OXIDATIVELY STRESSED OUT AT HIGH-ALTITUDE!

Free radical damage at high-altitude; isolating the source and implications for the pathophysiology of acute mountain sickness

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Introduction

What causes acute mountain sickness (AMS) and how can it be prevented? These questions are consistently asked by even the most up-to-date expedition doctor yet despite almost a century of investigation we are still far from understanding a syndrome that continues to command fear and respect amongst the climbing population. The anorexia, nausea, vomiting, headache, dizziness and insomnia associated with AMS can interfere with a mountaineer's ascent to high-altitude and in more serious cases can develop into the life-threatening condition of high-altitude cerebral oedema (HACE).

The most recent evidence suggests that AMS and HACE share a common pathophysiology characterised by a predominantly vasogenic oedema which incriminates a shift of fluid from intracellular to extracellular compartments due to blood-brain barrier leakage (4). But what affects the integrity of the blood-brain barrier at high-altitude? This paper presents preliminary evidence obtained from three separate studies that systematically addresses the potential contributory role for free radicals in the pathophysiology of altitude-illness.

What are free radicals?

Justice to the definition of the related terms and concepts involved in free radical biochemistry is beyond the scope of the present paper and the reader is directed to an elegant review by Halliwell (6). In short, electrons positioned around the nucleus of an atom are usually found in pairs occupying a defined space referred to as an atomic or molecular orbital. A *free* radical is defined as any species capable of independent existence that contains one or more unpaired electrons and is characterised in chemical terms by a superscript dot as illustrated in Table 1.

Table 1. Types of free radicals

Free Radical	Chemical Formula
Superoxide	$O_2^{\bullet -}$

Hydroxyl	$OH^{\bullet -}$
Peroxyl	$RO_2^{\bullet}$
Alkoxyl	$RO^{\bullet}$
Hydroperoxyl	$HO_2^{\bullet}$
Nitric oxide	$NO^{\bullet}$

An unavoidable consequence of cellular metabolism using molecular O_2, these highly energised free radicals are capable of abstracting hydrogen from a polyunsaturated fatty acid side-chain in membranes, a process referred to as lipid peroxidation. Propagation of this process sets up a “chain reaction” that ultimately causes membrane destabilisation and cell damage. The degree of oxidative damage is controlled by the body’s sophisticated antioxidant defense system which is armed with a plethora of chemical compounds and enzymes capable of stabilising or terminating the radical species. However, there is accumulating evidence to suggest that these defense mechanisms are to some extent overpowered during ascent to high-altitude; a consequence of the pro-oxidant effects of physical exercise, $UV_{-A/B}$ radiation, ambient temperature shifts, dehydration and anorexia (14). Whilst the mechanisms of free radical generation during physical exercise are numerous and the subject of continued interest, emerging evidence also defines a contributory role for hypoxia. Associated mechanisms implicate the release of oxygen radicals from erythrocytes (11) and also increased electron “leakage” from the mitochondrial respiratory chain. A build up of reducing equivalents that cannot be transferred to O_2 at cytochrome oxidase has been incriminated in the latter observation; a phenomenon termed “reductive stress” (8).

Why is the brain susceptible to oxidative damage?
The anatomical and metabolic characteristics of the human brain renders this organ particularly susceptible to oxidative stress. Its antioxidant defenses are modest compared with other tissues and neuronal membrane lipids are particularly rich in highly polyunsaturated fatty-acid side-chains that for reasons previously discussed are sensitive to oxidation reactions (2). The high rate of O_2 consumption per unit mass of tissue, dynamic calcium flux across neuronal membranes and autoxidation of neurotransmitters all represent potentially potent sources of free radical generation. Thus it would appear that the blood-brain barrier, so crucial for the maintenance of normal physiological function, is subjected to constant free radical attack! Indications that damage to the blood-brain barrier at high-altitude is linked to free radicals can be seen in studies demonstrating the relative success of dexamethasone in the prevention and treatment of AMS and HACE (7). The protective action of this potent glucorticoid has not been fully elucidated but it seems to act as a non-enzymatic antioxidant by suppressing lipid peroxidation and preventing the normal increase in permeability of cultured endothelial cell monolayers exposed to hypoxia (9). But before we consider the relative merits of antioxidant supplementation, what evidence is there that free radical generation actually increases at high-altitude and what are the potential sources? Studies 1 and 2 outlined below were designed with precisely these questions in mind.

Study 1: Is there any evidence of free radical damage at high-altitude?

Overview and aims:
A field-based study was designed to investigate changes in free radical "footprints" and to assess subsequent implications for muscle damage and muscle soreness during the 1998 British Mt Kanchenjunga Medical Expedition. A secondary aim was to determine whether these parameters were different in subjects who developed AMS compared to those who remained apparently healthy.

Methodology:
Design: Nineteen experienced male mountaineers aged 38 ± 12 years old were examined at rest and after a standardised cycling test to volitional exhaustion on three separate occassions at sea-level before (SL_1) and after (SL_2) an expedition and within 14-19 h of arrival at Mt Kanchenjunga basecamp (BC) located at 5100m. The trek to BC lasted 20 ± 5 days with subjects engaging in 250 ± 44 min of trekking at 68 ± 5% of their predicted sea-level maximal heart rate. All subjects were instructed to refrain from taking any vitamin/mineral supplements specifically antioxidant therapies, analgesics/anti-inflammatories or prophylactic medication against AMS (eg acetazolamide, spironolactone and dexamethasone) for at least 2 months prior to the start and for the duration of the study.

