

TOPICAL REVIEW

# Impact of extreme exercise at high altitude on oxidative stress in humans

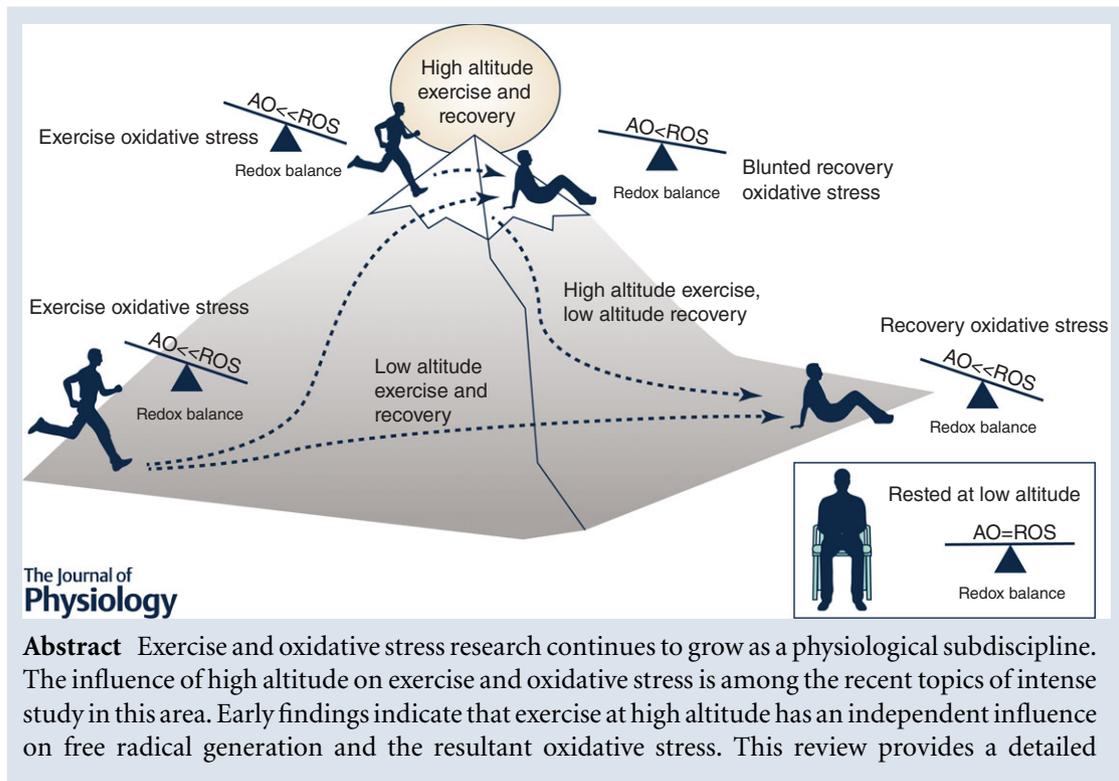
John Quindry<sup>1</sup>, Charles Dumke<sup>2</sup>, Dustin Slivka<sup>3</sup> and Brent Ruby<sup>2,4</sup>

<sup>1</sup>School of Kinesiology, Auburn University, Auburn, AL, USA

<sup>2</sup>Department of Health and Human Performance, University of Montana, Missoula, MT, USA

<sup>3</sup>School of Health, Physical Education and Recreation, University of Nebraska at Omaha, Omaha, NE, USA

<sup>4</sup>Montana Centre for Work Physiology and Exercise Metabolism, University of Montana, Missoula, MT, USA



**Abstract** Exercise and oxidative stress research continues to grow as a physiological subdiscipline. The influence of high altitude on exercise and oxidative stress is among the recent topics of intense study in this area. Early findings indicate that exercise at high altitude has an independent influence on free radical generation and the resultant oxidative stress. This review provides a detailed

**John Quindry** received a PhD from East Tennessee State University/Quillen College of Medicine, completed post-doctoral research at the University of Florida and was on faculty at Appalachian State University. He is currently at Auburn University, where he examines cardioprotection and oxidative stress. **Charles Dumke** received a PhD from the University of Wisconsin. He worked at Appalachian State University prior to the University of Montana. **Dustin Slivka** received a PhD from Ball State University, carried out post-doctoral research at the Montana Center for Work Physiology and Metabolism and now works at the University of Nebraska at Omaha. His main area of research is environmental physiology with focus on cellular mechanisms of the exercise response and adaptation to extreme environments. **Brent Ruby** received a PhD from the University of New Mexico. He works at the University of Montana where he investigates the physiological responses to extreme exercise in challenging environmental conditions.



summary of oxidative stress biochemistry as gleaned mainly from studies of humans exercising at high altitude. Understanding of the human response to exercise at altitude is largely derived from field-based research at altitudes above 3000 m in addition to laboratory studies which employ normobaric hypoxia. The implications of oxidative stress incurred during high altitude exercise appear to be a transient increase in oxidative damage followed by redox-sensitive adaptations in multiple tissues. These outcomes are consistent for lowland natives, high altitude acclimated sojourners and highland natives, although the latter group exhibits a more robust adaptive response. To date there is no evidence that altitude-induced oxidative stress is deleterious to normal training or recovery scenarios. Limited evidence suggests that deleterious outcomes related to oxidative stress are limited to instances where individuals are exposed to extreme elevations for extended durations. However, confirmation of this tentative conclusion requires further investigation. More applicably, altitude-induced hypoxia may have an independent influence on redox-sensitive adaptive responses to exercise and exercise recovery. If correct, these findings may hold important implications for athletes, mountaineers, and soldiers working at high altitude. These points are raised within the confines of published research on the topic of oxidative stress during exercise at altitude.

(Received 8 July 2015; accepted after revision 28 September 2015; first published online 10 October 2015)

**Corresponding author** J. C. Quindry: Cardioprotection Laboratory, School of Kinesiology, Auburn University, Auburn, AL 36830, USA. Email: jcq0001@auburn.edu

**Abstract figure legend:** Summary of oxidative stress responses to exercise and recovery at low and high altitudes. Independent of altitude oxidative stress is induced by acute exercise in proportion to either exercise intensity or exercise duration. Redox balance, as indicated by depletion of endogenous antioxidants (AO) and production of reactive oxygen species (ROS), shifts toward oxidative stress during and following exercise at low and high altitudes (hiker symbol). Recent findings suggest that recovery (reclined stick figure) from high altitude exercise or ascension from low-to-high altitude is marked by an attenuated oxidative stress response as compared with recovery at lower elevations. Moreover, early findings indicate that redox-sensitive adaptations to exercise-induced oxidative stress may be negatively impacted by high altitude recovery. In contrast to exercise, healthy rested (inset) individuals at low altitude are often in redox balance and without oxidative stress.

**Abbreviations** CAT, catalase; FRAP, ferric reducing antioxidant potential; GPx, glutathione peroxidase; GR, glutathione reductase; GSH, reduced glutathione; GSSG, oxidized glutathione; HIF-1 $\alpha$ , hypoxia-inducible factor 1 $\alpha$ ; HNE, 4-hydroxynonenal; HMOX1, haem oxygenase 1; NFE2L2, nuclear factor erythroid 2-like 2; ROS, reactive oxygen species; SOD, superoxide dismutase; MnSOD, manganese-dependent superoxide dismutase; VEGF, vascular endothelial growth factor.

