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TOPICAL REVIEW

Mitochondrial function at extreme high altitude

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Abstract At high altitude, barometric pressure falls and with it inspired P_{O_2}, potentially compromising O_2 delivery to the tissues. With sufficient acclimatisation, the erythropoietic response increases red cell mass such that arterial O_2 content (C_{aO_2}) is restored; however arterial P_{O_2} (P_{aO_2}) remains low, and the diffusion of O_2 from capillary to mitochondrion is impaired. Mitochondrial respiration and aerobic capacity are thus limited, whilst reactive oxygen species (ROS) production increases. Restoration of P_{aO_2} with supplementary O_2 does not fully restore aerobic capacity in acclimatised individuals, possibly indicating a peripheral impairment. With prolonged exposure to extreme high altitude (>5500 m), muscle mitochondrial volume density falls, with a particular loss of the subsarcolemmal population. It is not clear whether this represents acclimatisation or deterioration, but it does appear to be regulated, with levels of the mitochondrial biogenesis factor PGC-1α falling, and shows similarities to adapted Tibetan highlanders. Qualitative changes in mitochondrial function also occur, and do so at more moderate high altitudes with shorter periods of exposure. Electron transport chain complexes are downregulated, possibly mitigating the increase in ROS production. Fatty acid oxidation capacity is decreased and there may be improvements in biochemical coupling at the mitochondrial inner membrane that enhance O_2 efficiency. Creatine kinase expression falls, possibly impairing high-energy phosphate transfer from the mitochondria to myofibrils. In climbers returning from the summit of Everest, cardiac energetic reserve (phosphocreatine/ATP) falls, but skeletal muscle energetics are well preserved, possibly supporting the notion that mitochondrial remodelling is a core feature of acclimatisation to extreme high altitude.

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Abbreviations ^{31}P-MRS, ^{31}P magnetic resonance spectroscopy; BNIP3, BCL2/adenovirus E1B 19 kDa protein-interacting protein 3; ETC, electron transport chain; HIF, hypoxia-inducible factor; HOAD, 3-hydroxyacyl-CoA dehydrogenase; PGC, peroxisome proliferator-activated receptor gamma co-activator; P_{O_2}, partial pressure of O_2; PCr, phosphocreatine; PPARα, peroxisome proliferator-activated receptor alpha; ROS, reactive oxygen species; UCP3, uncoupling protein 3; V_{max}, maximum rate of mitochondrial oxygen consumption; $V_{O_2\,max}$, maximum rate of whole-body oxygen consumption.

Introduction

Barometric pressure decreases with altitude and thus inspired P_{O_2} falls, such that at high altitudes O_2 delivery from the lungs to oxidative tissues such as heart and skeletal muscle is challenged. Within the body, the majority of oxygen consumption occurs at cytochrome

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c oxidase (complex IV) of the mitochondrial electron transport chain (ETC). The concept of symmorphosis postulates that biological systems are economical, with structural parameters matched to functional capacity (Weibel *et al.* 1991). Accordingly, for the respiratory system, mitochondrial volume in the muscles would be proportional to total aerobic capacity (Weibel *et al.* 1991). Thus, at high altitude, when aerobic capacity becomes limited, alterations in mitochondrial volume and function could intuitively form part of the process of human acclimatisation (Murray, 2014). Alternatively, the mitochondria might be viewed as potential targets of damage during the less well understood process of high altitude deterioration (Ward, 1954) as they are sites of reactive oxygen species (ROS) production.

Over the past 25 years, several studies have reported changes in mitochondrial volume density, subcellular distribution, oxidative enzyme activity and protein expression in the muscles of climbers returning from extreme high altitude (>5500 m) and these findings are reviewed here (Fig. 1). Whilst no direct measures of mitochondrial function have yet been made above 5500 m, ^{31}P magnetic resonance spectroscopy (^{31}P-MRS) has been used by our research group to study heart and skeletal muscle energetics as an indirect measure of mitochondrial function in climbers at sea level, prior to and within a few weeks of their return from extreme altitude. In addition, a small number of informative studies of mitochondrial respiratory function have been made in permeabilised fibres of skeletal muscle biopsies at altitudes between 3400 and 4500 m using high-resolution respirometry, and these findings have also been included here. Finally, whilst the focus of this review is on human mitochondrial function at extreme high altitude, reference is made to relevant mechanistic insights gleaned from high altitude adapted species, experimental animal models and cells cultured under hypoxic conditions. For a more comprehensive account of the metabolic changes that occur in the skeletal muscle of rodents and humans in response to real or simulated altitudes >3000 m, readers are referred to our recent review (Horscroft & Murray, 2014).

What underlies the possible change in mitochondrial function at extreme high altitude?

Upon ascent to altitudes over 5500 m, barometric pressure, and thus inspired P_{O_2}, falls to less than half of that at sea level, with the inspired P_{O_2} on the summit of Mt Everest (8848 m) being less than one-third of that at sea level (43.1 *vs.* 148.0 mmHg) (West *et al.* 1983). The successful ascent of Everest without supplementary oxygen by Reinhold Messner and Peter Habeler in 1978 (a feat repeated by Messner and others since) demonstrated the remarkable capacity of the human body to perform at such extreme altitude. It is clear that successful acclimatisation during a gradual ascent is paramount to any such attempt to reach extreme altitudes.