Blood samples: Overnight fasted venous blood samples were collected at rest and immediately following the exercise test and evidence for lipid peroxidation was assessed by measuring plasma malondialdehyde (MDA) and serum lipid hydroperoxides (LH) according to established methods. Muscle damage assays included the serum activity of total creatine phosphokinase (CPK) determined using a standard diagnostic kit and the serum concentration of myoglobin and cardiac troponin I (cTnI) measured using an automated-chemiluminescence radioimmunoassay. All exercise blood samples were corrected for plasma volume shifts. Specific care was taken to minimise and standardise tourniquet constriction due to the potential increase in oxidative stress introduced by an ischemia-reperfusion manoeuvre. This was achieved by tightening the tourniquet so that its circumference was 8 cm less than the relaxed girth measured at the belly of the biceps brachi.

Quantification of perceived muscle soreness
Resting muscle soreness; pain threshold. The muscle belly and distal region of the vastus lateralis and gastrocnemius were located via palpation and marked with a permanent pen. A pressure algometer (Force Dial™ FDK2, Wagner Instruments, USA), which consisted of a round-ended metal probe with a 10mm diameter rubber tip was randomly applied to each site of both legs with the subject prone. The investigator gradually increased the force up to a maximum of 30 kg. Following ten practice trials on alternative sites, the subject was instructed to verbally indicate when the stimulus became "uncomfortable". The force was subsequently recorded. If no indication of discomfort was reported up to 30 kg, soreness was not considered to be present at that specific site. The pain threshold was quantified as the summed forces divided by the number of sites with soreness (maximum of 8 sites).

Exercise muscle soreness. Each subject was asked to perceive the intensity of lower

limb soreness using a modified Borg CR-10 scale during the last 10s of each 3 min stage of an incremental cycling test to volitional exhaustion. Subjects were familiarised with this procedure prior to the experiment and pointed to a digit or verbal descriptor rating muscle soreness from 0 (no soreness) to 10 (very very sore).

AMS symptoms. Symptoms of AMS were quantified using the Lake Louise Consensus scoring system (3) that was administered between 16:00-18:00h at least three hours after the subjects had eaten and/or performed any physical exercise. The cumulative AMS score was calculated for each day from a maximum of 28 points. Mean baseline AMS scores were calculated over a 7d period during sea-level tests and on a daily basis at BC. Subjects were retrospectively divided into those with AMS (Ž3 points at BC).

Results and discussion

Free radical footprints: Figure 1 demonstrates that exposure to high-altitude resulted in a selective increase in serum LH both at rest and after exercise. The increase in LH in the present study is one of the initial major reactants of lipid peroxidation and provides indirect evidence for increased molecular oxidative damage at high altitude.

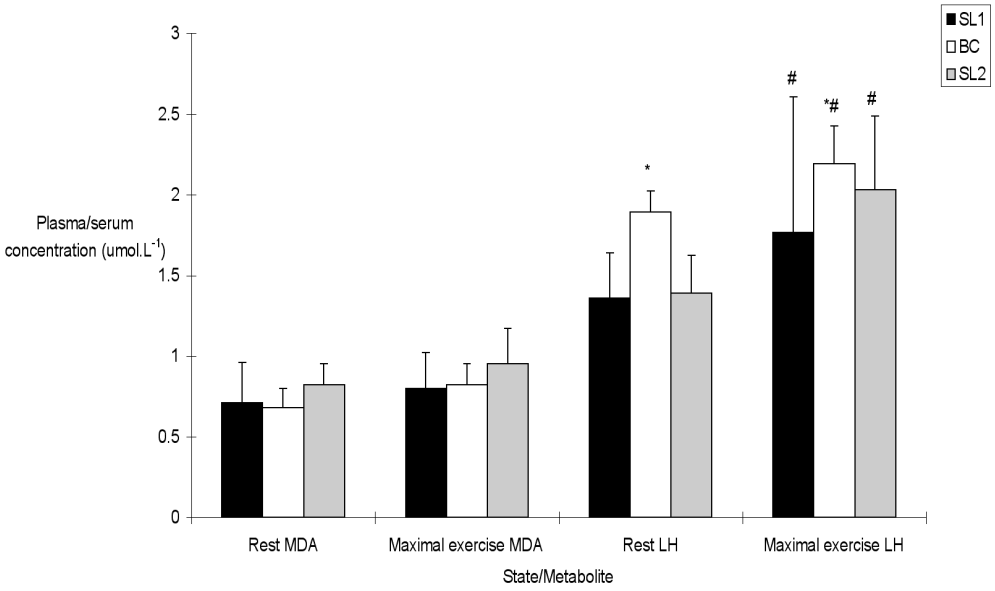

Figure 1. Lipid peroxidation at rest and after maximal exercise at sea-level and high altitude. Values are means ± SD; SL_1, sea-level before ascent to high altitude; BC, basecamp located at 5100m; SL_2, after return to sea-level. A main effect for state (rest v exercise) was established for MDA ($P < 0.05$, exercise > rest). A main effect for time (SL_1 v BC v SL_2), state and time x state interaction ($P < 0.05$) was established for LH. BC > SL_1/SL_2 and exercise > rest. * Significantly different from SL_1 and SL_2 value ($P < 0.05$) as a function of state. # Significantly different from rest ($P < 0.05$) as a function of time.

