Alterations in enzymes involved in fat metabolism after acute and chronic altitude exposure

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The purpose of this study was to examine the effect of acute (24 h) and chronic (5 wk) hypobaric hypoxic exposure equivalent to a simulated altitude of 4,300 m (446 mmHg) on the enzymes of fat metabolism. Heart, liver, and skeletal muscle were taken from 32 male Sprague-Dawley rats. Altitude exposure did not affect the activity of citrate synthase in any of the tissues, suggesting that mitochondrial content was unchanged. Carnitine palmitoyltransferase-I (CPT-I) activity was significantly reduced in the heart by both acute and chronic high altitude exposure compared with controls. A similar reduction was found for CPT-I activity in extensor digitorum longus after acute and chronic exposure compared with control animals. CPT-I activity was not affected by altitude exposure in the soleus muscle or the liver. 3-Hydroxyacyl-CoA dehydrogenase (β-HAD) activity was significantly depressed in the hearts of chronically exposed animals compared with controls. No difference between acute and control animals was found in the heart for β-HAD activity. Liver β-HAD activity was also significantly decreased in the acclimatized as well as in the acute animals compared with the control group. Quadriceps β-HAD activity was reduced for the chronic animals only compared with controls. These data suggest that acclimatization to high altitude selectively decreases key enzymes in fat utilization and oxidation in the heart, liver, and select skeletal muscles.

hypobaric hypoxia; 3-hydroxyacyl-CoA dehydrogenase; carnitine palmitoyltransferase-I

EXPOSURE TO HIGH ALTITUDE (hypobaric hypoxia) elicits a number of physiological and metabolic adjustments that have both clinical and scientific implications. Specifically, alterations in substrate metabolism have been reported with both acute and chronic high altitude exposure (6, 7, 14, 25, 28, 33, 34). Using primed continuous infusion of [6,6-2H2]glucose, Brooks et al. (6) concluded that acclimatization to high altitude (4,300 m) resulted in increased utilization of blood glucose both at rest and during submaximal exercise. More recently, Roberts et al. (26) reported similar findings, suggesting a greater dependence on blood glucose during prolonged altitude exposure (21 days at 4,300 m) in men. Consequently, it has been suggested that a greater dependence on glucose rather than fatty acids metabolism would assist in maintaining homeostasis by optimizing the energy yield per unit of oxygen (17, 20). Compared with fatty acid oxidation, carbohydrate (CHO) oxidation generates more ATP per molecule of oxygen consumed. CHO can also be metabolized non-oxidatively to yield ATP and lactate.

However, other studies have provided indirect evidence to suggest that acclimatization to high altitude results in a greater rate of fat utilization, thereby sparing muscle glycogen stores (14, 28, 34, 35). These conclusions are based on a slower rate of muscle glycogen utilization after acclimatization, elevated levels of free fatty acids (FFA) and glycerol in blood, and measurement of the respiratory exchange ratio (RER).

This lack of agreement concerning altitude effects on metabolism is confounded by variations in altitude and duration of exposure. Extreme altitudes, such as those over 7,000 m, provoke profound muscle degeneration, which confounds other metabolic changes. Chronic altitude periods range from 2 to 8 wk. The shorter duration studies may not allow time for mitogenesis or other mitochondrial adaptations. Also, a reduction in energy intake, as well as an elevation in basal metabolic rate, is frequently associated with ascent to high altitude. This results in a negative energy balance and weight loss that shift substrate utilization toward greater fat oxidation (8).

Given the disparate results regarding the influence of altitude exposure on substrate selection, it was the purpose of this study to examine the effects of both acute (24 h) and chronic (5 wk) altitude exposure on key enzymes involved in fat metabolism. Specifically, the activities of 3-hydroxyacyl-CoA dehydrogenase (β-HAD), a marker of β-oxidation, and carnitine palmitoyltransferase-I (CPT-I), a rate-limiting step in the translocation of FFA into mitochondria, were measured in heart, liver, and skeletal muscle in rats.

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METHODS

Animals. Male Sprague-Dawley rats (n = 32) were randomly assigned into three groups. The acute group (n = 11) resided in a hypobaric chamber at 482 mmHg (4,300 m) for 24 h, whereas the acclimatized group (n = 10) was killed after 5 wk of exposure under similar conditions (482 mmHg). The control group (n = 11) was housed in an identical chamber but at a normobaric pressure (sea level). Animals were given rat chow and water ad libitum. Rats were weighed at the beginning and completion of the study. Additionally, acute rats were weighed before altitude exposure. All animals were killed on the same day by an intraperitoneal injection of pentobarbital sodium (50 mg/kg body wt). Quadriceps, soleus, extensor digitorum longus (EDL), and gastrocnemius muscles, as well as the liver, heart, and adrenals, were harvested by dissection. Hearts and adrenal glands were weighed at time of death. Tissues were instantly frozen by liquid nitrogen immersion. The protocol was approved by the University of Colorado Institutional Animal Care and Use Committee.

