*  
731 *Biol Med*. 39:1460-1467; 2005.
- 732 54. Palomero, J.; Pye, D.; Kabayo, T.; Spiller, D.G.; Jackson, M.J. In situ detection and  
733 measurement of intracellular reactive oxygen species in single isolated mature skeletal  
734 muscle fibres by real-time fluorescence microscopy. *Antioxid Redox Signal*. 10:1463-  
735 1474; 2008.
- 736 55. Jackson, M.J.; Pye, D.; Palomero, J. The production of reactive oxygen and nitrogen  
737 species by skeletal muscle. *J Appl Physiol*. 102:1664-1670; 2007.
- 738 56. Pye, D.; Palomero, J.; Kabayo, T.; Jackson, M.J. Real-time measurement of nitric  
739 oxide in single mature mouse skeletal muscle fibres during contractions. *J Physiol*.  
740 581:309-318; 2007.
- 741 57. Finkel, T. Signal transduction by reactive oxygen species. *J Cell Biol*. 194:7-15; 2011.
- 742 58. Goncalves, R.L.S.; Quinlam, C.L.; Perevoshchikova, I.V.; Hey-Mogensen.; Brand,  
743 M.D. Sites of Superoxide and Hydrogen Peroxide Production by Muscle  
744 Mitochondria Assessed ex vivo Under Conditions Mimicking Rest and Exercise. *J*  
745 *Biol Chem*. DOI: jbc.M114.619072; 2014.

- 746 59. Murphy, M.P. How mitochondria produce reactive oxygen species. *Biochem J.* 417:1-  
747 13; 2009.
- 748 60. Sakellariou, G.K.; Vasilaki, A.; Palomero, J.; Kayani, A.; Zibrik, L.; McArdle, A.; et  
749 al. Studies of mitochondrial and non-mitochondrial sources implicate nicotinamide  
750 adenine dinucleotide phosphate oxidase (s) in the increase skeletal muscle superoxide  
751 generation that occurs following contractile activity. *Antioxid Redox Signal.* 18:603-  
752 621; 2013.
- 753 61. Sakellariou, G.K.; Jackson, M.J.; Vasilaki, A. Redefining the major contributors to  
754 superoxide production in contracting skeletal muscle. Role of NAD(P)H oxidases.  
755 *Free Radic Res.* 48:12-29; 2014.
- 756 62. Espinosa, A.; Leiva, A.; Pena, M.; Muller, M.; Debandi, A.; Hidalgo, C.; et al.  
757 Myotube depolarization generates reactive oxygen species through NAD(P)H  
758 oxidase; ROS-elicited Ca<sup>2+</sup> stimulates ERK, CREB, early genes. *J Cell Physiol.* 209:  
759 379–388; 2006.
- 760 63. Hidalgo, C.; Sanchez, G.; Barrientos, G.; Aracena-Parks, P. A transverse tubule  
761 NADPH oxidase activity stimulates calcium release from isolated triads via ryanodine  
762 receptor type 1 S-glutathionylation. *J Biol Chem* 281:26473–26482; 2006.
- 763 64. Xia, R.; Webb, J.A.; Gnall, L.L.; Cutler, K.; Abramson, J.J. Skeletal muscle  
764 sarcoplasmic reticulum contains a NADH-dependent oxidase that generates  
765 superoxide. *Am J Physiol Cell Physiol* 285:C215–C221; 2003.
- 766 65. Winterbourn, C.C.; Metodiewa, D. The reaction of superoxide with reduced  
767 glutathione. *Arch. Biochem. Biophys.* 314:284–290; 1994.
- 768 66. Forman, H.J.; Davies, K.J.A.; Ursini, F. How do nutritional antioxidants really work:  
769 Nucleophilic tone and para-hormesis versus free radical scavenging in vivo. *Free*  
770 *Radic Biol Med.* 66:24-35; 2014.
- 771 67. Gutteridge, J.M.C.; Halliwell. B. Antioxidants: molecules, medicines, and myths.  
772 *Biochem Biophys Res Commun.* 393:561-564; 2010.
- 773 68. Spencer, N.Y.; Engelhardt, J.F. The basic biology of redoxosomes in cytokine-  
774 mediated signal transduction and implications for disease-specific therapies.  
775 *Biochemistry.* 53:1551–1564; 2014.
- 776 69. Halliwell, B.; Gutteridge, J.M.C. *Free Radicals in Biology & Medicine.* Fourth  
777 Edition. Oxford University Press: Oxford; 2007.
- 778 70. Bloor, C.M. Angiogenesis during exercise and training. *Angiogenesis.* 8:263–271;  
779 2005.
- 780 71. Chinsomboon, J.; Raus, J.; Gupta, R.K.; Thom, R.; Shoag, J.; Rowe, G.C.; et al. The  
781 transcriptional coactivator PGC-1 $\alpha$  mediates exercise-induced angiogenesis in skeletal  
782 muscle. *Proc Natl Acad Sci USA.* 106:21401–21406; 2009.
- 783 72. Buettner, G.R. Superoxide Dismutase in Redox Biology: The roles of superoxide and  
784 hydrogen peroxide. *Anticancer Agents Med Chem.* 11:341–346; 2011.
- 785 73. Takahashi, M.; Asada, K. Superoxide anion permeability of phospholipid membranes  
786 and chloroplast thylakoids. *Arch Biochem Biophys.* 226:558-566; 1983.
- 787 74. Jiang, Z.; Yin, X.; Jiang, Q. Natural forms of vitamin and 130-carboxychromanol, a  
788 long-chain vitamin E metabolite, inhibit leukotriene generation from stimulated