Features of acclimatisation that improve O_2 delivery at altitude have been well documented. Upon arrival at altitude, resting heart rate increases and cardiac output is elevated for a given amount of physical work. The sea-level relationship between cardiac output and workload is later restored as arterial O_2 content (C_{aO_2}) returns to sea level values, although notably the maximum achievable cardiac output falls at higher altitudes (Peacock, 1998). Ventilatory aspects of acclimatisation show a distinct time course, with an initial increase in ventilation (the hypoxic ventilatory response) followed by a brief reduction back towards control values (hypoxic ventilatory decline) before a sustained period of acclimatisation, during which ventilation continues to increase for up to about 2 weeks (West *et al.* 2007). Meanwhile, an initial increase in haematocrit can result from a fall in plasma volume due to dehydration, but in response to sustained hypoxia the juxtaglomerular apparatus of the kidneys increases production of erythropoietin (EPO), which stimulates bone marrow to increase red cell output. EPO levels can rise within hours of arrival at altitude, but the increase in red cell mass can take several weeks (Peacock, 1998; West *et al.* 2007). In the face of falling arterial P_{O_2}, and therefore haemoglobin-O_2 saturation, the erythropoietic response allows C_{aO_2} to be maintained at, or even above, sea level values up to 7000 m, falling only at more extreme altitudes (Grocott *et al.* 2009).

Whilst the process of acclimatisation can take many weeks, a prolonged stay at extreme high altitude can result in high altitude deterioration. This process is rather less well understood than acclimatisation, but is characterised by weight loss (including muscle wasting), poor appetite, lethargy, slow recovery from fatigue and possibly low systemic blood pressure (Ward, 1954). Altitude exposure can decrease energy intake by as much as half (Guilland & Klepping, 1985; Rose *et al.* 1988), resulting in an energy deficit which underlies the loss of body mass (Westerterp & Kayser, 2006). The cachectic response to high altitude, whilst traditionally viewed as detrimental, may afford some protection through, for example, the release of metabolic substrates and signalling molecules (Murray & Montgomery, 2014; Levett *et al.* 2015), an increased ratio of capillaries to muscle fibre cross sectional area (Hoppeler *et al.* 1990*a*) or simply by decreasing whole-body O_2 demand due to the loss of body mass. Changes in diet and energy balance are therefore confounding factors, which must be considered in studies of metabolism at extreme altitude.

Even with adequate acclimatisation, however, and a C_{aO_2} equivalent to that at sea level, exercise capacity and $V_{O_2\,max}$

are impaired at altitude (Cerretelli, 1976; Levett *et al.* 2011, 2012). Moreover, the loss of $V_{O_2\,max}$ at any given altitude is not discernibly different between unacclimatised and fully acclimatised subjects (Cerretelli, 1976). Thus, whilst successful acclimatisation can help to stave off some of the potentially adverse consequences of high altitude (most notably, acute mountain sickness) (West *et al.* 2007), exercise capacity is not improved. This somewhat counterintuitive finding illustrates the importance of capillary P_{O_2}, as opposed to C_{aO_2}, in supporting O_2 transport and utilisation. Convective O_2 delivery is restored by acclimatisation, but because capillary P_{O_2} remains low, the capacity for diffusion between the capillary and mitochondrion is limited – a concept proposed by Peter Wagner and co-workers (Wagner, 1996, 2000), and supported by experimental evidence in hypoxic dogs (Hogan *et al.* 1988) and retrospective analysis of data collected from human subjects during Operation Everest II, in which subjects underwent progressive decompression to the equivalent of 8840 m in a chamber at sea level (Sutton *et al.* 1988; Wagner, 2010). Whilst muscle wasting at altitude might conceivably improve diffusion to the mitochondria by decreasing mean diffusion distance, in dogs with muscle wasting following leg immobilisation, O_2 conductance was not improved (Hepple *et al.* 2000).

Therefore, at extreme high altitude capillary P_{O_2} is decreased, even in fully acclimatised subjects. What then of the mitochondrial P_{O_2}? Measurements of the distribution of P_{O_2} within working skeletal muscle fibres suggest a sharp immediate fall in P_{O_2} at the interface between capillary and myocyte, but with the P_{O_2} across the remaining width of the myocyte being reasonably uniform and low, due to the presence of myoglobin in aiding distribution (Gayeski & Honig, 1986*a*,*b*). Within the mitochondrion itself, at cytochrome *c* oxidase (the terminal complex of the ETC and site of O_2 consumption) P_{O_2} is extremely low, probably less than 1 mmHg (0.13 kPa) even in the absence of environmental hypoxia (Chance, 1957;

