

Similar carbohydrate but enhanced lactate utilization during exercise after 9 wk of acclimatization to 5,620 m

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Van Hall, G., J. A. L. Calbet, H. Søndergaard, and B. Saltin. Similar carbohydrate but enhanced lactate utilization during exercise after 9 wk of acclimatization to 5,620 m. *Am J Physiol Endocrinol Metab* 283: E1203–E1213, 2002. First published July 30, 2002; 10.1152/ajpendo.00134.2001.—We hypothesized that reliance on lactate as a means of energy distribution is higher after a prolonged period of acclimatization (9 wk) than it is at sea level due to a higher lactate R_a and disposal from active skeletal muscle. To evaluate this hypothesis, six Danish lowlanders (25 ± 2 yr) were studied at rest and during 20 min of bicycle exercise at 146 W at sea level (SL) and after 9 wk of acclimatization to 5,260 m (Alt). Whole body glucose R_a was similar at SL and Alt at rest and during exercise. Lactate R_a was also similar for the two conditions at rest; however, during exercise, lactate R_a was substantially lower at SL ($65 \mu\text{mol} \cdot \text{min}^{-1} \cdot \text{kg body wt}^{-1}$) than it was at Alt ($150 \mu\text{mol} \cdot \text{min}^{-1} \cdot \text{kg body wt}^{-1}$) at the same exercise intensity. During exercise, net lactate release was ~ 6 -fold at Alt compared with SL, and related to this, tracer-calculated leg lactate uptake and release were both 3- or 4-fold higher at Alt compared with SL. The contribution of the two legs to glucose disposal was similar at SL and Alt; however, the contribution of the two legs to lactate R_a was significantly lower at rest and during exercise at SL (27 and 81%) than it was at Alt (45 and 123%). In conclusion, at rest and during exercise at the same absolute workload, CHO and blood glucose utilization were similar at SL and at Alt. Leg net lactate release was severalfold higher, and the contribution of leg lactate release to whole body lactate R_a was higher at Alt compared with SL. During exercise, the relative contribution of lactate oxidation to whole body CHO oxidation was substantially higher at Alt compared with SL as a result of increased uptake and subsequent oxidation of lactate by the active skeletal muscles.

glucose; isotopes; [$1\text{-}^{13}\text{C}$]lactate

CARBOHYDRATE AND LACTATE METABOLISM is altered by hypoxia. Carbohydrate oxidation under hypoxic conditions has been suggested to be the preferable metabolic pathway during exercise, because it provides the highest ATP yield per mole of oxygen (3, 15, 16). Thus any increase in the percentage of energy derived from carbohydrate sources will result in a more economical use of oxygen. Indeed, results from studies in high-altitude

natives (17) and lowlanders exposed to high altitude (5, 27) show a shift toward increased blood glucose utilization relative to normoxic conditions. However, McClelland et al. (23) reported that rats acclimatized to severe hypoxia did not increase carbohydrate utilization compared with normoxic conditions. Moreover, women acclimatized for 10 days to 4,300 m had a tendency for a lower contribution of carbohydrate oxidation to total energy expenditure during exercise, and they had a similar blood glucose disposal compared with sea level (2). In addition, Young et al. (35) suggested that, after chronic hypoxia exposure, increased mobilization and use of free fatty acids during exercise resulted in sparing of muscle glycogen. Thus there is no consensus in the literature with regard to metabolic fuel selection under hypoxic conditions. Some of the differences observed in fuel utilization after altitude exposure have been attributed to cachexia and energy deficiency (5, 8), gender (2), or limited carbohydrate stores (23).

With regard to lactate, it is well known that the blood lactate concentration at a given submaximal workload is larger under acute exposure to hypoxia compared with sea level. After several weeks of acclimatization to hypoxia, the blood lactate response to a given workload is blunted, i.e., the “lactate paradox” (33), although this concept has been challenged (22, 32). So far, only one study is available describing in more detail whole body and muscle carbohydrate and lactate metabolism at rest and during exercise after a relatively short period of 3 wk of acclimatization to altitude (3, 4, 5). In addition, a careful evaluation of the data reveals that the field study suffered from variability in the measurements, which potentially might explain the “disbalance” in glucose and lactate whole body vs. leg glucose and lactate utilization/production (3). Despite these limitations, it was concluded that active skeletal muscle as a source of lactate was not affected by acclimatization, and it was suggested that other tissues besides active skeletal muscle participate in producing the blood lactate concentration responses described as the lactate paradox (7). In addition, it was concluded that blood lactate was less important as a

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means of distributing energy between glucose/glycogen and complete oxidation when acclimated (4). However, evidence has been presented that the blunted blood lactate concentration in the course of acclimatization is transient. After 7 wk of acclimatization to high altitude (5,400 m), the blood lactate concentration was similar to what was observed in acute hypoxia (22). Furthermore, during submaximal and maximal bicycle work, net lactate release from the active legs has been reported to be lower with acute compared with chronic exposure to hypoxia (32). Therefore, we hypothesized that, after a prolonged period of acclimatization, the reliance on lactate as a means of distributing carbohydrate potential energy is higher than it is at sea level due to a higher lactate appearance and disposal from active skeletal muscle.

METHODS

Subjects. Six healthy, physically active subjects (5 males, 1 female, 25 ± 2 yr) residing in the Copenhagen area participated in the study. The subjects were informed about the possible risks and discomfort involved before their voluntary consent to participate was given. The study was performed according to the Declaration of Helsinki and was approved by the Ethics Committee of the Copenhagen and Frederiksberg Communities, Denmark.

Study periods. Each subject underwent two moderate-intensity bicycle exercise tests on a cycle ergometer, at 5,260 m after 9 wk of acclimatization (Chacaltaya, Bolivia) and at sea level (~ 0 m) in Copenhagen. The sea-level trial was performed 10 mo after return from Bolivia. The reason for performing the trial at sea level a long time after the sojourn was due to the following, in part ethical reasons. A slightly larger number of subjects left for Bolivia than those finally studied. Ahead of departure, it was difficult to foresee who would be able to handle the exposure and primitive life at 5,260 m. This experiment, as well as another study investigating the lactate paradox during incremental exercise until exhaustion (32), was quite invasive and should not be performed unless the results are going to be used. Thus the studies at sea level had to be performed after a prolonged period of deacclimatization and when the subjects had returned to their normal habits, i.e., activity level. Therefore, sea-level trials were performed in the same month as, but a year later than, when they left for Bolivia. Physical activity level, maximal oxygen uptake ($\dot{V}O_{2\max}$), body weight, and forearm venous blood lactate concentration at submaximal and maximal work were similar compared with sea-level values before the altitude studies (32).

Acclimatization. Upon arrival in Bolivia, the subjects spent ~ 5 days in La Paz ($\sim 3,700$ m). Subsequently, they went up to the laboratory at Chacaltaya (5,260 m) for 1 day and returned to La Paz for the night, followed by ~ 2 days at a base camp (4,700 m) before climbing Mt. Potosi (6,088 m). The subjects then moved to the laboratory at Chacaltaya and, except for a 4-day climb of Mt. Illimani (6,462 m), stayed there until weeks 9–10, when they participated in the studies. All subjects were given oral iron supplementation during the first 5–6 wk following their arrival in La Paz.