- 789 neutrophils by blocking calcium influx and suppressing 5-lipoxygenase activity,  
790 respectively. *J. Immunol.* 186:1173–1179; 2011.
- 791 75. Jiang, Q. Natural forms of vitamin E: metabolism, antioxidant and anti-inflammatory  
792 activities and their role in disease prevention and therapy. *Free Radic Biol Med.*  
793 72:69-90; 2014.
- 794 76. Halliwell, B. Vitamin C: poison, prophylactic or panacea? *Trends Biochem Sci.*  
795 24:255-559; 1999.
- 796 77. Carballal, S.; Bartesaghi, S.; Radi, R. Kinetic and mechanistic considerations to assess  
797 the biological fate of peroxynitrite. *Biochem Biophys Acta.* 1840:768-780; 2014.
- 798 78. Carr, A.C.; Bozonet, S.M.; Puller, J.M.; Simcock, J.W.; Vissers, M.C. Human skeletal  
799 muscle ascorbate is highly responsive to changes in vitamin C intake and plasma  
800 concentrations. *Am J Clin Nutr.* 97:800-807; 2013.
- 801 79. Mason, S.A.; Baptista, R.; DellaGatta, P.A.; Yousif, A.; Russell, A.P.; Wadley, G.D.  
802 High-dose vitamin C supplementation increases skeletal muscle vitamin C  
803 concentration and SVCT2 transporter expression but does not alter redox status in  
804 healthy males. *Free Radic Biol Med.* 77:130-138; 2014
- 805 80. Jones, D.P. Radical-free biology of oxidative stress. *Am J Physiol Cell Physiol.*  
806 295:C849-C868; 2008.
- 807 81. Jones, D.P.; Go, Y.M. Redox compartmentalization and cellular stress. *Diabetes Obes*  
808 *Metab.* 12:166-125; 2010;
- 809 82. Go, Y.M.; Jones, D.P. Redox compartmentalization in eukaryotic cells. *Biochim*  
810 *Biophys Acta.* 1780:1273-1290.
- 811 83. Albrect, S.C.; Barata; A.G.; Großhans, J.; Teleman, A.A.; Dick, T.P. In vivo mapping  
812 of hydrogen peroxide and oxidised glutathione reveals chemical and regional  
813 specificity of redox homeostasis. *Cell Metab.* 14:819-829; 2011.
- 814 84. Hansen, G.T.; Aggeler, R.; Oglesbee, D.; Cannon, M.; Capaldi, R.A.; Tsien, R.Y.;  
815 Remington, S.J. Investigating mitochondrial redox potential with redox-sensitive  
816 green fluorescent protein indicators. *J Biol Chem.* 279:13044-13053; 2004.
- 817 85. Patel, H.H.; Insel, P.A. Lipid rafts and caveolae and their role in compartmentation  
818 of redox signalling. *Antiox Redox Signal.* 11:1357-1372; 2009.
- 819 86. Ushio-Fukai, M. Compartmentalisation of redox signaling through NADPH oxidase-  
820 derived ROS. *Antiox Redox Signal.* 11:1289-1299; 2009.
- 821 87. Martinez-Ruiz, A.; Cadenas, S; Lamas, S. Nitric oxide signalling: Classical, less  
822 classical and nonclassical mechanisms. *Free Radic Biol Med.* 51:17-29; 2011.
- 823 88. Sakellariou, G.K.; Pye, D.; Vasilaki, A.; Zibrik, L.; Palomero, J.; McArdle, F.; et al.  
824 Role of superoxide–nitric oxide interactions in the accelerated age-related loss of  
825 muscle mass in mice lacking Cu,Zn superoxide dismutase. *Aging Cell.* 10:749–760;  
826 2011.
- 827 89. Stamler, J.S.; Meissner, G. Physiology of nitric oxide in skeletal muscle. *Physiol Rev.*  
828 81:209-237; 2001.
- 829 90. Palmer, R.M.J.; Ashton, D.S.; Moncada, S. Vascular endothelial cells synthesize  
830 nitric oxide from L-arginine. *Nature.* 333:664-666; 1988.