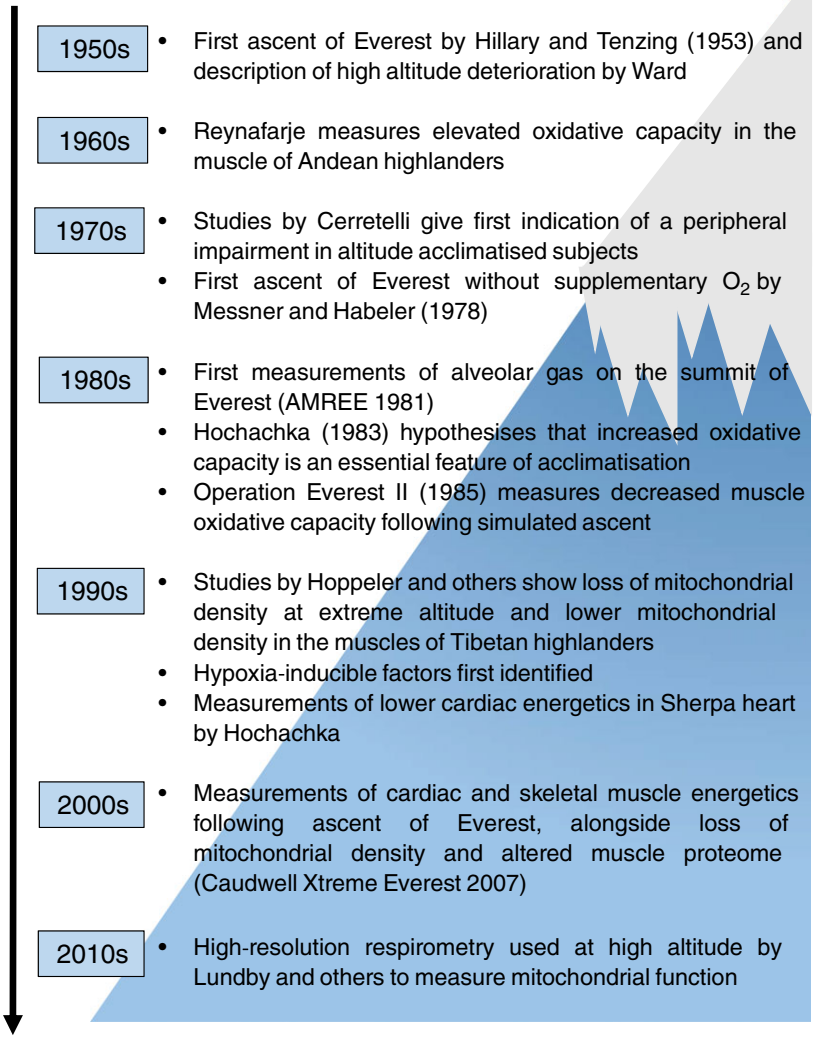

Figure 1. Timeline of key events and studies pertaining to the study of mitochondrial function in heart and skeletal muscle at extreme high altitude and discussed in this review

Chance *et al.* 1962). As such, mitochondrial P_{O_2} has often been considered negligible and thus assumed to be zero in many models of O_2 diffusion and consumption. This assumption cannot be correct, however, because oxidative phosphorylation is dependent upon O_2, and if P_{O_2} were zero then so too would be V_{O_2} (Cano *et al.* 2013). A recently refined model of whole-body O_2 transport and utilisation has allowed for greater heterogeneity in P_{O_2} across muscle regions, with mitochondrial P_{O_2} increasing under conditions where mitochondrial respiratory capacity (V_{max}) is relatively low compared with O_2 delivery (Cano *et al.* 2015). Using data from Operation Everest II, the model predicts a progressive fall in mitochondrial P_{O_2} of exercising muscle at altitude, from around 1 mmHg at sea level to a mean value of 0.119 mmHg on the summit of Everest (Cano *et al.* 2015). This is below measurements made *in vitro* of the P_{50} of mitochondrial respiration i.e. the P_{O_2} at which respiration would fall by half (Gnaiger *et al.* 1998) and as such respiration would be limited. In addition, another expected consequence of such low mitochondrial P_{O_2} at extreme altitude would be a sharp increase in ROS production (Cano *et al.* 2014), the implications of which are discussed later in this review.

The fall in arterial (and thus capillary) P_{O_2} at extreme altitude thus limits O_2 diffusion, exaggerating the mismatch between the capacity of the tissue to consume available O_2 (V_{max}) and the capacity to deliver it – a situation already present to some degree in humans at sea level (Boushel *et al.* 2011) and seeming to contradict the concept of symmorphosis (Weibel *et al.* 1991). At altitude, $V_{O_2\,max}$ can be improved by the use of O_2 supplementation to elevate arterial (and thus capillary) P_{O_2}, but curiously it is not completely restored to sea level values in acclimatised subjects, despite their elevated haematocrit which actually results in them having an enhanced C_{aO_2} compared with that at sea level (Cerretelli, 1976). This finding provided the earliest evidence for a peripheral impairment either in local O_2 delivery, as Paolo Cerretelli suggested at the time, or in the oxidative capacity of the muscle – a subject that was to form the basis of much of his later research (Ferretti, 2003). In further support of this, $V_{O_2\,max}$ does not return to pre-expedition values for several weeks after acclimatised subjects return to sea level (Grassi *et al.* 1996). Intriguingly, recent measures of impaired blood flow through the sublingual microvasculature using sidestream darkfield imaging suggest that local O_2 delivery might indeed be worsened in acclimatised subjects at altitude (Martin *et al.* 2009), perhaps due to increased blood viscosity secondary to a raised haematocrit or endothelial dysfunction. Note, however, that comparable measures have not yet been made in the skeletal muscle microcirculation and how generalisable this finding is remains of debate.

Morphological changes in mitochondria – volume density and subcellular distribution

Altitude thus poses a sustained challenge to oxidative phosphorylation. An interpretative hypothesis, based on observational data from the muscles from high altitude adapted animals, had previously suggested that an enhanced oxidative capacity was a necessary component of high altitude acclimatisation (Hochachka *et al.* 1983). This was supported by earlier evidence of high oxidative capacities and myoglobin concentrations in the sartorius muscle of some Andean highlanders compared with lowlanders (Reynafarje, 1962), although these measurements may have been confounded by differences in training status between subjects. Endurance training is a potent stimulant for mitochondrial biogenesis, and was found to be as effective in Andean natives as it is in lowland residents (Desplanches *et al.* 1996). Certainly in lowlanders acclimatising to high altitude this is not the case; oxidative capacities fall with prolonged exposure, and at extreme altitude the mitochondrial volume density of human skeletal muscle decreases. A loss of 20–30% of mitochondrial density has consistently been seen in climbers returning from the summit of Everest (Hoppeler *et al.* 1990; Levett *et al.* 2012), alongside the accumulation of lipofuscin, a substance believed to result from lipid peroxidation and thus indicating possible oxidative stress (Howald & Hoppeler, 2003). It is tempting to view this phenomenon as a feature of high altitude deterioration; interestingly, there was no apparent loss of mitochondria in the muscles of the subjects of Operation Everest II (MacDougall *et al.* 1991) whose rapid ascent to the equivalent altitude of the summit of Everest (over 40 days) and immediate return to normal barometric pressure (Houston *et al.* 1987) limited their exposure to extreme altitude in comparison with a traditional Everest expedition. Intriguingly, however, in muscle biopsies from Sherpas, a highly adapted subgroup of native Tibetan highlanders reputed to be strong performers at extreme altitude (Gilbert-Kawai *et al.* 2014), lower mitochondria densities have also been reported (Kayser *et al.* 1991) and this also appears to be the case in lowland-dwelling Tibetans who have never resided at altitude (Kayser *et al.* 1996). Therefore, the loss of mitochondrial density in acclimatising lowlanders may be an adaptive response that mimics the adaptations of high altitude natives, rather than simple deterioration.

