Tissue-specific changes in fatty acid oxidation in hypoxic heart and skeletal muscle

Andrea J. Morash, Aleksandra O. Kotwica, and Andrew J. Murray

Department of Physiology, Development and Neuroscience, University of Cambridge, Downing Site, Cambridge, United Kingdom

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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 305: R534–R541, 2013. First published June 19, 2013; doi:10.1152/ajpregu.00510.2012.—Exposure to hypobaric hypoxia is sufficient to decrease cardiac PCr/ATP and alters skeletal muscle energetics in humans. Cellular mechanisms underlying the different metabolic responses of these tissues and the time-dependent nature of these changes are currently unknown, but altered substrate utilization and mitochondrial function may be a contributory factor. We therefore sought to investigate the effects of acute (1 day) and more sustained (7 days) hypoxia (13% O2) on the transcription factor peroxisome proliferator-activated receptor α (PPARα) and its targets in mouse cardiac and skeletal muscle. In the heart, PPARα expression was 40% higher than in normoxia after 1 and 7 days of hypoxia. Activities of carnitine palmitoyltransferase (CPT) I and β-hydroxyacyl-CoA dehydrogenase (HOAD) were 75% and 35% lower, respectively, after 1 day of hypoxia, returning to normoxic levels after 7 days. Oxidative phosphorylation respiration rates using palmitoyl-carnitine followed a similar pattern, while respiration using pyruvate decreased. In skeletal muscle, PPARα expression and CPT I activity were 20% and 65% lower, respectively, after 1 day of hypoxia, remaining at this level after 7 days with no change in HOAD activity. Oxidative phosphorylation respiration rates using palmitoyl-carnitine were lower in skeletal muscle throughout hypoxia, while respiration using pyruvate remained unchanged. The rate of CO2 production from palmitate oxidation was significantly lower in both tissues throughout hypoxia. Thus cardiac muscle may remain reliant on fatty acids during sustained hypoxia, while skeletal muscle decreases fatty acid oxidation and maintains pyruvate oxidation.

In the healthy heart, 90% of ATP production is generated via mitochondrial oxidative phosphorylation with 60–70% of that energy being derived from lipid oxidation (36). The heart is therefore extremely reliant on a continual supply of oxygen and fatty acids. Despite this, the heart has remarkable metabolic plasticity and is able to maintain ATP production under diverse and nonoptimal physiological conditions, utilizing other substrates such as glucose, amino acids, lactate, and ketone bodies (52). In comparison, skeletal muscle ATP is more evenly derived from a combination of oxidative and substrate level phosphorylation, making it comparably more flexible in terms of substrate use and oxygen demand (18). Given the requirement of the heart for oxygen to drive oxidative phosphorylation, it is highly sensitive to changes in oxygen pressure, and this has a significant effect on substrate use. Hence, the oxygen-deprived heart exhibits decreased mitochondrial respiration and ATP production (10, 17). It has been suggested that this energy deprivation may result from an inhibition of fatty acid oxidation (25), coupled with an incomplete compensation by glucose oxidation (8, 37, 46, 49). It may be that hypoxia induces a substrate switch from fatty acids to carbohydrate oxidation, since complete oxidation of carbohydrates provides 8–11% more ATP per mole of oxygen than fatty acid oxidation (16). Meanwhile, a significant loss of skeletal muscle mass and mitochondrial density in hypoxia (9, 14) might be considered an adaptive modification decreasing the tissue’s demand for limited O2 and lowering the production of potentially harmful reactive oxygen species (ROS) (13). Changes in cardiac mitochondrial function have recently been described in the hypoxia-acclimatized rat heart (15); however, little is known about skeletal muscle metabolism following acclimatization and the shorter-term metabolic response to hypoxia of both heart and skeletal muscle remains unknown.

Substrate selection during hypoxia is dependent on energetic signals from within the cell along with substrate availability itself and is under the control of cellular enzymes and transcription factors. Acutely, metabolic pathways may be altered through enzymatic activation via phosphorylation or by regulation through metabolic inhibition, whereas chronically these changes may be amplified or adjusted through transcriptional mechanisms. Peroxisome proliferator-activated receptor α (PPARα), a transcription factor expressed in cardiac and skeletal muscle and activated by fatty acids, induces the expression of genes encoding lipid-oxidizing enzymes (12) and may be critical in mediating substrate selection in hypoxic skeletal muscle (1, 29) and heart (10). In vitro studies of acute (<24 h) or chemically induced hypoxia have indicated that hypoxia inducible factor (HIF)-1α, a transcriptional mediator of the hypoxic response (for review see Ref. 47), may suppress PPARα expression in a number of tissues (1, 35, 45), thereby
restricting fatty acid oxidation. In contrast, however, PPARα levels increased in human skeletal muscle after 66 days exposure to high altitude hypoxia (29).