## Introduction

Oxidative stress has been the focus of exercise-based research for more than three decades. In recent years, intense research efforts have been directed at understanding oxidative stress and exercise in climatic scenarios including high altitude. Exercise adaptations to altitude accrue in proportion to elevation, beginning around 1000–1500 m. A survey of the literature, however, reveals that responses to ‘high altitude’ are generally limited to elevations above 3000 m (Sinha *et al.* 2009a; Faiss *et al.* 2013; Miller *et al.* 2013; Lewis *et al.* 2014; McGinnis *et al.* 2014). Within the context of exercise, applications are often limited to endurance/ultra-endurance competitions, recreational and military-related mountain trekking, altitude considerations of living and training for elite endurance athletes, and vigorous activity as performed by high-altitude natives. What is currently known about extreme exercise and oxidative stress at high altitude

is generally derived from a limited number of field studies. Additional understanding is extracted from well-controlled laboratory studies of exercise performance and blood biomarker alteration in humans exposed to normobaric hypoxia in an environmental chamber. However, current knowledge of muscle level adaptations to hypoxic exercise is largely procured from a handful of animal-based research studies. Accordingly, understanding of oxidative stress related to exercise and high altitude is still unfolding.

The aim of this review is to examine what is currently known about exercise and oxidative stress at high altitude. The paucity of published investigations specific to exercise at high altitude is such that early conclusions may need to be contextualized to exercise and oxidative stress findings from all altitudes. Moreover, modern understanding about exercise and free radicals, independent of altitude, is sometimes limited due to common misconceptions about the biomarkers used to

quantify oxidative stress. Accordingly, a brief discussion of oxidative stress biomarkers is necessary to better understand their responses during exercise and recovery at altitude.

### Examination of oxidative stress during high altitude exercise

The oxidative biomarkers fundamental to altitude research are identical to those collected for sea-level exercise. The labile nature of oxidative stress is such that most researchers examine a composite response founded upon a biomarker panel. Since the inception of this research line in the late 1970s (Dillard *et al.* 1978), oxidative stress responses to exercise have been determined by (1) elevations in oxidative damage markers, (2) alterations in endogenous antioxidant systems (enzymatic and non-enzymatic antioxidants), and (3) alterations in redox-sensitive gene transcripts and their corresponding proteins.

Quantification of oxidative damage entails examination of relatively stable redox-sensitive lipid, protein and nucleotide end products (Powers *et al.* 2011). In addition to these 'finger print' biomarkers of oxidative damage, oxidative stress is quantified by examination of antioxidant content. Examination of antioxidant status is arguably more complex than quantification of oxidative damage markers due to the fact that enzymatic antioxidant protein content and activity can be measured. Moreover, the contribution of non-enzymatic antioxidants must also be considered (reviewed in Powers *et al.* 2011). In the latter instance, the contribution of low molecular mass antioxidants includes protein/non-protein, thiol/non-thiol, and lipid soluble/aqueous antioxidants. Thus, quantification of the antioxidant network is important due to the fact that a variety of antioxidants work in concert to quench a host of free radical parent molecules and their oxidized products. Within a biological fluid, oxidative stress occurs according to a biochemical pecking order such that aqueous antioxidants are depleted prior to lipid soluble antioxidants, which are then exhausted prior to the appearance of oxidative damage markers (Buettner, 1993). In reality, however, the pecking order of oxidative stress reactions is further complicated by the fact that oxidative chain reactions are not completely partitioned within or between cells and tissues. Accordingly, many researchers incorporate assays of antioxidant capacity (trolox equivalent antioxidant capacity) or radical quenching capacity (ferric reducing ability of plasma, oxygen radical absorbance capacity) as a comprehensive method of quantifying the antioxidant dynamic within a few variables (Cao & Prior, 1998). Finally, examination of redox-sensitive gene

transcripts related to mitochondrial biogenesis, endogenous antioxidant up-regulation, and cellular stress responses reflects the acute exercise stimulus and holds important implications for the adaptive response to exercise at altitude (Powers *et al.* 2011). Collectively, comparison of these oxidative stress biomarkers across low, moderate and high altitude scenarios is fundamental to current understanding and ongoing research initiatives.

### Preliminary studies to identify oxidative stress during high altitude exercise

Independent of exercise, exposure to a high altitude environment elicits oxidative stress as quantified by the various biometrics mentioned above. Importantly, both the hypobaric (Faiss *et al.* 2013) and hypoxic (Debevec *et al.* 2014) aspects of high altitude appear to have independent influences on the resultant oxidative stress, though the underlying mechanisms are not resolved. While research has been conducted in conjunction with a number of endurance and ultra-endurance events that included limited time at high altitude (Nieman *et al.* 2003; Quindry *et al.* 2008), the oxidative stress findings from these studies cannot be attributed to altitude alone and are excluded from the current discussion. Thus, early evidence of high altitude and oxidative stress is largely derived from field studies, expeditions, or simulated altitude lab studies where most or all of the physical effort is conducted above 3000 m (Sinha *et al.* 2009a; Faiss *et al.* 2013; Miller *et al.* 2013; Lewis *et al.* 2014; McGinnis *et al.* 2014).

The first human study related to high altitude and exercise-induced oxidative stress was conducted in 1988 (Simon-Schnass & Pabst, 1988). However, even after this first study, research on exercise and high altitude has been relatively infrequent. Accordingly, broader findings from exercise and oxidative stress at all altitudes were used to bridge early knowledge gaps relative to work conducted at high altitude. As a general rule, sea level participation in high intensity (Quindry *et al.* 2003) or extended duration (Mastaloudis *et al.* 2001) muscular exercise is marked by a prominent rise in circulating levels of oxidative stress biomarkers. Similar observations occur following acute bouts of physical activity performed at high altitude where short duration-high intensity exercise (Sinha *et al.* 2009a) and long duration-low intensity exercise (Vasankari *et al.* 1997; Miller *et al.* 2013; Krzeszowiak *et al.* 2014) result in a transient increase in various indices of oxidative stress. However, in regard to exercise intensity, it is important to note that due to the low  $P_{O_2}$  at high altitude, the relative intensity for a given work rate increases significantly at high altitude. In fact, slow speed hiking above 8000 m can approach 100% relative intensity as  $\dot{V}_{O_{2max}}$  significantly decreases in an elevation-dependent fashion (Buskirk *et al.* 1967).

### What are the implications of oxidative stress during high altitude exercise?

As described above, biomarker indices from multiple tissues, including blood plasma and skeletal muscle, clearly indicate that acute exercise at high altitude induces a readily identifiable oxidative stress. Oxidative stress findings, however, may be more directly related to exercise than altitude. In support, exposure to high altitude is associated with a modest increase in basal levels for multiple oxidative stress biomarkers, while acute exercise elicits an additive increase of greater magnitude (Sinha *et al.* 2009a).