Curiously, there appear to be differential effects of extreme altitude on two discrete subpopulations of mitochondria in human muscle. Within the cell, mitochondria do not generally exist as distinct, individual, rod-shaped organelles, but instead form a dynamic reticular network, which undergoes continual fusion and fission (Picard *et al.* 2011). Within skeletal and

cardiac muscle, this network forms spatially distinct pools, with a subpopulation of subsarcolemmal mitochondria lying alongside the cell membrane and interfibrillar mitochondria positioned in rows alongside the myofibrils (Ferreira *et al.* 2010; Hollander *et al.* 2014). In climbers returning from extreme altitude, there is a greater percentage loss of the less abundant subsarcolemmal mitochondria than the more populous, deep-lying interfibrillar mitochondria (Hoppeler *et al.* 1990; Levett *et al.* 2012) (Fig. 2). Correspondingly, whilst the lower mitochondrial density in Tibetans compared with lowland Nepalese appears to be driven by lower densities of both subsarcolemmal and interfibrillar mitochondria, a greater component of the difference is accounted for by the lower subsarcolemmal population (Kayser *et al.* 1996). These findings are somewhat counterintuitive, as a selective loss of the subsarcolemmal population would result in a potentially counterproductive increase in mean diffusion distance for O_2 between the capillary and remaining (largely interfibrillar) mitochondria. This may in part be mitigated by an increase in capillary density, due to muscle fibre wasting (Hoppeler *et al.* 1990*a*; Ferretti, 2003; Murray & Montgomery, 2014), and possibly by an increased myoglobin content to facilitate intracellular O_2 diffusion. Increased myoglobin levels have been reported in rodents exposed to environmental hypoxia (Clark *et al.* 1952; Vaughan & Pace, 1956) and dogs residing at high altitude (Hurtado *et al.* 1937). In humans, however, the picture is less clear. Elevated myoglobin levels were reported in some high altitude natives (Reynafarje, 1962), although again this observation may have been confounded by training status. Expression levels of myoglobin were not increased in climbers returning from the summit of Everest, however (Levett *et al.* 2015), and may only become upregulated when exercise training is imposed on top of the hypoxic stimulus (Hoppeler & Vogt, 2001; Desplanches *et al.* 2014). Moreover, in one study muscle levels of myoglobin fell by 35% in human subjects after 7–9 days at 4559 m, alongside the downregulation of other iron-related proteins and loss of total muscle iron content – effects that were attributed to the need to mobilise intramuscular iron to support the erythropoietic response (Robach *et al.* 2007).

The subcellular distribution of mitochondria is not, however, only important for the diffusion of O_2 but also for the exchange of the high energy phosphate products of respiration between the mitochondrial source and the myofibrils (Hoppeler & Billeter, 1991). The latter process is aided in highly oxidative tissues such as cardiac and skeletal muscle by the creatine kinase shuttle, which acts as a temporal and spatial buffer of cellular ATP by transferring phosphate from ATP to creatine at the mitochondrion, forming phosphocreatine (PCr) and ADP, and catalysing the reverse reaction at the myofibrils. These reactions maintain [ADP] at the mitochondrion, supporting respiration, and [ATP] at myosin, supporting contraction. The creatine kinase shuttle also allows a faster distribution of the high energy phosphate from the mitochondrion to myofibril as the smaller PCr molecule diffuses more rapidly than ATP. At altitude, however, creatine kinase is downregulated (Vigano *et al.* 2008; Levett *et al.* 2015), potentially impairing high energy phosphate transfer. Muscle fibre wasting may mitigate this

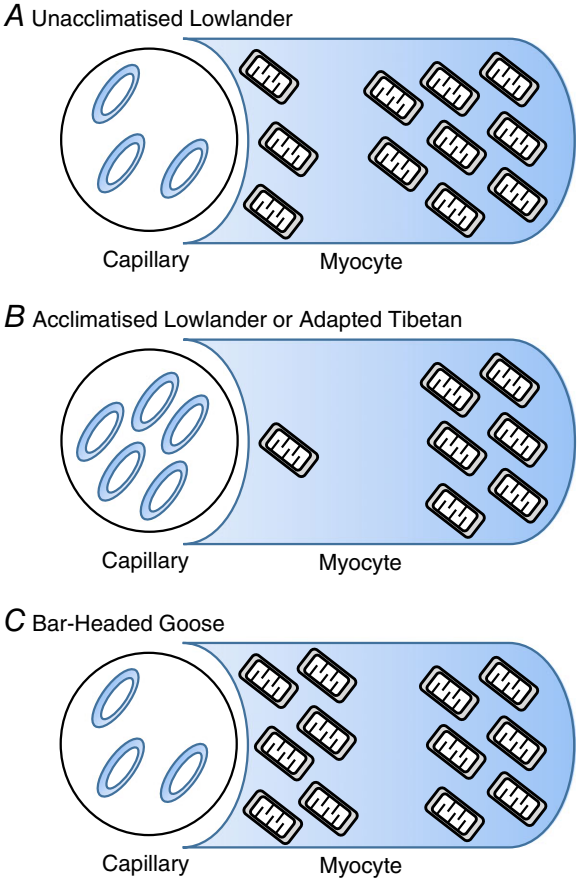

Figure 2. Subcellular distribution of mitochondria within the myocyte in *A*, unacclimatised lowlanders; *B*, acclimatised lowlanders or adapted Tibetan highlanders; and *C*, adapted bar-headed geese
Acclimatisation in humans is associated with a greater loss of subsarcolemmal mitochondria, compared with the interfibrillar population. Meanwhile high altitude adapted Tibetans have lower mitochondrial densities than lowlanders with a greater proportion of the difference being accounted for by a relatively low subsarcolemmal population. Hence, high altitude acclimatisation appears to mimic the adapted-highlander phenotype. In bar-headed geese, however, there is a preponderance of subsarcolemmal mitochondria, which appears to minimise the diffusion distance for O_2. Bar-headed geese appear to have an enhanced creatine kinase shuttle, to aid the distribution of high-energy phosphate metabolites throughout the myocyte, whereas in humans at altitude, creatine kinase is downregulated.

to some extent, by decreasing average diffusion distances, but it is possible that with a compromised capacity for PCr synthesis the preferred maintenance of mitochondria in intermyofibrillar regions circumvents some of the resulting limitations of high-energy phosphate delivery to myosin.