β-HAD. Spectrophotometric analysis of β-HAD was conducted to determine activity of the β-oxidation of fats. Frozen tissue (~200 mg) was pulverized and added to ice-cold 0.1 M potassium phosphate buffer (pH 7.9, 1:9 wt/vol) and homogenized over ice. It was diluted 1:1 with phosphofructokinase dilution mix (25 ml potassium phosphate buffer, 25 ml glyc- erin, 2 µl mercaptoethanol, 9.5 mg EDTA, and 10 mg bovine albumin) and stored on ice until assay time. One hundred microliters of ice-cold sample were mixed with a reaction mixture of 60 µl 1.67 M triethanolamine, 10 µl 500 mM EDTA, 100 µl 2 mM NADH, 100 µl 10 mM acetoacetyl-CoA, and 630 µl H2O (total 900 µl), and absorption followed at 340 nm for 5 min.

CPT-I assay. The activity of CPT-I was assayed radiochemically (12). The reaction mixture contained (in ml) 1.0 2 M KC1, 1.25 1 M OPS, 0.25 0.1 M EGTA, 0.5 10% BSA, 0.5 KCN, 0.5 H2O, and 0.5 0.2 M dithiothreitol at a pH of 7.5. A 5% homogenate was made by mixing 20 mg tissue with 150 mM NaNCl and 0.1 M EGTA. The reaction was run either with or without 0.2 mM malonyl-CoA to inhibit CPT-I activity and isolate CPT-I activity from residual carnitine palmitoyltransferase II (CPT-II) activity. The reaction was run in 50 µl reaction mix, 10 µl 1.25 M pmalonyl-CoA, 10 µl homogenate, and 145 µl H2O. The reaction was started by addition of 50 mM t-[3H]carnitine and allowed to run for 3 min. The reaction was stopped with 1.5 ml 1 N HCl. One milliliter of butanol saturated with water was added, vortexed, and centrifuged for 5 min. The butanol phase was removed, added to 2 ml of water saturated with butanol, and centrifuged, and 0.4 ml of the butanol phase was then counted. CPT-I activity was calculated as the CPT activity without malonyl-CoA less the activity with malonyl-CoA.

Citrate synthase assay. Spectrophotometric analysis of citrate synthase activity was conducted using the method of Srere (27). The tissues were homogenized in potassium phosphate buffer, pH 8.5 (1:9 wt/vol). The homogenate was diluted in Tris buffer (1:20). Homogenate (0.05 ml) was added to 0.65 ml Tris buffer, 0.20 ml equal volumes of 1 mM DTNB-3 mM acetyl-CoA, and 0.10 ml 5 mM and read for 5 min at 25°C at 412 nm.

Statistics. Data are presented as means ± SE. Statistical analysis consisted of a one-way factorial ANOVA to evaluate the main treatment effects (acute hypoxia, chronic hypoxia, and normoxia conditions). P < 0.05 was set as the alpha level. When significant difference was found, Tukey's post hoc analysis was used.

RESULTS

Animal characteristics. Animal body weights are reported in Table 1. Animal body weights were equivalent at the beginning of the study. Control rats gained significantly more weight throughout the study than acute or acclimatized rats (P < 0.05). The body weights of acute rats were equivalent to those of the control group before the 24 h of altitude exposure.

Altitude acclimatization induced hypertrophy of both the heart and adrenals (Table 1). Adrenals were significantly enlarged in the acclimatized rats compared with the acute (P < 0.05) and control rats. These differences are even greater when the adrenal weight-to-body weight ratios were compared. The ratios of the acclimatized rats were significantly higher than those of the control (P < 0.005) and acute (P < 0.05) groups. The acclimatized rats also had enlarged hearts compared with the acute (P < 0.0001) and control (P < 0.001) animals (Table 1). Heart weight-to-body weight ratios were also significantly greater in acclimatized animals compared with the other groups (P < 0.0001).

Citrate synthase activity. Citrate synthase activity, a marker of mitochondrial oxidative capacity, did not differ across any condition in the heart, liver, and EDL tissues (Table 2), suggesting that mitochondrial content was unaffected by altitude exposure.