Given the influence of exercise intensity and duration on reactive oxygen species (ROS) formation, findings of oxidative stress due to high altitude exercise may simply indicate that physical exertion at high altitude is demanding. Past notions that exercise-induced oxidative stress is deleterious to long term health are largely refuted today (reviewed in Quindry *et al.* 2014; Peake *et al.* 2015). In this regard it is important to contextualize exercise-induced oxidative stress relative to the specific biomarkers used to quantify oxidative stress in order to understand the acute and adaptive responses to exercise at high altitude. Moreover, a brief overview of oxidative stress biomarkers is helpful in overcoming common misconceptions about exercise and oxidative stress.

Because the majority of high altitude studies are conducted in human participants while at elevation in remote regions, much of the oxidative stress research reports only blood plasma markers. Categorization of oxidative damage markers from these field studies reveals that elevations in circulating levels of markers of lipid peroxidation, protein modification (usually carbonylation) and DNA damage are frequently reported. Table 1 summarizes oxidative stress findings from exercise research studies conducted either at high altitude or in laboratory settings that utilize hypoxic inspiratory gases. Biomarkers of lipid peroxidation increase as a result of exercise at high altitude, although the source of these lipids and the initiating reactions are rarely known in the context of exercise. Nonetheless, modification of F<sub>2</sub>-isoprostanes are likely to be related to arachidonic acid metabolism and signalling while an increase in lipid hydroperoxide formation is likely to result from cell membranes disrupted by oxidative reactions (Nourooz-Zadeh *et al.* 1994; Morrow & Roberts, 2002). While unconfirmed currently, there is reason to speculate that damaged red blood cells may be a primary source of increased circulating lipid hydroperoxides during exercise at high altitude. In relation to the current topic, red blood cell fragility and lipid peroxidation occur due to high altitude exposure, a response that is also associated with exercise participation (Sinha *et al.* 2009b; Vani *et al.* 2010). Another

possible source of lipid hydroperoxides is the vascular endothelium. For example, Lewis *et al.* examined physiological indices of blood flow in lowland natives introduced to 5000 m altitude (Lewis *et al.* 2014). They reported that flow-mediated dilatation and glyceryl trinitrate-mediated vasodilatation were diminished at altitude while pulse wave velocity increased. Notably, a rise in circulating lipid hydroperoxides was inversely correlated ( $r = -0.69$ ) with glyceryl trinitrate-mediated vasodilatation. This finding may indicate that acute oxidative stress is associated with a corresponding decline in vasoreactivity (Lewis *et al.* 2014).

Similar to lipid markers of oxidative damage, protein markers of oxidative damage remain undefined in terms of source. Protein carbonyl formation is due to direct oxidation of amino acid residues, although examination of the constituent proteins is rarely performed in the context of exercise. While it is confirmed that actin and myosin are oxidatively modified due to exercise at altitude (Radak *et al.* 1997), there is little reason to suspect that a post-exercise spike in plasma protein carbonyls is derived from muscle in appreciable amounts. More likely, plasma albumin, which contains a cysteine residue that is readily oxidized during physiological stress, is the primary target for protein carbonylation. Just as important, albumin represents the most abundant thiol in plasma (Torres *et al.* 2012). As such it is tempting to speculate that oxidation of circulating albumin accounts for much of the acute spike in circulating protein carbonyls following high altitude exercise. While this notion is currently unconfirmed relative to exercise, if correct it would mean that albumin could serve as a 'sacrificial' protein by directly quenching free radicals. Moreover, this notion is congruent with established understanding of the antioxidant milieu in blood plasma (Cao & Prior, 1998). In this regard it is clear that more sophisticated immunoblotting and immunoprecipitation experiments are needed to better understand the nature and severity of protein oxidation products due to high altitude exercise (Goto *et al.* 1999).

As summarized in Table 1, an outcome of high altitude exercise is that plasma antioxidant capacity is acutely altered. To bring order to the multitude of transient responses from available laboratory and field studies, low molecular mass antioxidants and antioxidant metrics will be discussed independently of endogenous antioxidant enzymes. Of the low molecular mass antioxidants, uric acid is the most important water soluble antioxidant contributing to blood antioxidant radical trapping capacity (Cao & Prior, 1998). Several published investigations of high altitude exercise report a rise in circulating plasma uric acid levels (Sinha *et al.* 2009a; Peters *et al.* 2015). Exercise-induced elevations in plasma uric acid occur due to purine metabolism in skeletal muscle. This phenomenon includes ROS production through the generation of hydrogen peroxide at two steps of the biochemical process, and as such, does not

**Table 1. Human-based field studies at altitude and laboratory studies at simulated high altitude**

		Field studies								
		Study design considerations			Blood plasma marker			Muscle markers		
Population examined	Exercise	Altitude	Sampling periods	Lipid peroxidation	Protein	DNA	Antioxidant	ROS/RONS	Gene transcripts	Reference
Lowland natives	9 day trek in the Alps	2000–4200 m	Baseline vs. 3 days exposure to altitude	↔ TBARS			↑ CAT ↔ SOD			(Krzyszowiak et al. 2014)
Lowland natives	2 week mountain trek Mt Everest region	3000–5000 m	Sea level baseline vs. 1 day and 7 days at altitude	↑ LOOH			↔ tocopherol ↔ carotene	↑ NOx		(Lewis et al. 2014)
Lowland natives	Trek on Mt Rainier	3000–4400 m	Baseline vs. 3000 m before and after summit, sea level recovery	↑ LOOH	↑ PC		↑ TEAC ↑ FRAP			(Miller et al. 2013)
Lowland natives	Cycle ergometer $V_{O_{2max}}$ test	4500 m	Baseline vs. post max test			↑ DNA				(Moller et al. 2001)
Lowland natives acclimated, highland natives	Cycle ergometer $V_{O_{2max}}$ test	4500 m	Baseline vs. post max test	↑ LOOH in lowland and highland natives	↑ 3NT in lowland and highland natives	↑ DNA in lowland and highland natives				(Sinha et al. 2010)
Lowland natives acclimated 1 week	Cross country ski competition	1600 m	Baseline vs. post competition	↔ MDA		↑ TAS	↑ TAS			(Vasankari et al. 1997)
		Laboratory studies								
		Study design considerations			Blood plasma marker			Muscle markers		
Population examined	Exercise	Simulated altitude	Sampling periods	Lipid peroxidation	Protein	DNA	Antioxidant	ROS/RONS	Gene transcripts	Reference
Lowland natives	90 min cycling at 60% $V_{O_{2max}}$	Normobaric normoxia vs. normobaric hypoxia during recovery only (5000 m)	Baseline vs. post, 2, 4, 6 h post	↑ LOOH	↑ PC		↑ TEAC, hypoxic lower than Normoxic recovery		↑ NFE2L2 normoxic only ↑ MnSOD normoxic only ↔ HMOX1	(Ballmann et al. 2014)
Lowland natives, altitude habituated	10 days' confinement to altitude chamber with/without cycle exercise ( $2 \times 60$ min 50% $V_{O_{2max}}$ )	Normobaric hypoxia 4000 m	Baseline vs. 24 h, 10 days exposure, 24 h reoxygenation		↑ AOPP, ↑ 3NT at reoxygenation without exercise group only		↑ SOD, ↑ FRAP with exercise group only			(Debevec et al. 2014)