In contrast with humans, in the muscles of certain species of bird adapted for flight at extreme high altitudes, such as the bar-headed goose, a greater overall aerobic capacity is accompanied by a preponderance of subsarcolemmal mitochondria (Fig. 2), an adaptation presumed to be beneficial in minimising O_2 diffusion distance (Scott, 2011). Training status may be a confounding factor here too though, because the low air density at these altitudes increases the work required to generate the lift and thrust to maintain flight (Bishop *et al.* 2015). Note, however, that mitochondrial ATP production in these birds is more strongly regulated by the creatine kinase shuttle than in species dwelling at low altitude (Scott *et al.* 2009). Perhaps this suggests that the geese have a more effective high energy phosphate delivery mechanism than humans, obviating the need for a more substantial population of interfibrillar mitochondria and allowing redistribution of mitochondria to the cell periphery. Moreover, these birds exhibit other adaptations, including high muscle capillarity and an enhanced hypoxic ventilatory response, which would partly counteract the fall in arterial P_{O_2} at these altitudes (Scott, 2011).

The physiological significance of the apparent selective loss of subsarcolemmal mitochondria in humans at extreme altitude remains unclear, whilst the mechanism regulating it is undetermined. Indeed little is firmly known about the functional importance of these two subpopulations, although in some rat muscles (but not all) they have different respiratory capacities (Palmer *et al.* 1977; Philippi & Sillau, 1994) and differences in the proteome of the two subpopulations have also been noted (Ferreira *et al.* 2010). In humans at sea level, it is notable that the subsarcolemmal population is associated with much greater changes in volume density in response to endurance training than the interfibrillar mitochondria (Desplanches *et al.* 1993). It is possible that the loss of mitochondrial density, and in particular the subsarcolemmal population, with prolonged exposure to extreme high altitude may therefore simply represent a detraining response accompanying the lethargy associated with high altitude deterioration, rather than true acclimatisation. Moreover, the cachectic response to high altitude and changes in diet may also affect mitochondrial density and distribution. In cells, however, and increasingly in animal models and human subjects, there is evidence of regulated transcriptional modification of metabolism and mitochondrial density associated with hypoxic exposure, and this evidence is reviewed in the next section.

Regulation of mitochondrial density in prolonged hypoxia

The cellular response to hypoxia is predominantly governed by the hypoxia-inducible factor (HIF) family of transcription factors. HIF-1α and 2α subunits are constitutively expressed, but degraded following O_2-dependent hydroxylation by prolyl-hydroxylase enzymes (PHD1–3) (Semenza, 2007). At low tissue P_{O_2}, the HIF α-subunits are thus stable and dimerise with the nuclear HIF-1β subunit, activating the transcription of genes that contain hypoxia-response elements in their promoter regions, including those encoding EPO and vascular endothelial growth factor (VEGF) (Semenza, 2007). There appears to be a timing-dependent component to this response, termed the HIF switch, whereby HIF-1 stabilisation underpins an initial response to hypoxia (<24 h) whilst the more sustained response is supported by HIF-2 (Koh & Powis, 2012). Systemically, HIF-2 activation underlies the erythropoietic response, whilst in cultured cells HIF-1-dependent measures to decrease O_2 demand include the downregulation of mitochondrial oxidative phosphorylation via inhibition of pyruvate dehydrogenase (Kim *et al.* 2006). An HIF-dependent upregulation of the pro-apoptotic protein BNIP3 (BCL2/adenovirus E1B 19 kDa protein-interacting protein 3) also underlies a process of mitochondrial autophagy, and consequent loss of mitochondrial density, in some cells (Zhang *et al.* 2008). An increase in BNIP3 appears to occur in the hypoxic rat heart (Regula *et al.* 2002), but was not seen in human muscle at extreme altitude, although markers of chaperone-mediated autophagy were present (Levett *et al.* 2015) (Fig. 3).

In skeletal muscle, there is a possible interaction between the transcriptional activity of HIF and that of the mitochondrial biogenesis factor, peroxisome proliferator-activated receptor γ co-activator 1α (PGC-1α). In cultured myocytes, PGC-1α overexpression increased mitochondrial biogenesis and thus cellular O_2 consumption, causing a fall in tissue P_{O_2}, which in turn stabilised HIF-1α (O'Hagan *et al.* 2009) (Fig. 3). Conversely, in renal carcinoma cells with constitutively stabilised HIF-1, mitochondrial biogenesis was suppressed secondary to repression of C-MYC activity and decreased expression of PGC-1β (Zhang *et al.* 2007). Thus, mitochondrial density is dynamically regulated in response to O_2 availability, with mitochondrial biogenesis supported when O_2 is available (and HIF-1 therefore not stabilised) but further mitochondrial biogenesis is inhibited if cellular O_2 consumption increases to the extent that P_{O_2} falls below a critical point and HIF-1 becomes stabilised. Levels of PGC-1α were decreased in climbers returning from the summit of Everest and biopsied at 5300 m, suggesting a suppression of mitochondrial biogenesis had occurred (Levett *et al.*

2012). HIF-1α levels were not elevated in these subjects, although in line with the HIF switch this would not perhaps have been expected after 66 days of exposure. Unfortunately, levels of HIF-2α, which mediates the more sustained response to hypoxia, were not measured in these subjects. In the same study, subjects biopsied within a few days of arrival at 5300 m following ascent did show enhanced HIF-1α, but no loss of mitochondrial density or PGC-1α at this stage (Levett *et al.* 2012).