- 831 91. Halliwell, B.; Zhao, K.; Whiteman, M. Nitric oxide and peroxynitrite. The ugly, the  
832 uglier and the not so good: a personal view of recent controversies. *Free Radic Res.*  
833 31:651-669; 1999.
- 834 92. May, J. M. How does ascorbic acid prevent endothelial dysfunction? *Free Radic. Biol.*  
835 *Med.* 28:1421-1429; 2000.
- 836 93. Traber, M.G.; Stevens, J.F. Vitamins C and E: Beneficial effects from a mechanistic  
837 perspective. *Free Radic Biol Med.* 51:1000-1013; 2011.
- 838 94. Stuehr, D.J.; Santolini, J.; Wang, Z.Q.; Wei, C.C.; Adak, S. Update on mechanism  
839 and catalytic regulation in the NO synthases. *J. Biol. Chem.* 279:36167-36170; 2000.
- 840 95. Brandes, R.P.; Fleming, I.; Busse, R. Endothelial aging. *Cardiovasc Res* 66:286-294.  
841 2005.
- 842 96. Patel, K.B.; Stratford, M.R.; Wardman, P.; Everett, S.A. Oxidation of  
843 tetrahydrobiopterin by biological radicals and scavenging of the trihydrobiopterin  
844 radical by ascorbate. *Free Radic. Biol. Med.* 32:203-211; 2002.
- 845 97. Beckman, J.H.; Beckman, T.W.; Chen, J.; Marshall, P.A.; Freeman, B.A.; Apparent  
846 hydroxyl radical production by peroxynitrite: Implications for endothelial injury from  
847 nitric oxide and superoxide. *Proc. Natl. Acad. Sci. USA.* 87:1620-1624; 1990.
- 848 98. Botti, H.; Moller, M.N.; Steinmann, D.; Nauser, T.; Koppenol, W.H.; Denicola, A.;  
849 *Radi. R.* Distance-dependent diffusion-controlled reaction of •NO and O<sub>2</sub> •- at  
850 chemical equilibrium with ONOO, *J. Phys. Chem. B.* 114:16584-16593; 2011.
- 851 99. Ferrer-Sueta, G.; Radi, R. Chemical biology of peroxynitrite: kinetics, diffusion, and  
852 Radicals. *ACS Chem. Biol.* 4:161-177; 2009.
- 853 100. Radi, R. Peroxynitrite, a stealthy biological oxidant. *J Biol Chem.*  
854 288:26464:26472; 2013.
- 855 101. Beckman, J.H.; Koppenol, W.H. Nitric oxide, superoxide, and peroxynitrite:  
856 the good, the bad, and ugly. *Am. J. Physiol.* 271:C1424-C1437; 1996.
- 857 102. Pearson, T.; McArdle, A.; Jackson, M.J. Nitric oxide availability is increased  
858 in contracting skeletal muscle from aged mice, but does not differentially decrease  
859 muscle superoxide. *Free Radic Biol Med.* 78:82-88; 2015.
- 860 103. Bryk, R.; Griffin, P.; Nathan, C. Peroxynitrite reductase activity of bacterial  
861 peroxiredoxins. *Nature.* 407:211-215; 2000.
- 862 104. Denicola, A.; Freeman, B. A.; Trujillo, M.; Radi, R. Peroxynitrite reaction  
863 with carbon dioxide/bicarbonate: kinetics and influence on peroxynitrite-mediated  
864 oxidations, *Arch. Biochem. Biophys.* 333:49-58; 1996.
- 865 105. Dubussion, M.; Vander Stricht, D.; Clippe, A.; Etienne, F.; Nauser, T.;  
866 Kissner, R.; et al. Human peroxiredoxin 5 is a peroxynitrite reductase. *FEBS letters.*  
867 571:161-165; 2004.
- 868 106. Trujillo, M.; Clippe, M.; Manta, A.; Ferrer-Sueta, B.; Smeets, G.; Declercq,  
869 A.; et al. Pre-steady state kinetic characterization of human peroxiredoxin 5: taking  
870 advantage of Trp84 fluorescence increase upon oxidation. *Arch Biochem Biophys.*  
871 467:95-106; 2007.
- 872 107. Radi, R.; Peluffo, G.; Alvarez, M.N.; Naviliat, M.; Cayota, A. Unravelling  
873 peroxynitrite formation in biological systems. *Free Radic. Biol. Med.* 30:463-488;  
874 2001.