A significant correlation was observed between the cumulative AMS score (days 1 + 2 after arrival at BC) and the exercise LH response ($r = 0.69$, $P < 0.05$) which may implicate exercise-induced oxidative stress as a constitutional risk factor for the pathogenesis of AMS. This may provide an alternative reason to explain why physical exercise has long been recognised as an important risk factor for altitude

illness. Another intriguing correlation was observed between the increase in resting LH at BC and the increase in plasma cholecystokinin (CCK), a short-term satiety neuropeptide which was also shown to increase markedly at BC and was associated with a marked caloric deficit (1). Subjects with AMS also presented with a greater increase in CCK at BC relative to their healthy counterparts, perhaps suggestive of a common mechanism. These data may also suggest at least a contributory role for free radicals in the hypophagia and cachexia typically experienced at high-altitude by altering the hypothalamic control of appetite.

Muscle damage and soreness: Figure 2 illustrates the marked elevation of selected serum myofibre proteins suggestive of an increase in sarcolemmal membrane permeability at high altitude. These changes appeared to be more pronounced in those subjects diagnosed with AMS compared to those who remained apparently healthy (Figure 3).
The specificity and stability of cTnI would tend to discount the possibility of myocardial injury which when combined with the marked lower limb soreness (Figures 4 and 5) would support the contention that the major site of global tissue damage at altitude was confined to skeletal muscle.

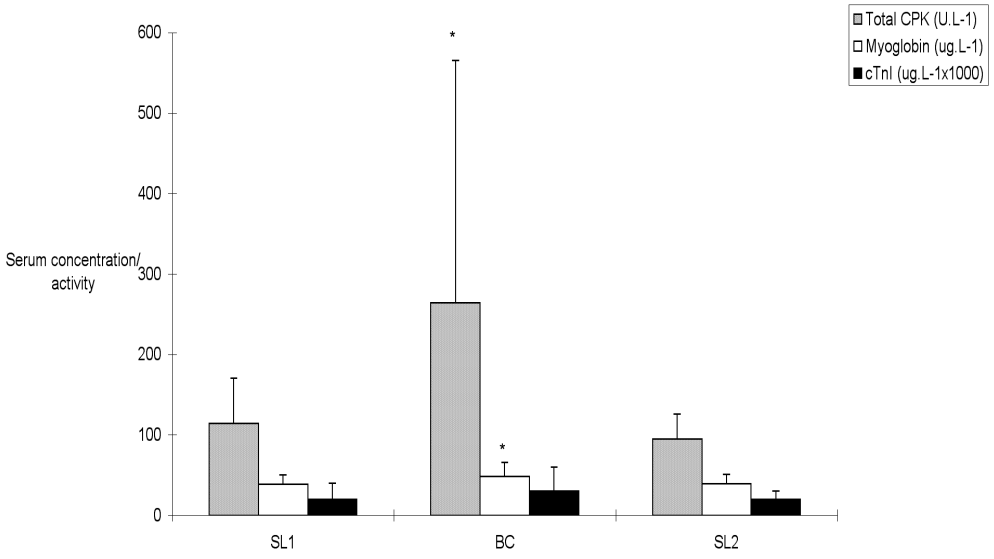

Figure 2. Resting serum concentration/activity of selected myofibre proteins at sea-level and high altitude. CPK, total creatine phosphokinase; cTnI, cardiac troponin I. * Significantly different from SL_1 and SL_2 ($P < 0.05$).

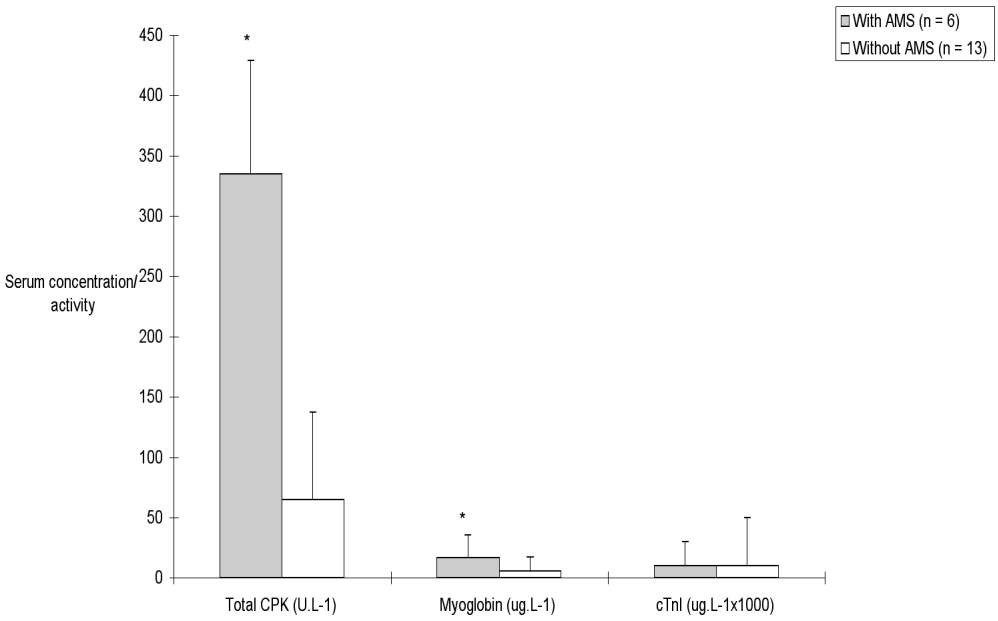

Figure. 3. Changes (BC minus SL_1) in selected myofibre proteins in subjects with and without AMS. AMS was defined as a Lake Louise score of Ž 3 points on day 2 at BC. * Significantly different from group without AMS ($P < 0.05$).