Protocol for continuous cycle exercise at a constant moderate workload. Each subject underwent 20 min of cycle exercise (Monark 818, Varberg, Sweden) at 146 ± 14 W at sea level breathing ambient air (Sea Level) and 40 min of cycle exercise at the same workload at 5,260 m after 9 wk of acclimatization (Altitude), the first 20 min breathing ambi-

ent air, immediately followed by 20 min of breathing 47% O_2 in N_2 (Altitude, normoxia). The workload at sea level was $46 \pm 2\%$ of the sea level $\dot{V}O_{2\max}$. At altitude, the workload while breathing ambient air was $82 \pm 2\%$ of the altitude $\dot{V}O_{2\max}$ and an estimated $62 \pm 4\%$ of the altitude $\dot{V}O_{2\max}$ while breathing 47% O_2 in N_2 . $\dot{V}O_{2\max}$ was determined during an incremental bicycle test, as described elsewhere (32). A relatively high workload at altitude and, as a consequence, a relatively short exercise time were chosen in the present study to get relatively large arterial-femoral venous differences for concentration and tracer measurements of lactate and glucose, thereby reducing variability in the leg exchange data. This was especially important for the Sea Level trial, carried out at the same absolute workload, because leg net lactate release and leg lactate uptake and release can be expected to be lower the lower the exercise intensity. On the day of the experiment, the subjects reported to the laboratory at 8 AM. The subjects changed clothes and remained supine for the next 3 h. After 10 min in the supine position, catheters were placed in a femoral artery and vein under local anesthesia (lidocaine, 20 mg/ml) for blood sampling and blood flow measurements. The tip of the each catheter was advanced 6 cm proximal to the inguinal ligament. The arterial catheter was 20 G and 20 cm (Ohmeda, Swindon, UK), and the venous catheter was a radiopack TFE catheter with side holes (Cook, Bjaevershou, Denmark). A thermistor was inserted through the venous catheter for blood flow measurements by the constant infusion thermomodulation technique (1). Thirty minutes after placement of the catheters, breath and blood samples were obtained for assessment of background enrichment of breath and blood CO_2 and blood lactate and glucose. Immediately after the background samples had been obtained, a primed constant infusion of [$1-^{13}C$]lactate ($1.4 \mu\text{mol}\cdot\text{min}^{-1}\cdot\text{kg body wt}^{-1}$, prime $22 \mu\text{mol/kg body wt}$) and [$6,6-^2H_2$]glucose ($0.25 \mu\text{mol}\cdot\text{min}^{-1}\cdot\text{kg body wt}^{-1}$, prime $17 \mu\text{mol/kg body wt}$) was started, along with a prime of bicarbonate ($1.5 \mu\text{mol/kg body wt}$). For each individual, the actual rate of infusion for each tracer was determined from the tracer concentration and infusion rate of the pump. All isotopes were purchased from Cambridge Isotope Laboratories (Andover, MA) and were dissolved in 0.9% sterile saline and passed through a 0.2- μm filter into a 250-ml sterile saline bag immediately before infusion. At minutes 90, 105, and 120 after the start of the constant infusion, blood samples were taken for analysis of the concentration and enrichment of lactate and glucose, and the blood flow was measured. In addition, the blood samples were analyzed for hematocrit, hemoglobin, and O_2 saturation (OSM3 hemoxymeter, Radiometer, Copenhagen, Denmark), and blood pH, O_2 , and CO_2 tension (ABL5, Radiometer). While the blood samples were being taken, indirect calorimetry measurements were performed (Medgraphics, St. Paul, MN) and, in a separate 5-l bag at rest or 20-l bag during exercise (Hans Rudolph, Kansas City, KS), expired air was collected, from which a 20-ml vacutainer was filled via a needle for measuring CO_2 enrichment. At the start of exercise, the infusion rate of [$1-^{13}C$]lactate was increased threefold (Sea Level) or fourfold (chronic hypoxia and acute normoxia), whereas that for [$6,6-^2H_2$]glucose was increased twofold for each of the three conditions. Blood and breath samples were taken and blood flow measured every 5 min from the start of exercise.

Analytical procedures. Part of the blood sample was collected in ice-cold tubes that contained 10 μl of 0.33 M EDTA per milliliter of blood. The blood sample was collected in an ice-cold tube that contained 28 μl of 70% perchloric acid ($HClO_4$) per milliliter of blood. Blood samples were vortex-mixed and centrifuged at 4°C for 10 min. The $HClO_4$ extract

was stored at -50°C . Before analysis, the HClO_4 extracts were neutralized with $22\ \mu\text{l}$ of 2 M potassium bicarbonate (KHCO_3) per $100\ \mu\text{l}$ of extract and analyzed for the concentration and enrichment of lactate and glucose. Lactate and glucose enrichments were measured by gas chromatography-mass spectrometry (GC-MS, Finnigan Automass II and III, Paris, France). In preparation of the GC-MS analysis, samples were processed to make a trimethylsilyl derivative of lactate and a butylboronic acid acetate derivative of glucose. For the preparation of the trimethylsilyl derivative of lactate, 1 ml of ethanol was added to $200\ \mu\text{l}$ of blood extracts and centrifuged for 10 min, and the supernatant was transferred to a new screw-capped tube and evaporated to dryness under a stream of nitrogen. For lactate enrichment, $50\ \mu\text{l}$ of pyridine and $50\ \mu\text{l}$ of *N,O*-bis(trimethylsilyl)trifluoroacetamide with 1% trimethylchlorosilan (Pierce, Rockford, IL) were added, and the solution was incubated for 30 min at room temperature. The lactate enrichment was determined by split injection (ratio 1:25) of $1\ \mu\text{l}$ into the GC-MS (GC column, CP-SIL 8CB, Chrompack, Middelburg, The Netherlands). The isotopic enrichment was determined using electron impact ionization, with ions at mass-to-charge ratio (m/z) 219 and 220, representing the molecular ions of unlabeled and labeled derivatives, respectively.

For determination of glucose enrichment, $250\ \mu\text{l}$ of water and 3 ml of chloroform-methanol (2.3:1) were added to $150\ \mu\text{l}$ of blood extract, vortex-mixed for 10 min, and centrifuged at 4°C for 15 min. The upper layer was washed once by adding 1 ml of water (pH 2, with HCl) and 2 ml of chloroform and centrifuged at 4°C for 15 min. The upper layer was then evaporated to dryness, after which $250\ \mu\text{l}$ of butylboronic acid (100 mg/10 ml pyridine) were added to the dry residue and incubated for 30 min at 95°C . After the addition of $250\ \mu\text{l}$ of acetic anhydride and incubation for 90 min at room temperature, the solution was evaporated to dryness and redissolved in $100\ \mu\text{l}$ of ethyl acetate. The deuterium enrichment of glucose was determined by split injection (ratio 1:30) of $1\ \mu\text{l}$ into the GC-MS (GC column, CP-SIL 8CB, Chrompack). The isotopic enrichment was determined using electron impact ionization. Ions at m/z 297 and 299, representing the molecular ions of unlabeled and labeled glucose derivatives, respectively, were selectively monitored, and their corresponding peaks were integrated.