- 875 108. Lymar, S. V.; Hurst, J. K. Rapid reaction between peroxonitrite ion and carbon  
876 dioxide: Implications for biological activity. *J Am Chem Soc.* 117:8867–8868; 1995.
- 877 109. Augusto, O.; Bonini, M.G.; Amanso, A.M.; Linares, E; Santos, C.C.; De  
878 Menezes, S.L. Nitrogen dioxide and carbonate radical anion: two emerging radicals in  
879 biology. *Free Radic. Biol. Med.* 32:841–859; 2002.
- 880 110. Franco, M.C.; Ye, Y.; Refakis, C.A.; Feldman, J.L.; Stokes, A.L.; Basso, M.;  
881 et al. Nitration of Hsp90 induces cell death. *Proc Natl Acad Sci USA.* 110:E1102-  
882 E1111; 2013.
- 883 111. Asahi, M.; Fujii, J.; Suzuki, K.; Seo, H.G.; Kuzuya, T.; Hori, M.; et al.  
884 Inactivation of glutathione peroxidase by nitric oxide: implication for cytotoxicity. *J*  
885 *Biol. Chem.* 270: 21035–21039; 1995.
- 886 112. Padjama, S.; Squadrito, G.L; Pryor, W.A. Inactivation of glutathione  
887 peroxidase by peroxynitrite. *Arch Biochem. Biophys.* 349:1–6; 1998.
- 888 113. Yamakura, F.; Taka, H.; Fujimura, T.; Murayam, K. Inactivation of human  
889 manganese-superoxide dismutase by peroxynitrite Is Caused by exclusive nitration of  
890 Tyrosine 34 to 3-Nitrotyrosine. *J. Biol. Chem.* 273:14085–14089; 1998.
- 891 114. Wood, Z.A.; Poole, A.B.; Karplus, P.A. Peroxiredoxin evolution and the  
892 regulation of hydrogen peroxide signalling. *Science.* 300:650-653; 2003.
- 893 115. Woo, H.A.; Yim, S.H.; Shin, D.H.; Kang, D.; Yu, D.; Rhee, S.G. Inactivation  
894 of peroxiredoxin I by phosphorylation allows localized H<sub>2</sub>O<sub>2</sub> Accumulation for cell  
895 signalling. *Cell.* 140:517–528; 2010.
- 896 116. Niki, E. Role of vitamin E as a lipid-soluble peroxy scavenger: in vitro and in  
897 vivo evidence. *Free Radic Biol Med.* 66:3-12; 2014.
- 898 117. Botti, H.; Batthyany, C.; Trostchansk, A.; Radi, R.; Freeman, B.A.; Rubbo, H.  
899 Peroxynitrite-mediated oxidation of  $\alpha$ -tocopherol in low density lipoprotein: A  
900 mechanistic approach. *Free Radic Biol Med.* 32:152-162; 2004.
- 901 118. D’Autreaux, B.; Toldeano, M.B. ROS as signalling molecules: mechanisms  
902 that generate specificity in ROS homeostasis. *Nat Rev Mol Cell Biol.* 8:813-824;  
903 2007.
- 904 119. Giorgio, M.; Trineli, M.; Migliaccio, E.; Pelicci, P.G. Hydrogen peroxide: a  
905 metabolic by-product or common mediator of ageing signals? *Nat Rev Mol Cell Biol.*  
906 8:722-728; 2007.
- 907 120. Sies, H. Role of metabolic H<sub>2</sub>O<sub>2</sub> generation: redox signalling and oxidative  
908 stress. *J Biol Chem*, DOI:10.1074/jbc.R113.544635; 2014.
- 909 121. Winterbourn, C.C. The biological chemistry of hydrogen peroxide. *Methods*  
910 *Enzymol.* 528:3-25; 2013.
- 911 122. Toppo, S.; Flohe, L.; Ursini, F.; Vanin, S.; Maiorino, M. Catalytic  
912 mechanisms and specificities of glutathione peroxidases: variations of a basic scheme.  
913 *Biochim Biophys Acta.* 1790:1486–1500; 2009.
- 914 123. Chance, B.; Greenstein, D.S.; Roughton, F.J.W. The mechanism of catalase  
915 action. I. Steady-state analysis. *Arch Biochem Biophys.* 37:301–339; 1952.
- 916 124. Karplus, P.A. A primer on peroxiredoxin biochemistry. *Free Radic Biol Med.*  
917 DOI: doi: 10.1016/j.freeradbiomed.2014.10.009; 2014.

- 918 125. Marhino, H.S.; Real, C.; Cryne, L.; Soares, H.; Antunes, F. Hydrogen  
919 peroxide sensing, signalling and regulation of transcription factors. *Redox Biol.*  
920 2:535-562; 2014.
- 921 126. Kang, S.W.; Chang, T.S.; Lee, T.H.; Kim, E.S.; Yu, D.Y.; Rhee, S.G.  
922 Cytosolic peroxiredoxin attenuates the activation of Jnk and p38 but potentiates that  
923 of Erk in Hela cells stimulated with tumor necrosis factor-alpha. *J Biol Chem.*  
924 279:2535-2543, 2004.
- 925 127. Sobatto, M.C.; Liou, W.; Stocker, S.; Talwar, D.; Oehler, M.; Ruppert, T.; et  
926 al. Peroxiredoxin-2 and STAT3 form a redox relay for H<sub>2</sub>O<sub>2</sub> signaling. *Nat Chem*  
927 *Biol.* 11:64-70; 2015.
- 928 128. Bae, Y.S.; Oh, H.; Rhee, S.G.; Yoo, Y.D. Regulation of reactive oxygen  
929 species generation in cell signaling. *Mol. Cells.* 32:491-509; 2011.
- 930 129. Meng, T.C.; Fukada, T.; Tonks, N.K. Reversible oxidation and inactivation of  
931 protein tyrosine phosphatases in vivo. *Mol. Cell.* 9:387-399; 2002.
- 932 130. Rhee, S.C. Cell signalling: H<sub>2</sub>O<sub>2</sub>, a necessary evil for cell signalling. *Science.*  
933 312:1882-1883; 2006.
- 934 131. Kwon, J.; Lee, S. R.; Yang, K. S.; Ahn, Y.; Kim, Y. J.; Stadtman, E. R.; Rhee,  
935 S. G. Reversible oxidation and inactivation of the tumor suppressor PTEN in cells  
936 stimulated with peptide growth factors. *Proc Natl Acad Sci USA.* 101:16419-16424;  
937 2004.
- 938 132. Rhee, S.C.; Kang, S.W.; Jeong, W.; Chang, T.S.; Kang, K.S.; Woo, H.A.  
939 Intracellular messenger function of hydrogen peroxide and its regulation by  
940 peroxiredoxins. *Curr Opin Cell Biol.* 17:183-189; 2005.
- 941 133. Murphy, M.P.; Holmgren, A.; Larsson, N.; Halliwell, B.; Chang, C.J.;  
942 Kalyanaraman, B.; et al. Unravelling the biological roles of reactive oxygen species.  
943 *Cell Metab.* 13:361-366; 2011.
- 944 134. McCord, J.M.; Fridovich, I. Superoxide dismutase: an enzymatic function for  
945 erythrocyte (Hemocuprein). *J Biol Chem.* 244:6049-6055; 1969.
- 946 135. Li, W.G.; Miller, F.J.; Zhang, H.J.; Spitz, D.R.; Oberley, L.W.; Weintraub, N.L.  
947 H<sub>2</sub>O<sub>2</sub>-induced O<sub>2</sub> production by a non-phagocytic NAD(P)H oxidase causes  
948 oxidant injury. *J. Biol. Chem.* 276:29251-29256; 2001.
- 949 136. Sun, Q.A.; Hess, D.T.; Nogueira, L.; Yong, S.; Bowles, D.E.; Eu, J.; et al.  
950 Oxygen-coupled redox regulation of the skeletal muscle ryanodine receptor-Ca<sup>2+</sup>  
951 release channel by NADPH oxidase4. *Proc Natl Acad Sci USA.* 108:16098-16103;  
952 2011.
- 953 137. Halliwell, B. The antioxidant paradox. *Br J Clin Pharmacol.* 75:637-644;  
954 2012.
- 955 138. Halliwell, B. Free radicals and antioxidants: updating a personal view. *Nutr*  
956 *Rev.* 70:257-265; 2012.
- 957 139. Hess, D.T.; Mastumoto, A.; Kim, S.; Marshall, H.E.; Stamler, J.S. Protein S-  
958 nitrosylation: purview and parameters. *Nat Rev Mol Cell Biol.* 6:150-166; 2005.
- 959 140. Zhang, Y.; Hogg, N. S-Nitrosothiols: cellular formation and transport. *Free*  
960 *Radic Biol Med.* 38:831-838; 2005.