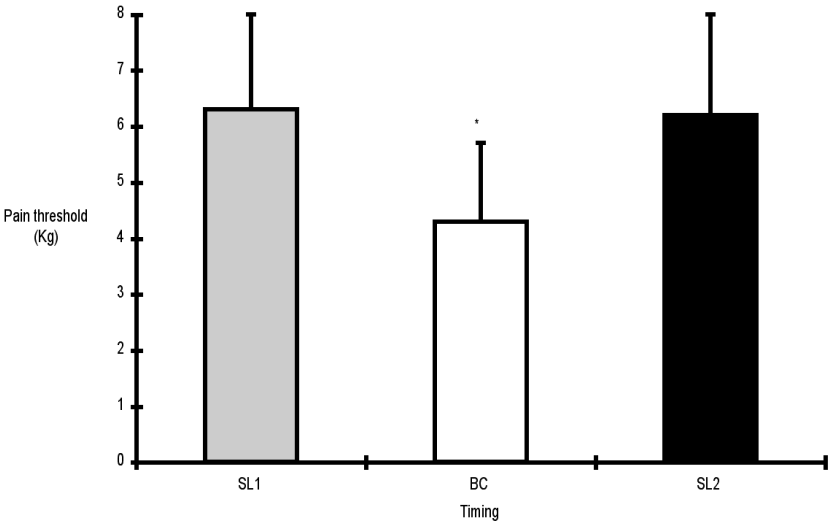

Figure 4. Resting pain threshold at sea-level and high altitude. A decrease in the pain threshold indicates that subjects were more sensitive to the controlled application of an external force. * Significantly different from SL_1 and SL_2 ($P < 0.05$).

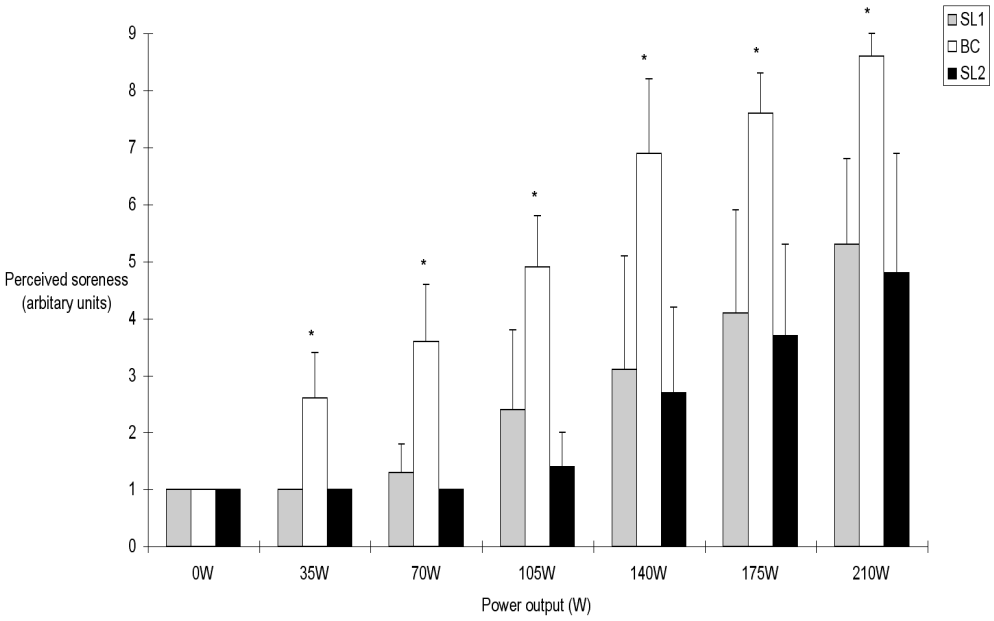

Figure 5. Rating of perceived lower limb muscle soreness during an incremental exercise test at sea-level and high altitude
A main effect for both timing ($P < 0.05$) and power output was established ($P < 0.05$) as well as a timing x power output interaction ($P < 0.05$). BC > SL_1/SL_2.
* Significantly different from SL_1/SL_2 ($P < 0.05$) as a function of power output.

A correlation was observed between the cumulative AMS score (day 1 + day 2 at BC) and Δ (BC minus SL_1) mean exercise muscle soreness score ($r = 0.84$, $P < 0.05$). While there was no apparent association between the metabolic markers of oxidative stress and muscle damage, an association was observed between Δ (BC minus SL_1) mean perceived lower limb soreness during exercise and Δ exercise LH ($r = 0.96$, $P < 0.05$); a phenomenon which, like the acute phase response, may implicate PGE_2 which has been shown to sensitize type III/IV pain afferents leading to the sensation of soreness (12).