We therefore investigated the expression of PPARα and its downstream targets, alongside fatty acid oxidation during acute and more sustained hypoxic exposure in mouse heart and skeletal muscle. We hypothesized that fatty acid oxidation would remain a crucial facet of the hypoxia-acclimatized myocardium, whereas oxidative metabolism in skeletal muscle would be suppressed driving an increased reliance on anaerobic pathways for ATP production.

METHODS

Experimental animals. C57/B16 mice (starting weight 20 g; n = 18) were obtained from a commercial breeder (Harlan, Oxfordshire, UK) and housed under controlled conditions for light, humidity, and temperature with standard rodent chow and water available ad libitum throughout the experiment. All experiments conformed to UK Home Office guidelines under the Animals in Scientific Procedures Act and were reviewed by the University of Cambridge Animal Welfare and Ethical Review Committee. All procedures were carried out by a personal license holder, under UK Home Office project license no. 80/2428.

Hypoxia exposure and tissue collection. Mice were exposed to either normoxia (21% O2) or normobaric hypoxia (13% O2) in a flexible-film isolator (PFI Systems, Milton Keynes, UK) for 1 or 7 days. Postexposure mice were anesthetized with a lethal intraperitoneal injection of pentobarbital, and the heart and skeletal muscle tissues were removed. For mitochondrial isolation, the left ventricle and muscles from the whole lower hindlimb were excised, snap frozen in liquid N2, and kept at −80°C for further analysis.

Mitochondrial isolation. Mitochondria from both cardiac and skeletal muscle were isolated according to Campbell et al. (4). Briefly, tissues were diced in chilled mitochondrial isolation buffer and mitochondrial fractions were pelleted using differential centrifugation and kept in BIOPS buffer (10 mM Ca-EGTA buffer, 0.1 μM free calcium, 20 mM imidazole, 20 mM taurine, 50 mM K-MES, 0.5 mM dithiorthiol, 6.56 mM MgCl2, 5.77 mM ATP, 15 mM phosphocreatine, pH 7.1). For protein and enzyme analysis, ventricle and skeletal muscle tissues were homogenized in a chilled mitochondrial isolation buffer to a final concentration of 50 μM Tris-HCl, 5 mM MgSO4, 5 mM EDTA, 1 mM ATP, pH 7.4). For respiratory analysis, the ventricle and soleus muscles were extracted and kept in BIOPS buffer (10 mM Ca-EGTA buffer, 0.1 μM free calcium, 20 mM imidazole, 20 mM taurine, 50 mM K-MES, 0.5 mM dithiorthiol, 6.56 mM MgCl2, 5.77 mM ATP, 15 mM phosphocreatine, pH 7.1). For protein and enzyme analysis, ventricle and muscles from the whole lower hindlimb were excised, snap frozen in liquid N2, and kept at −80°C for further analysis.

Mitochondrial isolation. Mitochondria from both cardiac and skeletal muscle were isolated according to Campbell et al. (4). Briefly, tissues were diced in chilled mitochondrial isolation buffer and homogenized using a chilled Potter-Elvehjem homogenizer. Mitochondrial fractions were pelleted using differential centrifugation and resuspended in chilled isolation buffer lacking BSA and kept on ice for use in a carnitine palmitoyltransferase (CPT) I assay and fatty acid oxidation assay.

CPT I activity. CPT I activity was determined using [14C]carnitine according to McGarry and Brown (31). The assay buffer contained 117 mM Tris-HCl, 0.28 mM reduced glutathione, 4.4 mM MgCl2, 16.7 mM KCl, 2.2 mM KCN, 300 μM palmitoyl-CoA, 5 mM carnitine, 4.4 mM ATP, 40 mg/ ml rotenone, 0.5% BSA, and 1 μCi [14C]carnitine. The reaction was initiated with the addition of diluted mitochondrial homogenates to a final concentration of 50 μg/mil and incubated for 8 min at 37°C. The reaction was terminated by the addition of 60 μl of HCl. The palmitoyl-[14C]carnitine formed during the reaction was separated by the method of Starratt et al. (51) and then radioactivity was counted to determine CPT I activity.