- 961 141. Gould, N.; Doulias, P.; Tenopoulou, M.; Raju, K.; Ischiropoulos, H.  
962 Regulation of protein function and signalling by reversible cysteine S-nitrosylation. *J*  
963 *Biol Chem.* 288:26473-26479; 2013.
- 964 142. Benhar, M.; Forrester, M.T.; Stamler, J.S. Protein denitrosylation: enzymatic  
965 mechanisms and cellular functions. *Nat Rev Mol Cell Biol.* 10:721-732; 2009.
- 966 143. Smith, B.C.; Marletta M.A. Mechanisms of S-nitrosothiol formation and  
967 selectivity in nitric oxide signaling. *Curr Opin Chem Biol.* 16:498-506; 2012.
- 968 144. Yasinska, I.M.; Sumbayev, V.V. S-nitrosation of Cys-800 of HIF-1 $\alpha$  protein  
969 activates its interaction with p300 and stimulates its transcriptional activity. *FEBS*  
970 *Lett.* 549:105–109; 2003.
- 971 145. Schonhoff, C. M.; Daou, M. C.; Jones, S. N.; Schiffer, C. A.; Ross, A. H.  
972 Nitric oxide-mediated inhibition of Hdm2–p53 binding. *Biochemistry* . 41:13570–  
973 13574; 2002.
- 974 146. Eu, J.P.; Sun, J.; Xu, L.; Stamler, J.S.; Meissner, G. The skeletal muscle  
975 calcium release channel: coupled O<sub>2</sub> sensor and NO signaling functions.  
976 *Cell* .102:499–509; 2000.
- 977 147. Huang, B.; Chen, C. An ascorbate-dependent artifact that interferes with the  
978 interpretation of the biotin switch assay. *Free Radic. Biol. Med.* 41:562–567; 2006.
- 979 148. Forrester, M.T.; Foster, M.W.; Benhar, M.; Stamler, J.S. Detection of protein  
980 S-nitrosylation with the biotin-switch technique. *Free Radic Biol Med.* 46:119-126;  
981 2009.
- 982 149. Holmes, A.J.; Williams, D.L.H. Reaction of ascorbic acid with S-nitrosothiols:  
983 clear evidence for two distinct reaction pathways. *J Chem Soc, Perkin Trans.* 2:1639–  
984 1644; 2000.
- 985 150. Seth, D.; Stamler, J.S. The S-NO proteome causation and classification. *Curr*  
986 *Opin Chem Biol.* 15:129:136; 2011.
- 987 151. Niki, E. Lipid peroxidation: physiological levels and dual biological effects.  
988 *Free Radic Biol Med.* 47:469–484; 2009.
- 989 152. Niki, E. Do antioxidants impair signaling by reactive oxygen species and  
990 lipidoxidation products? *Febs Letters.* 586:3767-3770; 2012.
- 991 153. Chen, Z.H.; Saito, Y.; Yoshida, Y.; Serkine, A.; Noguchi, N.; Niki, E. 4-  
992 Hydroxynonenal induces adaptive response and enhances PC12 cell tolerance  
993 primarily through induction of thioredoxin reductase 1 via activation of Nrf2. *J. Biol.*  
994 *Chem.* 280:41921–41927; 2005.
- 995 154. Chen, Z.H.; Yoshida, Y.; Saito, Y.; Serkine, A.; Noguchi, N.; Niki, E.  
996 Induction of adaptive response and enhancement of PC12 cell tolerance by 7-  
997 hydroxycholesterol and 15- deoxy-delta(12,14)-prostaglandin J<sub>2</sub> through up-  
998 regulation of cellular glutathione via different mechanisms. *J. Biol. Chem.*  
999 281:14440–14445; 2006.
- 1000 155. Kasper, J.W.; Niture, S.K.; Jaiswal, A.K. Nrf2:INrf2 (Keap1) signalling in  
1001 oxidative stress. *Free Radic Biol Med.* 47:1304-1309; 2009.
- 1002 156. Brandes, R.P., Weissman., N.; Schroder, K. Nox family NADPH oxidases:  
1003 Molecular mechanisms of activation. 76:208-226; 2014.

