Extreme Physiology & Medicine

Skeletal muscle energy metabolism in environmental hypoxia: climbing towards consensus

James A Horscroft[*] and Andrew J Murray

Abstract

Skeletal muscle undergoes metabolic remodelling in response to environmental hypoxia, yet aspects of this process remain controversial. Broadly, environmental hypoxia has been suggested to induce: (i) a loss of mitochondrial density; (ii) a substrate switch away from fatty acids and towards other substrates such as glucose, amino acids and ketone bodies; and (iii) a shift from aerobic to anaerobic metabolism. There remains a lack of a consensus in these areas, most likely as a consequence of the variations in degree and duration of hypoxic exposure, as well as the broad range of experimental parameters used as markers of metabolic processes. To attempt to resolve some of the controversies, we performed a comprehensive review of the literature pertaining to hypoxia-induced changes in skeletal muscle energy metabolism. We found evidence that mass-specific mitochondrial function is decreased prior to mass-specific mitochondrial density, implicating intra-mitochondrial changes in the response to environmental hypoxia. This loss of oxidative capacity does not appear to be matched by a loss of glycolytic capacity, which on the whole is not altered by environmental hypoxia. Environmental hypoxia does however induce a selective attenuation of fatty acid oxidation, whilst glucose uptake is maintained or increased, perhaps to support glycolysis in the face of a downregulation of oxidative metabolism, optimising the pathways of ATP synthesis for the hypoxic environment.

Keywords: Hypoxia, High altitude, Skeletal muscle, Mitochondria, Metabolism

Review
Background

Skeletal muscle, like all oxidative tissues of the body, is critically dependent on a supply of oxygen to maintain energetic and redox homeostasis. ATP can be synthesised in the skeletal muscle in an oxygen-dependent manner in the mitochondria via oxidative phosphorylation, utilising substrates such as glycolytically derived pyruvate, fatty acids, amino acids and ketone bodies, but also in an oxygen-independent manner in the cytosol, via glycolysis with the resulting pyruvate converted to lactate (Figure 1). Under conditions of a plentiful oxygen supply, however, oxidative phosphorylation would normally meet the majority of the cell's ATP requirements [1], due to the greater range of substrates available and the much higher yield of ATP derived from glucose.

Environmental hypoxia, either in a hypobaric/normobaric hypoxia chamber or at high altitude, decreases the partial pressure of arterial oxygen ($Pa(O_2)$). In order to compensate for this, oxygen delivery is improved via changes in resting ventilation rate, circulating haemoglobin concentration and capillary density [3], whilst metabolic remodelling at the tissues might alter oxygen utilisation. Studies in cultured cells suggest that the transcription factor, hypoxia-inducible factor 1-alpha (HIF1α), is upregulated in hypoxia, increasing glycolysis [4] and thereby attenuating oxygen utilisation and ATP synthesis [5]. A loss of cellular mitochondrial content may be driven by the downregulation of mitochondrial biogenesis factors such as peroxisome proliferator-activated receptor γ co-activator 1 alpha or beta (PGC1α/β) in tandem with the upregulation of mitochondrial autophagy factors such as BCL2/adenovirus E1B 19 kDa interacting protein (BNIP3) [6]. Meanwhile, the upregulation of pyruvate dehydrogenase kinase (PDK) isoforms deactivates pyruvate dehydrogenase, which impairs pyruvate entry into the TCA cycle, resulting in a high rate of glycolysis

* Correspondence: jah212@cam.ac.uk
Department of Physiology, Development and Neuroscience, University of Cambridge, Downing Street, CB2 3EG, Cambridge, UK
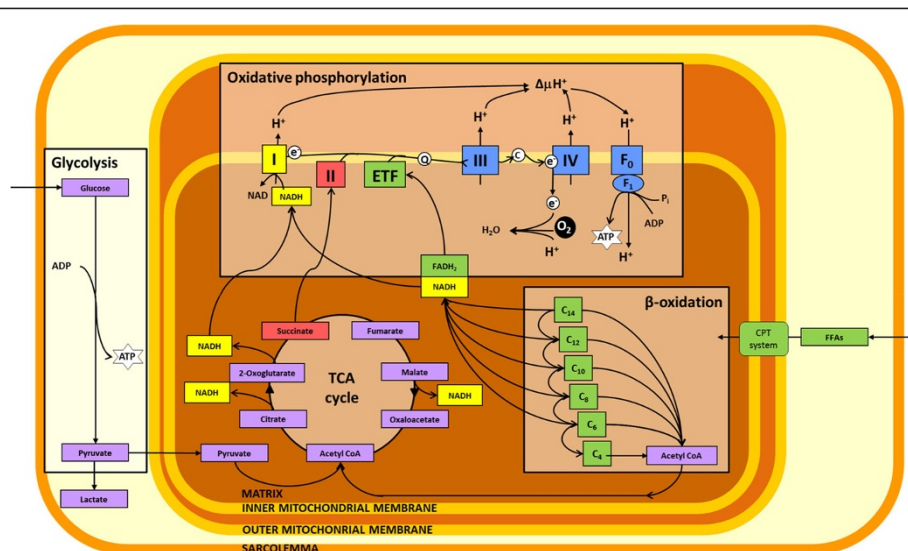
Figure 1 Energy metabolism in the skeletal muscle. Glycolysis represents an oxygen-independent source of ATP and pyruvate. Pyruvate is reduced in the cytosol to form lactate or oxidised in the mitochondrial matrix to form acetyl CoA, which feeds into the TCA cycle. β-oxidation of fatty acids and the TCA cycle produce reduced intermediates, NADH and FADH$_2$, which are oxidised by complexes of the electron transport chain. Electrons are transferred to the final oxygen acceptor, O$_2$, and the free energy from this process is used to pump H$^+$ ions into the intermembrane space. The resulting electrochemical gradient is the driving force for the oxidative phosphorylation of ADP. *ETF* electron-transferring flavoprotein, *I-IV* complexes of the electron transport chain, *F$_0$* and *F$_1$* subunits of the ATP synthase, *NADH* β-nicotinamide adenine dinucleotide reduced, *NAD* β-nicotinamide adenine dinucleotide, *C$_n$* acetyl CoA with carbon chain length *n*, *FFA* free fatty acids. Figure adapted from [2].

relative to oxidative phosphorylation, the Warburg effect [7,8]. Finally, the efficiency of mitochondrial electron transfer and thus oxygen utilisation is improved by a HIF1α-dependent switch in subunits at complex IV [9].

Despite this valuable mechanistic work in cell cultures, there remains a paucity of research into the effects of environmental hypoxia on energy metabolism in different mammalian tissues *in vivo*. The skeletal muscle is an interesting model tissue, as it has a relatively high capacity for respiration, with metabolic rates altered acutely by exertion and numerous metabolic features (for example, mitochondrial density and/or substrate preference) altered chronically by, e.g. training [10], diet [10] and environmental factors [11]. In humans, the muscle is easily accessible for biopsy, even under field conditions.

The aim of this review was to collate evidence pertaining to the remodelling of metabolic processes in mammalian skeletal muscle *in vivo* in response to environmental hypoxia, accounting for variations in degree and duration of hypoxic exposure.

Methods
Search strategy
A search protocol was developed to identify relevant research articles with unbiased results. The search term '(altitude OR hypoxia) AND "skeletal muscle" AND (mitochondria OR glycolysis OR "fatty acid" OR "oxidative phosphorylation")' was entered into the database PubMed in June 2014, and the titles and abstracts of all results were assessed for relevance. The reference lists of review articles arising from this initial search were reviewed for research papers which did not appear in the original search, and any relevant articles were also included. Any publication date or animal model was accepted for inclusion, providing that a skeletal muscle was studied. Finally, any type (e.g. ascent to altitude, habitation of a hypoxic chamber, ischaemia and anaemia), intensity, duration and frequency of hypoxic exposure was considered acceptable for more thorough analysis.

Search results
The search returned 343 results in June 2014. A further 21 papers cited in reviews found by the initial search term were added due to relevance. Of these 364 papers, 251 were excluded as irrelevant and 113 reviewed in detail. An aim of this review was to investigate the consequences of variations in degree and duration of hypoxic exposure on mammalian muscle energy metabolism. Thus, from the articles identified as relevant, we selected those in which a mammal was exposed to continuous environmental hypoxia of greater than 1 day and aspects of skeletal muscle energy metabolism were assessed. Where possible, observations that may have been influenced by confounding factors were excluded. To this end, studies using genetically manipulated animal models, pre-acclimatised or evolutionarily adapted human cohorts, or confounding interventions such as exercise or pharmacological agents, were excluded. This left 33 articles, of which 14 used

human *m. vastus lateralis*, 6 used a mouse skeletal muscle and 13 used a rat skeletal muscle. A flowchart of the selection process is shown in Figure 2, and further details of the reasons for exclusion are given in Additional file 1: Table S1.

Data extraction

In the remaining 33 articles, we recorded all reported observations that could be used as a marker of one of four metabolic processes of interest (glycolysis, β-oxidation, TCA cycle and oxidative phosphorylation) plus mitochondrial density. Ketolysis, amino acid metabolism and high-energy phosphate transfer were excluded, as there were very few observations of biomarkers of these processes. Expression, levels or activity of appropriate enzymes; expression and levels of regulating transcription factors; and functional respirometry data were considered as markers (Table 1).