Whilst we did not demonstrate whether oxidative stress was a direct cause or merely a consequence of myocellular enzyme release initiated by other chemical, hypoxic or mechanical events, considerable evidence suggests that damaged tissue peroxidises more rapidly than healthy tissue which can significantly impede recovery (5). Tissue damage and muscle soreness was more apparent in subjects with AMS despite the fact that these sensations are not featured in the present scoring system employed to quantify AMS symptomatology. Whilst purely speculative, individual susceptibility to AMS may be partially attributable to a deficiency in antioxidant defenses. The chronic tissue damage of AMS may over time contribute to enhanced membrane peroxidation and increase susceptibility to the more malignant condition of HACE.

Closing remarks
The physical demands of an ascent to high-altitude are usually quite exceptional and it is possible that the oxidative stress observed in the present study was merely a consequence of vigorous physical exercise. There appears to be no studies in the

literature which have attempted to isolate the potentially pro-oxidant effects of acute hypoxia and physical exercise *in vivo*; this was the focus of the second study.

Study 2: Are acute hypoxia and physical exercise independent sources of oxidative stress?

Overview and aims:
A randomised double-blind placebo-controlled study was designed to independently quantify the effects of acute hypoxia and physical exercise for free radical generation and to address subsequent implications for peripheral vascular function. Some preliminary evidence isolating *the species* of free radical using the technique of spin-trapping and electron paramagnetic resonance (EPR) spectroscopy will also be briefly presented (direct versus indirect methods of assessing free radical damage).

Methodology:
Design: Eighteen University undergraduate students aged 22 ± 3 years old were randomly assigned double-blind to perform a test in normobaric normoxia (F_IO_2 = 0.21) and moderate normobaric hypoxia (F_IO_2 = 0.16) following two months of abstention from any vitamin supplemements. Each test was separated by a 48 h recovery period and consisted of 30 mins of seated exposure (rest) followed by a standardised incremental cycling test to volitional exhaustion (exercise).

Blood samples: Overnight fasted venous samples were obtained at the end of the rest and exercise periods for the measurement of LH, MDA and the plasma concentration of a variety of lipid soluble antioxidant vitamins (LSA) which included retinol (vitamin A), α-tocopherol (vitamin E) and the carotenoids, lycopene and α/β -carotene. Arterial distensibility was derived from pulse-wave velocity (PWV) which is inversely related to the square root of distensibility. The conduction velocities of arterial pressure waveforms between the right brachial and radial arteries were determined at the end of the passive and active phases using a technique described by Ramsey *et al*. (10).

Results and discussion
Free radical footprints: Figure 6 demonstrates that both acute hypoxia and physical exercise were *independent* sources of free radical-mediated lipid peroxidation. Lipid peroxidation during the hypoxic insult was clearly evident despite a selective mobilisation of α-tocopherol (Figure 7), a chain-breaking antioxidant which inhibits lipid peroxidation by scavenging peroxyl and hydroxyl radicals (Table 1). Whilst not providing complete protection, mobilisation of α-tocopherol from adipose tissue and the liver may serve to limit the degree of oxidative damage inflicted by free radicals.

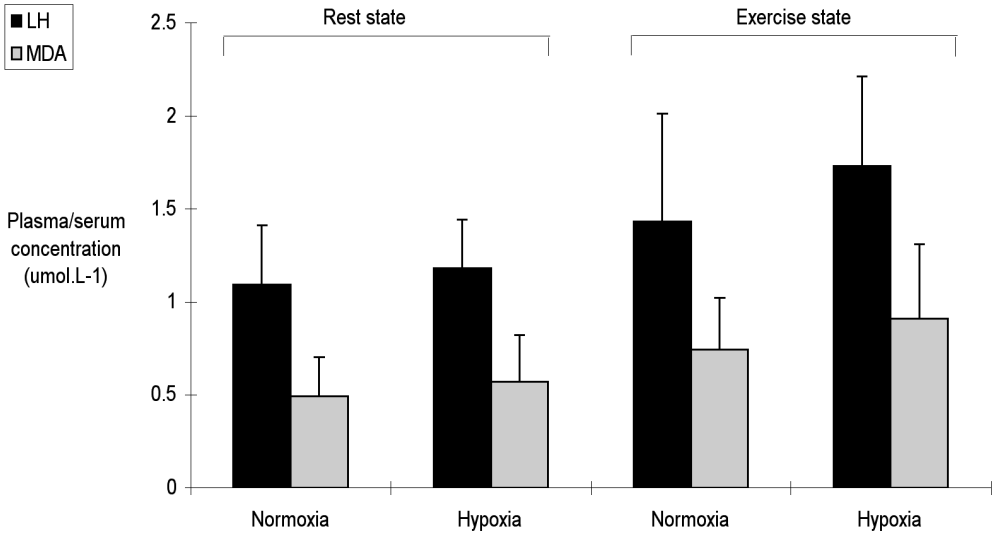

Figure 6. Lipid peroxidation after 30 minutes of rest and immediately after maximal exercise in normobaric normoxic and hypoxic conditions. Main effects for condition (normoxia *v* hypoxia) and state (rest *v* exercise) were established for both dependent variables ($P < 0.05$).