Samples of arterial and venous blood and expired breath for measurement of ^{13}C enrichment were analyzed by gas chromatography-isotope ratio mass spectrometry (GC-IRMS; Delta^{plus}, Finnigan MAT, Bremen, Germany). The ^{13}C -to- ^{12}C ratio was determined by split injection (ratio 1:4) of $20\ \mu\text{l}$ of the expired air on the GC-IRMS (GC-column, Poraplot Q, Chrompack). For the determination of blood CO_2 enrichment, $0.5\ \text{ml}$ of 2.5 M phosphoric acid was added to $0.5\ \text{ml}$ of blood in a 10-ml vacutainer to release all of the CO_2 . The tubes were brought to pressure with pure helium. The ^{13}C -to- ^{12}C ratio was determined by split injection (ratio 1:10) of $20\ \mu\text{l}$ of the headspace on the GC-IRMS.

Calculations. The measured pH, PO_2 , and PCO_2 were corrected for temperature by using the blood temperature as measured in the femoral vein. Blood CO_2 content was calculated according to Douglas et al. (11a).

Systemic and leg carbohydrate oxidation rates were calculated using stoichiometric equations (12, 26). Carbohydrate oxidation in micromoles per minute was determined by converting the rate of carbohydrate oxidation to its molecular equivalent by dividing it by the glucose molecular weight of $180\ \text{g/mol}$

$$\text{carbohydrate oxidation} = \frac{4.585 \times \dot{V}\text{CO}_2 - 3.226 \dot{V}\text{O}_2}{180}$$

Whole body tracer measurements. The whole body rate of appearance (R_a) at rest of lactate and glucose was calculated using the steady-state equation

$$R_a = R_d = \frac{F}{E}$$

where F is the isotopic infusion rate ($\mu\text{mol/min}$) and E is the arterial whole blood isotope enrichment in tracer-to-tracee ratio (TTR). Whole body measurements of the R_a and rate of disappearance (R_d) during exercise of lactate and glucose were calculated using the non-steady-state equations of Steele (30) adapted for stable isotopes (34)

$$R_a = \frac{F - pV[(C_2 + C_1)/2][(E_2 - E_1)/(t_2 - t_1)]}{(E_2 + E_1)/2}$$

$$R_d = R_a - pV[(C_2 - C_1)/(t_2 - t_1)]$$

where E_1 and E_2 are the arterial whole blood isotope enrichments at sample times 1 (t_1) and 2 (t_2) (min), respectively; C_1 and C_2 are the arterial whole blood concentrations at t_1 and t_2 (mmol/l), respectively; and pV is the volume of distribution of 0.10 and 0.18 l/kg body wt for lactate and glucose, respectively (29). Although pV for both lactate and glucose has been used previously in studies of lactate and glucose kinetics, the assumption that this value is appropriate for resting and exercise conditions is obviated by the presence of a near-steady-state condition. Lactate oxidation rates were calculated

$$\text{lactate oxidation rate} = \frac{E_{\text{CO}_2} \times V_{\text{CO}_2}}{E \times k}$$

$$\%R_d \text{ lactate oxidized} = \frac{\text{lactate oxidation rate}}{R_d} \times 100\%$$

where E_{CO_2} is the breath ^{13}C -to- ^{12}C ratio; V_{CO_2} is the volume of CO_2 ($\mu\text{mol/min}$), and the conversion factor is $1\ \text{mol}\ \text{CO}_2 = 22.4\ \text{l}$; and k is the correction factor for retention of $^{13}\text{CO}_2$ in the bicarbonate pools [0.80 at rest and 1.00 during exercise (30)].

Leg tracer measurements.

$$\text{net leg balance} = (C_a - C_v) \times \text{blood flow}$$

$$\text{leg fractional lactate extraction} = \frac{(C_a \times E_a) - (C_v \times E_v)}{C_a \times E_a}$$

$$\text{leg lactate uptake} = \text{leg fractional lactate extraction} \times C_a \times \text{blood flow}$$

$$\text{leg lactate release} = \text{leg lactate uptake} - \text{net lactate balance}$$

%lactate uptake oxidized

$$= \frac{(C_{v,\text{CO}_2} \times E_{v,\text{CO}_2}) - (C_{a,\text{CO}_2} \times E_{a,\text{CO}_2})}{(C_a \times E_a) - (C_v \times E_v)} \times 100\%$$

Blood flow was used to calculate net leg uptake of lactate and glucose. C_{v,CO_2} , C_{a,CO_2} and E_{v,CO_2} , E_{a,CO_2} are the blood CO_2 concentration and enrichment in TTR in the femoral vein and artery, respectively. C_a , C_v and E_a , E_v are the blood lactate concentration and enrichment in TTR in the femoral artery and vein, respectively (Table 1).

Statistical analysis. All data are expressed as means \pm SE of six subjects. The nonparametric Wilcoxon signed rank test

Table 1. Summary of variables at rest and the moment of exhaustion during bicycle exercise at 146 W at sea level and at altitude

	Sea Level		9 wk at 5,260 m		
	Rest	20-min Exercise	Altitude		Normoxia
			Rest	20-min Exercise	20-min Exercise
PCO _{2,a} , mmHg	39 ± 1	41 ± 1	22 ± 1*	20 ± 1*	28 ± 1†‡
PCO _{2,v} , mmHg	47 ± 1	67 ± 1	30 ± 1*	42 ± 1*	57 ± 1†‡
PO _{2,v} , mmHg	98 ± 3	97 ± 2	51 ± 2*	43 ± 3*	152 ± 4†‡
PO _{2,v} , mmHg	31 ± 2	21 ± 7	28 ± 1	13 ± 2*	20 ± 1‡
O ₂ sat _a , %	98 ± 0.3	97 ± 0.2	86 ± 1*	75 ± 2*	98 ± 0‡
O ₂ sat _v , %	64 ± 3	20 ± 2	49 ± 3*	9 ± 1*	15 ± 2‡
pH _a	7.421 ± 0.007	7.400 ± 0.010	7.476 ± 0.005*	7.395 ± 0.021	7.383 ± 0.011
pH _v	7.386 ± 0.007	7.294 ± 0.008	7.432 ± 0.006*	7.288 ± 0.019	7.262 ± 0.011
HCO _{3,a} , mmol/l	24.9 ± 0.6	26.7 ± 0.5	15.8 ± 0.6*	11.5 ± 0.8*	15.8 ± 0.8†‡
HCO _{3,v} , mmol/l	27.8 ± 0.6	34.2 ± 0.4	19.6 ± 0.6*	18.9 ± 1.1*	24.3 ± 0.7†‡
Blood flow, l/min	0.4 ± 0.1	4.2 ± 0.4	0.5 ± 0.3	4.4 ± 0.3	3.8 ± 0.3
Leg O ₂ uptake, ml/min	39 ± 5	674 ± 59	47 ± 6	789 ± 88	814 ± 73

Values are means ± SE of 6 subjects. Altitude, at 5,260 m after 9 wk of acclimatization; PCO_{2,a}, arterial PCO₂; PCO_{2,v}, femoral venous PCO₂; PO_{2,a}, arterial PO₂; PO_{2,v}, femoral venous PO₂; O₂sat_a, arterial O₂ saturation; O₂sat_v, femoral venous O₂ saturation; pH_a, arterial pH; pH_v, femoral venous pH; HCO_{3,a}, arterial plasma bicarbonate concentration; HCO_{3,v}, venous plasma bicarbonate concentration. *Significant differences between altitude and sea level, †significant differences between 9 wk at 5,260 m breathing normoxia and sea level, ‡significant differences between 9 wk at 5,260 m breathing normoxia and altitude.

was applied to determine differences between data obtained at each time point. Statistical significance was set at $P < 0.05$.