Data analysis

The degree and duration of hypoxic exposure was noted and has been described uniformly in this review. Degree is reported as an estimate of the minimum atmospheric partial pressure of oxygen $p(O_2)_{min}$ reached by every member of the cohort during each study. Duration is reported as the total time spent in an environment with a $p(O_2) <15.0$ kPa (equivalent to being >3,000 m above sea

Figure 2 Selection process for identifying relevant papers in the literature.

level). Where hypoxic degree was not reported in $p(O_2)$, conversions were made to estimate the $p(O_2)_{min}$ in the reported condition using the following formula, adapted from West 1996 [12] where h is the height above sea level in kilometres.

$$p(O_2)(kPa) = 0.02793\,e^{\left(6.63268\,-0.1112\,h\,-0.00149\,h^2\right)}$$

If appropriate, the results reported in each paper were sub-divided into those pertaining to different experimental "settings". We define a setting as a uniform hypoxic challenge (degree and duration), exerted on one particular species and muscle or muscle group within a single study.

For each setting, all biomarkers described in Table 1 were considered and are reported here. In addition, a single result for each of the four metabolic processes and mitochondrial density was inferred from each setting as follows: *increase* (where at least one biomarker of a process was significantly increased by hypoxia, and none decreased); *decrease* (where at least one biomarker of a process was significantly decreased by hypoxia, and none increased); *unchanged* (where at least one biomarker was measured and no biomarkers were significantly altered by hypoxia); and *unclear* (where at least one biomarker of a process was significantly increased and another significantly decreased). In the case of a conflict in results, however, where a direct measurement was taken (e.g. mitochondrial density by electron microscopy), this was given priority over an established indirect proxy (e.g. mitochondrial density by citrate synthase activity) [13], which in turn was given priority over expression, levels or activity of known regulators of that process (e.g. PGC1α). This occurred in one instance in the study by Chaillou *et al.* [14], where two established markers of mitochondrial density (citrate synthase activity and complex IV activity) decreased in a rat *plantaris* muscle, whilst one upstream regulator of mitochondrial biogenesis (PGC1α) increased. This setting was thus labelled as a *decrease*.

To untangle the effects of different degrees and durations of hypoxia, observations were sub-categorised by severity in terms of atmospheric partial pressure of O_2 ($p(O_2)$): high ($11.7 < p(O_2) \leq 15.0$ kPa, ca. 3,000–5,000 m above sea level), very high ($10.0 < p(O_2) \leq 11.7$ kPa, ca. 5,000–6,250 m above sea level) or extreme ($p(O_2) \leq 10.0$ kPa, ca. 6,250+ m above sea level); and duration (t): short term ($0 < t \leq 14$ d in hypoxia), medium term ($14 < t \leq 42$ d) and long term ($t > 42$ d).

Results
Glycolysis

For biomarkers of glycolysis, 25 hypoxic settings were identified across 15 papers, the results of which are summarised in Table 2. The markers of glycolysis in human
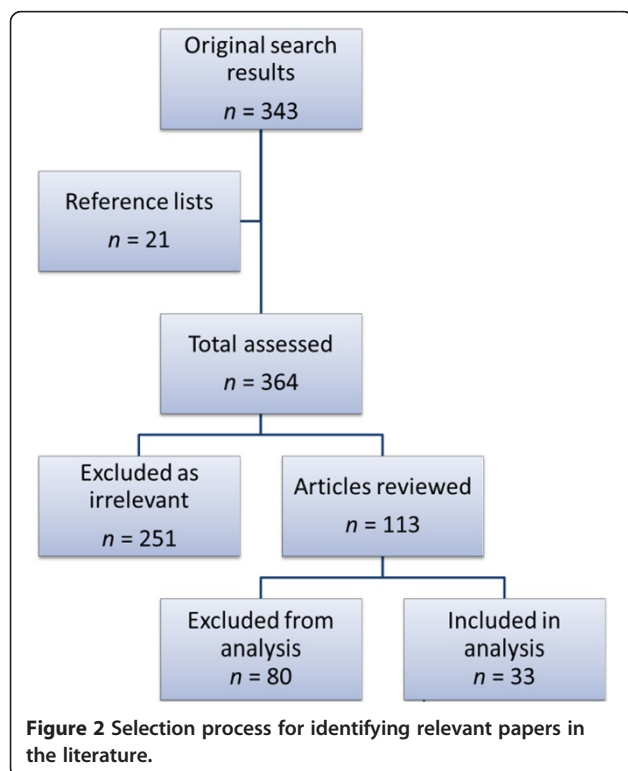
Table 1 Accepted biomarkers for glycolysis, β-oxidation, TCA cycle function, oxidative phosphorylation and mitochondrial density

Aspect of metabolism	Biomarkers			
	Expression, levels or activity of enzymes/transporters	Expression, levels or activity of regulators	Rate measurements	Other validated markers [13]
Glycolysis				
	Monocarboxylate transporters (MCT)			
	Hexokinase (HK)			
	Phosphoglucose isomerase (PGI)			
	Phosphofructokinase (PFK)			
	Aldolase (ALD)			
	Triose phosphate isomerase (TPI)			
	Glyceraldehyde 3-phosphate dehydrogenase (G3PDH)			
	Phosphoglycerate kinase (PGK)			
	Phosphoglycerate mutase (PGM)			
	Enolase (ENO)			
	Pyruvate kinase (PyK)			
	Lactate dehydrogenase (LDH)			
			Glucose oxidation	
β-oxidation				
	Carnitine acylcarnitine translocase (CACT)			
	Carnitine palmitoyl transferases (CPT)			
	Acyl CoA dehydrogenases			
	Enoyl CoA hydratase (ECAH)			
	Enoyl CoA isomerase (ECAI)			
	L-3-hydroxyacyl CoA dehydrogenase (HOAD)			
	Thiolase (THI)			
		PPARα		
			Uptake/utilisation of fatty acids	
			Oxidative phosphorylation with fatty acid substrates	
TCA cycle				
	Pyruvate dehydrogenase			
	Citrate synthase			
	Aconitase			
	Isocitrate dehydrogenase			
	α-ketoglutarate dehydrogenase			
	Succinyl CoA synthetase			
	Succinate dehydrogenase			
	Fumarase			
	Malate dehydrogenase			
Oxidative phosphorylation				
	Complex I			
	Complex II			
	Complex III			
	Complex IV			

Table 1 Accepted biomarkers for glycolysis, β-oxidation, TCA cycle function, oxidative phosphorylation and mitochondrial density (Continued)

	Complex V	
	Electron transferring flavoprotein (ETF)	
		Oxidative phosphorylation
Mitochondrial density (mitochondrial density measurements by electron microscopy)		
	Bax	
	Bcl-2*	
	BNIP3*	
	PGC-1α	
		Citrate synthase activity
		Complex IV activity

*biomarkers used as negative indicators of the process.

m. vastus lateralis decreased in four settings [15-18], increased in two [19,20], remained unchanged in five [18,20-22] and were unclear in one [15]. Similar patterns were found in rodents [23-28] and appeared to be unrelated to the degree of hypoxic exposure. The effect of hypoxia on individual glycolytic enzymes does not reveal a striking pattern, with most unchanged, significantly increased or significantly decreased in one of the studies.

β-oxidation

For biomarkers of β-oxidation, 22 hypoxic settings were identified across 15 papers, the results of which are summarised in Table 3. There was a tendency towards a decrease in β-oxidation following a hypoxic stimulus, with a decrease in at least one biomarker reported in 8/22 settings [16,18,23,28,30-32] and none showing an increase. A commonly used marker of β-oxidation was the activity of 3-hydroxyacyl-CoA dehydrogenase (HOAD). HOAD activity was unchanged in five settings [15,17,18,33] and decreased in one setting [18] in humans, with a similar ratio of results in rodents [23,24,28,31,32,34]. Assessment of levels and/or activity of proteins associated with mitochondrial fatty acid import, e.g. carnitine-acylcarnitine translocase (CACT) [16] and carnitine pamitoyltransferase 1 (CPT1) [32] suggested that these are decreased by sustained hypoxia, an effect possibly mediated through the HIF-PPARα signalling axis, as levels of peroxisome proliferator-activated receptor alpha (PPARα) were lowered by environmental hypoxia in mice [31]. Acyl-carnitine-supported respirometry rates were lower following hypoxic exposure, when malate plus palmitoyl carnitine [31,32], but not octanoyl carnitine [35,36], were used as substrates.

TCA cycle

For biomarkers of TCA cycle function, 29 hypoxic settings were identified across 20 papers, the results of which are summarised in Table 4. A decrease in biomarkers of TCA cycle activity was measured in 3/10 settings in humans [16-18] and 8/19 settings in rodents [14,23,27,28,34,37,38], whilst none reported an increase in either group. Moreover, the loss of TCA cycle enzyme activity appears to be dependent on the degree of hypoxic exposure, with 1/14 (7%), 7/15 (47%) and 3/3 (100%) observations at high, very high and extreme degrees of hypoxia, respectively, showing such a loss. This appears to be unrelated to the particular enzyme assayed with activity of aconitase (1 decreased, 2 unchanged), citrate synthase (5 decreased, 13 unchanged), malate dehydrogenase (2 decreased, 4 unchanged) and succinate dehydrogenase (2 decreased, 3 unchanged) either falling or not changing following hypoxic exposure.