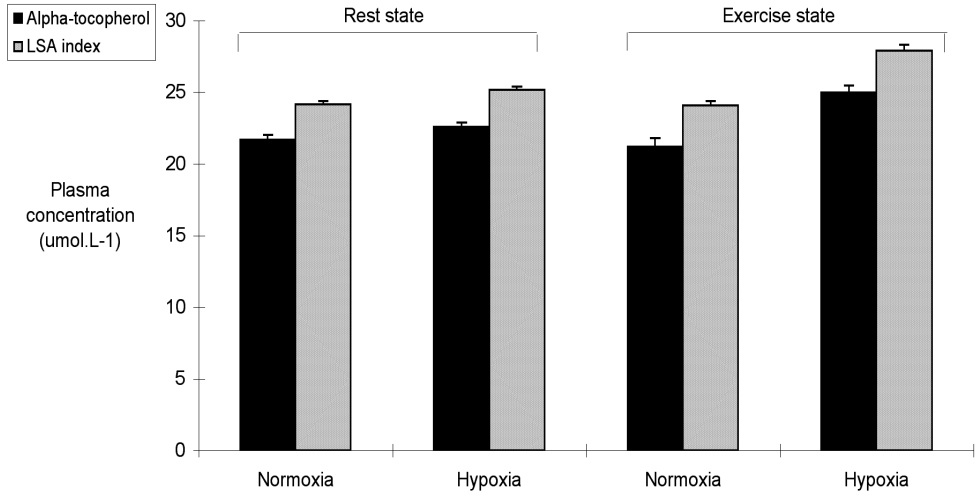

Figure 7. Changes in α-tocopherol and cumulative lipid soluble antioxidants (LSA index) after 30 minutes of rest and immediately after maximal exercise in normobaric normoxic and hypoxic conditions. A main effect for condition (normoxia *v* hypoxia) was identified for both dependent variables ($P < 0.05$).

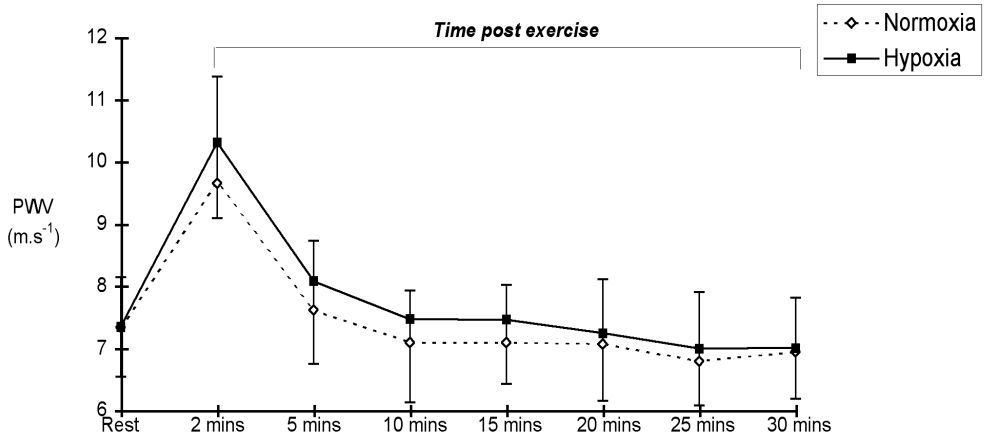

Figure 8. Conduction velocities of arterial pressure waveforms between the right brachial and radial arteries at rest and following recovery from hypoxic exercise. Main effects for time and condition ($P < 0.05$)

Inverse correlations were observed between the increase in LH/α-tocopherol (hypoxic minus normoxic maximal values) and the decrease (hypoxic minus normoxic maximal values) in arterial oxygen saturation [($Sa\mathrm{O_2}$), ΔLH α $\Delta Sa\mathrm{O_2}$, $r = -0.75$, $P < 0.05$ and Δ α-tocopherol α $\Delta Sa\mathrm{O_2}$, $r = -0.71$, $P < 0.05$]. These intriguing findings, whilst clearly not establishing cause and effect may suggest that free radical generation and antioxidant mobilisation are to some extent regulated by changes in tissue $P\mathrm{O_2}$. It would therefore be of interest to examine if a dose-response relationship exists between ambient $P\mathrm{O_2}$ and free radical generation *in vivo*, particularly at lower $P\mathrm{O_2}$'s than that encountered in the present study.

Physical exercise and hypoxia both decreased arterial distensibility as indicated by the pulse wave velocity data (Figure 8). Whether free radicals were implicated in these subtle alterations of peripheral vascular function remains speculative but nonetheless a possibility. The acute downregulation of endothelial function may have been caused by nitric oxide depletion following its reaction with superoxide radicals (Table 1) to form the reactive nitrogen oxide species, peroxynitrite ($ONOO^-$) which in itself is a potent oxidant that possesses "$OH^{\bullet -}$-like activity". This mechanism has recently been established as the major cause for enhanced leucocyte-endothelial adhesive interactions during acute hypoxia ($F\mathrm{IO_2}$ ~0.10) in the mesenteric circulation of the rat (15). Whether impaired endothelial function is a cause or consequence of AMS is an important question that is currently the focus of our research.