- 1004 157. Halliwell, B.; Whiteman, M. Measuring reactive species and oxidative damage  
1005 in vivo and in cell culture: how should you do it and what do the results mean? *Br J*  
1006 *Pharmacol.* 142:231-255; 2004.
- 1007 158. Margaritelis, N.V.; Kyparos, A.; Paschalis, V.; Paschalis, A.A.; Panayiotou,  
1008 G.; Zafeiridis, Z.; et al. Reductive stress after exercise: The issue of redox  
1009 individuality. *Redox Biol.* 2:520–528; 2014.
- 1010 159. Nikolaidis, M.G.; Jamurtas, A.Z. Blood as a reactive species generator and  
1011 redox status regulator during exercise. *Arch Biochem Biophys.* 490:77-84; 2009.
- 1012 160. Negre-Salvayre, A.; Auge, N.; Ayala, V.; Basaga, H.; Boada, J.; Brenke, R.; et  
1013 al. Pathological aspects of lipid peroxidation. *Free Radic Res.* 44:1125–1171; 2010.
- 1014 161. Jones, D.P. Redefining oxidative stress. *Antiox Redox Signal.* 8:1865-1879;  
1015 2006.
- 1016 162. Day, B.J. Antioxidant therapeutics: Pandora's box. *Free Radic Biol Med.*  
1017 66:58-64; 2014.
- 1018 163. Murphy, M.P. Antioxidants as therapies: can we improve on nature? *Free*  
1019 *Radic Biol Med.* 66:20-23; 2014.
- 1020 164. Chen, A.F.; Chen, D.D.; Daiber, A.; Faraci, F.M.; Li, H.; Rembold, C.M.;  
1021 Laher, I. Free radical biology of the cardiovascular system. *Clin Sci.* 123:73-91; 2012.
- 1022 165. Paschalis, V.; Theodorou, A.A.; Kyparos, A.; Dipla, K.; Zafeiridis, A.;  
1023 Panayiotou, G.; et al.; Low vitamin C values are linked with decreased physical  
1024 performance and increased oxidative stress: reversal by vitamin C supplementation.  
1025 *Eu J Nutr.* Epub ahead of print; 2014.
- 1026 166. Stepanyan, V.; Crowe, M.; Haleagrahara, N.; Bowden, B. Effects of vitamin E  
1027 supplementation on exercise-induced oxidative stress: a meta-analysis. *Appl. Physiol.*  
1028 *Nutr. Metab.* 39:1029–1037; 2014.
- 1029 167. Halliwell, B. Vitamin C and genomic instability. *Muta Res.* 475:29-35; 2001.
- 1030 168. Chen, Q.; Espey, M.G.; Krishna, M.C.; Mitchell, J.B.; Corpe, C.P.; Buettner,  
1031 G.R.; et al. Pharmacologic ascorbic acid concentrations selectively kill cancer cells:  
1032 action as a pro-drug to deliver hydrogen peroxide to tissues. *Proc. Natl. Acad. Sci. U.*  
1033 *S. A.* 102:13604–13609; 2005.
- 1034 169. Du, J.; Cullen, J.J.; Buettner, G.R. Ascorbic acid: Chemistry, biology and the  
1035 treatment of cancer. *Biochem et Biophys Acta.* 1826:443-457; 2012.
- 1036 170. Coyle, P., Philcox, J.C., Carey, L.C. & Rofe, A.M. Metallothionein: the  
1037 multipurpose protein. *Cell Mol Life Sci.* 59:627-647; 2002.
- 1038 171. Dixon, S.J.; Stockwell. B.R. The role of iron and reactive oxygen species in  
1039 cell death. *Nature Chem Biol.* 10:9-17; 2014.
- 1040 172. Mahoney, D.J.; Praise, G.; Melov, S.; Safdar, A.; Tarnopolsky, M.A. Analysis  
1041 of global mRNA expression from human skeletal muscle during recovery from  
1042 endurance exercise. *FASEB J.* 19:1498-1500; 2005.
- 1043 173. Kerkweg, U.; Pamp, K.; Fieker, J.; Petrat, F.; Hider, R.C.; de Groot, H.  
1044 Release of redox-active iron by muscle crush trauma: no liberation into the  
1045 circulation. *Shock.* 33:513-518; 2010.
- 1046 174. Theodorou, A.A.; Nikolaidis, M.G.; Paschalis, S.; Sakellariou, G.K.; Fatouros,  
1047 I.G.; Koutedakis, Y.; et al. Comparison between glucose-6-phosphate dehydrogenase-