Oxidative phosphorylation

For biomarkers of oxidative phosphorylation, 19 hypoxic settings were identified across 14 papers, the results of which are summarised in Table 5. Markers of oxidative phosphorylation decreased in 3/4 human settings [16,18,36] and 8/15 rodent settings [14,25,27,29,38,41], with an increase in 1 of the 15 rodent settings [42]. Complexes I [18,27], III [16], IV [18], V [16,18,27] and the electron-transferring flavoprotein [16] were each shown to be diminished after exposure in various studies. Respirometry performed at high altitude revealed a decrease in oxidative capacity in the presence of both complexes I and II substrates [36].

Mitochondrial density

For biomarkers of mitochondrial density, 34 hypoxic settings were identified across 23 papers, the results of

Table 2 The effects of environmental hypoxia on biomarkers of glycolysis in skeletal muscle

First author	Year	Organism	Muscle model	Hypoxia model	$p(O_2)_{min}$ (kPa)	Duration (d)	Marker	Change
Green [15]	1992	Human	vl	4,300 m	12.8	1	Phosphofructokinase activity	↓
							Hexokinase activity	=
Roberts [20]	1996	Human	vl	4,300 m	12.8	1	Glucose oxidation	=
Pastoris [29]	1995	Rat	gnm	10% O_2	10.1	3	Hexokinase activity	=
							Phosphofructokinase activity	↑
							Lactate dehydrogenase activity	=
							Pyruvate kinase activity	↓
Pastoris [29]	1995	Rat	sol	10% O_2	10.1	3	Hexokinase activity	=
							Phosphofructokinase activity	↓
							Lactate dehydrogenase activity	=
							Pyruvate kinase activity	=
Dutta [28]	2009	Rat	mix	349 mmHg	10.3	7	Lactate dehydrogenase activity	↑
Vigano [16]	2008	Human	vl	4,559 m	12.4	8	Enolase levels	↓
van Hall [21]	2009	Human	vl	4,100 m	13.1	14	Lactate dehydrogenase activity	=
De Palma [27]	2007	Rat	gnm	10% O_2	10.1	14	β-enolase levels	↓
							Phosphoglyercomutase 2 levels	↓
							Pyruvate kinase levels	↑
							Triose phosphate isomerase levels	↓
Young [22]	1984	Human	vl	4,300 m	12.8	18	Hexokinase activity	=
							Lactate dehydrogenase activity	=
Levett [18]	2012	Human	vl	5,300 m	11.3	19	Hexokinase activity	=
Roberts [30]	1996	Human	vl	4,300 m	12.8	21	Glucose oxidation	↑
Green [15]	1992	Human	vl	4,300 m	12.8	21	Phosphofructokinase activity	↓
							Hexokinase activity	↑
Daneshrad [24]	2000	Rat	sol	10% O_2	10.1	21	Hexokinase activity	↑
							Lactate dehydrogenase activity	=
							Phosphofructokinase activity	=
							Pyruvate kinase levels	=
Green [19]	2000	Human	vl	6,194 m	10.1	21	Lactate dehydrogenase activity	↑
Green [17]	1989	Human	vl	8,848 m	7.1	40	Hexokinase activity	↓
							α-GPDH activity	=
							Lactate dehydrogenase activity	=
							Phosphofructokinase activity	=
							Pyruvate kinase levels	=
van Hall [21]	2009	Human	vl	4,100 m	13.1	56	Lactate dehydrogenase activity	=
McClelland [25]	2002	Rat	sol	4,300 m	12.8	56	Lactate dehydrogenase levels	=
							Monocarboxylate transporter 1 levels	=
							Monocarboxylate transporter 4 levels	↓
McClelland [25]	2002	Rat	pla	4,300 m	12.8	56	Lactate dehydrogenase levels	=
							Monocarboxylate transporter 1 levels	↓
							Monocarboxylate transporter 4 levels	↓
McClelland [25]	2002	Rat	gnm	4,300 m	12.8	56	Lactate dehydrogenase levels	=
							Monocarboxylate transporter 1 levels	=
							Monocarboxylate transporter 4 levels	=

Table 2 The effects of environmental hypoxia on biomarkers of glycolysis in skeletal muscle (Continued)

Abdelmalki [23]	1996	Rat	sol	13% O_2	13.1	64	Lactate dehydrogenase activity	=
							Phosphofructokinase activity	↑
							Hexokinase activity	=
Abdelmalki [23]	1996	Rat	pla	13% O_2	13.1	64	Lactate dehydrogenase activity	=
							Phosphofructokinase activity	=
							Hexokinase activity	=
Abdelmalki [23]	1996	Rat	rq	13% O_2	13.1	64	Lactate dehydrogenase activity	=
							Phosphofructokinase activity	=
Abdelmalki [23]	1996	Rat	wq	13% O_2	13.1	64	Lactate dehydrogenase activity	=
							Phosphofructokinase activity	↑
Levett [18]	2012	Human	vl	8,848 m	7.1	66	Hexokinase activity	↓
Ou [26]	2004	Rat	edl	5,500 m	11.0	90	Lactate dehydrogenase activity	=

↑ Change in biomarker is indicative of an *increase* in β-oxidation in hypoxia.
= No change in biomarker in hypoxia.
↓ Change in biomarker is indicative of a *decrease* in β-oxidation in hypoxia.
Abbreviations: *edl* extensor digitorum longus, *mix* mixed skeletal, *pla plantaris*, *q quadriceps*, *rq* red *quadriceps*, sol *soleus*, vl *vastus lateralis*, wq white *quadriceps*.

which are summarised in Table 6. Considering only direct observations of mitochondrial density in human *m. vastus lateralis*, 19 d at 5.300 m [18] and 40 d progressive decompression to the equivalent of 8,000 m [44] proved insufficient to induce detectable changes, whilst 56 d at 5,000 m [45] and 66 d spend above 6,600 m [18] resulted in a decrease in mitochondrial density. Considering all biomarkers of mitochondrial density, 4/13 (31%) measures at high, 6/14 (43%) measures at very high and 4/7 (57%) measures in extreme hypoxia, resulted in a significant decrease in biomarkers compared with baseline.

Summary of results
The effect of each hypoxic setting on glycolysis, β-oxidation, TCA cycle, oxidative phosphorylation and mitochondrial density is represented graphically in Figure 3, for all organisms and in Figure 4 for human *m. vastus lateralis* only.

Discussion
In this review, we set out to understand the remodelling of metabolic processes in the mammalian skeletal muscle *in vivo* in response to environmental hypoxia, accounting for variations in degree and duration of hypoxic exposure. To do so, we reviewed the literature considering a broad range of biomarkers pertinent to mitochondrial energy metabolism and glycolysis and collated the results to gauge whether a consensus exists within the literature. Whilst both human and rodent studies were included, we initially considered all findings together for completion, followed by data from human *m. vastus lateralis* in isolation for clarity.

Environmental hypoxia induces a loss of mitochondrial density in human *m. vastus lateralis* after long-term [18,48] but not short-term [35] exposure. Although studies involving adapted populations were excluded from our analysis, it

is interesting to note that the skeletal muscle of highland Tibetans is less rich in mitochondria than that of lowlanders [49], as this supports the idea that this is an adaptive trait. Attenuation of oxidative processes, such as β-oxidation [16,18,20,23,28,31,32], the TCA cycle [14,16,17,23,27-29,34,38] and oxidative phosphorylation [14,16,18,25,27,29,36,38,41], also seems to be induced by environmental hypoxia. The effect of hypoxia on glycolytic capacity is less clear, with some studies showing increased [19,20] and others decreased [15-18] levels of biomarkers.

The hypoxia-induced downregulation of β-oxidation, TCA cycle function and oxidative phosphorylation may be secondary to a loss of mitochondrial density, as in short-term (≤14 d) hypoxic settings, all were diminished in at least some studies of human *m. vastus lateralis*, whilst mitochondrial density remained unchanged (Table 7). Some medium-term (≤42 d) and most long-term (>42 d) settings resulted in a significant loss of skeletal muscle mitochondrial density. This therefore suggests that hypoxia-induced remodelling of mitochondrial pathways precedes a loss of mitochondrial density. This notion receives support from Jacobs and colleagues, who measured a loss of oxidative capacity, which persisted when respiration was corrected to citrate synthase activity [36], an established marker of mitochondrial density in human muscle [13]. A possible mechanism underpinning this might be that the mismatch in oxygen supply and demand results in ROS production at complexes I and III. This ROS production within the mitochondrion may result in damage to intra-mitochondrial machinery and thus result in loss of function. Alternatively, ROS are known to stabilise HIF, which in the long term may induce changes in mitochondrial density (through BNIP3 and PGC1α) [6,48] and muscle mass, but may also remodel metabolic pathways in the short term. Indeed, complex I and aconitase,

Table 3 The effects of environmental hypoxia on biomarkers of β-oxidation in skeletal muscle