Direct detection of the free radical species in hypoxia

Direct detection of the free radical species during hypoxic exercise is currently under investigation in our laboratory using the spectroscopic technique of EPR combined with spin trapping. The latter incorporates a specific trap molecule such as α-phenyl-*tert*-butylnitrone (PBN) which on reaction with a free radical forms a stable product which can subsequently be measured. EPR assesses the physical properties of radicals as they spin and measures the strength of the signal emitted; the greater the

resonance signal peak, the greater the radical production and close inspection of the morphological characteristics of the EPR spectra (nuclear hyperfine strucure) facilitates the quantification of the *species*. Figure 9 illustrates typical EPR spectra obtained at rest in hypoxia (F_IO_2 ~0.16) and following 2 hours of hypoxic exercise at a 60% of an individual's normoxic maximal oxygen uptake ($\dot{V}O_{2MAX}$). Note the increase in peak height; subsequent measurement of the hyperfine coupling constants would suggest that the species detected are secondary oxygen-centred alkoxyl radicals ($RO^{\bullet}$) most likely derived from membrane phospholipids. Direct detection coupled with the *footprinting* method provides convincing evidence that the damage inflicted during hypoxic exercise is in fact free radical-mediated.

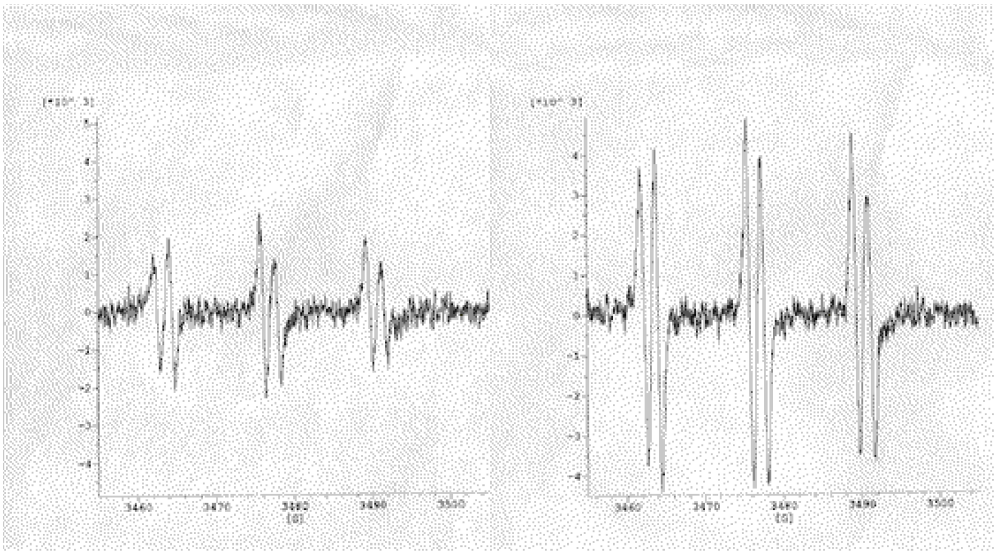

Figure 9. Examples of EPR spectra at rest (left-hand panel) and immediately after 2 hours of cycling exercise (right-hand panel) in normobaric hypoxia (F_IO_2 ~0.15) at an intensity equivalent to 60% normoxic $\dot{V}O_{2MAX}$. n = 1. Ordinate = free radical concentration in arbitrary units; Abscissa = magnetic field in Gauss.

Closing remarks

Studies 1 and 2 have demonstrated indirect evidence for free radical damage at high-altitude; a consequence in part of the environmental hypoxia and vigorous physical exercise that mountaineers typically indulge in. Antioxidant supplementation at high-altitude would appear the next "logical" step and if found to be of any prophylactic benefit would further add support to the contributions made by free radicals in the pathophysiology of altitude illness. This constitutes the third and final study.

Study 3. Are there any prophylactic benefits of antioxidant supplementation at high-altitude?

Overview and aims:

A randomised double-blind placebo-controlled study was designed to determine the prophylactic benefits of an antioxidant "cocktail" during an ascent to Mt Everest

basecamp.

Methodology:
Physiological testing: Eighteen mountaineers (16 males, 2 females) aged 35 ± 10 years participated in a battery of physiological tests at sea-level and within 15 h of arrival to Mt Everest basecamp (~5180 m). Lake Louise AMS score and resting SaO_2 were measured on a daily basis immediately on waking and prior to retiring. Hunger (how strong is your desire to eat?) and satiety (how full do you feel?) were quantified before and after a standardised meal using a visual analogue scale that ranged from 0 to 100mm. A microvascular fragility test was conducted according to the methods described by Stirrups *et al.* (13). Briefly, a 1cm diameter barrel of a 2ml syringe was applied to two adjacent sites of the buccal mucous membrane of the lower lip and a subatmospheric pressure of 200 mmHg subsequently generated. The petechiae at each respective site were counted and summed.

Supplementation: Subjects were matched for age and physical activity levels and were randomly assigned double-blind to either an antioxidant (n = 9) or placebo group (n = 9). The antioxidant group ingested 4 vegetable-based capsules per day *each* containing 250 mg of L-ascorbic acid, 100 IU of dl-α-tocopherol acetate and 150 mg of α-lipoic acid. The placebo group ingested 4 capsules of identical external appearance that each contained an equal quantity of plant cellulose (Cultech Ltd, UK). Supplementation was enforced for 3 weeks at sea-level prior to departure to Kathmandhu and during an 11 day ascent to Everest basecamp (5180 m). Subjects were instructed to refrain from any prophylactic medication against AMS as described in Study 1.