Fatty acid oxidation assay. The rate of palmitate oxidation was measured according to previously published methods (2, 32) using [14C]palmitate. Briefly, modified Krebs-Ringer buffer (115 mM NaCl, 2.6 mM KCl, 1.2 mM KH2PO4, 10 mM NaHCO3, 10 mM HEPES, pH 7.4) supplemented with 5 mM ATP, 1 mM NAD+, 0.5 mM carnitine, 0.1 mM coenzyme A, 25 μM cytochrome C, and 0.5 mM malate was incubated at 37°C in a 20-ml glass scintillation vial with 150 μl of benzethonium hydroxide in a suspended microcentrifuge tube to trap the 14CO2 produced during the reaction. Mitochondria were added to the system, which was then sealed with a rubber cap. The reaction was initiated by the addition of 1 μCi of [14C]palmitate (made up as a 6:1 palmitate-BSA complex) to a final palmitate concentration of 100 μM via syringe through the rubber cap. The vial was incubated for 30 min at 37°C before termination with 50 μl of HClO4. The microcentrifuge tube containing the benzethonium hydroxide and trapped 14CO2 was then removed to a scintillation vial and the radioactivity counted.

Tissue permeabilization and respirometry. Excised cardiac and soleus tissues were dissected into small fiber bundles for respirometric analysis according to previously published protocols (26). Muscle fibers were incubated with 50 μM saponin in BIOPS medium for 20 min, which selectively permeabilizes the plasma membrane but leaves mitochondrial membranes intact. Fibers were washed three times (5 min each; 4°C) in respiration medium (0.5 mM EGTA, 3 mM MgCl2-6H2O, 20 mM taurine, 10 mM KH2PO4, 20 mM HEPES, 60 mM potassium-lactobionate, 110 mM mannitol, 0.3 mM dithiothreitol, and 1 g/l BSA, pH 7.1) to remove any endogenous substrates from the tissues. Mitochondrial respiration in the permeabilized tissues was then measured in Clark-type oxygen electrodes (Strathkelvin Instruments) at 37°C. Respiration was stimulated by the addition of glutamate (10 mM) and malate (5 mM) from which the leak rate (state II) was measured. Oxidative phosphorylation (OXPHOS; state III) rate was measured after the addition of ADP (2 mM). Complex I-stimulated respiration was then inhibited using rotenone (0.5 μM) to obtain complex II respiration rates stimulated by succinate (10 mM). Complex II-stimulated respiration was then inhibited using antimycin and complex IV respiration stimulated via the addition of N,N,N',N'-tetramethyl-1,4-phenylenediamine (TMPD, 0.5 mM) and ascorbate (2 mM). To determine the respiration rate for specific substrates, glutamate, malate, and both pyruvate (20 mM) or palmitoyl-carnitine (100 μM) were added to initiate respiration and determine leak rate. ADP was added to determine maximal OXPHOS rates for each substrate. All respiration rates were normalized to dry tissue weight obtained after 48 h at 80°C.

Enzyme analysis. Frozen tissues were powdered under liquid nitrogen with a mortar and pestle and then homogenized in potassium phosphate buffer (100 mM KH2PO4, 5 mM EDTA, 0.1% Triton X-100, pH 7.2) using a polytron homogenizer for citrate synthase (CS) and β-hydroxyacyl-CoA dehydrogenase (HOAD). Frozen tissue for the aconitase assay was homogenized using the same method but using the assay buffer from an aconitase assay kit (Abcam, Cambridge, UK).

HOAD activity was assayed according to McClelland et al. (30). The assay buffer contained 50 mM imidazole (pH 7.4), 0.1 mM acetoacetyl-CoA, and 0.15 mM NADH, an 0.1% Triton X-100. The reaction was initiated by the addition of 0.5 mM oxaloacetate (omitted from controls), and absorbance was monitored at 412 nm for 3 min.

Aconitase activity was measured using a commercially available kit (ab83459, Abcam). Briefly, powdered tissues were homogenized with the assay buffer and assayed in a 96-well plate by measuring the rate of isocitrate production from citrate (omitted from controls) at 450 nm. Aconitase activity was then determined using an isocitrate standard curve.