CPT-I activity. The activity of CPT-I, a marker of the capacity for fatty acid transport into the mitochondria, was significantly reduced in the heart for both acute and chronic high altitude exposure compared with control (1.35 ± 0.11, 1.32 ± 0.10, and 1.69 ± 0.09 µmol·g−1·min−1, respectively) (Fig. 1A). A similar reduction was found for CPT-I activity in EDL after acute and chronic (exposure 0.44 ± 0.06 and 0.28 ± 0.07 µmol·g−1·min−1, respectively) compared with control animals (0.74 ± 0.08 µmol·g−1·min−1) (Fig.

<table>
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<th>Table 1. Physical characteristics</th>
<th>Initial Body Wt, g</th>
<th>Final Body Wt, g</th>
<th>Adrenal Wt, mg</th>
<th>Adrenal/Body Wt, mg/g</th>
<th>Heart Weight, mg</th>
<th>Heart/Body Wt, mg/g</th>
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<tr>
<td>Chronic</td>
<td>194 ± 2</td>
<td>304 ± 4</td>
<td>24.9 ± 1.0†</td>
<td>0.082 ± 0.003†</td>
<td>1,030 ± 19†</td>
<td>3.39 ± 0.06†</td>
</tr>
<tr>
<td>Acute</td>
<td>192 ± 1</td>
<td>303 ± 6</td>
<td>22.3 ± 0.61†</td>
<td>0.074 ± 0.003†</td>
<td>870 ± 19</td>
<td>2.88 ± 0.06</td>
</tr>
<tr>
<td>Control</td>
<td>190 ± 2</td>
<td>316 ± 5†</td>
<td>22.1 ± 0.63</td>
<td>0.070 ± 0.002</td>
<td>913 ± 24</td>
<td>2.88 ± 0.05</td>
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</tbody>
</table>

Values are means ± SE; n = 10 for chronically exposed rats, n = 11 for acute and control. Prealtitude weight for acute animals was 309.8 ± 2.7 g. †Significant difference from acute animals, P < 0.05. ‡Significant difference from control animals, P < 0.05. ‡Significant difference from chronic animals, P < 0.05.
B). CPT-I activity was not affected by altitude exposure in the soleus muscle or the liver (Fig. 1).

**β-HAD activity.** β-HAD activity was significantly depressed in the hearts of chronically exposed animals compared with controls (7.0 ± 0.7 vs. 8.7 ± 0.4 μmol·g⁻¹·min⁻¹, respectively). No difference between acute (9.3 ± 0.8 μmol·g⁻¹·min⁻¹) and control animals was found in the heart for β-HAD activity (Fig. 2A).

Liver β-HAD activity was also significantly decreased in the chronic and acute animals compared with the control group (1.2 ± 0.1 and 1.4 ± 0.1 vs. 2.2 ± 0.5 μmol·g⁻¹·min⁻¹, respectively) (Fig. 2B). Quadriceps β-HAD activity was reduced for the chronic animals only compared with controls (9.1 ± 0.1, 12.0 ± 0.1, and 13.2 ± 0.2 μmol·g⁻¹·min⁻¹ for chronic, acute, and control groups, respectively) (Fig. 2B).

**DISCUSSION**

As there has been much debate about the alterations in substrate utilization in response to altitude exposure (6, 7, 14, 15, 25, 34, 35), this study investigated alterations in fat metabolism at key controlling steps. The main findings were that 1) CPT-I activity was significantly depressed in the hearts of animals exposed to both acute and chronic altitude compared with controls and 2) β-HAD activity was decreased in the heart and liver after chronic exposure. These findings

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**Table 2. Citrate synthase activity in heart, liver, and skeletal muscle**

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<thead>
<tr>
<th></th>
<th>Chronic</th>
<th>Acute</th>
<th>Control</th>
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<tr>
<td>Heart</td>
<td>28.5 ± 4.8</td>
<td>32.3 ± 1.6</td>
<td>33.9 ± 2.4</td>
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<tr>
<td>Liver</td>
<td>9.3 ± 0.6</td>
<td>10.1 ± 0.7</td>
<td>9.3 ± 0.4</td>
</tr>
<tr>
<td>EDL</td>
<td>15.0 ± 1.5</td>
<td>17.4 ± 1.1</td>
<td>16.8 ± 1.3</td>
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Values are means ± SE; n = 10 for acclimatized rats, 11 for control and acute. All values are expressed as pmol·g⁻¹·min⁻¹. EDL, extensor digitorum longus muscle.
are consistent with previous tracer studies in humans that have shown an elevation in CHO metabolism with altitude exposure and an associated depression of fat utilization.