Interestingly, the altitude-dependent loss of mitochondria and remodelling of mitochondrial function do appear to be dependent on length of exposure or altitude reached (Horscroft & Murray, 2014), perhaps reflecting a time-dependent regulation of the transcriptional response in line with the HIF switch. Notably, in two studies from Carsten Lundby's group, which used high-resolution respirometry to measure respiratory function in skeletal muscle biopsies, relatively short-term exposure (9–11 days) to 4559 m had no discernible effect on mitochondrial respiratory capacity (Jacobs *et al.* 2013), whilst 28 days of exposure to a lower altitude (3454 m) did result in a diminished respiratory capacity, albeit in the absence of a change in mitochondrial content (Jacobs *et al.* 2012). The findings from these studies are discussed in more detail in the next section.

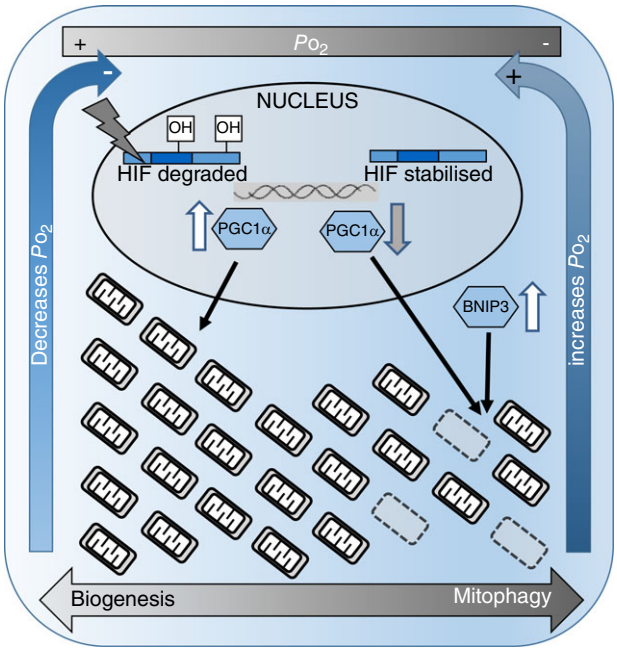

Figure 3. Possible mechanism underlying the regulation of mitochondrial volume density in response to cellular hypoxia at extreme high altitude
Under normoxic conditions (relatively high P_{O_2}), the hypoxia-inducible factors (HIFs) are hydroxylated and degraded, whilst under hypoxic conditions (low P_{O_2}) they are stabilised, with increased mitochondrial ROS production enhancing HIF stability. HIF binds to hypoxia response elements on target genes activating transcription. Hypoxia can therefore increase mitophagy factors (e.g. BNIP3) and lead to the downregulation of factors that stimulate mitochondrial biogenesis (e.g. PGC-1α). Thus, in sustained hypoxia, mitochondrial density falls, whereas in normoxic conditions mitochondrial biogenesis can be supported. An expanded population of mitochondria will consume O_2 at a greater rate, decreasing cellular P_{O_2} and leading to HIF stabilisation, whereas a loss of mitochondria will conversely restore P_{O_2} leading to HIF degradation. Thus, the cell's capacity for oxygen consumption (i.e. mitochondrial density) is dynamically coupled to tissue P_{O_2}, and oxygen demand therefore matched to supply.

Mitochondria themselves play a further, critical role in hypoxia signalling, forming the nexus of a feedback loop that maintains cellular redox homeostasis. Under hypoxic conditions, electron leak, particularly from complexes I and III of the highly reduced mitochondrial ETC, leads to increased production of ROS, such as the superoxide radical ($O_2^{\cdot -}$), potentially damaging proteins, lipids and DNA and thus impairing cellular function (Guzy & Schumacker, 2006; Tsutsui, 2006). ROS production is predicted to rise with altitude, increasing rapidly at altitudes above 7000 m (Cano *et al.* 2014). Oxidative stress enhances HIF stabilisation in cells (Chandel *et al.* 2000; Guzy *et al.* 2005; Guzy & Schumacker, 2006), thereby activating responses that in turn curb ROS generation, albeit at the possible cost of impaired ATP synthesis (Murray, 2009). An HIF-regulated process of mitophagy or repression of mitochondrial biogenesis, in matching tissue O_2 demand to a diminished supply, is thought to limit ROS production, thereby protecting the remaining mitochondria and preserving their respiratory function. Paradoxically, there is also evidence that ROS are necessary mediators for training-induced mitochondrial biogenesis (Gomez-Cabrera *et al.* 2008). These apparently opposing effects may be determined by the duration of the ROS stimulus, with an acute increase in ROS during exercise eliciting mitochondrial biogenesis, but sustained ROS production with chronic exposure to extreme altitude repressing biogenesis and activating mitophagy.