RESULTS

Body mass, hemoglobin, and $\dot{V}O_2$. Body weight of the subjects at sea level was 74 ± 3 kg before acclimatization and 76 ± 4 kg 10 mo after acclimatization when the Sea Level trials were carried out. Despite adequate and nutritional food supply, the subjects had under-eaten, as they all lost weight (7.3 ± 1.0 kg) during their 9-wk stay at 5,260 m. Magnetic resonance imaging revealed that the leg lean area was reduced by an average of $\sim 9\%$ (36). $\dot{V}O_{2\max}$ was 4.3 l/min at sea level and 2.8 l/min at altitude. The subject's maximal performance was maintained at Altitude despite the loss in body weight. In fact, performance was even increased when expressed per kilogram of body weight, as indicated by the $\dot{V}O_{2\max}$ with acute exposure to hypoxia equivalent to 5,260 m ($35 \text{ ml} \cdot \text{min}^{-1} \cdot \text{kg body wt}^{-1}$) and after 9 wk at 5,260 m ($40 \text{ ml} \cdot \text{min}^{-1} \cdot \text{kg body wt}^{-1}$) (32). The 20 min of continuous cycle work at 146 W was $46 \pm 2\%$ of sea level $\dot{V}O_{2\max}$. After 9 wk of acclimatization, the workload was $82 \pm 2\%$ of altitude $\dot{V}O_{2\max}$, and altitude breathing normoxia was an estimated $62 \pm 4\%$ of the altitude breathing normoxia $\dot{V}O_{2\max}$. Hemoglobin concentration increased from 14.3 ± 0.5 g/dl at sea level to 18.7 ± 0.5 g/dl at altitude with a corresponding increase in hematocrit.

Whole body and leg glucose metabolism. The arterial glucose concentration at rest was significantly higher at sea level than at altitude (Fig. 1). After 10 min of exercise, however, this changed, and arterial glucose concentration at sea level was lower than at altitude. Despite the difference in arterial glucose concentration, the R_a was similar, as was the net glucose uptake by the active leg.

Whole body lactate metabolism. At rest, arterial lactate concentration and lactate R_a were similar at sea level and altitude (Fig. 2). At the onset of exercise at a constant workload of 146 W, the arterial lactate concentration increased nearly twofold at sea level, whereas at altitude the lactate concentration increased more than sixfold. The arterial lactate concentration decreased with exercise duration, although quantitatively more so at altitude. With normoxia, the arterial lactate concentration immediately decreased substantially, and after 20 min of exercise under this condition, lactate concentration was lower than after 20 min of exercise at sea level. The same pattern of changes was observed for the systemic lactate R_a ; however, the absolute changes in R_a from rest to exercise were far more pronounced, with 4- and 11-fold increases at sea level and at altitude, respectively. The percentage of [^{13}C]lactate that disappeared from the circulation and was recovered as $^{13}\text{CO}_2$ in the breath at rest was similar with 52–63% at sea level and altitude. During exercise at sea level, the amount of lactate leaving the circulation was nearly completely oxidized; however, at altitude, this was significantly lower (80%). Nearly the same differences were observed for the amount of lactate taken up by the active leg and recovered as $^{13}\text{CO}_2$ in the blood, suggesting that the active skeletal muscle was responsible for a lower percentage of lactate being oxidized at altitude. In contrast, the relative contribution of lactate oxidation to carbohydrate oxidation was significantly higher during exercise at altitude than it was at sea level (Table 2).

Leg lactate metabolism. At rest, no significant net uptake or release of lactate from the leg could be observed (Fig. 3). During exercise at sea level, the net lactate release gradually decreased with exercise duration, and, after 20 min of exercise, a small net lactate release was observed in three subjects and a

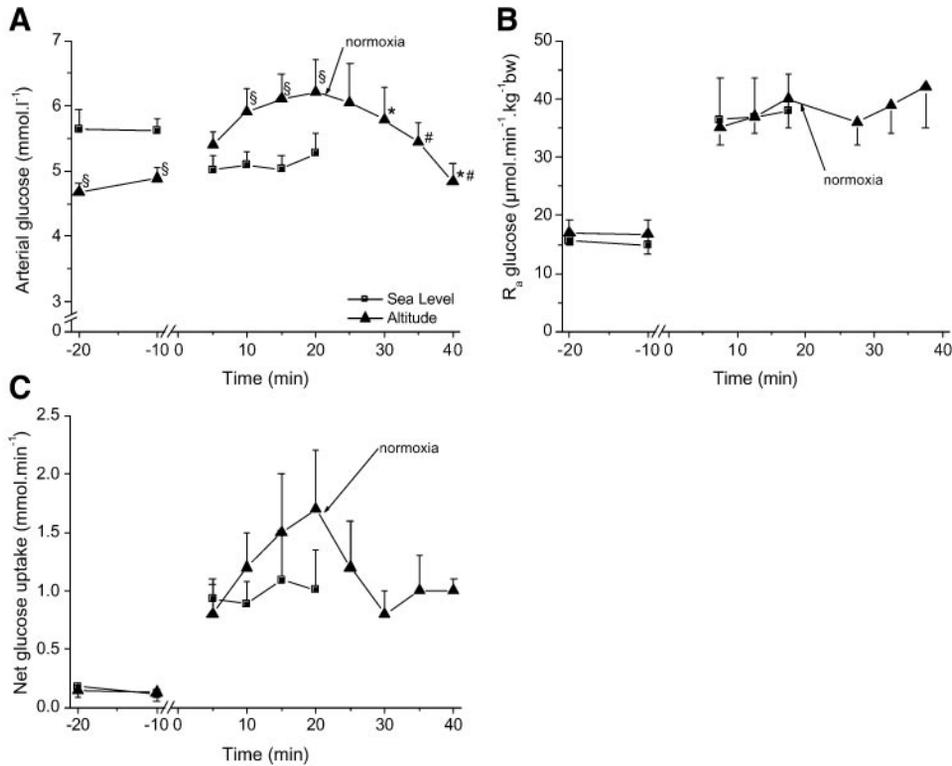


Fig. 1. Arterial glucose concentration (A), whole body rate of appearance (R_a) of glucose (B), and leg glucose uptake (C) at rest and during 20 min of bicycle exercise at sea level (Sea Level) and at 5,260 m after 9 wk of acclimatization (Altitude) breathing ambient air immediately followed by 20 min at the same workload breathing a normoxic gas mixture (normoxia). Values are means ± SE of 6 subjects. §Significant differences between altitude and sea level; *significant differences between altitude breathing normoxia and sea level; #significant differences between normoxia and ambient air at altitude.

