Lactate metabolism during exercise in patients with mitochondrial myopathy

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Abstract

Patients with mitochondrial DNA mutations often have elevated plasma lactate at rest and during exercise, but it is unknown whether the high lactate levels are caused by a high production, an impaired oxidation or a combination. We studied lactate kinetics in 10 patients with mtDNA mutations and 10 matched healthy control subjects at rest and during cycle exercise with a combination of femoral arterio-venous differences of lactate, and lactate tracer dilution methodology. During exercise, lactate concentration and production rates were several-fold higher in patients, but despite mitochondrial dysfunction, lactate was oxidized in muscle to the same extent as in healthy control subjects. This surprisingly high ability to burn lactate in working muscle with defective mitochondria, probably relates to the variability of oxidative capacity among muscle fibers. The data suggests that lactate is not solely an indicator of impaired oxidative capacity, but an important fuel for oxidative metabolism, even in muscle with severely impaired mitochondrial function.

Keywords: Mitochondrial myopathy; Lactate kinetics; Lactate acidosis; Premature fatigue

1. Introduction

In patients with mutations of mitochondrial DNA (mtDNA), oxidative phosphorylation, and thus mitochondrial production of adenosine triphosphate (ATP), is impaired due to dysfunction of proteins in the mitochondrial respiratory chain. Patients with mutation of mtDNA carry a unique composition of both mutated and normal (wild type) mtDNA copies in each cell, a condition called heteroplasmy. The fraction of mutated vs. wild type mtDNA determines the mutation load in the tissue, which varies among and within tissues of a patient. Patients with mutations of mtDNA often have symptoms from multiple organs, but given that the mutation load of mtDNA generally is high in skeletal muscle, and that oxygen demand increases dramatically during exercise in healthy muscle, the most common symptom in patients is exercise intolerance. Other common features in patients with mtDNA mutations are premature fatigue and elevated resting plasma lactate [1]. It has been speculated if the elevated plasma lactate found in patients with mitochondrial myopathy may contribute to the severe exercise intolerance and premature fatigue found in these conditions. Lowering of plasma lactate with dichloracetate has shown both no change [2,3] and improvement [4,5] of exercise capacity in patients with mtDNA mutations. In healthy control subjects, studies have shown that lactate is taken up and oxidized by the exercising muscle [6,7]. Considering that lactate is being used as a fuel for muscle during exercise in healthy individuals, the question is whether the high lactate levels found in patients with mitochondrial...
dysfunction reflect impaired oxidation or increased production. The elevated plasma lactate levels in patients with dysfunctional mitochondria have generally been ascribed to a high lactate production [8–10], but lactate kinetics have not been studied in these conditions. Thus, the aim of this study was to investigate the kinetics of lactate metabolism in patients with well-characterized molecular defects of mtDNA and impaired oxidative capacity. Lactate uptake, release and oxidation were studied across a resting and exercising muscle, using stable isotope-labeled lactate.

2. Subjects and methods

2.1. Subjects

Ten patients (four men and six women) with well-characterized molecular defects of mtDNA (see Table 1) were included in the study. In order to study the effect of mitochondrial impairment on lactate kinetic, we included patients with high mtDNA mutation loads in skeletal muscle, irrespective of mtDNA mutation type. Due to the invasive nature of the study, and that mitochondrial disease is a relative rare disease, it was not possible to include a high number of patients carrying the same mtDNA mutation type. Results from the patients were compared to 10 healthy subjects. Inclusion criteria for these control subjects were that there had to be an individual match between one patient and one healthy control subject in regard to gender and age, and the healthy control subjects did not perform any aerobic or strength training on a regular basis (more than 1 h/week) at the time of the study, and two months prior to conduction of the study. Exercise capacity (maximal oxygen uptake, VO$_{2\text{max}}$) in patients and healthy control subjects are shown in Table 1.

None of the patients in the study were related. Three patients had type II diabetes, and were treated with sugar-restricted diet, and two were treated with oral antidiabetic drugs. One patient received antiepileptic treatment for myoclonic seizures. No other subject took any medication.

The study was approved by the Scientific-ethical committee of Copenhagen and Frederiksberg Committee in Denmark (No. KF 01-046/01). The subjects were all informed about the nature and risks of the study, and gave written consent to participate.

2.2. Experimental protocol

Pre-experimental testing: Between one and two weeks before the experimental day, all subjects had their VO$_{2\text{max}}$ determined by an incremental exercise test to exhaustion, as previously described [11].

Protocol: On the day of the experiment, subjects reported to the laboratory at 9 a.m., 1 h after ingesting a breakfast, low in fat, and containing 75–125 g of carbohydrates. They were instructed not to perform exercise for 24 h before testing, and not to ingest caffeine or alcohol 12 h before the experiment. Under local anesthesia, all subjects had catheters inserted anterograde (Seldinger technique) in the right femoral artery and vein, 1–2 cm distal of the inguinal ligament. The catheterization procedure lasted approximately 30 min. A cubital venous catheter was inserted for infusion of glucose and lactate tracers.