First author	Year	Organism	Muscle model	Hypoxia model	$p(O_2)_{min}$ (kPa)	Duration (d)	Marker	Change
Green [15]	1992	Human	vl	4,300 m	12.8	1	HOAD activity	=
Roberts [30]	1996	Human	vl	4,300 m	12.8	1	Fatty acid oxidation	=
Morash [31]	2013	Mouse	mix	13% O_2	13.1	1	PPARα levels	↓
							CPT-1 levels	↓
							CPT-1 activity	↓
							HOAD activity	=
							Palmitate oxidation	↓
							Palmitoyl carnitine OXPHOS	↓
Dutta [28]	2009	Rat	mix	349 mmHg	10.3	7	CPT-1 activity	=
							Fatty acid oxidation	↓
							HOAD activity	↓
Morash [31]	2013	Mouse	mix	13% O_2	13.1	7	PPARα levels	↓
							CPT-1 levels	↓
							CPT-1 activity	↓
							HOAD activity	=
							Palmitate oxidation	↓
							Palmitoyl carnitine OXPHOS	↓
Vigano [16]	2008	Human	vl	4,559 m	12.4	8	CACT levels	↓
							ECAH levels	↓
							ECAI levels	↓
Jacobs [35]	2013a	Human	vl	4,559 m	12.4	10	Octanoyl carnitine OXPHOS	=
Levett [18]	2012	Human	vl	5,300 m	11.3	19	HOAD activity	=
Daneshrad [24]	2000	Rat	sol	10% O_2	10.1	21	HOAD activity	=
Green [15]	1992	Human	vl	4,300 m	12.8	21	HOAD activity	=
Roberts [30]	1996	Human	vl	4,300 m	12.8	21	Fatty acid oxidation	↓
Takahashi [34]	1993	Rat	pla	10% O_2	10.1	28	HOAD activity	=
Takahashi [34]	1993	Rat	sol	10% O_2	10.1	28	HOAD activity	=
Jacobs [36]	2013b	Human	vl	3,454 m	14.2	28	Octanoyl carnitine OXPHOS	=
Galbes [32]	2008	Rat	q	4,000 m	13.3	35	CPT-1 activity	↓
							CPT-1 levels	↓
							HOAD activity	↓
							Palmitoyl carnitine OXPHOS	↓
Green [17]	1989	Human	vl	8,848 m	7.1	40	HOAD activity	=
Abdelmalki [23]	1996	Rat	sol	13% O_2	13.1	64	HOAD activity	=
Abdelmalki [23]	1996	Rat	pla	13% O_2	13.1	64	HOAD activity	↓
Abdelmalki [23]	1996	Rat	rq	13% O_2	13.1	64	HOAD activity	=
Abdelmalki [23]	1996	Rat	wq	13% O_2	13.1	64	HOAD activity	=
Levett [18]	2012	Human	vl	8,848 m	7.1	66	HOAD activity	↓
Mizuno [33]	2008	Human	vl	5,250 m	11.4	75	HOAD activity	=
Ou [26]	2004	Rat	edl	5,500 m	11.0	90	Palmitate uptake	↓
							Palmitate oxidation	↑

↑ Change in biomarker is indicative of an *increase* in glycolysis in hypoxia.
= No change in biomarker in hypoxia.
↓ Change in biomarker is indicative of a *decrease* in glycolysis in hypoxia.
Abbreviations: *edl* extensor digitorum longus, *mix* mixed skeletal, *pla* plantaris, *rq* red quadriceps, *sol* soleus, *vl* vastus lateralis, *wq* white quadriceps.

Table 4 The effects of environmental hypoxia on biomarkers of TCA cycle function in skeletal muscle

First author	Year	Organism	Muscle model	Hypoxia model	$p(O_2)_{min}$ (kPa)	Duration (d)	Marker	Change
Morash [31]	2013	Mouse	mix	13% O_2	13.1	1	Citrate synthase activity	=
							Aconitase activity	=
Green [15]	1992	Human	vl	4,300 m	12.8	1	Succinate dehydrogenase activity	=
Magalhaes [38]	2005	Mouse	mix	8,500 m	7.4	2	Aconitase activity	↓
Pastoris [29]	1995	Rat	gnm	5,860 m	10.1	3	Citrate synthase activity	↓
							Malate dehydrogenase activity	=
Pastoris [29]	1995	Rat	sol	5,860 m	10.1	3	Citrate synthase activity	=
							Malate dehydrogenase activity	=
Morash [31]	2013	Mouse	mix	13% O_2	13.1	7	Citrate synthase activity	=
							Aconitase activity	=
Dutta [28]	2009	Rat	mix	349 mmHg	10.3	7	Citrate synthase activity	↓
							Malate dehydrogenase activity	↓
							Succinate dehydrogenase activity	↓
Vigano [16]	2008	Human	vl	4,559 m	12.4	8	Aconitase levels	↓
							α-ketoglutarate dehydrogenase levels	↓
							Malate dehydrogenase levels	↓
Chaillou [14]	2013	Rat	pla	5,500 m	11.0	9	Citrate synthase activity	↓
De Palma [27]	2007	Rat	gnm	10% O_2	10.1	14	Aconitase levels	↓
							Malate dehydrogenase levels	↓
							Pyruvate dehydrogenase levels	↓
							Succinyl coenzyme A synthetase levels	↓
Young [22]	1984	Human	vl	4,300 m	12.8	18	Malate dehydrogenase activity	=
Levett [18]	2012	Human	vl	5,300 m	11.3	19	Citrate synthase levels	=
							Citrate synthase expression	=
Green [15]	1992	Human	vl	4,300 m	12.8	21	Succinate dehydrogenase activity	=
Green [19]	2000	Human	vl	6,194 m	10.1	21	Citrate synthase activity	=
Daneshrad [24]	2000	Rat	sol	10% O_2	10.1	21	Citrate synthase activity	=
Takahashi [34]	1993	Rat	pla	10% O_2	10.1	28	Malate dehydrogenase activity	↓
Takahashi [34]	1993	Rat	sol	10% O_2	10.1	28	Malate dehydrogenase activity	=
Beaudry [39]	2010	Mouse	gnm	480 mmHg	13.4	28	Citrate synthase activity	=
Wuest [40]	2009	Rat	pla	410 mmHg	11.5	28	Succinate dehydrogenase activity	=
Jacobs [36]	2013b	Human	vl	3,454 m	14.2	28	Citrate synthase activity	=
Galbes [32]	2008	Rat	q	4,000 m	13.3	35	Citrate synthase activity	=
Green [17]	1989	Human	vl	8,848 m	7.1	40	Citrate synthase activity	↓
							Succinate dehydrogenase activity	↓
Chaillou [14]	2013	Rat	pla	5,500 m	11.0	63	Citrate synthase activity	↓
Abdelmalki [23]	1996	Rat	sol	13% O_2	13.1	64	Citrate synthase activity	=
Abdelmalki [23]	1996	Rat	pla	13% O_2	13.1	64	Citrate synthase activity	↓
Abdelmalki [23]	1996	Rat	rq	13% O_2	13.1	64	Citrate synthase activity	=
Abdelmalki [23]	1996	Rat	wq	13% O_2	13.1	64	Citrate synthase activity	=
Levett [18]	2012	Human	vl	8,848 m	7.1	66	Citrate synthase levels	↓
Mizuno [33]	2008	Human	vl	5,250 m	11.4	75	Citrate synthase activity	=

↑ Change in biomarker is indicative of an *increase* in TCA cycle function in hypoxia.
= No change in biomarker in hypoxia.
↓ Change in biomarker is indicative of a *decrease* in TCA cycle function in hypoxia.
Abbreviations: edl extensor digitorum longus, gnm gastrocnemius, mix mixed skeletal, pla plantaris, q quadriceps, rq red quadriceps, sol soleus, vl vastus lateralis, wq white quadriceps.

Table 5 The effects of environmental hypoxia on biomarkers of oxidative phosphorylation in skeletal muscle

First author	Year	Organism	Muscle model	Hypoxia model	$p(O_2)_{min}$ (kPa)	Duration (d)	Marker	Change
Morash [31]	2013	Mouse	mix	13% O_2	13.1	1	Complex I OXPHOS	=
							Complex II OXPHOS	=
							Complex IV OXPHOS	=
Magalhaes [38]	2005	Mouse	mix	8,500 m	7.4	2	Complex II OXPHOS	↓
Pastoris [29]	1995	Rat	sol	5,860 m	10.1	3	Complex III activity	=
							Complex IV activity	=
Pastoris [29]	1995	Rat	gnm	5,860 m	10.1	3	Complex III activity	=
							Complex IV activity	↓
Morash [31]	2013	Mouse	mix	13% O_2	13.1	7	Complex I OXPHOS	=
							Complex II OXPHOS	=
							Complex IV OXPHOS	=
Vigano [16]	2008	Human	vl	4,559 m	12.4	8	Complex III levels	↓
							Complex V levels	↓
							ETF levels	↓
Chaillou [14]	2013	Rat	pla	5,500 m	11.0	9	Complex IV activity	↓
Jacobs	2013a	Human	vl		12.4	10	Complex I OXPHOS	=
							Complex II OXPHOS	=
							Complex I+II OXPHOS	=
De Palma [27]	2007	Rat	gnm	10% O_2	10.1	14	Complex V levels	↓
Daneshrad [42]	2001	Rat	sol	10% O_2	10.1	21	OXPHOS	↑
Beaudry [39]	2010	Mouse	gnm	480 mmHg	13.4	28	Complex IV activity	=
Gamboa [41]	2010	Mouse	gnm	10% O_2	10.1	28	Complex II levels	↓
							Complex IV levels	↓
							Complex V levels	↓
Gamboa [43]	2012	Mouse	mix	10% O_2	10.1	28	Complex IV levels	↓
							Complex V activity	↑
							Complex I OXPHOS	↓
Jacobs [36]	2013b	Human	vl	3,454 m	14.2	28	Complex I OXPHOS	↓
							Complex II OXPHOS	↓
							Complex I+II OXPHOS	↓
							Complex IV activity	=
McClelland [25]	2002	Rat	sol	4,300 m	12.8	56	Complex IV activity	↓
McClelland [25]	2002	Rat	pla	4,300 m	12.8	56	Complex IV activity	↓
McClelland [25]	2002	Rat	gnm	4,300 m	12.8	56	Complex IV activity	=
Chaillou [14]	2013	Rat	pla	5,500 m	11.0	63	Complex IV activity	↓
Levett [18]	2012	Human	vl	8,848 m	7.1	66	Complex I expression	=
							Complex I levels	↓
							Complex II levels	=
							Complex III levels	=
							Complex IV expression	=
							Complex IV levels	↓
							Complex V expression	=
							Complex V levels	↓

↑ Change in biomarker is indicative of an *increase* in oxidative phosphorylation in hypoxia.
= No change in biomarker in hypoxia.
↓ Change in biomarker is indicative of a *decrease* in oxidative phosphorylation in hypoxia.
Abbreviations: gnm gastrocnemius, mix mixed skeletal, pla plantaris, sol soleus, vl vastus lateralis.