- 1048 deficient and normal individuals after eccentric exercise. *Med Sci Sport Exerc.*  
1049 42:1113-1121; 2010.
- 1050 175. Michels, A.J.; Frei, B. Myths, Artifacts, and Fatal Flaws: Identifying  
1051 Limitations and Opportunities in Vitamin C Research. *Nutrients.* 5:5161-5192; 2013.
- 1052 176. Bowry, V.W.; Mohr, D.; Cleary, J.; Stocker, R. Prevention of tocopherol-  
1053 mediated peroxidation in ubiquinol-10-free human low density lipoprotein. *J.*  
1054 *Biol.Chem.* 270:5756–5763; 1995.
- 1055 177. Pompella, A.; Visvikis, A.; Paolicchi, A.; De Tata, V.; Casini, A.F. The  
1056 changing face of glutathione, a cellular protagonist. *Biochem Pharmacol.*  
1057 66:1499:1503; 2003.
- 1058 178. Kalyanaraman, B.; Usmar, V.; Davies, K.J.A.; Dennery, P.A.; Forman, R.J.;  
1059 Grisham, M.B.; et al. Measuring reactive oxygen and nitrogen species with  
1060 fluorescent probes: challenges and limitations. *Free Radic Biol Med.* 52:1-6; 2012.
- 1061 179. Wardman, P. Fluorescent and luminescent probes for measurement of  
1062 oxidative and nitrosative species in cells and tissues: Progress, pitfalls, and prospects.  
1063 *Free Radic Biol Med.* 43:995-1022; 2007.
- 1064 180. Winterbourn, C.C. The challenges of using fluorescent probes to detect and  
1065 quantify specific reactive oxygen species in living cells. *Biochim Biophys Acta.*  
1066 1840:730-738; 2014.
- 1067 181. Halliwell, B. Oxidative stress in cell culture: an under-appreciated problem?  
1068 *FEBS Lett.* 540:3-6; 2003.
- 1069 182. Halliwell, B. Cell culture, oxidative stress, and antioxidants: avoiding pitfalls.  
1070 *Biomed J.* 37: 99-105; 2014.
- 1071 183. Burniston, J.G.; Hoffman, E.P. Proteomic responses of skeletal and cardiac  
1072 muscle to exercise. *Expr Rev Proteomics.* 8:361-367; 2011.
- 1073 184. Burniston, J.G.; Gray, S.; Kenyani, J.; Harafuji, N.; Jarman, I.H.; Cogley, J.N.;  
1074 et al. Conditional independence mapping of DiGE data reveals PDIA3 protein species  
1075 as key nodes associated with muscle aerobic capacity. *J Proteomics.* 106:230-245;  
1076 2014.
- 1077 185. Malik, Z.A.; Cogley, J.N.; Morton, J.P.; Close, G.L.; Edwards, B.J.; Koch,  
1078 L.G.; et al.; Label-free LC-MS profiling of skeletal muscle reveals heart-type fatty  
1079 acid binding protein as a candidate marker of aerobic capacity. *Proteomes.* 1:290-308;  
1080 2013.
- 1081 186. McDonough, B.; Sakellariou, G.K.; Jackson, M.J. Application of redox  
1082 proteomics to skeletal muscle aging and exercise. *Biochem Soc Trans.* 42:965-970;  
1083 2014.
- 1084 187. Mermelekas G.; Makridakas, M.; Koech, T; Valhou, A. Redox proteomics:  
1085 from residue modifications to putative biomarker identification by gel- and LC-MS-  
1086 based approaches. *Exp Rev Proteomics.* 10:537-549; 2013.
- 1087 188. Collins, Y.; Chouchani, E.T.; James, A.M.; Menger, K.E.; Cocheme, H.M.;  
1088 Murphy, M.P. Mitochondrial redox signalling at a glance. *J Cell Sci.* 125:801-806;  
1089 2012.