(Continued)

Table 1. Continued

		Laboratory studies										
		Study design considerations					Blood plasma marker					Muscle markers
Population examined	Exercise	Simulated altitude	Sampling periods	Lipid peroxidation	Protein	DNA	Antioxidant	ROS/RONS	Gene transcripts	Reference		
Lowland natives	Cycle ergometer $\dot{V}_{O_{2max}}$ test	Normobaric hypoxia and hypobaric hypoxia at 3000 m for 1, 8, 16, and 24 h exposures	Baseline vs. post 1, 8, 16, and 24 h exposures	↔ MDA ↔ HNE	↑ AOPP hypobaric hypoxia group ↔ 3NT		↔ GPx ↔ SOD	↓ NOx in hypobaric hypoxia group		(Faiss <i>et al.</i> 2013)		
Lowland natives	Cycle ergometer $\dot{V}_{O_{2max}}$ test	Normobaric hypoxia at 4300 m	Baseline vs. post	↔ MDA ↔ HNE	↑ PC ↔ 3NT					(Gatterer <i>et al.</i> 2013)		
Lowland natives	60 min cycling at 60% $\dot{V}_{O_{2max}}$	Normobaric normoxia vs normobaric hypoxia during exercise and recovery (3000 m)	Baseline vs. post, 2, 4 h post	↑ LOOH hypoxic trial only	↔ PC		↓ Ascorbic acid ↔ TEAC			(McGinnis <i>et al.</i> 2014)		
Lowland natives	60 min cycling at 70% $\dot{V}_{O_{2max}}$	Normobaric normoxia vs normobaric hypoxia during recovery only (0 m, 1667m, 3333m and 5000 m)	Baseline vs. post, 1, 5 h post	↑ LOOH 0 m and 1667m only	↑ PC		↑ UA ↑ TEAC ↑ FRAP		↔ HMOX1 ↔ NFE2L2 ↔ SOD	(Peters <i>et al.</i> 2015)		
Acclimatized lowland natives vs. highland natives	Cycle ergometer $\dot{V}_{O_{2max}}$ test	3500–4500 m	Baselines vs. post exercise	↔ LOOH			↑ GSH & GPx highland natives only ↑ TAS ↑ UA ↔ CAT ↔ GR			(Sinha <i>et al.</i> 2009a)		

AOPP, advanced oxidative protein products; CAT, catalase; FRAP, ferric reducing antioxidant potential; GPx, glutathione peroxidase; GR, glutathione reductase; GSH, reduced glutathione; GSSG, oxidized glutathione; HNE, 4-hydroxynonenal; HMOX1, haem oxygenase 1; LOOH, lipid hydroperoxides; MDA malondialdehyde; MnSOD, manganese-dependent superoxide dismutase; NFE2L2 nuclear factor erythroid 2-like 2; NOx, nitrogen oxides (nitric oxide and nitrogen dioxide); 3NT, 3-nitrotyrosine; PC, protein carbonyl; SOD, superoxide dismutase; TAS, total antioxidant status; TBARS, thiobarbituric acid reactive substances; TEAC, trolox equivalent antioxidant capacity; UA, uric acid.

appear to be a mechanism of compensatory antioxidant fortification as some speculate. Thus, exercise-induced oxidative damage may still result concomitant to a rise in plasma uric acid and corresponding increase of plasma ferric reducing antioxidant potential (FRAP). In support, it was observed following an ultra-marathon with a peak elevation around 3000 m that the post-exercise rise in plasma uric acid was correlated with corresponding elevations in FRAP ( $r = 0.621$ ) (Quindry *et al.* 2008). This outcome supports biochemical understanding of these antioxidant and radical trapping metrics that are largely influenced by acute changes in circulating uric acid (Cao & Prior, 1998). Moreover, exercise that elicits significant catecholamine release is associated with a spike in circulating levels of the water soluble antioxidant vitamin C (Quindry *et al.* 2003), although this response has not been measured or observed in the context of high altitude exercise and oxidative stress. Collectively, the increase in antioxidant capacity and radical quenching capacity observed following exercise at high altitude (Vasankari *et al.* 1997; Sinha *et al.* 2009a; Miller *et al.* 2013; Ballmann *et al.* 2014; Peters *et al.* 2015) is likely to be due to acute alterations in the low molecular mass water soluble antioxidants uric acid and vitamin C. While long term elevation in these water soluble antioxidants appears to prevent altitude-induced damage to red blood cells (Devi *et al.* 2007), there is no evidence to suggest that exercise-induced alterations in these antioxidants directly impact performance or recovery during exercise at altitude.

Circulating levels of endogenous antioxidant enzymes also increase acutely in response to exercise at high altitude. Specifically, circulating levels of superoxide dismutase (SOD) and catalase (CAT) are increased in altitude-habituated lowlanders (Debevec *et al.* 2014; Krzeszowiak *et al.* 2014). Notably, the isoform of SOD is not defined in the three key studies listed currently and examination of the published methods does not indicate that red blood cell isolates were collected for selective antioxidant enzyme analysis (Faiss *et al.* 2013; Debevec *et al.* 2014; Krzeszowiak *et al.* 2014). Nonetheless, it is reasonable to assume that these findings for endogenous antioxidant enzymes largely represent rupture of red blood cells or vascular endothelium cells. Interestingly, acute exercise at altitude does not appear to increase red blood cell concentration of these enzymes (Joanny *et al.* 2001), suggesting that acclimatization to altitude is a necessary co-stimulus. In support, animal studies of habituation to altitudes exceeding 5000 m and 6300 m elicit consistent increases in red blood cell content of CAT, SOD and glutathione peroxidase (GPx) (Asha Devi *et al.* 2005; Devi *et al.* 2007). Whether these findings are due to altitude-induced polycythaemia and/or cellular level adaptations is currently uncertain.

Final consideration of high altitude exercise and oxidative stress in humans raises the question of muscle level oxidative damage and adaptations. To date, no published investigations report muscle level tissue oxidative damage in humans exercising at high altitude. This fact is likely to reflect the difficulty of obtaining muscle biopsy samples, a task that may be more challenging at extreme altitude in remote locations where permanent labs do not exist and portable labs cannot be easily delivered.

To date, only two laboratory-based investigations report findings from muscle biopsies (vastus lateralis) during exercise performed in normobaric hypoxia to simulate high altitude (Ballmann *et al.* 2014; Peters *et al.* 2015). Due to the limited sample volumes from muscle biopsies, only gene transcripts for redox-sensitive markers were examined. First, the study by Ballmann *et al.* reported a significantly elevated transcript expression for nuclear factor erythroid 2-like 2 (NFE2L2) and SOD. In addition, a non-significant increase in haem oxygenase (HMOX1) was found (Ballmann *et al.* 2014). Collectively these findings from human studies are supported by a related investigation employing laboratory rats acclimatized to elevations above 4000 m where it was observed that manganese-dependent superoxide dismutase (MnSOD) protein content was up-regulated in both soleus and gastrocnemius (Radak *et al.* 1997). Notably, this and other animal-based research studies of multiple body tissues acclimatized to extreme high altitude (> 6000 m) suggest that long term exposure is associated with depletion of endogenous antioxidant enzyme content and a corresponding elevation in markers of oxidative damage (Radak *et al.* 1994; Radak *et al.* 1997). Notably, these extreme altitude findings may be associated with redox-sensitive muscle wasting processes that occur in normoxic environment muscle investigations (Edwards *et al.* 2010). The notion that extended duration exposure to extreme high altitude may be detrimental to multiple organs merits further investigation as applied to human application such as mountaineering. The reader is directed to an insightful review on the topic of muscle level responses to altitude exposure (Dosek *et al.* 2007), but cautioned about interpretation of findings from extreme altitudes ('death zone') as potentially having fundamentally different outcomes from exercise performed at lower altitudes.