Mitochondrial and tissue protein was assayed using the Bradford method (3) with a Bio-Rad protein assay kit.

Immunoblotting. Levels of CPT I and PPARα were measured in whole muscle homogenates by standard SDS-PAGE and Western blotting techniques using polyclonal rabbit anti-mouse CPT I (SC-20670; Santa Cruz Biotechnology) and polyclonal rabbit anti-mouse PPARα (SC-9000; Santa Cruz Biotechnology), respectively. Goat anti-rabbit IgG horse radish peroxidase-conjugate secondary antibody (20320,
Alpha Diagnostics International) was used for both proteins. Protein expression levels were normalized to total protein using Ponceau S stain (Sigma, St. Louis, MO). Blots quantified using Un-Scan-It Gel 6.1 (Silk Scientific).

Statistical analysis. Data are presented as the means ± SE. Differences were determined using a one-way ANOVA and Tukey’s post-hoc test (Prism 5, GraphPad Software) with significance set at $P < 0.05$.

RESULTS

Physical characteristics. Exposure to 13% O$_2$ did not cause a significant change in body mass of the mice after 1 or 7 days (Table 1). After 1 day of hypoxia, hemoglobin levels were not different from those in normoxic mice, however, hemoglobin levels were 14% higher after 7 days exposure (Table 1; $P < 0.05$).

Fatty acid uptake and oxidation. PPAR$_{a}$ protein level was 30% higher in cardiac muscle after 1 and 7 days of hypoxia compared with the hearts of normoxic mice (Fig. 1; $P < 0.05$). Conversely, skeletal muscle PPAR$_{a}$ was 30% lower after 1 and 7 days of hypoxic exposure (Fig. 1; $P < 0.05$). Correspondingly, levels of CPT I, which is under the transcriptional control of PPAR$_{a}$, were 60% lower in hypoxic skeletal muscle compared with normoxia (Fig. 2A; $P < 0.05$). Interestingly, cardiac muscle CPT I levels were 50% lower after 1 day of hypoxia but returned to normoxic levels after 7 days of hypoxia (Fig. 2A; $P < 0.05$). CPT I activity followed a similar pattern with significantly lower activity in hypoxic cardiac and skeletal muscle after 1 day of hypoxia and a return to normoxic activity level after 7 days of hypoxia in cardiac muscle (Fig. 2B; $P < 0.05$). HOAD activity, also under the regulatory control of PPAR$_{a}$, was not significantly different in hypoxic skeletal muscle compared with normoxic animals (Fig. 2C). Although not statistically significant, HOAD activity in the cardiac muscle followed a similar pattern to that of CPT I (Fig. 2C).

To estimate the total flux rate of palmitate through the entire fatty acid oxidation pathway, the rate of $^{14}$CO$_2$ production from isolated mitochondria provided with $[^{14}$C]palmitate as a substrate was measured. After 1 day of hypoxia, the rate of palmitate oxidation was 70% lower than in normoxia in both cardiac and skeletal muscle, and this decrease was maintained after 7 days of hypoxic exposure in both tissues (Fig. 2D; $P < 0.05$).

Mitochondrial enzymes and respiration. CS activity, a marker of mitochondrial density and tricarboxylic acid cycle (TCA) flux, was not significantly different between normoxia and hypoxia in either tissue despite an upward trend as the duration of hypoxia increased (Table 2). Aconitase, the second enzyme in the TCA cycle, was 65% lower in hypoxic cardiac muscle than in normoxic hearts ($P < 0.01$) but was not altered in skeletal muscle (Table 2). When corrected for mitochondrial density, using CS activity as a mitochondrial marker, the same pattern emerged in cardiac muscle, indicating the decreased activity is irrespective of mitochondrial content (Table 2).

Respiration rates via individual electron transport system (ETS) complexes were compared between normoxic and hypoxic cardiac and skeletal muscle and showed no significant differences (Table 3). However, the ratio of complex I to complex II respiration was significantly higher in cardiac muscle after 7 days of hypoxia (Table 3; $P < 0.001$) indicating a greater reliance on electron entry through complex I in this group.