Qualitative changes in mitochondrial function – oxidative enzymes and respiratory coupling

Mitochondrial respiratory function itself exhibits plasticity under prolonged/severe hypoxia, beyond a modulation of mitochondrial content, with changes in substrate metabolism, ETC activity and respiratory coupling occurring in response to extreme high altitude, but also at more moderate high altitude over shorter periods of exposure when there may not yet be a loss of mitochondrial density (Horscroft & Murray, 2014). Regarding extreme altitude, the activity and/or expression of several tricarboxylic acid cycle enzymes have been found to be downregulated following real or simulated ascent above 5500 m, including citrate synthase (Green *et al.* 1989; Howald *et al.* 1990; Levett

et al. 2012), isocitrate dehydrogenase (Levett *et al.* 2015), α-ketoglutarate dehydrogenase (Levett *et al.* 2015) and succinate dehydrogenase (Green *et al.* 1989; Levett *et al.* 2015), which also comprises complex II of the ETC. Of the other ETC complexes, decreased protein levels or activity of subunits of complex I (Levett *et al.* 2012), complex III (Levett *et al.* 2015) and complex IV (Howald *et al.* 1990; Levett *et al.* 2012) have all been reported following exposure to extreme altitude. Furthermore, in high-resolution respirometry studies of permeabilised muscle fibres, respiration capacity for both complex I and complex II substrates was decreased following 28 days at 3454 m with no loss in citrate synthase activity (Jacobs *et al.* 2012), but no such changes were seen with short-term exposure to 4559 m (Jacobs *et al.* 2013). Downregulation of ETC complexes under hypoxic conditions may mitigate the potential rise in ROS production via electron leak, by restricting electron entry into the chain. In hypoxic human fibroblasts, upregulation of an HIF-regulated microRNA, miR-210, suppressed the expression of ETC complex assembly proteins, decreasing the levels and respiratory capacity of complexes I and IV (Colleoni *et al.* 2013). This effect was also seen in human placentas at 3100 m (Colleoni *et al.* 2013) and may explain the downregulation of these two complexes in the muscle of climbers returning from the summit of Everest (Levett *et al.* 2012) (Fig. 4).

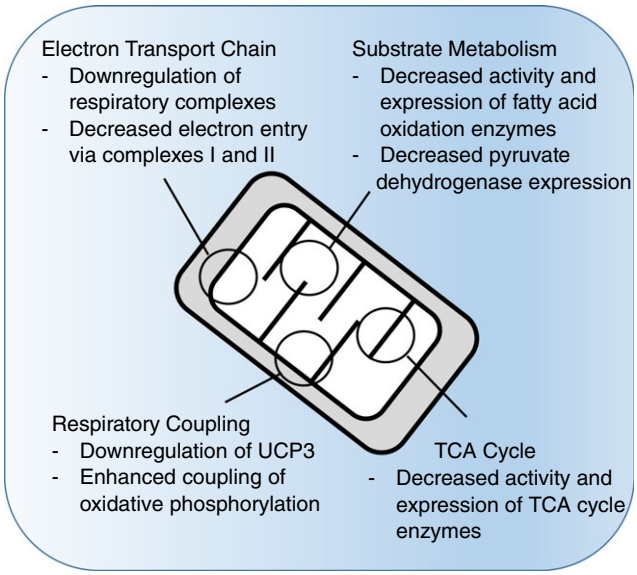

Figure 4. Summary of intramitochondrial changes that occur during acclimatisation to extreme high altitude
Studies at extreme high altitude have shown changes in enzyme activities and protein levels that suggest downregulation of electron transport chain (ETC) complexes, tricarboxylic acid (TCA) cycle enzymes and substrate oxidation enzymes, alongside decreased levels of uncoupling protein 3 (UCP3). High resolution respirometry of human muscle fibres at more moderate high altitude has shown that sustained hypoxia is associated with decreased electron entry into the ETC and improved mitochondrial efficiency.

A further possible adaptive response might be one of improved mitochondrial O_2 efficiency, resulting from a substrate switch away from fatty acid oxidation towards more O_2-efficient glucose oxidation, and a possible improvement in mitochondrial coupling. The capacity for fatty acid oxidation tends to fall at high altitude (Horscroft & Murray, 2014), with decreased activity of 3-hydroxyacyl-CoA dehydrogenase (HOAD) (Levett *et al.* 2012) and protein levels of several other β-oxidation enzymes (Levett *et al.* 2015) reported in climbers following ascent of Everest (Fig. 4). HOAD activities were also lower in Tibetans compared with lowland Nepalese (Kayser *et al.* 1996). Several of these enzymes are transcriptional targets for a fatty acid-activated transcription factor, peroxisome proliferator-activated receptor α (PPARα), which, at least in some tissues, is downregulated in hypoxia in an HIF-dependent manner (Narravula & Colgan, 2001). Notably, however, protein levels of pyruvate dehydrogenase were also lower in these subjects following ascent of Everest, perhaps arguing against a switch towards glucose oxidation and instead supporting a possible increased role for glycolytic ATP production and lactate production instead, bypassing the mitochondria.

With the downregulation of oxidative enzymes and loss of mitochondrial density, it is conceivable that anaerobic glycolysis might make a greater contribution to muscle ATP demands at extreme altitude, particularly during exercise. The genes for many glycolytic enzymes contain hypoxia-response elements in their promoter regions (Semenza *et al.* 1994), and in hypoxic cells, the inhibition of pyruvate dehydrogenase (Kim *et al.* 2006) supports a Pasteur effect, in which glycolytically derived lactate is expelled from the cell. In humans, however, the evidence to support increased glycolysis at altitude is limited (Horscroft & Murray, 2014). Indeed, in muscle biopsies from humans returning from extreme altitude, the levels of several glycolytic enzymes were decreased (Levett *et al.* 2015) as was hexokinase activity (Levett *et al.* 2012). These observations might reflect the so-called 'lactate paradox'. In this phenomenon, acute exposure to high altitude is accompanied by greater blood lactate levels ($[La_b]$) at a given submaximal workload than at sea level, although following acclimatisation over a period of weeks, the same exercise challenge results in a lower $[La_b]$, more comparable with that at sea level (West, 1986). Thus, acclimatisation may decrease the initial dependence on glycolysis to meet cellular ATP demand, perhaps through multiple adjustments that optimise O_2 delivery and utilisation or through better coupling of pyruvate production and oxidative phosphorylation. Some studies, however, have suggested that the 'lactate paradox' is a more transient feature of acclimatisation and not applicable to those spending longer durations at extreme altitude (Lundby *et al.* 2000; van Hall *et al.* 2001, 2009).