**Section summary.** In summary, oxidative stress occurs in both blood and muscle due to exercise at high altitude. Acute oxidative stress responses to exercise at high altitude are transient by nature, lasting from a few hours for short duration exercise (Moller *et al.* 2001; Sinha *et al.* 2010; Gatterer *et al.* 2013; Ballmann *et al.* 2014; McGinnis *et al.* 2014; Peters *et al.* 2015), to a few days for extended trekking

expeditions (Faiss *et al.* 2013; Miller *et al.* 2013; Debevec *et al.* 2014; Lewis *et al.* 2014). The time required for the various biomarkers of oxidative stress to return to baseline highlights the scientific need for multiple exercise recovery sampling points following acute exercise at altitude. Moreover, given that mountain trekking is associated with a significant amount of lengthening muscle contractions, which are linked to prolonged elevations in oxidative stress (Quindry *et al.* 2011), several days of follow-up sampling may be warranted depending on the volume of downhill walking, running, or other activity involving lengthening contractions. Finally, outside of a handful of animal-based studies that examine prolonged exposure to extreme altitude (Radak *et al.* 1994), there is currently no evidence to suggest that the oxidative stress incurred during routine acute exercise or exercise training at high altitude is deleterious to long term health or performance. In contrast, and congruent with understanding of sea level exercise, the transient oxidative stress associated with exercise is a potent stimulus for beneficial adaptations (Gomez-Cabrera *et al.* 2008). Preliminary findings from a recent investigation also appear to support the role of oxidative stress in promoting adaptations to exercise performed at altitude (Ballmann *et al.* 2014).

### Oxidative stress in highland natives and altitude acclimatized lowlanders

Based on findings from recent studies, there appears to be subtle, but important differences in lowland dwelling *versus* native highland people. Differences are particularly marked for native peoples of the Himalayan region where generations have lived and worked at altitudes considered to be extreme. One notable difference is that high altitude natives appear to have higher blood concentrations of reduced glutathione (GSH)/oxidized glutathione (GSSG) and SOD as compared with altitude acclimated or unacclimated lowlanders (Sinha *et al.* 2009a,b). However, firm conclusions about these populations may not be warranted currently as the collective blood antioxidant profile, in particular for low molecular weight fat soluble and water soluble antioxidants, was not consistently elevated in highlanders as compared with their lowland counterparts (Sinha *et al.* 2009a,b). Whether these findings are influenced by diet in addition to genetic and other environmental factors is not currently resolved and also merits further investigation before establishing firm conclusions on the matter. Nonetheless, findings from a related study by the research group referenced above reveal that basal levels of plasma DNA oxidation products, 3-nitrotyrosine and lipid hydroperoxides were lower in native highlanders as compared with their altitude acclimated or sea level acclimated lowland counterparts

(Sinha *et al.* 2010). Moreover, while acute maximal intensity cycle ergometer exercise at 4500 m elicited a rise in plasma oxidative stress markers in all three groups, the magnitude of the response for DNA oxidation products and lipid hydroperoxides was significantly lower in the high altitude natives (Sinha *et al.* 2010).

Lowland natives who are acclimated to altitude appear to have a modulated oxidative stress response in both unstressed resting and post-exercise scenarios. Several important studies reveal that basal levels of plasma oxidative damage markers and antioxidant content are elevated by 4–5 weeks of acclimation to altitude above 3500 m. In one investigation native lowland volunteers were habituated to elevation by residence at 3500 m for 1 week and then at 4500 m for 3 weeks thereafter. At the end of the habituation period plasma glutathione content was increased with GSH/GSSG being modestly but significantly higher. Related concentrations of GPx and glutathione reductase were also higher following a month at high altitude. Plasma levels of uric acid were also elevated due to acclimation, while vitamin C concentrations were lower than at sea level (Sinha *et al.* 2009a). Similar findings were reported by the same group of researchers, who exposed subjects to 3500 m for 1 week followed by 4 weeks at 4500 m. Increased plasma SOD, CAT, uric acid, GSH/GSSG and glutathione reductase were observed after 5 weeks at high altitude. However, lipid hydroperoxides and protein carbonyls were also elevated in these acclimatized subjects (Sinha *et al.* 2009b). A separate report using an identical acclimation protocol examined the acute oxidative stress response to maximal intensity cycle ergometry. Findings revealed that basal levels in plasma 3-nitrotyrosine and lipid hydroperoxides were elevated in acclimatized lowlanders, but the magnitude of exercise-induced oxidative damage was attenuated for DNA, protein and lipid biomarkers (Sinha *et al.* 2010). Collectively, these responses appear to support the aforementioned findings from animal studies where skeletal muscle adaptations to moderate and high altitude include antioxidant fortification to quench the increased free radical load associated with altitude (Radak *et al.* 1994, 1997).

**Section summary.** Carefully conducted examinations of native lowland and highland populations suggest that exposure to high altitude has an independent effect on elevated markers of oxidative damage. Concurrent to this finding, adaptations in endogenous antioxidant capacity are likely to protect native highlanders as compared with their lowland counterparts. Nonetheless, acclimatized lowlanders exhibit similar adaptations in endogenous antioxidant capacity, though these outcomes are less robust than in highland natives. The influence of altered diet on these findings cannot be ascertained currently, but

there is little doubt that the acute oxidative stress responses to exercise at altitude can be attenuated by acclimatization to altitude.

### Altitude considerations for living and training

Given the established link between high altitude exercise and oxidative stress, combinations of living elevation and training elevation must be addressed. While the primary known ergogenic advantage to living at or above 1500 m is erythropoiesis, the advantage of training at moderate to high altitudes may include cellular adaptations to the hypoxic stimulus as well. Accordingly, combinations of living and training at high *versus* low altitude have been examined, though not in the context of oxidative stress.

As compared with sea level dwelling athletes, there does not appear to be an overall ergogenic advantage to living high and training high (Bailey *et al.* 1998; Roels *et al.* 2006). As concluded above there is no reason to suspect that the oxidative stress and the corresponding adaptive responses to living and exercising at moderately high altitudes are ergogenic or deleterious as compared with sea level. Thus, until a testable scientific rationale for or against living high and training high is formulated, the matter does not seem pertinent to oxidative stress and exercise at high altitude.