Leak rate and oxidative phosphorylation rate were determined using pyruvate and palmitoyl-carnitine to assess differences in the respiratory capacity for each substrate (Fig. 3). The leak rate was not significantly different in hypoxic cardiac muscle with palmitoyl-carnitine as the substrate (Fig. 3A), but the maximal oxidative phosphorylation respiration rate was 45% higher after 7 days of hypoxia compared with normoxia (Fig. 3B; $P < 0.05$). In skeletal muscle, the leak and maximal oxidative phosphorylation respiration rates were significantly lower after 1 day of hypoxia with palmitoyl-carnitine as substrate but not after 7 days (Fig. 3, A and B; $P < 0.05$). With pyruvate, the leak and maximal oxidative phosphorylation respiration rates were significantly lower after 7 days of hypoxia in the heart (Fig. 3, C and D; $P < 0.05$) but were unchanged in the skeletal muscle (Fig. 3, C and D).

<table>
<thead>
<tr>
<th>Physical Characteristic</th>
<th>Normoxic</th>
<th>1 Day Hypoxic</th>
<th>7 Days Hypoxic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial body mass, g</td>
<td>25.4 ± 0.3</td>
<td>20.6 ± 0.5</td>
<td>22.9 ± 0.5</td>
</tr>
<tr>
<td>Final body mass, g</td>
<td>25.4 ± 0.3</td>
<td>20.5 ± 0.5</td>
<td>22.3 ± 0.7</td>
</tr>
<tr>
<td>Hemoglobin, g/l</td>
<td>134 ± 4.5*</td>
<td>129 ± 9.7*</td>
<td>153 ± 4.2*</td>
</tr>
</tbody>
</table>

Values are means ± SE; $n = 6$ per group. Symbols denote significance between normoxic and hypoxic groups ($P < 0.05$).

Fig. 1. Peroxisome proliferator-activated receptor α (PPAR$_{a}$) protein expression in cardiac (A) and skeletal (B) muscle from normoxic, 1 day, and 7 day hypoxic mice. *Significance ($P < 0.05$) between hypoxic and normoxic muscle. $n = 6$ per group.
DISCUSSION

The selection of metabolic substrates by heart and muscle during hypoxia is regulated by a complex signaling network to maintain ATP synthesis while minimizing cellular damage due to ROS production (34). We show here that cardiac and skeletal muscle respond with a similar metabolic pattern during acute hypoxia by decreasing the uptake and use of lipids as a substrate to fuel ATP production. As the duration of hypoxia increases, however, cardiac muscle, unlike skeletal muscle, reverts back to normoxic rates of lipid oxidation. Furthermore, the capacity for pyruvate oxidation is suppressed in cardiac mitochondria after 7 days of hypoxia yet is maintained in skeletal muscle. Correspondingly, PPARα protein levels were lowered in skeletal muscle during acute and chronic environmental hypoxia, suggesting a suppression of fat oxidation but in contrast increased in cardiac muscle, suggesting that fatty acid oxidation may continue to contribute to myocardial ATP synthesis during hypoxia.

The activity and expression patterns of CPT I, one of the main regulatory enzymes of mitochondrial lipid uptake, appeared to follow changes in PPARα levels in skeletal muscle, though this did not seem to be the case in the heart. It has