Table 6 The effects of environmental hypoxia on biomarkers of mitochondrial density in skeletal muscle

First author	Year	Organism	Muscle model	Hypoxia model	$p(O_2)_{min}$ (kPa)	Duration (d)	Marker	Change
Morash [31]	2013	Mouse	mix	13% O_2	13.1	1	Citrate synthase activity	=
							Complex IV OXPHOS	=
Magalhaes [38]	2005	Mouse	mix	8,500 m	7.4	2	Complex II OXPHOS	↓
Magalhaes [46]	2007	Mouse	mix	8,500 m	7.4	2	Bax expression	↑
							Bcl-2 expression	↓
Pastoris [29]	1995	Rat	sol	5,860 m	10.1	3	Complex IV activity	=
							Citrate synthase activity	=
Pastoris [29]	1995	Rat	gnm	5,860 m	10.1	3	Complex IV activity	↓
							Citrate synthase activity	↓
Chaillou [14]	2013	Rat	pla	5,500 m	11.0	3	BNIP3 expression	=
							PGC-1α expression	=
Morash [31]	2013	Mouse	mix	13% O_2	13.1	7	Citrate synthase activity	=
							Complex IV OXPHOS	=
Dutta [28]	2009	Rat	mix	349 mmHg	10.3	7	Citrate synthase activity	↓
Chaillou [14]	2013	Rat	pla	5,500 m	11.0	9	Complex IV activity	↓
							Citrate synthase activity	↓
							PGC-1α expression	↑
							BNIP3 expression	=
Jacobs [35]	2013a	Human	vl	4,559 m	12.4	10	Complex I OXPHOS capacity	=
							Complex II OXPHOS capacity	=
							Complex I+II OXPHOS capacity	=
Levett [18]	2012	Human	vl	5,300 m	11.3	19	**Mitochondrial density**	=
							PGC-1α levels	=
Green [19]	2000	Human	vl	6,194 m	10.1	21	Citrate synthase activity	=
Daneshrad [24]	2000	Rat	sol	10% O_2	10.1	21	Citrate synthase activity	=
Daneshrad [42]	2001	Rat	sol	10% O_2	10.1	21	OXPHOS	↑
Beaudry [39]	2010	Mouse	gnm	480 mmHg	13.4	28	Complex IV activity	=
							Citrate synthase activity	=
Gamboa [41]	2010	Mouse	gnm	10% O_2	10.1	28	**Mitochondrial density**	=
							BNIP3 expression	=
							Complex IV levels	↓
							PGC-1α levels	=
Gamboa [43]	2012	Mouse	mix	10% O_2	10.1	28	Complex I OXPHOS	↓
Jacobs [36]	2013b	Human	vl	3,454 m	14.2	28	Complex I OXPHOS	↓
							Complex II OXPHOS	↓
							Complex I+II OXPHOS	↓
							Complex IV activity	=
							Citrate synthase activity	=
Galbes [32]	2008	Rat	q	4,000 m	13.3	35	Citrate synthase activity	=
Green [17]	1989	Human	vl	8,848 m	7.1	40	Citrate synthase activity	↓
MacDougall [44]	1991	Human	vl	8,848 m	7.1	40	**Mitochondrial density**	=
van Ekeren [47]	1992	Rat	edl	8% O_2	8.1	45	**Mitochondrial density**	↑
van Ekeren [47]	1992	Rat	sol	8% O_2	8.1	45	**Mitochondrial density**	↓
Hoppeler [45]	1990	Human	vl	5,000 m	11.7	56	**Mitochondrial density**	↓

Table 6 The effects of environmental hypoxia on biomarkers of mitochondrial density in skeletal muscle *(Continued)*

McClelland [25]	2002	Rat	sol	4,300 m	12.8	56	Complex IV activity	↓
McClelland [25]	2002	Rat	gnm	4,300 m	12.8	56	Complex IV activity	=
McClelland [25]	2002	Rat	pla	4,300 m	12.8	56	Complex IV activity	↓
Chaillou [14]	2013	Rat	pla	5,500 m	11.0	63	Complex IV activity	↓
							Citrate synthase activity	↓
Abdelmalki [23]	1996	Rat	sol	13% O$_2$	13.1	64	Citrate synthase activity	=
Abdelmalki [23]	1996	Rat	pla	13% O$_2$	13.1	64	Citrate synthase activity	↓
Abdelmalki [23]	1996	Rat	rq	13% O$_2$	13.1	64	Citrate synthase activity	=
Abdelmalki [23]	1996	Rat	wq	13% O$_2$	13.1	64	Citrate synthase activity	=
Levett [18]	2012	Human	vl	8,848 m	7.1	66	**Mitochondrial density**	↓
							PGC-1α levels	↓
							PGC-1α expression	=
Mizuno [33]	2008	Human	vl	5,250 m	11.4	75	Citrate synthase activity	=

↑ Change in biomarker is indicative of an *increase* in mitochondrial density in hypoxia.
= No change in biomarker in hypoxia.
↓ Change in biomarker is indicative of a *decrease* in mitochondrial density in hypoxia.
Abbreviations: *gnm* gastrocnemius, *mix* mixed skeletal, *pla* plantaris, *q* quadriceps, *rq* red quadriceps, *sol* soleus, *vl* vastus lateralis, *wq* white quadriceps.

an enzyme of the TCA cycle, are known to be particularly susceptible to HIF-mediated loss of function via miR-210 upregulation [50,51].

It has been hypothesised that environmental hypoxia could alter the balance of substrate utilisation, with an enhanced use of carbohydrates and a correspondingly diminished use fatty acids [11]. Indeed in the hypoxic rat heart, a downregulation of fatty acid oxidation has been reported [52,53]. Such a substrate switch would be expected to be beneficial, as the oxidation of fatty acids requires more O$_2$ per ATP synthesised than the complete oxidation of carbohydrates [54]; thus, an increased reliance on carbohydrates may improve oxygen efficiency. If such a hypoxia-induced switch did occur, it might be expected that biomarkers for β-oxidation would be attenuated more frequently than biomarkers for oxidative phosphorylation. However, this does not appear to be the case, as 8/22 (36%) hypoxic settings induced a significant decrease in a biomarker of β-oxidation whilst 11/19 (58%) altered oxidative phosphorylation. Of those settings in which biomarkers of both β-oxidation and oxidative phosphorylation were measured, 1/4 showed a decrease in oxidative phosphorylation with no change in β-oxidation [36], 2/4 showed a decrease in both [16,18] and 1/4 reported no change in either [35]. Work from our laboratory in rat soleus found that oxygen consumption in the presence of an acyl-carnitine was lower following hypoxic exposure, whilst respiration when complexes I and II were activated directly was unaltered [31], which is indicative of a substrate switch. In humans, however, the opposite was found to be true, as acyl-carnitine-driven oxygen consumption was unchanged by hypoxia, whilst complex I + II-driven respiration was diminished [36]. Roberts *et al.*

showed that 21 d at 4,300 m increased glucose uptake [20] and decreased fatty acid oxidation [30] in human *m. vastus lateralis*. It is unclear, however, whether this increase in glucose uptake supported increased lactate production through lactate dehydrogenase (LDH) or pyruvate oxidation via pyruvate dehydrogenase (PDH) and the TCA cycle. Research into PDH activity following hypoxic exposure is limited, though LDH activity has been reported to rise following hypoxic exposure in humans [19] and rats [28]. A direct comparison of activities of LDH and PDH following hypoxia would be revealing.