small net lactate uptake in the other three subjects. At altitude, a more than sixfold higher net lactate release from the leg was observed compared with sea level, with a marked decrease from 5 to 20 min of exercise. However, the decrease in net lactate release was not directly reflected in a fall in the arte-

rial lactate concentration in either condition. The tracer-calculated lactate uptake and release were 0.16–0.18 mmol/min at rest under both conditions. With exercise, the leg lactate uptake increased ~13-fold at sea level and ~32-fold at altitude. The lactate uptake remained nearly constant during 20 min of

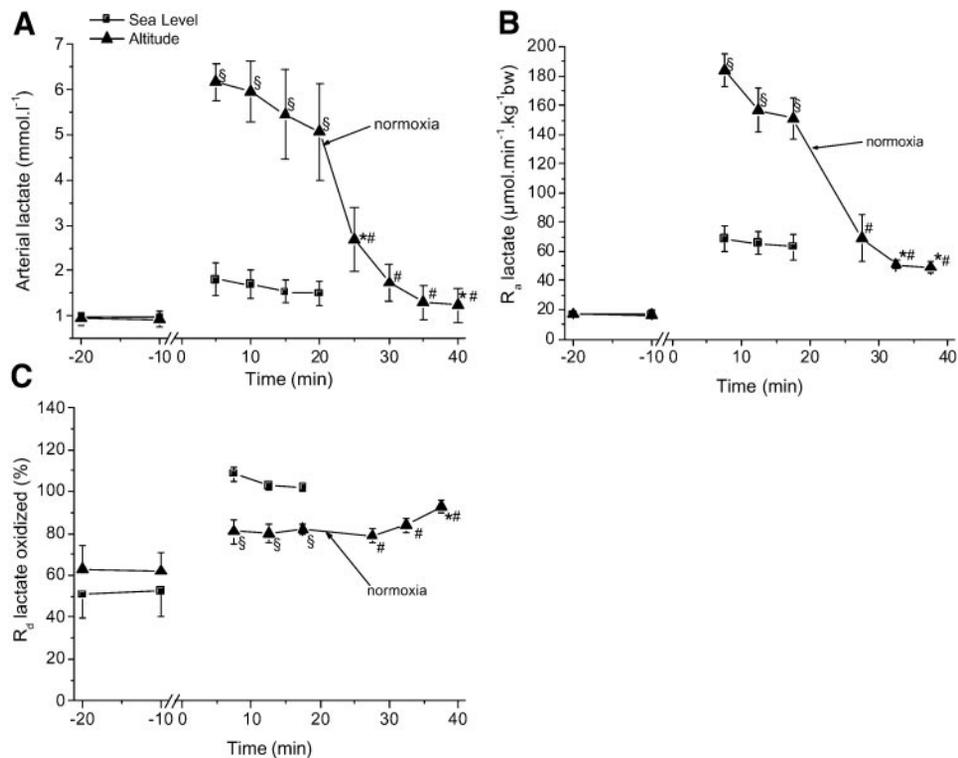


Fig. 2. Whole body lactate metabolism at rest and with continuous bicycle exercise at sea level and at altitude. Arterial lactate concentration (A), whole body lactate R_a (B), and the percentage of ¹³C from [1-¹³C]lactate disappearing (R_d) from the circulation being recovered as ¹³CO₂ in the breath (C) during 20 min of bicycle exercise at a constant workload of 146 W at sea level and at altitude breathing ambient air immediately followed by 20 min breathing a normoxic gas mixture. For further details see Fig. 1.

Table 2. Whole body and leg carbohydrate, glucose, and lactate utilization at rest and during continuous exercise at sea level and at altitude

		Rest	Exercise	Exercise Normoxia
Pulmonary O ₂ uptake, l/min	Sea Level	0.39 ± 0.05	2.26 ± 0.19	
	Altitude	0.42 ± 0.12	2.31 ± 0.06	ND
Respiratory exchange ratio	Sea Level	0.85 ± 0.02	0.92 ± 0.02	
	Altitude	0.82 ± 0.02	0.92 ± 0.01	ND
WB CHO oxidation	Sea Level	1.2 ± 0.3	12.3 ± 1.9	
	Altitude	1.1 ± 0.3	12.8 ± 0.8	ND
WB Glc R _d	Sea Level	1.2 ± 0.4	2.8 ± 0.5	
	Altitude	1.1 ± 0.2	2.4 ± 0.1	2.4 ± 0.1
WB Lact R _a	Sea Level	1.2 ± 0.1	5.0 ± 0.6	
	Altitude	1.1 ± 0.1	10.6 ± 0.4*	3.2 ± 0.1†‡
WB Lact oxidation	Sea Level	0.7 ± 0.1	5.2 ± 0.6	
	Altitude	0.8 ± 0.1	9.0 ± 0.2*	3.2 ± 0.2†‡
%WB Lactate/WB CHO oxidation	Sea Level	33 ± 6	21 ± 2	
	Altitude	33 ± 6	38 ± 3*	
One-leg blood flow, l/min	Sea Level	0.43 ± 0.06	4.24 ± 0.36	
	Altitude	0.47 ± 0.03	4.38 ± 0.31	3.70 ± 0.23‡
Two-leg O ₂ uptake, l/min	Sea Level	0.07 ± 0.02	1.4 ± 0.08	
	Altitude	0.09 ± 0.01	1.5 ± 0.07	1.6 ± 0.09
Leg respiratory quotient	Sea Level	0.90 ± 0.03	0.96 ± 0.08	
	Altitude	0.85 ± 0.01	0.93 ± 0.01	0.91 ± 0.02
Two-leg CHO oxidation	Sea Level	0.32 ± 0.10	10.8 ± 3.4	
	Altitude	0.37 ± 0.05	9.3 ± 0.6	9.3 ± 0.8
Two-leg Glc uptake	Sea Level	0.34 ± 0.11	1.79 ± 0.87	
	Altitude	0.32 ± 0.04	2.33 ± 0.42	2.07 ± 0.28
Two-leg Lact release	Sea Level	0.32 ± 0.04	4.1 ± 0.4	
	Altitude	0.46 ± 0.02	14.7 ± 1.4*	3.4 ± 0.7‡
Two-leg Lact oxidation	Sea Level	0.05 ± 0.01	3.3 ± 0.9	
	Altitude	0.15 ± 0.04*	7.5 ± 0.7*	3.8 ± 0.4‡
%Leg Lact/leg CHO oxidation	Sea Level	7 ± 3	18 ± 8	
	Altitude	21 ± 5	44 ± 5*	23 ± 3‡
%Two-leg CHO/WB CHO oxidation	Sea Level	33 ± 18	88 ± 27	
	Altitude	34 ± 11	78 ± 6	
%Two-leg Glc uptake/WB Glc R _d	Sea Level	28 ± 5	69 ± 18	
	Altitude	30 ± 9	91 ± 8	84 ± 6
%Two-leg Lact release/WB Lact R _a	Sea Level	27 ± 4	81 ± 9	
	Altitude	45 ± 5*	123 ± 8*	90 ± 6‡