### Table 2. Mitochondrial TCA cycle enzyme activities in normoxic, 1 day and 7 day hypoxic mice

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Treatment</th>
<th>CS Activity, μmol·min⁻¹·mg protein⁻¹</th>
<th>Aconitase Activity, mmol·min⁻¹·mg protein⁻¹</th>
<th>Aconitase/CS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiac</td>
<td>Normoxic</td>
<td>171.9 ± 22.4</td>
<td>30.0 ± 6.3*</td>
<td>0.198 ± 0.035*</td>
</tr>
<tr>
<td></td>
<td>1D hypoxic</td>
<td>235.0 ± 47.92</td>
<td>11.2 ± 1.6†</td>
<td>0.052 ± 0.008†</td>
</tr>
<tr>
<td></td>
<td>7D hypoxic</td>
<td>271.4 ± 17.8</td>
<td>6.8 ± 1.5†</td>
<td>0.035 ± 0.011†</td>
</tr>
<tr>
<td>Skeletal</td>
<td>Normoxic</td>
<td>180.2 ± 21.1</td>
<td>248 ± 59.2</td>
<td>0.98 ± 0.13</td>
</tr>
<tr>
<td></td>
<td>1D hypoxic</td>
<td>181.1 ± 15.9</td>
<td>273.4 ± 87.5</td>
<td>1.43 ± 0.49</td>
</tr>
<tr>
<td></td>
<td>7D hypoxic</td>
<td>165.8 ± 22.4</td>
<td>188.9 ± 47.7</td>
<td>1.14 ± 0.29</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 6 per group. CS, citrate synthase. Symbols denote significance between normoxic and hypoxic groups within the same tissue (P < 0.05).
however, been suggested that PPARα activity, rather than expression, is HIF-1α regulated, since in cultured cardiac myocytes exposed to 48 h of hypoxia, PPARα levels did not change, while levels of its obligate cofactor the retinoic acid X receptor (RxR) were diminished, restricting transcriptional activity (22). In acute hypoxia, despite an increase in PPARα, we observed a decrease in CPT I expression and activity in cardiac muscle corresponding with findings in both cultured cardiac myocytes and following regional myocardial ischemia in dogs (22, 41). The oxidation enzyme, HOAD, which is also under the transcriptional control of PPARα, did not change in hypoxic skeletal muscle despite a 65% loss of CPT I activity. In cardiac muscle, however, we saw a similar pattern in HOAD activity to that exhibited by CPT I, a fall in acute hypoxia followed by a recovery to normoxic levels. Despite an initial increase in HIF-1α stabilization, in both rats and hum...
mans, a loss of HIF-1α protein and activity has been noted after 1 wk of hypobaric hypoxia (27, 54), and therefore it may be possible that an acute, HIF-dependent inhibition of PPARα activity may be relieved following more sustained hypoxia, at least in the heart, allowing CPT I expression and activity as well as HOAD activity to return to normoxic levels.

The return to normoxic activity levels of CPT I and HOAD may suggest an inability of cardiac mitochondria to maintain ATP production during more sustained hypoxia without reverting to fatty acid oxidation, whereas skeletal muscle can sustain ATP production using anaerobic pathways. Alternatively, the erythropoietic response to hypoxia, which increased circulating hemoglobin levels after 7 days in hypoxia, may have relieved tissue hypoxia at this time point in the heart but perhaps not in skeletal muscle where blood flow is more intermittent than it is through the coronary arteries. Interestingly, however, while the capacity for mitochondrial fatty acid uptake and β-oxidation was restored after 7 days of hypoxia, there was no corresponding restoration in the amount of CO2 produced from the acetyl-CoA liberated from the oxidized fatty acids in the heart. Typically, CO2 is released during the production of NADH by isocitrate dehydrogenase and α-ketoglutarate dehydrogenase in the TCA cycle, and this might suggest that the amount of NADH produced is also lower. Thus, in cardiac tissue, the routine flow of acetyl-CoA into the TCA cycle appears to be disrupted after 7 days of hypoxia, which could lead to excess acetyl-CoA being converted to other compounds such as malonyl-CoA, or providing acetyl groups for acetylation reactions. Acetylation is a major posttranslational regulatory mechanism for many proteins and is also important in histone function and gene transcription. Acetylation of proteins and regulation of gene expression via this mechanism during hypoxia warrants further research.

To further investigate routine TCA cycle flux, we measured the activity of the first two enzymes of the TCA cycle, CS and aconitase. Curiously, CS activity was normal in both tissues at both durations of hypoxia, however, aconitase activity was more than 50% lower in hypoxic cardiac muscle at both time points, in agreement with a recent finding in the cardiac mitochondria of chronically hypoxic rats (15). Previous studies in lung (43), skeletal muscle (55), brain (5), and nerve cells (53) have indicated that aconitase is particularly susceptible to ROS-mediated inhibition, as are other TCA cycle enzymes such as isocitrate dehydrogenase (28). Aconitase inhibition during hypoxic stress has been hypothesized to occur via several different mechanisms. First, iron-sulfur cluster assembly proteins may be subject to ROS-mediated disruption thereby reducing total aconitase protein as suggested by work in various cell lines (7, 11). Alternatively, aconitase has also been shown to undergo reversible modulation by frataxin, an iron-binding protein (40). Previous reports on rat cardiac tissue indicate that total aconitase protein concentration does not change after 2 wk of hypoxia and propose that it is a posttranslational modification that decreases aconitase activity (15). Hypoxic tissues produce ROS as a result of attenuated electron flow through the ETS when oxygen, the final electron acceptor, is limited (33). In the heart, ROS generation during both acute and chronic hypoxia may therefore inhibit aconitase activity, disrupting TCA cycle flux. Acutely, when ATP production from glycolysis is high, this inhibition may be beneficial, decreasing the production of electron donors from the TCA cycle so as to not overwhelm the ETC and thereby further exacerbate ROS generation. During more sustained hypoxia, however, when glycolytic production of ATP can no longer meet the demands of the myocardium, the aconitase inhibition and consequent loss of NADH/FADH2 from the TCA cycle may force an increased reliance on NADH/FADH2 derived from β-oxidation of fatty acids. This might explain the importance of returning CPT I and HOAD activity to normoxic levels in the chronically hypoxic heart. Mechanistically, a rise in intracellular fatty acids during the initial phases of hypoxia due to increased sympathetic drive and enhance fatty acid uptake (6, 48) may stimulate PPARα (44) inducing the expression of fatty acid oxidation enzymes.