Whilst oxidative processes are selectively downregulated in the skeletal muscle following exposure to environmental hypoxia, in contrast to studies in cultured cells, glycolytic markers appear to remain largely unchanged. It is noteworthy, however, that there has been a distinct lack of direct measurements of glycolytic flux *in vivo* or *ex vivo* following hypoxic exposure. These would be revealing, as glycolytic flux can increase in skeletal muscle by up to 1,000-fold upon the onset of high-intensity exercise [55]. Resting glycolytic flux is thus significantly below capacity, and as such measures of capacity, by protein expression or enzyme activity, would not accurately reflect flux *in vivo* at normal levels of exertion. Even so, our analysis of biomarkers of glycolytic capacity suggests that the relative contribution of glycolytic *versus* oxidative ATP production is increased by a hypoxic stimulus and this might be exaggerated upon exertion. An increased dependence on glycolysis would improve oxygen economy but would limit the scope for ATP production in the respiring muscle and result in inefficient use of fuel reserves. The 'lactate paradox' originally described by West [56] states that short-term environmental hypoxia does not alter concentrations
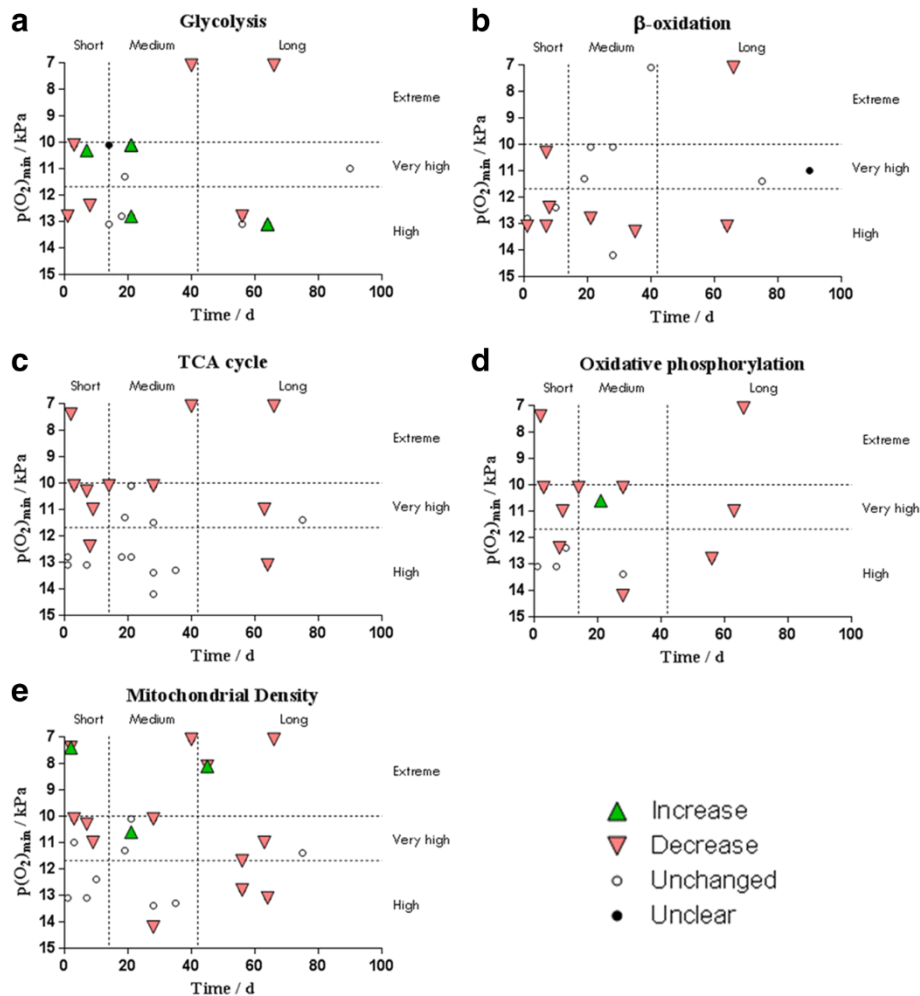
Figure 3 The effects of environmental hypoxia, in studies of rodent and human skeletal muscle, on (a) glycolysis, (b) β-oxidation, (c) TCA cycle, (d) oxidative phosphorylation and (e) mitochondrial density with varying duration and estimated environmental p(O₂) of the hypoxic setting. *Increase* indicates settings where at least one biomarker of the process was significantly increased by hypoxia and none decreased; *decrease* indicates settings where at least one biomarker of the process was significantly decreased by hypoxia and none increased; *unchanged* indicates settings where no biomarker was significantly altered by hypoxia; and *unclear* indicates settings where at least one biomarker was increased and another decreased by hypoxia.

of blood lactate ([La$_b$]) during any given submaximal exercise workload, yet work capacity decreases markedly in hypoxic environments; hence, [La$_b$] is lower at maximal workloads. The literature might support this assertion, as glycolytic flux is on the whole unaffected by hypoxic exposure. Today, the lactate paradox is more commonly defined as the phenomenon in which an acute sojourn at altitude induces an increase in blood-lactate accumulation during exercise in the short term, yet this decreases after chronic exposure [21,57,58]. However, whilst this may reflect some aspect of metabolic remodelling following hypoxic acclimation, current explanations for this phenomenon remain controversial and probably involve factors beyond the mere capacity for substrate utilisation [59,60].

The primary strength of our approach is that we provide a thorough and, as far as possible, objective analysis of the literature to date. By collating the available data from a range of animal models and different muscles, it is easy to identify clear, repeatable trends in the effects of environmental hypoxia on aspects of skeletal muscle energy metabolism. Moreover, the exclusion of datasets with confounding factors (e.g. explicit exercise training or pharmacological therapy) maximises the likelihood that these trends are a consequence of environmental hypoxia alone, with the caveat that a sojourn to altitude in itself inevitably introduces confounding variables other than hypoxia, e.g. cold, altered nutrition and possibly infection or gastrointestinal upset. Organising observations of biomarkers into hypoxic 'settings' allows for the fact that these
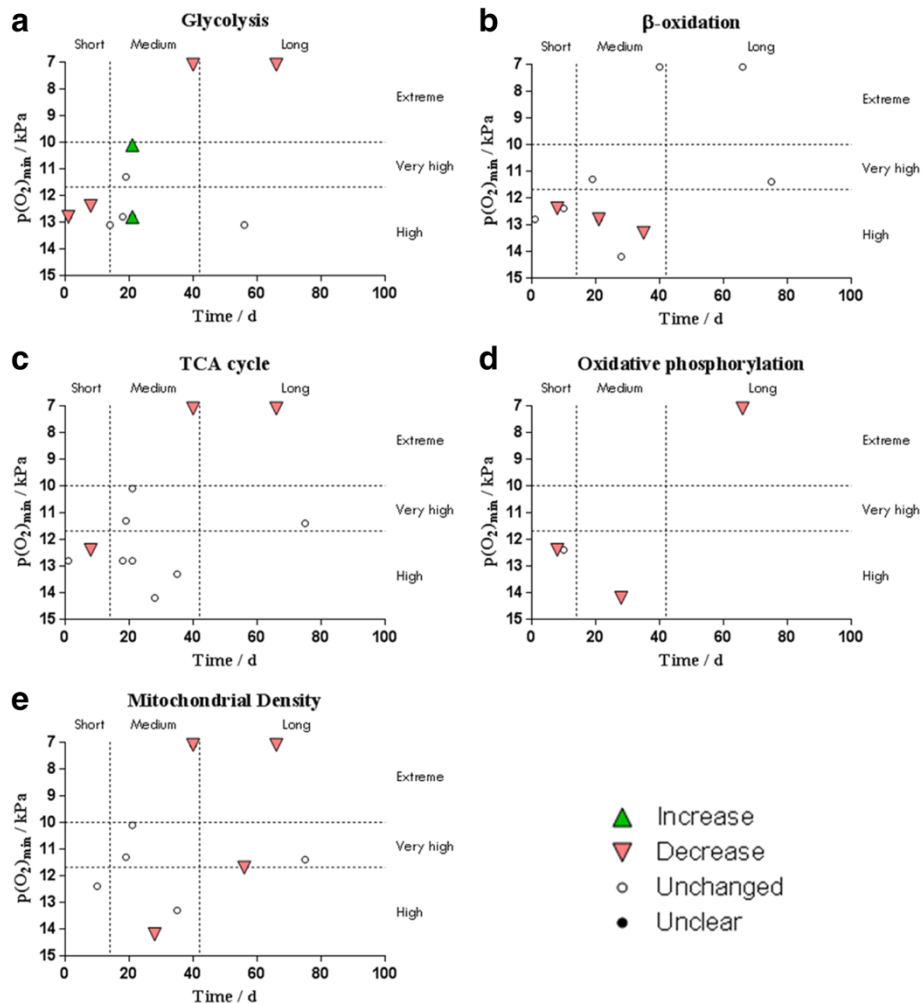
Figure 4 The effects of environmental hypoxia, in human *m. vastus lateralis* only, on (a) glycolysis, (b) β-oxidation, (c) TCA cycle, (d) oxidative phosphorylation and (e) mitochondrial density with varying duration and estimated environmental p(O$_2$) of the hypoxic setting. *Increase* indicates settings where at least one biomarker of the process was significantly increased by hypoxia and none decreased; *decrease* indicates settings where at least one biomarker of the process was significantly decreased by hypoxia and none increased; *unchanged* indicates settings where no biomarker was significantly altered by hypoxia; and *unclear* indicates settings where at least one biomarker was increased and another decreased by hypoxia.

observations are unlikely to be independent and sub-categorising these settings by duration and degree of hypoxic exposure and human *versus* rodent studies gives insight into the process of acclimation to hypoxic environments.