Values are means ± SE in mmol/min, unless stated otherwise; *n* = 6 subjects. WB, whole body; CHO, carbohydrate; Glc, glucose; Lact, lactate; R_a, rate of appearance; R_d, rate of disappearance; ND, not determined. *Significant differences between altitude and sea level; †significant differences between altitude breathing normoxia and sea level; ‡significant differences between normoxia and ambient air at altitude.

exercise, whereas leg lactate release decreased at sea level. At altitude, the leg lactate uptake also decreased with exercise duration but to a far less extent than the decrease in lactate release, as reflected by the changes in net lactate release from the leg. At rest, only 16 and 32% of the [1-¹³C]lactate taken up was released as ¹³CO₂. At 5 min of exercise, on the other hand, 100% of the lactate taken up by the leg was oxidized. At rest, oxidation is likely to be underestimated by underestimating ¹³CO₂ production as a result of a disequilibrium between the slow turnover of muscle bicarbonate pools and the ¹³CO₂ produced from lactate oxidation. During the initial period of exercise, however, ¹³CO₂ production is overestimated as a result of ¹³CO₂ "stored" at rest and appearing during the initial period of exercise due to changes in the size and turnover rates of the muscle bicarbonate pools (31). During the last 15 min of exercise, an apparently steady oxidation rate of ~90% was observed. The relative contribution of leg lactate oxidation to the leg carbohydrate oxidation is higher at altitude compared with sea level (Table 2);

however, this was significant only during exercise, despite a lower percentage of lactate taken up being oxidized.

Whole body vs. leg carbohydrate, glucose, and lactate metabolism and the relative contribution of lactate to carbohydrate oxidation. The contribution of the legs to whole body carbohydrate utilization was about one-third at rest and increased to ~80% with exercise, and no difference was observed between sea level and altitude (Table 2). The contribution of glucose uptake of the legs to R_d of glucose during exercise tended to be higher at altitude than at sea level. This was caused by a nonsignificantly higher leg glucose uptake, since R_d was similar in both conditions (Fig. 1). Both at rest and during exercise, the contribution of the lactate release of the legs to R_a of lactate was higher at altitude than it was at sea level. The relative contribution of lactate oxidation to carbohydrate oxidation was ~33% at whole body level and between 7 and 32% for the leg, no differences between sea level and at altitude. However, during exercise, the contribution of lactate oxidation to carbohydrate oxidation was significantly higher at al-

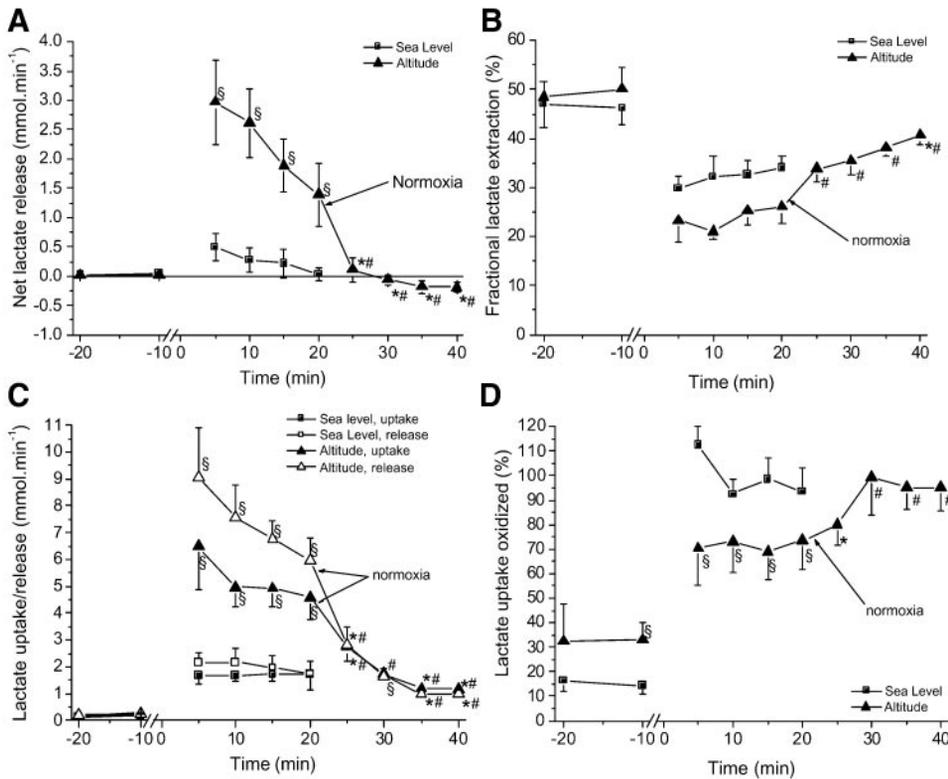


Fig. 3. Leg lactate metabolism at rest and with continuous bicycle exercise at sea level and at altitude. *A*: net lactate release from the leg (femoral a-v lactate concentration multiplied by blood flow). *B*: fractional extraction in percentage of lactate by the leg. *C*: calculated uptake and release of lactate by the leg (for tracer [1-¹³C]lactate calculation see METHODS). *D*: percentage of ¹³C from [1-¹³C]lactate tracer taken up by the leg recovered as ¹³CO₂ released by the leg at rest and during 20 min of bicycle exercise at a constant workload of 146 W at sea level and at altitude breathing ambient air immediately followed by 20 min breathing a normoxic gas mixture. For further details see Fig. 1.

titude compared with sea level, caused by an increased contribution of lactate to carbohydrate oxidation of the active legs.

Catecholamine concentration. During continuous bicycle exercise at moderate intensity, the norepinephrine and epinephrine concentrations were about sixfold higher at altitude than they were at sea level (Fig. 4). When switched acutely from ambient air to normoxia, catecholamine concentrations immediately dropped but were still threefold higher than sea level values.