The increased reliance on fatty acids following more sustained hypoxia is reflected in the rate of O2 consumption at the level of the ETS. After 7 days of hypoxia, cardiac muscle consumed almost 50% more O2 per minute with palmitoyl-carnitine than the 1 day and normoxic tissues, whereas the oxygen consumption rate using pyruvate was 30% lower. In contrast, in hypoxic skeletal muscle, respiration using pyruvate was maintained at normoxic levels, but respiration was significantly lower with palmitoyl-carnitine.

Despite changes in respiration with pyruvate and palmitoyl-carnitine substrates, there were few apparent changes in the activities of the ETS complexes. In cardiac muscle there was an increased reliance on electron entry into the ETS via complex I after 7 days of hypoxia. Hypoxia induces a loss of complex I levels in some tissues including skeletal muscle, and this is thought to prevent the production of damaging levels of ROS (15, 29). In these mice, however, an increase in electron flux through complex I in chronic hypoxia may be necessary to maintain myocardial ATP production, though this could also theoretically increase ROS production.

In contrast to the heart, skeletal muscle is able to undergo metabolic suppression or reduce the total mass of metabolically active tissue. Indeed, humans at high altitude experience a significant loss of muscle mass and individual fiber size (19, 20), as well as decreased mitochondrial density following sustained exposure (20, 29). A reduction in protein synthesis would itself lower the tissue’s energetic demand, sparing the limited oxygen for basal metabolism. Additionally, production of ATP from anaerobic glycolysis may better match this lower requirement. Specifically, a decrease in lipid oxidation may also help to maintain ATP homeostasis promoting the use of more oxygen-efficient carbohydrates for ATP production. With regard to electron transport chain function, our data agree well with that shown previously in human skeletal muscle, following 9 days of exposure to ~4,600 m (24). Curiously, this study, unlike ours, did not report a repression of fatty acid oxidation in hypoxic skeletal muscle. This apparent discrepancy might be explained by the choice of fatty acid substrate used in the two studies. Since palmitate is the most abundant fatty acid in animal biology, we investigated palmitoyl-carnitine oxidation and found an impairment. In the human study, however, Jacobs et al. (24) studied octanoyl-carnitine oxidation and found no such effect most likely because octanoyl-carnitine import into the mitochondrion is independent of CPT (39), and the alternative carnitine octanoyltransf erase system is perhaps not affected by hypoxia. The significance of these different findings is unclear but is perhaps worthy of further investigation.
Finally, there is an interesting increase in complex I-to-complex IV ratio in skeletal muscle after 7 days of hypoxia, although this remains much lower than the normoxic ratio in the heart. A decrease in complex IV activity has the potential to cause an increase in ROS production by preventing normal electron flow. Given that this ratio is lower than that of the heart in normoxia, we would predict that this change does not have adverse effects in the skeletal muscle and may be part of an overall decrease in activity of the tissue.

**Perspectives and Significance**

Collectively, therefore, our data suggest that during chronic hypoxia, fatty acid oxidation is maintained in the heart to provide the necessary electron donors for oxidative phosphorylation. This reliance on fatty acid oxidation is likely due to the increased expression of PPARα in the cardiac muscle in conjunction with a loss of TCA cycle flux. Conversely, PPARα expression is low in hypoxic skeletal muscle, promoting an increased use of carbohydrates to meet energetic demands. The different metabolic responses of these two tissues may explain why human skeletal muscle is apparently able to maintain resting ATP levels following hypoxic exposure, while the heart is energetically impaired (9, 17).

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

**AUTHOR CONTRIBUTIONS**


**REFERENCES**