Results and discussion:
Figures 10 and 11 clearly indicate the physiological protection conferred by antioxidant supplementation at high-altitude. The antioxidant cocktail attenuated the normal increase in AMS at altitude (Figure 10) and resulted in a higher mean resting SaO_2 during the altitude sojourn (antioxidant group = 89 ± 5% *v* placebo group = 85 ± 5%, $P < 0.05$). These responses did not appear to be due to changes in *localised* microvascular fragility as no differences in the petechiae count were observed between the antioxidant and placebo groups. Supplementation appeared to exert a positive effect on appetite control. Subjects in the antioxidant group appeared to be hungrier and less satiated after a standardised meal (data not shown) which ultimately translated into a higher caloric intake relative to the placebo group (Figure 11).

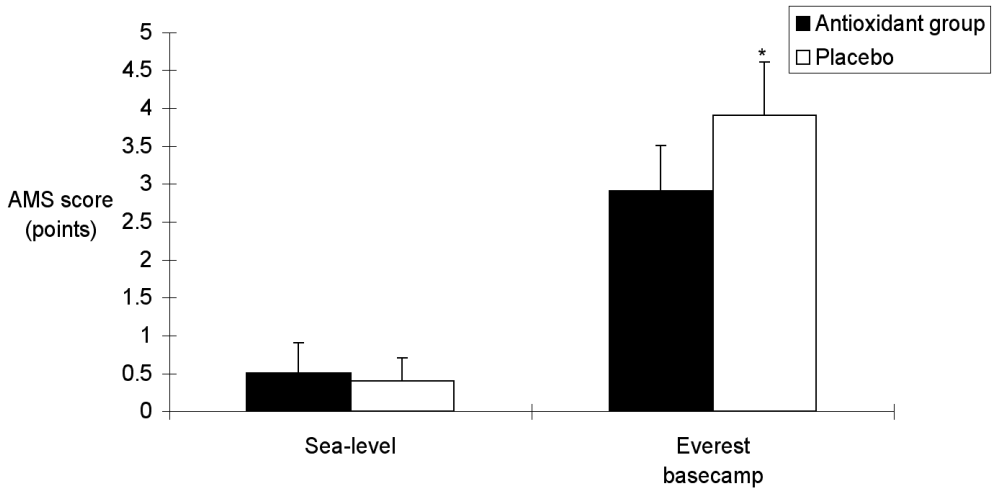

Figure 10. Implications of antioxidant supplementation for acute mountain sickness (AMS) at high-altitude. Sea-level/altitude data represent mean scores obtained over a 7 day and 11 day period respectively. Main effects observed for time (sea-level *v* 5180 m), group (antioxidant *v* placebo) and time x group interaction. * Significantly different between groups as a function of time ($P < 0.05$)

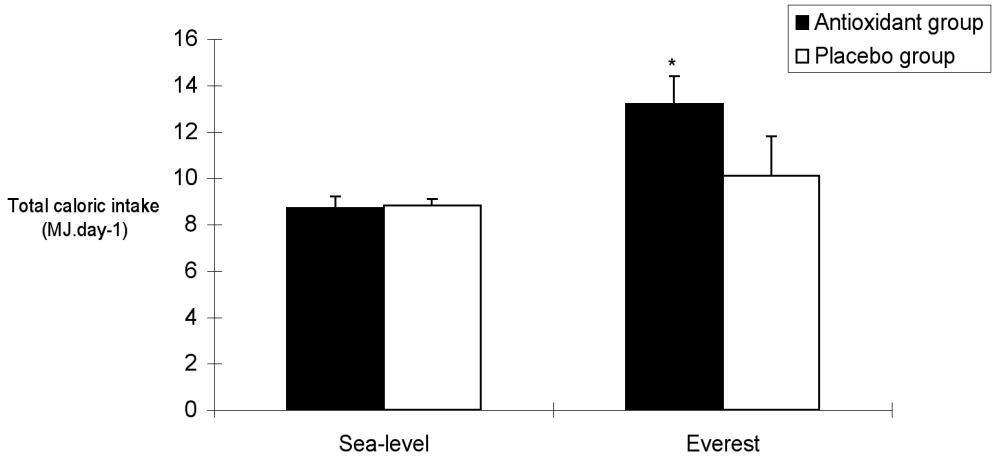

Figure 11. Hyperphagic effects of antioxidant supplementation at altitude. Altitude data obtained during the last 7 days of ascent to Everest basecamp. Main effects observed for time, group and time x group interaction. * Significantly different between groups as a function of time ($P < 0.05$).

Conclusions:

These data have systematically defined a potential role for free radicals in the pathophysiology of AMS and other potentially adverse metabolic reactions at high-altitude. The sources of oxidative damage are most likely multifactorial with physical exercise and environmental hypoxia known contributors. The hypophagia and subsequent weight loss during prolonged sojourns appears to be linked to alterations in the hypothalamic release of anorexigenic molecules which may also have a free radical basis. Exogenous antioxidant supplementation would appear to be a safe and effective strategy to confer *some* degree of physiological protection

against the ravages of high-altitude.

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