DISCUSSION

The major findings of the present study investigating carbohydrate and lactate metabolism in lowland na-

tives at sea level and after a prolonged period of acclimatization to high altitude (9 wk at 5,260 m) are the following. 1) At rest, arterial lactate concentration and the R_a of lactate in blood were similar at sea level and after acclimatization to hypoxia. During exercise, however, the arterial lactate concentration and the rate of lactate appearance in blood were severalfold higher after acclimatization to hypoxia. 2) The contribution of skeletal muscle to blood lactate appearance and disposal was higher after acclimatization compared with sea level both at rest and during exercise. 3) The contribution of lactate oxidation to carbohydrate oxidation was substantially higher after acclimatization compared with sea level, due mainly to an increased

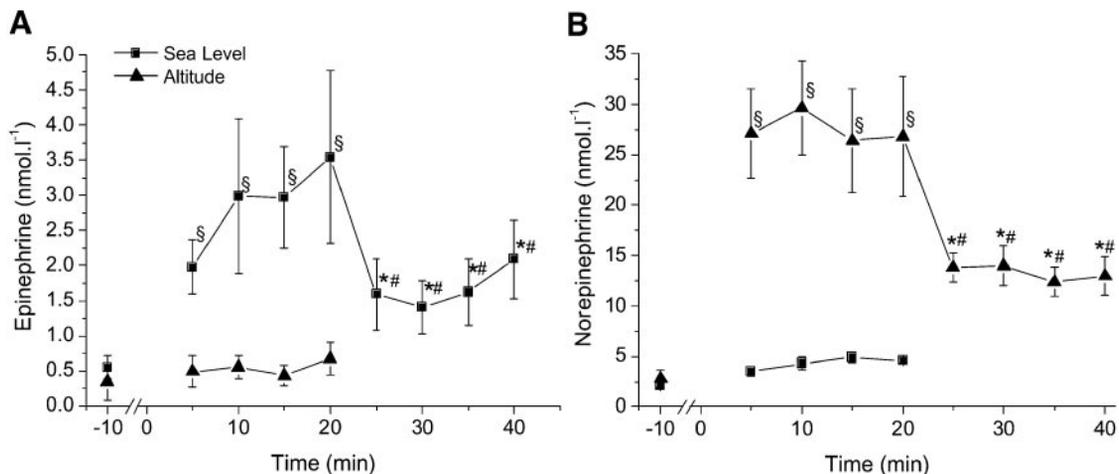


Fig. 4. Arterial epinephrine (*A*) and norepinephrine (*B*) concentration at rest and with continuous bicycle exercise at sea level and at altitude. For further details see Fig. 1.

uptake and subsequent oxidation of lactate by skeletal muscle.

The present study showed that, after 9 wk of acclimatization to 5,260 m, the lactate concentration and whole body lactate R_a and oxidation were increased during continuous exercise at the same absolute workload compared with sea level. These findings are similar to those after 3 wk at 4,300 m (4). However, in the present study, a severalfold increase during exercise of leg net lactate release, leg lactate uptake/release, leg lactate oxidation, and a higher contribution of the leg lactate release to whole body lactate R_a was observed at altitude compared with sea level. Previously, no change in the leg net lactate release (6, 7), lactate release, and contribution of leg lactate release to whole body lactate R_a was observed after 3 wk of acclimatization to 4,300 m compared with sea level (7). It was suggested that active skeletal muscle as a source of lactate was not affected by acclimatization and that other tissues besides active skeletal muscle participate in producing the blood lactate concentration responses described as the lactate paradox (7). Of note, no lactate paradox phenomenon was observed during incremental exercise in the same subjects of the present study; i.e., at submaximal and maximal exercise intensities, the lactate concentration was similar with acute exposure to hypoxia equivalent to 5,260 m compared with 5,260 m after 9 wk of acclimatization (32). Furthermore, at any absolute exercise intensity, the lactate concentration and leg lactate release were considerably higher at altitude compared with sea level. However, lactate concentration and leg lactate release were similar at any relative exercise intensity (32). The findings from the incremental maximal exercise study may imply that continuous submaximal exercise at the same relative exercise intensity as at sea level would have elicited similar lactate utilization at altitude compared with sea level. Thus the relative exercise intensity muscle lactate production relation was unchanged with prolonged acclimatization to high altitude. Whether the longer period and/or the higher altitude of acclimatization in the present study compared with the studies of Brooks and colleagues (4, 7) may have caused the differences in whole body and muscle lactate metabolism remains to be resolved.

Whole body vs. muscle lactate turnover: methodological considerations. In the present study, whole body lactate R_a was calculated from the dilution of the lactate tracer, infused in a forearm vein, in an artery (the a-v infusion/sample mode). However, lactate R_a is 20–30% higher when the generally accepted appropriate infusion in the aortic arch and blood sampled from the vena cava (the a-v infusion/sample mode) is used (21). With the underestimation in lactate R_a taken into account, lactate R_a would be 1.5 and 6.3 $\mu\text{mol}/\text{min}$ at rest and during exercise at sea level, respectively. Total skeletal muscle mass is $\sim 40\%$ of the body weight (~ 30 kg) and the muscle mass of two legs ~ 14 kg. Therefore, under resting conditions, the rate of lactate uptake and release by total skeletal muscle is ~ 0.7 mmol/min at sea level. Thus $\sim 45\%$ of the systemic

lactate R_a originates in skeletal muscle. The contribution of skeletal muscle to systemic lactate R_a is similar to the 41% previously reported when forearm lactate release was extrapolated to systemic lactate R_a (11). With exercise, the contribution of lactate release from the active legs to the corrected lactate R_a is $\sim 65\%$ at sea level. At rest 1.2 mmol/min and during exercise 2.2 mmol/min of lactate originate from nonleg tissues. This does not necessarily mean that other tissues than skeletal muscle have doubled in both lactate R_a and R_d . With bicycle exercise, not only the muscles in the legs directly involved in bicycling but also muscles in the rest of the body, such as the back, trunk, and, to some extent, arm muscles, will become more active.

Systemic lactate R_a or two-leg lactate release at rest was not significantly different at sea level and altitude. However, the corrected 65% contribution of the two-leg lactate release to lactate R_a at rest was significantly higher at altitude compared with sea level. During exercise, whole body lactate R_a , leg lactate release, and the relative contribution of the legs to whole body lactate R_a were higher at altitude compared with sea level. In fact, leg lactate release accounted for virtually all lactate appearing in the blood compared with $\sim 65\%$ at sea level. Thus, after acclimatization, lactate R_a during exercise is higher compared with sea level. In addition, resting and active skeletal muscles are more important in lactate R_a and R_d after acclimatization to hypoxia than at sea level.