- 1090 189. Cobley, J.N.; Moulton, P.R.; Burniston, J.G.; Morton, J.P.; Close, G.L. Exercise  
1091 improves mitochondrial and redox-regulated stress responses in the elderly: better late  
1092 than never! *Biogerontology*. DOI: 10.1007/s10522-014-9546-8; 2014.
- 1093 190. Kaczmarek, M.; Timofeeva, O. A.; Karaczyn, A.; Malyguine, A.; Kasprzak,  
1094 K. S.; Salnikow, K. The role of ascorbate in the modulation of HIF-1 $\alpha$  protein and  
1095 HIF dependent transcription by chromium(VI) and nickel(II). *Free Radic. Biol. Med.*  
1096 42:1246–1257; 2007.
- 1097 191. Kuiper, C.; Molenaar, I. G.; Dachs, G. U.; Currie, M. J.; Sykes, P. H.; Vissers,  
1098 M. C.; et al. ascorbate levels are associated with increased hypoxia-inducible factor-1  
1099 activity and an aggressive tumor phenotype in endometrial cancer. *Cancer Res.*  
1100 70:5749–5758; 2010.
- 1101 192. Jaakkola, P.; Mole, D.R.; Tian, Y.M.; Wilson, M.I.; Gielbert, J.; Gaskell, S. J.;  
1102 et al. Targeting of HIF- $\alpha$  to the von Hippel-Lindau ubiquitylation complex by O<sub>2</sub>-  
1103 regulated prolyl hydroxylation. *Science*. 292:468–472; 2001.
- 1104 193. Schofield, C. J.; Ratcliffe, P. J. Oxygen sensing by HIF hydroxylases. *Nat.*  
1105 *Rev. Mol. Cell Biol.* 5:343–354; 2004.
- 1106 194. Cyr, A.R.; Domann, F.E. The redox basis of epigenetic modifications: from  
1107 mechanisms to functional consequences, *Antioxid. Redox Signal.* 15:551–589; 2011.
- 1108 195. Esteban, M.A.; Pei, D. Vitamin C improves the quality of somatic cell  
1109 reprogramming. *Nat. Genet.* 44:366–367; 2012.
- 1110 196. Barres, R.; Yan, J.; Egan, B.; Treebak, J.T.; Rasmussen, M.; Fritz, T; et al.  
1111 Acute exercise remodels promoter methylation in human skeletal muscle *Cell Metab.*  
1112 15:405–411; 2012.
- 1113 197. Azzi, A.; Ricciarelli, R.; Zingg, J.M. Non-antioxidant molecular functions of  
1114  $\alpha$ -tocopherol (vitamin E). *FEBS Lett.* 519:8-10. 2002.
- 1115 198. Azzi, A.; Gysin, R.; Kempna, P.; Munteanu, A.; Villacorta, L.; Visarius, T.;  
1116 Zingg, J.M. Regulation of gene expression by  $\alpha$ -tocopherol. *Biol Chem.* 385:585-591;  
1117 2004.
- 1118 199. Zingg, J.M.; Azzi, A. Non-antioxidant activities of vitamin E. *Curr Med*  
1119 *Chem.* 11:1113-1133; 2004.
- 1120 200. Timmons, J.A. Variability in training-induced skeletal muscle adaptation. *J*  
1121 *Appl Physiol.* 110:846-853; 2011.
- 1122 201. Cobley, J.N.; McGlory, C.; Morton, J.P.; Close, G.L. N-Acetylcysteine's  
1123 attenuation of fatigue after repeated bouts of intermittent exercise: practical  
1124 implications for tournament situations. *Int J Sports Nutr Exerc Metab.* 21:451-461;  
1125 2011.

## 1126 **Figure Legends**

1127 **Figure 1:** A) A current general scheme. In this generic model, exercise increases ROS/RNS  
1128 generation and this is associated with kinase activation. Ascorbate and  $\alpha$ -tocopherol are  
1129 proposed to reduce ROS/RNS generation to interfere with phosphatase inactivation. Note that  
1130 in this general model the specific species are not identified underscoring a significant  
1131 limitation of this generic model. From this scheme it is not possible to appraise whether this

1132 redox dependent mode of action is feasible. B) Proposed specific scheme. In this model,  
1133 exercise activates NADPH oxidases resulting in increased superoxide production. Superoxide  
1134 is then dismutated to hydrogen peroxide in a reaction catalysed by SOD isoforms. Hydrogen  
1135 peroxide then reacts, in a two electron reaction, with the phosphatase PTP1B, possibly  
1136 relieving kinase inhibition. Whether this is possible given the peroxiredoxin kinetic  
1137 bottleneck is discussed in text. Nevertheless, ascorbate could inhibit this signalling response  
1138 by competing with SOD isoforms and NO (not shown for clarity) for reaction with  
1139 superoxide.

1140 **Figure 2:** Reduction of potentially bioactive oxidised macromolecule adducts. In this model,  
1141 exercise increases superoxide, NO, peroxynitrite and H<sub>2</sub>O<sub>2</sub> generation resulting in the  
1142 generation of bioactive oxidised adducts, such as 4-hydroxynoneneal. This could lead to Nrf-  
1143 2 activation and the induction of a cyto-protective response via S-alkylation of KEAP1, a  
1144 negative regulator of Nrf-2. Any ascorbate and  $\alpha$ -tocopherol mediated reduction in bioactive  
1145 oxidised macromolecule adducts could attenuate Nrf-2 activation. However, this possibility is  
1146 speculative for several reasons that are discussed in text.

1147 **Figure 3:** Summary of the limited reaction of ascorbate and  $\alpha$ -tocopherol with specific  
1148 reactive species implicated in exercise-induced redox signalling. Of note, ascorbate can react  
1149 with superoxide (O<sub>2</sub><sup>•-</sup>) and this could have implications for exercise-induced redox signalling.  
1150 The existence of kinetically favourable out-competing reactions for nitric oxide, hydrogen  
1151 peroxide and peroxynitrite might restrict any interference via a scavenging mechanism at  
1152 least for these species. It is possible for nitrogen dioxide and carbonate radical, but the roles  
1153 of these radicals in redox signalling is not well established.

## 1154 **Box**

1155 **Box 1. Assumptions implicit in a redox dependent mechanism of action.**

Assumptions implicit in a redox dependent mechanism of action.

1. Specific ROS/RNS are involved in redox signalling.
2. Ascorbate and  $\alpha$ -tocopherol react chemically with the relevant ROS/RNS.
3. The localisation of ascorbate and  $\alpha$ -tocopherol makes interference in cellular microdomains implicated in redox signalling likely (e.g. lipid rafts).
4. Ascorbate and  $\alpha$ -tocopherol out-compete enzymes and/or other ROS/RNS for reaction with the relevant ROS/RNS.

1156