Higher concentrations of FFA have frequently been associated with increased lipid metabolism, thus implying elevated fat metabolism at altitude (25, 34, 35). However, the increase in plasma FFA does not necessarily indicate an increased rate of oxidation of fats as a substrate. It is generally accepted that fatty acid oxidation is largely regulated by the activity of CPT-I and the transport of long chain fatty acyl groups into the mitochondria (4, 22, 32). The present investigation is the first to examine the effects of altitude exposure on CPT-I activity. We observed a significant reduction in CPT-I activity in the heart for both acute and chronic high-altitude exposure animals compared with controls (Fig. 1A). A similar pattern was also demonstrated for the EDL muscle. This downregulation of CPT-I suggests a decreased capacity for fatty acid oxidation after altitude exposure and also suggests a subsequent increase in dependence on CHO as a fuel. In support of this observation, Bass et al. (2) reported that the myocardium of rats exposed to high altitude demonstrated a reduced ability to utilize fatty acids after prolonged exposure. Furthermore, in men exposed to a similar altitude (4,300 m), Roberts et al. (25) found that, despite increases in plasma FFA levels, altitude resulted in a significant decrease in FFA uptake and glycerol release by resting muscle. This decreased reliance on fat as a fuel was accompanied by an increased dependence on blood glucose. The results reported by Roberts et al. (25) are consistent with the downregulation of enzymes of fat metabolism studied in the present investigation.

Other studies have supported the observation that altitude and hypoxia alter regulation of substrate metabolism to favor CHO utilization (6, 8, 16, 17, 25, 26). Brooks et al. (6) found that exposure to altitude resulted in an increased rate of blood glucose uptake by muscle. This was recently supported by Roberts et al. (26), who, using isotopic tracers, concluded that altitude exposure increases muscle glucose utilization. It has been shown in perfused rat hindlimb muscles that hypoxia causes an increase in glucose transporters in the plasma membrane, thereby stimulating glucose transport in muscle (9). On the basis of these reports on enhanced CHO dependence at high altitude, it was postulated that this adaptation would assist in maintaining homeostasis by optimizing the energy yield per unit of oxygen (17, 20). CHO oxidation generates more ATP per molecule of oxygen than fat oxidation; thus, under conditions of limited oxygen availability, it would be more efficient to increase the percentage of energy derived from CHO sources (17, 20). Again, these studies are consistent with the reported downregulation of CPT-I and β-HAD activity in the rat tissues of the present investigation.

β-HAD activity was significantly depressed in both the hearts and livers of the chronically exposed animals. A reduction of β-HAD activity was previously noted in heart and skeletal muscle (2, 19); however, these results were obtained after exposure to altitude equivalent to Mt. Everest (altitude peaking at 8,000 m, remaining over 5,300 m for 5–6 wk), which causes muscle and mitochondrial damage. It can be inferred, by the consistent activity of the mitochondrial enzyme citrate synthase, that muscle and mitochondrial damage were not factors in this study. Furthermore, cardiac hypertrophy renders it unlikely the cardiac muscle was damaged by hypoxia. A study by Bass et al. (2) utilized 24 or 72 intermittent exposures of 4–8 h/day, gradually reaching an altitude equivalent to 7,000 m. A depression of β-HAD activity in both ventricles after 72 exposures, without alteration in citrate synthase activity, was found.

Not all studies demonstrate a decrease in fat utilization with altitude exposure. In fact, several studies suggest that acclimatization to altitude elicits an increase in the capacity to utilize fats as a primary energy source (14, 28, 34, 35). Several studies demonstrated that acclimatization to high altitude results in glycogen sparing, which is also associated with elevated levels of plasma FFA. Together these results are interpreted as enhanced utilization of fats, although no direct measurements of fat metabolism were made. Recently, it was found that blood glucose utilization rates and whole body CHO utilization were significantly lower in women after 10 days acclimatization to 4,300 m compared with sea-level values (3). In that study, a greater reliance on fats was further supported by significantly lower RER values, both at rest and during submaximal exercise. Finally, McClelland et al. (23) were unable to find any increase in CHO utilization in female rats acclimatized to high altitude.