The notion of improved respiratory coupling, resulting in enhanced O_2 efficiency at extreme altitude, is also appealing, although mitochondrial uncoupling is likely to afford some antioxidant protection and may thus be beneficial. Levels of uncoupling protein 3 (UCP3), another PPARα target, decreased in the muscle of climbers following an ascent of Everest (Levett *et al.* 2012), and whilst the possible effects of UCP3 on mitochondrial efficiency do remain controversial, it is notable that in high-resolution respirometry studies LEAK state respiration (a functional measure of uncoupling) decreased in subjects after a month at 3454 m (Jacobs *et al.* 2012) (Fig. 4). UCP3 levels correlated negatively with some measures of cycling efficiency in athletes at sea level (Mogensen *et al.* 2006), but the question of whether improved mitochondrial coupling at altitude translates into enhanced exercise efficiency remains controversial, with some studies showing improvements (Gore *et al.* 2007; Latshang *et al.* 2013) and others showing no change in economy (Lundby *et al.* 2007). The regulation of mitochondrial efficiency, and indeed exercise efficiency, may occur independently of changes in gene transcription but with improvements in phosphorylation efficiency measured in rat liver mitochondria (Gnaiger *et al.* 2000) and human muscle mitochondria (Schiffer *et al.* 2014) when respiration was measured under acute hypoxic conditions. In contrast to these mitochondrial changes, however, in the same study of human subjects gross efficiency actually decreased with exercise in acute hypoxia (Schiffer *et al.* 2014), perhaps suggesting an influence from factors other than mitochondrial efficiency, such as different muscle fibre recruitment or the additional energetic cost of ventilation under hypoxic conditions.

Energetic and functional consequences of mitochondrial remodelling at extreme high altitude

The energetic and functional consequences of altitude-induced mitochondrial remodelling are difficult to establish firmly. Using ^{31}P-MRS, we found altered skeletal muscle energetics in climbers within 48 h of returning to sea level from altitudes >7950 m, following a total of 10–17 days of descent. Changes included an increase in the free energy available from ATP hydrolysis, and lower resting ADP concentrations (Edwards *et al.* 2010). There was no change in resting PCr levels, nor PCr recovery half-time (taken to represent mitochondrial function) following a plantar flexion exercise, but this re-synthesis of PCr appeared to be more tightly regulated, with no overshoot in PCr production following exercise (Edwards *et al.* 2010). Overall, however, it is remarkable how well preserved energy metabolism was in these subjects throughout an exercise challenge, in the face of significant skeletal muscle atrophy (Edwards *et al.* 2010), loss of mitochondrial density (Levett *et al.* 2012) and downregulation of creatine kinase (Levett *et al.* 2015). Whilst enhanced glycolytic ATP production may contribute, muscle pH following exercise was no lower following altitude exposure (Edwards *et al.* 2010). Our findings may instead indicate that the loss of mitochondrial density in these subjects was within the excess capacity known to exist in human muscle at sea level (Boushel *et al.* 2011). Interestingly, many of the changes seen following exposure to extreme altitude were recapitulated in subjects ascending no higher than 5300 m and spending less than 3 weeks at altitude (Edwards *et al.* 2010), an ascent profile that one would not expect to have resulted in a loss of mitochondria. Such maintenance of energetic function would support a notion of acclimatisation, rather than detrimental deterioration.

In parallel experiments on some of the same climbers returning from the summit of Everest, a loss of cardiac energetic reserve (PCr/ATP) was also seen, in association with impaired left ventricular filling during diastole (Holloway *et al.* 2011). Whilst it would be tempting to view this as deterioration, a similar loss of PCr/ATP has also been seen in trekkers returning from a relatively short sojourn to 5300 m (Holloway *et al.* 2011, 2014). Meanwhile, the Sherpa heart was found to have a lower PCr/ATP than that of lowlanders, perhaps indicating that this lower energetic reserve results from a beneficial adaptation (Hochachka *et al.* 1996). For obvious practical reasons, it has not been possible to establish whether a loss of cardiac mitochondrial density or qualitative changes in cardiac mitochondrial function occur in humans at extreme altitude, but studies in rats have shown that 14 days of hypoxic exposure (at 11 or 13% O_2) decreases cardiac ATP levels, fatty acid oxidation capacity and ETC complex I activity, with no loss in cardiac mitochondrial content over this time (Heather *et al.* 2012; Ashmore *et al.* 2014).

Conclusions

Prolonged exposure to extreme high altitude results in a loss of mitochondrial volume density in skeletal muscle, with a modulation in function of the remaining mitochondria characterised by a loss of oxidative capacity, particularly for fatty acid substrates, and a possible improvement in biochemical coupling at the inner mitochondrial membrane. Such qualitative changes in mitochondrial function are also seen at more moderate high altitudes, in the absence of changes in mitochondrial volume density. It is not clear whether such changes should be considered a feature of acclimatisation or deterioration, and indeed both aspects may be present, but the changes do appear to be regulated and similarities between the metabolic phenotype of the acclimatised lowlander and

adapted Tibetan highlander (e.g. decreased mitochondrial density particularly from subsarcolemmal mitochondria, decreased fatty acid oxidation) add support to this notion. Whilst impaired cardiac energetics have been reported in climbers returning from extreme high altitude, it is notable that skeletal muscle energetics are largely preserved during an exercise challenge, despite significant muscle atrophy, further supporting the concept of mitochondrial remodelling as a core feature of acclimatisation to extreme high altitude.

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Additional information

Competing Interests

The authors declare that they have no competing interests.

Author Contributions

Both authors conceived and planned the review. J.A.H. carried out a review of the literature. A.J.M. wrote the manuscript and generated the figures. Both authors reviewed the manuscript and edited it for content.

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