There are, however, a number of limitations to the methods used in this review. First, a wide range of animal and muscle models were accepted for analysis in this review, which, whilst a strength in itself, would have led to the inclusion of a number of different control groups across different studies, introducing baseline variation. Second, the time-dependence of rodent and human responses would likely be different, though we

Table 7 Time course of hypoxic response

Duration	Glycolysis			β-oxidation			TCA cycle function			Oxidative phosphorylation			Mitochondrial density		
	↑	=	↓	↑	=	↓	↑	=	↓	↑	=	↓	↑	=	↓
Short	0%	50%	50%	0%	75%	25%	0%	50%	50%	0%	50%	50%	0%	100%	0%
Medium	33%	50%	17%	0%	67%	33%	0%	86%	14%	0%	0%	100%	0%	60%	40%
Long	0%	50%	50%	0%	50%	50%	0%	50%	50%	0%	0%	100%	0%	33%	67%

The percentage of hypoxic settings in which biomarkers report a significant decrease (↓), a significant increase (↑) or unchanged/unclear results (=) in human *m. vastus lateralis*, following short- (0–14 d), medium- (15–42 d) or long- (43–90 d) term exposure to an environmental p(O$_2$) <15 kPa.

have considered data from human *m. vastus lateralis* separately where possible. Third, metabolic studies of muscles are beset by confounding factors relating to prior training status, species, fibre types and possibly even the specific skeletal muscle studied [61,62]. Fourth, whilst hypoxic settings taken from the same study are treated as independent in this review, the same equipment, experimenters and techniques were most likely used in each setting and thus a directional change in a biomarker might be more likely to be observed in two settings from the same paper than in two settings from different papers. Indeed, five rodent studies looked at different muscles presumably within the same animals in most cases, generating multiple settings (by our definition) which were clearly not independent. An alternative approach might have arbitrarily excluded one or more sets of data or attempted to combine findings or find consensus across different muscles; however, these approaches would each have been problematic in terms of presenting a complete set of findings or introducing bias.

Conclusions

The literature suggests that skeletal muscle oxidative metabolism is lowered by exposure to environmental hypoxia, which may precede a loss in muscle mitochondrial density. Meanwhile, the total capacity for skeletal muscle glycolysis is not consistently altered by environmental hypoxia. Taken together, the literature is not clear on whether a hypoxia-induced substrate switch from fatty acid oxidation to glucose oxidation occurs within the mitochondria of skeletal muscle as it does in the hypoxic rat heart, for instance. Environmental hypoxia does however induce a selective attenuation of whole muscle fatty acid oxidation, whilst glucose uptake is maintained or increased, perhaps to support glycolytic flux in the face of a downregulation of oxidative metabolism, optimising the pathways of ATP synthesis for the hypoxic environment.

Additional file

Additional file 1: Table S1. A list of all articles reviewed, their inclusion status and reasons for exclusion, where applicable.

Abbreviations

Edl: *Extensor digitorum longus*; gnm: *Gastrocnemius*; mix: *Mixed skeletal*; pla: *Plantaris*; rq: *Red quadriceps*; sol: *Soleus*; vl: *Vastus lateralis*; wq: *White quadriceps*; ADP: Adenosine diphosphate; ATP: Adenosine triphosphate; Bax: Bcl-2-associated X protein; Bcl-2: B-cell lymphoma 2; BNIP3: BCL2/adenovirus E1B protein-interacting protein 3; CACT: Carnitine acylcarnitine translocase; CPT: Carnitine palmitoyl transferase; ECAH: Enoyl CoA hydratase; ECAI: Enoyl CoA isomerase; ETF: Electron-transferring flavoprotein; HIF: Hypoxia-inducible factor; HOAD: L-3-hydroxyacyl CoA dehydrogenase; LDH: Lactate dehydrogenase; OXPHOS: Oxidative phosphorylation; PDH: Pyruvate dehydrogenase; PGC1α: Peroxisome proliferator-activated receptor gamma coactivator 1-alpha; PPARα: Peroxisome proliferator-activated receptor alpha; ROS: Reactive oxygen species; TCA: Tricarboxylic acid.

References

1. Mason S, Johnson RS: The role of HIF-1 in hypoxic response in the skeletal muscle. *Adv Exp Med Biol* 2007, **618:**229–244.
2. Murray AJ, Edwards LM, Clarke K: Mitochondria and heart failure. *Curr Opin Clin Nutr Metab Care* 2007, **10:**704–711.
3. Peacock AJ: ABC of oxygen: oxygen at high altitude. *BMJ* 1998, **317:**1063–1066.
4. Semenza GL, Roth PH, Fang HM, Wang GL: Transcriptional regulation of genes encoding glycolytic enzymes by hypoxia-inducible factor 1. *J Biol Chem* 1994, **269:**23757–23763.
5. Wheaton WW, Chandel NS: Hypoxia. 2. Hypoxia regulates cellular metabolism. *Am J Physiol Cell Physiol* 2011, **300:**C385–C393.
6. Zhang H, Bosch-Marce M, Shimoda LA, Tan YS, Baek JH, Wesley JB, Gonzalez FJ, Semenza GL: Mitochondrial autophagy is an HIF-1-dependent adaptive metabolic response to hypoxia. *J Biol Chem* 2008, **283:**10892–10903.
7. Kim JW, Tchernyshyov I, Semenza GL, Dang CV: HIF-1-mediated expression of pyruvate dehydrogenase kinase: a metabolic switch required for cellular adaptation to hypoxia. *Cell Metab* 2006, **3:**177–185.
8. Papandreou I, Cairns RA, Fontana L, Lim AL, Denko NC: HIF-1 mediates adaptation to hypoxia by actively downregulating mitochondrial oxygen consumption. *Cell Metab* 2006, **3:**187–197.
9. Fukuda R, Zhang H, Kim JW, Shimoda L, Dang CV, Semenza GL: HIF-1 regulates cytochrome oxidase subunits to optimize efficiency of respiration in hypoxic cells. *Cell* 2007, **129:**111–122.
10. Kiens B, Alsted TJ, Jeppesen J: Factors regulating fat oxidation in human skeletal muscle. *Obes Rev* 2011, **12:**852–858.
11. Murray AJ: Metabolic adaptation of skeletal muscle to high altitude hypoxia: how new technologies could resolve the controversies. *Genome Med* 2009, **1:**117.
12. West JB: Prediction of barometric pressures at high altitude with the use of model atmospheres. *J Appl Physiol (1985)* 1996, **81:**1850–1854.
13. Larsen S, Nielsen J, Hansen CN, Nielsen LB, Wibrand F, Stride N, Schroder HD, Boushel R, Helge JW, Dela F, Hey-Mogensen M: Biomarkers of mitochondrial content in skeletal muscle of healthy young human subjects. *J Physiol* 2012, **590:**3349–3360.
14. Chaillou T, Koulmann N, Meunier A, Malgoyre A, Serrurier B, Beaudry M, Bigard X: Effect of hypoxia exposure on the phenotypic adaptation in remodelling skeletal muscle submitted to functional overload. *Acta Physiol (Oxf)* 2013, **209:**272–282.
15. Green HJ, Sutton JR, Wolfel EE, Reeves JT, Butterfield GE, Brooks GA: Altitude acclimatization and energy metabolic adaptations in skeletal muscle during exercise. *J Appl Physiol (1985)* 1992, **73:**2701–2708.
16. Vigano A, Ripamonti M, De Palma S, Capitanio D, Vasso M, Wait R, Lundby C, Cerretelli P, Gelfi C: Proteins modulation in human skeletal muscle in the early phase of adaptation to hypobaric hypoxia. *Proteomics* 2008, **8:**4668–4679.
17. Green HJ, Sutton JR, Cymerman A, Young PM, Houston CS: Operation Everest II: adaptations in human skeletal muscle. *J Appl Physiol (1985)* 1989, **66:**2454–2461.

18. Levett DZ, Radford EJ, Menassa DA, Graber EF, Morash AJ, Hoppeler H, Clarke K, Martin DS, Ferguson-Smith AC, Montgomery HE, Grocott MPW, Murray AJ: **Acclimatization of skeletal muscle mitochondria to high-altitude hypoxia during an ascent of Everest.** *FASEB J* 2012, **26**:1431–1441.

19. Green H, Roy B, Grant S, Otto C, Pipe A, McKenzie D, Johnson M: **Human skeletal muscle exercise metabolism following an expedition to mount denali.** *Am J Physiol Regul Integr Comp Physiol* 2000, **279**:R1872–R1879.

20. Roberts AC, Reeves JT, Butterfield GE, Mazzeo RS, Sutton JR, Wolfel EE, Brooks GA: **Altitude and beta-blockade augment glucose utilization during submaximal exercise.** *J Appl Physiol (1985)* 1996, **80**:605–615.

21. van Hall G, Lundby C, Araoz M, Calbet JA, Sander M, Saltin B: **The lactate paradox revisited in lowlanders during acclimatization to 4100 m and in high-altitude natives.** *J Physiol* 2009, **587**:1117–1129.

22. Young AJ, Evans WJ, Fisher EC, Sharp RL, Costill DL, Maher JT: **Skeletal muscle metabolism of sea-level natives following short-term high-altitude residence.** *Eur J Appl Physiol Occup Physiol* 1984, **52**:463–466.

23. Abdelmalki A, Fimbel S, Mayet-Sornay MH, Sempore B, Favier R: **Aerobic capacity and skeletal muscle properties of normoxic and hypoxic rats in response to training.** *Pflugers Arch* 1996, **431**:671–679.

24. Daneshrad Z, Garcia-Riera MP, Verdys M, Rossi A: **Differential responses to chronic hypoxia and dietary restriction of aerobic capacity and enzyme levels in the rat myocardium.** *Mol Cell Biochem* 2000, **210**:159–166.