The aforementioned evaluation of the contribution of skeletal muscle to lactate R_a during exercise is based on the average of the 20 min of exercise (Table 2). However, it is clear from Fig. 3 that, with exercise duration, the decrease in lactate release from the legs is more pronounced than the decline in lactate R_a and that the leg lactate uptake decreased far less compared with the release. These changes are also reflected in the marked decrease in the net lactate release compared with a modest decline in arterial lactate concentration, as described previously (14, 19, 20). This implies that, with exercise duration, the active muscles become less important as a tissue for lactate clearance but maintain or even slightly increase their role in lactate clearance. Consequently, other tissues, including nonleg skeletal muscle, become more important in terms of lactate release. The decline in net lactate release and, as shown here, in lactate release as well has been suggested to originate from a shift in fiber type recruitment (25). The very early release of lactate upon the start of exercise may be due to activation of type II fibers with a high glycolytic capacity. During continued moderate-intensity exercise, these fibers may not be recruited, but rather type I fibers with a lower glycolytic capacity. However, most likely, the low workload of 146 W in the present study did not result in type II fiber recruitment. The higher lactate release during the initial phase of moderate-intensity exercise has also been attributed to a mismatch between the rate of pyruvate production and utilization in the tricarboxylic acid (TCA) cycle. However, the pyruvate dehydrogenase (PDH) activity and the TCA cycle inter-

mediate concentration did not change between 1 and 15 min of moderate-intensity exercise, suggesting that optimal activation may have been reached shortly after the start of exercise (13). Recently, Parolin et al. (24) showed that PDH activity at rest was similar under normoxic and hypoxic conditions. After 1 min of exercise at the same absolute work intensity, PDH activity was lower in hypoxia compared with normoxia. After 15 min of exercise, PDH activity did not further increase in normoxia, but PDH activity in hypoxia further increased compared with 1 min, resulting in a significantly higher PDH activity in hypoxia compared with normoxia. Despite a higher PDH activity in hypoxia, muscle lactate was severalfold higher in hypoxia compared with normoxia. Therefore, it appears that pyruvate oxidation is not limited in hypoxia, supported by the observation in the present study that lactate uptake and subsequent oxidation by active skeletal muscle are already high at the onset of exercise at altitude, and no differences could be observed in carbohydrate oxidation between sea level and altitude. This may indicate that hypoxia elicits an upregulation of glycolysis far above the rate of pyruvate production needed for oxidation. Still another alternative that cannot be excluded is that, locally in muscle intracellular compartments, a lack of oxygenation exists, and supplementary ATP is generated from glycolysis.

Contribution of lactate oxidation to carbohydrate oxidation. At rest, the contribution of lactate oxidation to whole body carbohydrate oxidation was 33% with sea level and at altitude. With exercise, the absolute rate of lactate oxidation increased more than sevenfold, but the relative contribution of lactate oxidation to carbohydrate oxidation decreased at sea level. In contrast, the relative contribution of lactate oxidation to carbohydrate oxidation at altitude was slightly higher compared with rest and significantly higher compared with sea level. The higher contribution of leg lactate oxidation to leg carbohydrate oxidation suggests that active skeletal muscle is responsible for the higher contribution of whole body lactate oxidation to whole body carbohydrate oxidation during exercise at altitude compared with sea level. If we assume that there is no net change in muscle glycogen at rest, about one-half of the carbon of glucose is converted to lactate at whole body level and by skeletal muscle. With exercise, leg lactate uptake was two- and sixfold higher than leg glucose uptake at sea level and at altitude, respectively. Thus lactate equals glucose in delivering carbon from the circulation to the muscle at sea level and threefold higher at altitude. Because lactate, especially during exercise, is nearly completely oxidized, this suggests that lactate is an important fuel for muscular activity. However, at rest, and certainly during exercise, a net lactate release is observed. This implies that, despite a substantial lactate uptake and oxidation by the leg, in a "net sense" lactate does not add carbon for oxidation, in contrast to glucose.

Carbohydrate and glucose utilization. Lowlanders acutely exposed to high altitude showed a shift toward increased blood glucose utilization relative to normoxic

conditions (5, 28). After 3 wk of acclimatization, reliance on blood glucose increased even further (5) or decreased (28). In the latter case, blood glucose disposal and oxidation were decreased after acclimatization compared with an acute hypoxic condition, but still higher than at sea level. In the present study, total carbohydrate oxidation tended to be lower at altitude at rest but similar during exercise, whereas glucose R_a was similar at sea level and altitude, as was previously shown in diet- and weight-controlled women after 10 days at 4,300 m (2). Hypoxia causes severe disturbances of sea-level homeostasis at rest but a more profound disturbance under a metabolic challenge like exercise. On the basis of the present study and the study of Roberts et al. (27), one could argue that the enhanced utilization of carbohydrates and blood glucose seen in acute hypoxic conditions and in the 1st wk of acclimatization is overcome by a prolonged period of acclimatization and that glucose homeostasis is reestablished to the sea-level condition. However, we cannot exclude the possibility that energy imbalance (5, 8) and change in dietary carbohydrate and fat intake may have affected our findings. Noteworthy, however, is the much higher blood lactate R_a and net lactate release from the active leg at altitude compared with sea level, indicating that muscle glycogen stores were sufficient despite reduced body weight. Moreover, muscle glycogen availability was not limited either by energy deficiency or by change in diet, as the same subjects had similar lactate concentrations at submaximal and maximal work during graded incremental exercise when acutely exposed to hypoxia and after 9 wk at 5,260 m (32).

Brooks et al. (5) observed small differences in epinephrine and norepinephrine concentrations during exercise at sea level and during exercise acutely and after 3 wk at 4,300 m. The norepinephrine concentration correlated with glucose R_a . In the present study, more than sixfold higher epinephrine and norepinephrine concentrations were observed during exercise at Altitude without a change in glucose R_a . The role of adrenergic stimulation on hepatic glucose output during exercise is unclear; however, the data of the present study support the notion that hepatic adrenergic stimulation does not play a major role in glucose homeostasis during exercise (9, 10, 18).

Altitude normoxia after 9 wk of acclimatization to 5,260 m. To get some insight into the effect of oxygen availability during exercise after acclimatization, the 20 min of breathing ambient air was immediately followed by 20 min of exercise while breathing a normoxic gas mixture (altitude normoxia) simulating sea-level conditions. Clearly, the comparison between normoxia and ambient air at altitude and sea level has its limitation. The values on carbohydrate, glucose, and lactate metabolism during normoxia are affected by the previous 20 min of exercise. The previous exercise (Altitude, ambient air) had caused a substantially enhanced arterial lactate concentration, thereby affecting lactate utilization, and furthermore, lipolysis had increased, thereby affecting carbohydrate utilization.

In addition, because lactate release from an active limb decreases with exercise duration, as is clearly shown in the present study, it is difficult to discriminate between the effects of exercise duration and normoxia. However, with these limitations kept in mind, it seems that, after 20 min of exercise while subjects breathed a normoxic gas mixture, most parameters, such as arterial lactate concentration, lactate R_a , percent lactate R_d oxidized, leg lactate uptake and release, and leg lactate uptake being oxidized, reached values similar to those at sea level. Immediate normalization of lactate metabolism with supplementary oxygen to sea-level values may originate from oxygen per se and/or from oxygen and adaptive changes caused by acclimatization.

In summary, After 9 wk of acclimatization to 5,260 m, the lactate concentration and whole body lactate R_a are increased during exercise compared with sea level, as previously observed after 3 wk at 4,300 m (4). However, different from previous work (7) were the findings of a higher leg net lactate release, leg lactate release, and higher contribution of the leg lactate release to whole body lactate R_a at altitude compared with sea level. In addition, the relative contribution of lactate oxidation to whole body carbohydrate oxidation was substantially higher at altitude compared with sea level as a result of an increased uptake and subsequent oxidation of lactate by the active skeletal muscles. Whether the differences between the present study and previous work are due to the longer duration and/or higher altitude of acclimatization is unclear.

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