In contrast to living and training high, living low and training high does raise interesting scientific questions related to oxidative stress. This training rationale persists despite the fact that high altitude exercise workloads are limited by altitude-dependent declines in  $\dot{V}_{O_2\max}$ . The most investigated cellular mechanism in regard to a hypoxic stimulus during exercise is hypoxia-inducible factor-1 $\alpha$  (HIF-1 $\alpha$ ). Examination of skeletal muscle in humans exposed acutely to hypoxic exercise reveals that HIF-1 $\alpha$  and vascular endothelial growth factor (VEGF) appear to be central to altitude-dependent adaptive responses (Hoppeler & Vogt, 2001*a,b*). Elevation in related blood markers for HIF-1 $\alpha$  and VEGF also occurs following acute hypoxic exercise as compared with normoxic conditions (Mounier *et al.* 2009). Reductionist work in skeletal muscle from mouse models links this adaptive HIF-1 $\alpha$  response to redox stimuli and also implicates beneficial adaptive outcomes related to metabolism and mitochondrial biogenesis (Mason & Johnson, 2007). Recent examination of mitochondrial biogenesis gene transcripts in human vastus lateralis obtained following an acute bout of hypoxic exercise reinforces the notion that adaptive responses to high altitude exercise are redox sensitive (Slivka *et al.* 2014). Nonetheless, human performance studies of hypoxic exercise on sea level performance are equivocal (Ventura *et al.* 2003; Dufour *et al.* 2006). Thus, as related to oxidative stress and exercise at high altitude, foundational research is needed in order to understand the role of oxidative stress and exercise adaptations as they may apply to performance and recovery outcomes.

**Live high, train low.** Living at high altitude and training at low altitude is a strategy for improving endurance exercise performance (Levine & Stray-Gundersen, 1997). While not currently examined in the context of oxidative stress research, there is scientific rationale to suspect that living at high altitude and training at sea level may be advantageous. First and foremost, the erythropoiesis observed with exercise altitude may limit oxidative damage by improved oxygen binding capacity independent of other adaptive responses (Vani *et al.* 2010). Moreover, high altitude living stimulates fortification of endogenous antioxidant levels in tissue and blood (Radak *et al.* 1997; Sinha *et al.* 2009*a,b*, 2010). Because cellular antioxidant status impacts muscular performance (reviewed in Powers *et al.* 2011), the live high and train low rationale may hold merit, although a need for foundational studies remains.

**A new twist: sleep high, train and recover low.** Several recent high altitude exercise and oxidative studies raise new insights on the concept of live high–train low and are presented conceptually in the Abstract figure. A series of three investigations from a collaborative group employed normobaric hypoxia in a laboratory setting to examine oxidative stress and muscle adaptive responses to cycle ergometer exercise and recovery (Ballmann *et al.* 2014; McGinnis *et al.* 2014; Peters *et al.* 2015). The first investigation employed a crossover study design during a 60 min exercise bout followed by 4 h of recovery (McGinnis *et al.* 2014). Exercise and recovery were maintained at low altitude or simulated high altitude (3000 m) and exercise workloads were matched for 60% of  $\dot{V}_{O_2\max}$  at the respective elevations. Given the impact of hypoxia on exercise intensity, a control trial with the prescribed workload from 3000 m, was performed at the base elevation. Findings from blood assay of oxidative damage and antioxidant markers revealed an altitude-dependent oxidative stress (McGinnis *et al.* 2014) similar to outcomes described above. The authors postulated that hypoxia may have an independent effect on oxidative stress outcomes as examined during exercise *versus* recovery.

Accordingly, in a nearly identical follow-up study by Ballmann *et al.* (90 min exercise at 60%  $\dot{V}_{O_2\max}$ ), exercise was performed at the base elevation while exercise recovery occurred at either base elevation or simulated 5000 m. Findings for several biomarkers revealed that both exercise sessions elicited an identifiable blood oxidative stress, although the magnitude of the response was attenuated when recovery occurred at 5000 m (Ballmann *et al.* 2014). This interesting outcome might indicate that altitude also influences the post-exercise recovery. Moreover, given that exercise adaptations are redox sensitive (Gomez-Cabrera *et al.* 2008), there is reason to suspect that acute adaptive responses to exercise may be attenuated in high altitude recovery. Indeed, Ballmann *et al.* examined vastus

lateralis muscle biopsies for redox-sensitive gene transcripts. Findings revealed that exercise-induced elevations NFE2L2 and SOD mRNA were attenuated during recovery at a simulated 5000 m.

In order to better understand the elevation at which this response occurs, a subsequent investigation by Peters *et al.* employed an identical crossover study design (60 min exercise at 70%  $\dot{V}_{O_{2max}}$ ), except that recovery was at simulated altitudes of 0, 1667, 3333, or 5000 m (Peters *et al.* 2015). Examination of oxidative stress blood biomarkers revealed that attenuation of the exercise-induced elevation in oxidative stress markers occurred between 1667 and 3333 m. Examination of thigh muscle biopsies produced non-significant numerical decreases in redox-sensitive transcripts at the two highest recovery altitudes (Peters *et al.* 2015). In support of these findings, the Operation Everest III study revealed persistent elevation in blood oxidative stress during reoxygenation at sea level (Joanny *et al.* 2001). Moreover, elevation in blood levels of advanced oxidation protein products persisted for at least 24 h following a 10 day exercise training regimen at high altitude (Debevec *et al.* 2014). These compelling findings require further examination, but might suggest that training and recovery should ideally occur at a lower altitude. Moreover, given that indices of oxidative stress can persist for more than a day, a balance in time spent at high elevation *versus* low elevation may need to be explored in order to elicit erythropoiesis but not attenuate redox-sensitive adaptations to exercise. One possible solution could be to exercise and recover at low altitude, while sleeping at high altitude. In support of this notion, some studies report that athletes who slept at high altitude (11 h) received the benefit of erythropoiesis (Robach *et al.* 2006). As with the scientific questions raised above, additional research is needed to formulate preliminary conclusions related to low altitude exercise and recovery in combination with limited high altitude living.

**Section summary.** Among the most important applications of high altitude exercise and oxidative stress are scenarios related to endurance athletes, recreational mountaineers, and military fighters who complete missions in mountainous terrain. The influences of oxidative stress outcomes are virtually untested in the context of high altitude exercise and recovery. As future research is conceived and executed, serious consideration should be given to incorporation of multiple biosampling times during exercise recovery. In addition, inclusion of muscle biopsy data in addition to blood biomarkers of oxidative stress is warranted. Finally, these investigations should be conducted in well-defined populations in order to minimize confounding outcomes from heterogeneous participant groups that often result from convenience sampling. These recommendations are essential for

understanding highly variable tissue level responses that are sensitive to fitness level and exercise habituation (Mounier *et al.* 2009; Slivka *et al.* 2014; Peters *et al.* 2015).