25. McClelland GB, Brooks GA: **Changes in MCT 1, MCT 4, and LDH expression are tissue specific in rats after long-term hypobaric hypoxia.** *J Appl Physiol (1985)* 2002, **92**:1573–1584.

26. Ou LC, Leiter JC: **Effects of exposure to a simulated altitude of 5500 m on energy metabolic pathways in rats.** *Respir Physiol Neurobiol* 2004, **141**:59–71.

27. De Palma S, Ripamonti M, Vigano A, Moriggi M, Capitanio D, Samaja M, Milano G, Cerretelli P, Wait R, Gelfi C: **Metabolic modulation induced by chronic hypoxia in rats using a comparative proteomic analysis of skeletal muscle tissue.** *J Proteome Res* 2007, **6**:1974–1984.

28. Dutta A, Vats P, Singh VK, Sharma YK, Singh SN, Singh SB: **Impairment of mitochondrial beta-oxidation in rats under cold-hypoxic environment.** *Int J Biometeorol* 2009, **53**:397–407.

29. Pastoris O, Foppa P, Catapano M, Dossena M: **Effects of hypoxia on enzyme activities in skeletal muscle of rats of different ages. An attempt at pharmacological treatment.** *Pharmacol Res* 1995, **32**:375–381.

30. Roberts AC, Butterfield GE, Cymerman A, Reeves JT, Wolfel EE, Brooks GA: **Acclimatization to 4,300-m altitude decreases reliance on fat as a substrate.** *J Appl Physiol (1985)* 1996, **81**:1762–1771.

31. Morash AJ, Kotwica AO, Murray AJ: **Tissue-specific changes in fatty acid oxidation in hypoxic heart and skeletal muscle.** *Am J Physiol Regul Integr Comp Physiol* 2013, **305**:R534–R541.

32. Galbes O, Goret L, Caillaud C, Mercier J, Obert P, Candau R, Py G: **Combined effects of hypoxia and endurance training on lipid metabolism in rat skeletal muscle.** *Acta Physiol (Oxf)* 2008, **193**:163–173.

33. Mizuno M, Savard GK, Areskog NH, Lundby C, Saltin B: **Skeletal muscle adaptations to prolonged exposure to extreme altitude: a role of physical activity?** *High Alt Med Biol* 2008, **9**:311–317.

34. Takahashi H, Kikuchi K, Nakayama H: **Effect of chronic hypoxia on oxidative enzyme activity in rat skeletal muscle.** *Ann Physiol Anthropol* 1993, **12**:363–369.

35. Jacobs RA, Boushel R, Wright-Paradis C, Calbet JA, Robach P, Gnaiger E, Lundby C: **Mitochondrial function in human skeletal muscle following high-altitude exposure.** *Exp Physiol* 2013, **98**:245–255.

36. Jacobs RA, Siebenmann C, Hug M, Toigo M, Meinild AK, Lundby C: **Twenty-eight days at 3454-m altitude diminishes respiratory capacity but enhances efficiency in human skeletal muscle mitochondria.** *FASEB J* 2013, **26**:5192–5200.

37. Pastoris O, Dossena M, Foppa P, Arnaboldi R, Gorini A, Villa RF, Benzi G: **Modifications by chronic intermittent hypoxia and drug treatment on skeletal muscle metabolism.** *Neurochem Res* 1995, **20**:143–150.

38. Magalhaes J, Ascensao A, Soares JM, Ferreira R, Neuparth MJ, Marques F, Duarte JA: **Acute and severe hypobaric hypoxia increases oxidative stress and impairs mitochondrial function in mouse skeletal muscle.** *J Appl Physiol* 2005, **99**:1247–1253.

39. Beaudry JL, McClelland GB: **Thermogenesis in CD-1 mice after combined chronic hypoxia and cold acclimation.** *Comp Biochem Physiol B Biochem Mol Biol* 2010, **157**:301–309.

40. Wust RC, Jaspers RT, van Heijst AF, Hopman MT, Hoofd LJ, van der Laarse WJ, Degens H: **Region-specific adaptations in determinants of rat skeletal muscle oxygenation to chronic hypoxia.** *Am J Physiol Heart Circ Physiol* 2009, **297**:H364–H374.

41. Gamboa JL, Andrade FH: **Mitochondrial content and distribution changes specific to mouse diaphragm after chronic normobaric hypoxia.** *Am J Physiol Regul Integr Comp Physiol* 2010, **298**:R575–R583.

42. Daneshrad Z, Novel-Chate V, Birot O, Serrurier B, Sanchez H, Bigard AX, Rossi A: **Diet restriction plays an important role in the alterations of heart mitochondrial function following exposure of young rats to chronic hypoxia.** *Pflugers Arch* 2001, **442**:12–18.

43. Gamboa JL, Andrade FH: **Muscle endurance and mitochondrial function after chronic normobaric hypoxia: contrast of respiratory and limb muscles.** *Pflugers Arch* 2012, **463**:327–338.

44. MacDougall JD, Green HJ, Sutton JR, Coates G, Cymerman A, Young P, Houston CS: **Operation Everest II: structural adaptations in skeletal muscle in response to extreme simulated altitude.** *Acta Physiol Scand* 1991, **142**:421–427.

45. Hoppeler H, Kleinert E, Schlegel C, Claassen H, Howald H, Kayar SR, Cerretelli P: **Morphological adaptations of human skeletal muscle to chronic hypoxia.** *Int J Sports Med* 1990, **11**(Suppl 1):S3–S9.

46. Magalhaes J, Ferreira R, Neuparth MJ, Oliveira PJ, Marques F, Ascensao A: **Vitamin E prevents hypobaric hypoxia-induced mitochondrial dysfunction in skeletal muscle.** *Clin Sci (Lond)* 2007, **113**:459–466.

47. van Ekeren GJ, Sengers RC, Stadhouders AM: **Changes in volume densities and distribution of mitochondria in rat skeletal muscle after chronic hypoxia.** *Int J Exp Pathol* 1992, **73**:51–60.

48. Hoppeler H, Vogt M, Weibel ER, Fluck M: **Response of skeletal muscle mitochondria to hypoxia.** *Exp Physiol* 2003, **88**:109–119.

49. Kayser B, Hoppeler H, Desplanches D, Marconi C, Broers B, Cerretelli P: **Muscle ultrastructure and biochemistry of lowland Tibetans.** *J Appl Physiol (1985)* 1996, **81**:419–425.

50. Favaro E, Ramachandran A, McCormick R, Gee H, Blancher C, Crosby M, Devlin C, Blick C, Buffa F, Li JL, Vojnovic B, Pires das Neves R, Glazer P, Iborra F, Ivan M, Ragoussis J, Harris AL: **MicroRNA-210 regulates mitochondrial free radical response to hypoxia and krebs cycle in cancer cells by targeting iron sulfur cluster protein ISCU.** *PLoS One* 2010, **5**:e10345.

51. Chan SY, Zhang YY, Hemann C, Mahoney CE, Zweier JL, Loscalzo J: **MicroRNA-210 controls mitochondrial metabolism during hypoxia by repressing the iron-sulfur cluster assembly proteins ISCU1/2.** *Cell Metab* 2009, **10**:273–284.

52. Essop MF, Razeghi P, McLeod C, Young ME, Taegtmeyer H, Sack MN: **Hypoxia-induced decrease of UCP3 gene expression in rat heart parallels metabolic gene switching but fails to affect mitochondrial respiratory coupling.** *Biochem Biophys Res Commun* 2004, **314**:561–564.

53. Heather LC, Cole MA, Tan JJ, Ambrose LJ, Pope S, Abd-Jamil AH, Carter EE, Dodd MS, Yeoh KK, Schofield CJ, Clarke K: **Metabolic adaptation to chronic hypoxia in cardiac mitochondria.** *Basic Res Cardiol* 2012, **107**:268.

54. Hinkle PC, Kumar MA, Resetar A, Harris DL: **Mechanistic stoichiometry of mitochondrial oxidative phosphorylation.** *Biochemistry* 1991, **30**:3576–3582.

55. Frayn KN: *Metabolic Regulation: A Human Perspective.* 3rd edition. Oxford, UK: Wiley-Blackwell; 2011.

56. West JB: **Lactate during exercise at extreme altitude.** *Fed Proc* 1986, **45**:2953–2957.

57. Reeves JT, Wolfel EE, Green HJ, Mazzeo RS, Young AJ, Sutton JR, Brooks GA: **Oxygen transport during exercise at altitude and the lactate paradox: lessons from Operation Everest II and Pikes Peak.** *Exerc Sport Sci Rev* 1992, **20**:275–296.

58. Kayser B: **Lactate during exercise at high altitude.** *Eur J Appl Physiol Occup Physiol* 1996, **74**:195–205.

59. Noakes TD: **Evidence that reduced skeletal muscle recruitment explains the lactate paradox during exercise at high altitude.** *J Appl Physiol (1985)* 2009, **106**:737–738.

60. Noakes TD: **Last word on viewpoint: evidence that reduced skeletal muscle recruitment explains the lactate paradox during exercise at high altitude.** *J Appl Physiol (1985)* 2009, **106:**745.

61. Murray AJ: **Of mice and men (and muscle mitochondria).** *Exp Physiol* 2013, **98:**879–880.

62. Jacobs RA, Diaz V, Meinild AK, Gassmann M, Lundby C: **The C57Bl/6 mouse serves as a suitable model of human skeletal muscle mitochondrial function.** *Exp Physiol* 2013, **98:**908–921.