Discrepancies between studies pertaining to differences in substrate selection at altitude may be explained, in part, by gender differences. Our study, demonstrating downregulation of key enzymes in fat metabolism with altitude exposure, used male rats. The tracer studies by Brooks et al. (6, 7) and Roberts et al. (25, 26), which indicate increased CHO utilization and decreased fat utilization at 4,300 m, were conducted on men. The studies that suggest either no change in or a decrease in CHO utilization at altitude were performed primarily with female subjects. A number of studies clearly indicate that, in response to a variety of conditions (exercise, hypoglycemia, altitude), women rely more on fats as a fuel compared with men (1, 11, 13, 18, 29, 30). Because the ovarian hormones estrogen and progesterone can have both direct and indirect effects on both CHO and fat metabolism, it is possible that substrate selection at altitude may be gender dependent.

Altitude exposure, particularly acute exposure, can induce anorexia; this fasting then induces changes in substrate selection, generally increasing the body’s reliance on fat metabolism. In the previous studies on men reporting glycogen sparing and elevated plasma FFA levels at 4,300 m, subjects lost several kilograms of body weight (34). Thus findings related to substrate selection were confounded by a negative energy bal-
ance. Most likely, the acutely exposed animals of the present study were fasting, as shown by the overnight decrease of 7 g in body weight. Modulation of muscle and liver CPT-I differs between the tissues. Liver CPT-I activity demonstrates an increase in activity in a fasted state (4, 10), whereas the CPT-I activity found in heart mitochondria seems unaffected by fed or fasted status (10, 12, 24). However, the findings in the present study of either no change (liver) or a decrease (heart and EDL) in CPT-I activity suggest that the anorexia induced by acute altitude exposure did not significantly alter the effects of altitude. Thus a lack of increased CPT-I activity in the livers of altitude-exposed rats, even acute, demonstrates an effect of altitude that is independent of fed or fasting state. It is unlikely that acclimatized rats were in a fasting state, as their weights were equivalent to the acute rats, indicating that weight stabilized after the initial 24-h weight loss.

Finally, a more convincing argument can be made for the decreased reliance on fat metabolism during acclimatization if measurements of whole body had been performed on these animals. Logistically, this was not feasible in the hypobaric chamber in which the rats were housed. However, we have cited other investigations that support a greater reliance on CHO metabolism during the acclimatization process in men (6, 7, 25, 26). It remains unknown whether acclimatization elicits a preferential increase in CHO metabolism in vivo by male rats. Our purpose was to examine possible mechanisms for potential substrate alterations as they were specifically related to key enzymes in fat metabolism.

It is important to note that the CPT-I activity in the soleus muscle was ~15–30% of the activity measured in the EDL. The soleus is a slow oxidative muscle that is richer in mitochondria than the fast glycolytic EDL. Thus one would expect to see a higher activity of CPT-I in the soleus muscle. Tikkanen et al. (31) compared CPT-I activity between fast- and slow-twitch muscles and found similar values among the rat gastrocnemius (mixed), the tibialis anterior (fast glycolytic), and the soleus (slow oxidative) muscles (0.55 ± 0.07, 0.53 ± 0.12, and 0.70 ± 0.10 μmol·g protein⁻¹·min⁻¹, respectively), despite the usual two- to threefold differences for α-ketoglutarate dehydrogenase and PFK between the three muscles. Tikkanen and co-workers (31) did not assay the EDL. Kerner et al. (21) noted total CPT activity (measured in the direction of palmitoylcarnitine formation) was ~59% higher in the soleus than in the EDL of rats; however, they used a spectrophotometric assay and did not separate the activity of CPT-I from the activity of CPT-II. In vivo, CPT-II catalyzes the net flux of long-chain fatty acylcarnitines to long-chain fatty acyl-CoAs; however, in vitro, the enzyme also catalyzes the reverse reaction. Unlike CPT-I, CPT-II is not inhibited by malonyl-CoA. In the present study, we used the radioenzymatic assay of Fiol et al. (12) and calculated the maximal activity of CPT-I as the rate of conversion of palmitoyl-CoA to palmitoylcarnitine minus the residual CPT-II activity measured by completely inhibiting CPT-I with malonyl-CoA. In any case, we were surprised by the differences between the soleus and EDL in the present study; therefore, we reexamined our observation by repeating the CPT-I assays on an additional six male Sprague-Dawley rats. We obtained similar results for the soleus and EDL muscles (0.13 ± 0.03 and 0.77 ± 0.11 μmol·g⁻¹·min⁻¹, respectively). Thus our results show that the activity of CPT-I is higher in the predominantly fast-twitch EDL than in the slow-twitch soleus.

In summary, the results of the present investigation suggest that, in response to a simulated altitude of 4,300 m, male rats demonstrated downregulation of CPT-I activity in the heart and EDL and of β-HAD activity in the heart and liver. These data are consistent with the concept of a greater reliance on CHO during the altitude conditions studied.

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