## Conclusion

Both intense and long duration exercise elicits oxidative stress, a response that is exacerbated at high altitude. Several dozen studies have directly investigated the oxidative stress response to exercise performed at high altitude, while other human and animal-based studies provide additional insight into the impact of altitude-derived hypoxia on oxidative stress and exercise adaptations. Collective understanding suggests that acute exercise performed at moderate to high altitude elicits an oxidative stress which serves as a potent adaptive stimulus for endogenous antioxidant fortification. In this regard, mechanistic links between redox and hypoxic stimuli on tissue level adaptations are likely to be redundant, although direct links to high altitude exercise have yet to be verified. Nonetheless, significant insights can be gleaned from studies of native highlanders *versus* lowlanders. Acclimatization to altitude is also revealing in this regard, with human- and animal-based studies providing strong evidence that exposure to high altitude is advantageous to many exercise and training scenarios. In contrast, detrimental effects attributed to high altitude exposure appear to be limited to extended duration exposures conducted at the highest ('death zone') elevations. Understanding of the independent and combined influence of hypoxic and oxidative stress stimulus of exercise at high altitude is still in the formative stages. This conclusion is perhaps most notable for the adaptive responses induced during exercise and the exercise recovery phase. Future research should be conducted in both human- and animal-based reductionist investigations of oxidative stress in order to understand the cellular level responses that underpin physiological outcomes to exercise at high altitude.

## References

- Asha Devi S, Subramanyam MV, Vani R & Jeevaratnam K (2005). Adaptations of the antioxidant system in erythrocytes of trained adult rats: impact of intermittent hypobaric-hypoxia at two altitudes. *Comp Biochem Physiol C Toxicol Pharmacol* **140**, 59–67.
- Bailey DM, Davies B, Romer L, Castell L, Newsholme E & Gandy G (1998). Implications of moderate altitude training for sea-level endurance in elite distance runners. *Eur J Appl Physiol Occup Physiol* **78**, 360–368.
- Ballmann C, McGinnis G, Peters B, Slivka D, Cuddy J, Hailes W, Dumke C, Ruby B & Quindry J (2014). Exercise-induced oxidative stress and hypoxic exercise recovery. *Eur J Appl Physiol* **114**, 725–733.

- Buettner GR (1993). The pecking order of free radicals and antioxidants:  $\alpha$ -tocopherol, and ascorbate. *Arch Biochem Biophys* **300**, 535–543.
- Buskirk ER, Kollias J, Akers RF, Prokop EK & Reategui EP (1967). Maximal performance at altitude and on return from altitude in conditioned runners. *J Appl Physiol* **23**, 259–266.
- Cao G & Prior RL (1998). Comparison of different analytical methods for assessing total antioxidant capacity of human serum. *Clin Chem* **44**, 1309–1315.
- Debevec T, Pialoux V, Mekjavic IB, Eiken O, Mury P & Millet GP (2014). Moderate exercise blunts oxidative stress induced by normobaric hypoxic confinement. *Med Sci Sports Exerc* **46**, 33–41.
- Devi SA, Vani R, Subramanyam MV, Reddy SS & Jeevaratnam K (2007). Intermittent hypobaric hypoxia-induced oxidative stress in rat erythrocytes: protective effects of vitamin E, vitamin C, and carnitine. *Cell Biochem Funct* **25**, 221–231.
- Dillard CJ, Litov RE, Savin WM, Dumelin EE & Tappel AL (1978). Effects of exercise, vitamin E, and ozone on pulmonary function and lipid peroxidation. *J Appl Physiol Respir Environ Exerc Physiol* **45**, 927–932.
- Dosek A, Ohno H, Acs Z, Taylor AW & Radak Z (2007). High altitude and oxidative stress. *Respir Physiol Neurobiol* **158**, 128–131.
- Dufour SP, Ponsot E, Zoll J, Doutreleau S, Lonsdorfer-Wolf E, Geny B, Lampert E, Fluck M, Hoppeler H, Billat V, Mettauer B, Richard R & Lonsdorfer J (2006). Exercise training in normobaric hypoxia in endurance runners. I. Improvement in aerobic performance capacity. *J Appl Physiol (1985)* **100**, 1238–1248.
- Edwards LM, Murray AJ, Tyler DJ, Kemp GJ, Holloway CJ, Robbins PA, Neubauer S, Levett D, Montgomery HE, Grocott MP, Clarke K & Caudwell Xtreme Everest Research Group (2010). The effect of high-altitude on human skeletal muscle energetics: P-MRS results from the Caudwell Xtreme Everest expedition. *PLoS One* **5**, e10681.
- Faiss R, Pialoux V, Sartori C, Faes C, Deriaz O & Millet GP (2013). Ventilation, oxidative stress, and nitric oxide in hypobaric versus normobaric hypoxia. *Med Sci Sports Exerc* **45**, 253–260.
- Gatterer H, Greilberger J, Philippe M, Faulhaber M, Djukic R & Burtscher M (2013). Short-term supplementation with alpha-ketoglutaric acid and 5-hydroxymethylfurfural does not prevent the hypoxia induced decrease of exercise performance despite attenuation of oxidative stress. *Int J Sports Med* **34**, 1–7.
- Gomez-Cabrera MC, Domenech E & Vina J (2008). Moderate exercise is an antioxidant: upregulation of antioxidant genes by training. *Free Radic Biol Med* **44**, 126–131.
- Goto S, Nakamura A, Radak Z, Nakamoto H, Takahashi R, Yasuda K, Sakurai Y & Ishii N (1999). Carbonylated proteins in aging and exercise: immunoblot approaches. *Mech Ageing Dev* **107**, 245–253.
- Hoppeler H & Vogt M (2001a). Hypoxia training for sea-level performance. Training high-living low. *Adv Exp Med Biol* **502**, 61–73.
- Hoppeler H & Vogt M (2001b). Muscle tissue adaptations to hypoxia. *J Exp Biol* **204**, 3133–3139.
- Joanny P, Steinberg J, Robach P, Richalet JP, Gortan C, Gardette B & Jammes Y (2001). Operation Everest III (Comex'97): the effect of simulated severe hypobaric hypoxia on lipid peroxidation and antioxidant defence systems in human blood at rest and after maximal exercise. *Resuscitation* **49**, 307–314.
- Krzeszowiak J, Zawadzki M, Markiewicz-Gorka I, Kawalec A & Pawlas K (2014). The influence of a 9-day trekking in the Alps on the level of oxidative stress parameters and blood parameters in native lowlanders. *Ann Agric Environ Med* **21**, 585–589.
- Levine BD & Stray-Gundersen J (1997). “Living high-training low”: effect of moderate-altitude acclimatization with low-altitude training on performance. *J Appl Physiol (1985)* **83**, 102–112.
- Lewis NC, Bailey DM, Dumanoir GR, Messinger L, Lucas SJ, Cotter JD, Donnelly J, McEneny J, Young IS, Stembridge M, Burgess KR, Basnet AS & Ainslie PN (2014). Conduit artery structure and function in lowlanders and native highlanders: relationships with oxidative stress and role of sympathoexcitation. *J Physiol* **592**, 1009–1024.
- Mason S & Johnson RS (2007). The role of HIF-1 in hypoxic response in the skeletal muscle. *Adv Exp Med Biol* **618**, 229–244.
- Mastaloudis A, Leonard SW & Traber MG (2001). Oxidative stress in athletes during extreme endurance exercise. *Free Radic Biol Med* **31**, 911–922.
- McGinnis G, Kliszczewicz B, Barberio M, Ballmann C, Peters B, Slivka D, Dumke C, Cuddy J, Hailes W, Ruby B & Quindry J (2014). Acute hypoxia and exercise-induced blood oxidative stress. *Int J Sport Nutr Exerc Metab* **24**, 684–693.
- Miller LE, McGinnis GR, Kliszczewicz B, Slivka D, Hailes W, Cuddy J, Dumke C, Ruby B & Quindry JC (2013). Blood oxidative-stress markers during a high-altitude trek. *Int J Sport Nutr Exerc Metab* **23**, 65–72.
- Moller P, Loft S, Lundby C & Olsen NV (2001). Acute hypoxia and hypoxic exercise induce DNA strand breaks and oxidative DNA damage in humans. *FASEB J* **15**, 1181–1186.
- Morrow JD & Roberts LJ 2nd (2002). Mass spectrometric quantification of F2-isoprostanes as indicators of oxidant stress. *Methods Mol Biol* **186**, 57–66.
- Mounier R, Pialoux V, Schmitt L, Richalet JP, Robach P, Coudert J, Clottes E & Fellmann N (2009). Effects of acute hypoxia tests on blood markers in high-level endurance athletes. *Eur J Appl Physiol* **106**, 713–720.
- Nieman DC, Dumke CI, Henson DA, McAnulty SR, McAnulty LS, Lind RH & Morrow JD (2003). Immune and oxidative changes during and following the Western States Endurance Run. *Int J Sports Med* **24**, 541–547.
- Nourooz-Zadeh J, Tajaddini-Sarmadi J & Wolff SP (1994). Measurement of plasma hydroperoxide concentrations by the ferrous oxidation-xyleneol orange assay in conjunction with triphenylphosphine. *Anal Biochem* **220**, 403–409.
- Peake JM, Markworth JF, Nosaka K, Raastad T, Wadley GD & Coffey VG (2015). Modulating exercise-induced hormesis: does less equal more? *J Appl Physiol (1985)* **119**, 172–189.

- Peters B, Ballmann C, McGinnis G, Epstein E, Hyatt H, Slivka D, Cuddy J, Hailes W, Dumke C, Ruby B & Quindry J (2015). Graded hypoxia and blood oxidative stress during exercise recovery. *J Sports Sci* **14**, 1–11.
- Powers SK, Ji LL, Kavazis AN & Jackson MJ (2011). Reactive oxygen species: impact on skeletal muscle. *Compr Physiol* **1**, 941–969.
- Quindry J, Kavazis A & Powers S (2014). Exercise-induced oxidative stress: Are supplemental antioxidants warranted? In *Sports Nutrition*, ed. Maughan RJ, pp. 263–276. Wiley Blackwell, Chichester, UK.
- Quindry J, Miller L, McGinnis G, Irwin M, Dumke C, Magal M, Triplett NT, McBride J & Urbiztondo Z (2011). Muscle-fiber type and blood oxidative stress after eccentric exercise. *Int J Sport Nutr Exerc Metab* **21**, 462–470.
- Quindry JC, McAnulty SR, Hudson MB, Hosick P, Dumke C, McAnulty LS, Henson D, Morrow JD & Nieman D (2008). Oral quercetin supplementation and blood oxidative capacity in response to ultramarathon competition. *Int J Sport Nutr Exerc Metab* **18**, 601–616.
- Quindry JC, Stone WL, King J & Broeder CE (2003). The effects of acute exercise on neutrophils and plasma oxidative stress. *Med Sci Sports Exerc* **35**, 1139–1145.
- Radak Z, Asano K, Lee KC, Ohno H, Nakamura A, Nakamoto H & Goto S (1997). High altitude training increases reactive carbonyl derivatives but not lipid peroxidation in skeletal muscle of rats. *Free Radic Biol Med* **22**, 1109–1114.
- Radak Z, Lee K, Choi W, Sunoo S, Kizaki T, Oh-ishi S, Suzuki K, Taniguchi N, Ohno H & Asano K (1994). Oxidative stress induced by intermittent exposure at a simulated altitude of 4000 m decreases mitochondrial superoxide dismutase content in soleus muscle of rats. *Eur J Appl Physiol Occup Physiol* **69**, 392–395.
- Robach P, Schmitt L, Brugniaux JV, Nicolet G, Duvallat A, Fouillot JP, Moutereau S, Lasne F, Pialoux V, Olsen NV & Richalet JP (2006). Living high–training low: effect on erythropoiesis and maximal aerobic performance in elite Nordic skiers. *Eur J Appl Physiol* **97**, 695–705.
- Roels B, Hellard P, Schmitt L, Robach P, Richalet JP & Millet GP (2006). Is it more effective for highly trained swimmers to live and train at 1200 m than at 1850 m in terms of performance and haematological benefits? *Br J Sports Med* **40**, e4.
- Simon-Schnass I & Pabst H (1988). Influence of vitamin E on physical performance. *Int J Vitam Nutr Res* **58**, 49–54.
- Sinha S, Dutta A, Singh SN & Ray US (2010). Protein nitration, lipid peroxidation and DNA damage at high altitude in acclimatized lowlanders and native highlanders: relation with oxygen consumption. *Respir Physiol Neurobiol* **171**, 115–121.
- Sinha S, Ray US, Saha M, Singh SN & Tomar OS (2009a). Antioxidant and redox status after maximal aerobic exercise at high altitude in acclimatized lowlanders and native highlanders. *Eur J Appl Physiol* **106**, 807–814.
- Sinha S, Ray US, Tomar OS & Singh SN (2009b). Different adaptation patterns of antioxidant system in natives and sojourners at high altitude. *Respir Physiol Neurobiol* **167**, 255–260.
- Slivka DR, Heesch MW, Dumke CL, Cuddy JS, Hailes WS & Ruby BC (2014). Human skeletal muscle mRNA response to a single hypoxic exercise bout. *Wilderness Environ Med* **25**, 462–465.
- Torres MJ, Turrell L, Botti H, Antmann L, Carballa S, Ferrer-Sueta G, Radi R & Alvarez B (2012). Modulation of the reactivity of the thiol of human serum albumin and its sulfenic derivative by fatty acids. *Arch Biochem Biophys* **521**, 102–110.
- Vani R, Reddy CS & Asha Devi S (2010). Oxidative stress in erythrocytes: a study on the effect of antioxidant mixtures during intermittent exposures to high altitude. *Int J Biometeorol* **54**, 553–562.
- Vasankari TJ, Kujala UM, Rusko H, Sarna S & Ahotupa M (1997). The effect of endurance exercise at moderate altitude on serum lipid peroxidation and antioxidative functions in humans. *Eur J Appl Physiol Occup Physiol* **75**, 396–399.
- Ventura N, Hoppeler H, Seiler R, Binggeli A, Mullis P & Vogt M (2003). The response of trained athletes to six weeks of endurance training in hypoxia or normoxia. *Int J Sports Med* **24**, 166–172.

## Additional information

### Competing interests

None declared.

### Author contributions

All authors have approved the final version of the manuscript and agree to be accountable for all aspects of the work. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

### Acknowledgements

The authors acknowledge the efforts of Dr Lindsey Miller, Dr Graham McGinnis, Dr Christopher Ballmann, Dr Bridget Peters, John Cuddy and Walter Hailes as important collaborators in the body of research underpinning this review. The authors acknowledge Dr L. Bruce Gladden for his editorial input in the revision of this manuscript.