After placement of the catheters, blood samples were obtained for assessment of background enrichment of lactate, glucose and carbon dioxide (CO$_2$). In addition, a
breath sample was obtained for background enrichment of CO₂. After background samples were drawn, a bolus of [13C]-labeled bicarbonate (H[13CO₃]₂) (1.5 μmol/kg) was infused IV, immediately followed by a primed constant infusion (0.79 μmol min⁻¹ kg⁻¹, prime 1.5 μmol/kg) of [13C]-labeled lactate ([1-13C] lactate) and (0.38 μmol min⁻¹ kg⁻¹, prime 17.6 μmol/kg) of 6,6H-labeled glucose ([6,6-²H₂] glucose). All stable isotopes were obtained from Cambridge Isotope Laboratories (Andover, MA, USA). Ninety minutes after the start of infusion, three resting blood and breath samples were obtained with 10 min interval. After a total of 2 h of rest with the stable isotope infusion, subjects cycled on a stationary cycle ergometer at an intensity of 65% of the patients VO₂max. The matched, healthy control subjects exercised at the same absolute workload as the corresponding patient (60 ± 7 W; range 35–106 W). The duration of the cycle test was initially set at 40 min, but four of the 10 patients experienced fatigue after 30 min. Correspondingly, data are only shown for the first 30 min of exercise for all subjects.

Intra-arterial pressure was monitored continuously via a pressure transducer (Pressure Monitoring Kit, Baxter) connected to the femoral artery catheter, and data was recorded via a MaclabTM/16s Ad instrument transducer coupled to a computer. Heart rate was derived continuously from the arterial pressure curve. VO₂ and CO₂ exchange was measured online (Cosmed Quark b2, Italy). Respiratory exchange ratio (RER) was calculated via CO₂/O₂ ratio. RER can be used as an indicator of which fuel (carbohydrate or fat) is being used to supply the skeletal muscle with energy. In general, an RER of 0.70 indicates that fat is the predominant fuel source, while an RER of 0.85 suggests a mix of fat and carbohydrates, and a value of 1.00 or above is indicative of carbohydrate being the predominant fuel source. Rate of perceived exertion (Borg Scale) [12] was monitored every other minute during exercise.

2.3. Blood samples and analyses

Arterial and venous blood samples were drawn simultaneously. Blood gases and pH were measured in whole blood, and sampled in syringes containing dry lithium and heparin. Hemoglobin and oxygen saturation measurements were performed on an OSM 3 analyzer (Radiometer, Denmark), while partial pressure of oxygen (pO₂), bicarbonate (pCO₂) and pH were analyzed on an ABL blood gas analyzer 65O (Radiometer, Denmark) immediately after sampling. Blood for plasma samples was obtained in ice-cold tubes containing 10 μmol of 0.33 ethylene-diamine-tetraacetic acid (EDTA)/ml of blood and was immediately centrifuged at 4°C for 5 min at 4400 rpm. Plasma lactate and glucose concentrations were measured immediately after centrifugation (YSI stat 2300, YSI incorporated, Yellow Springs, USA) while samples for catecholamines, free fatty acids (FFA) and insulin were stored at −80°C until analyses, using methods as previously described [13]. Lactate enrichments were measured by gas chromatography–mass spectrometry (GC–MS, Finnigan Automass II, Paris, France) [7], glucose by liquid chromatography–mass spectrometry (LC–MS, Finnigan aQa, Manchester, UK) [14] and blood and breath CO₂ enrichment were analyzed by gas chromatography–isotope ratio mass-spectrometry (GC–C–IRMS, Finnigan Delta+, Bremen, Germany) [7].

2.4. Stable isotope calculations

The whole body rates of appearance (Ra) and disappearance (Rd) of lactate and glucose at rest and during exercise and the lactate and glucose kinetics across the leg were calculated using the non steady state equations of Steele [15] adapted for stable isotopes [16] as previously described [7]. For calculations of arterial and venous plasma lactate concentrations, limb blood flow was calculated by dividing systemic VO₂ with Leg a-VO₂ difference, estimating leg VO₂ during cycling as (systemic VO₂ during cycling – resting systemic VO₂)/2. Thus, it was assumed that all the increase in systemic VO₂ during cycling was accounted for by the increase in VO₂ in the two exercising legs.

2.5. Statistical analysis

Significant difference from rest to exercise in the two groups, and between the patient and healthy control groups were analyzed with a 2-tailed paired and non-paired Student’s t test, respectively. In order to evaluate whether differences between the group of patients and healthy controls were due to variance in time, a 2 × 2 mixed ANOVA was performed. The significance level was set at p < 0.05. Data are presented as mean ± SE.

3. Results

Since glucose intolerance, diabetes and anti-epileptic medicine theoretically could alter the lactate and glucose kinetics, findings in the patients with diabetes and epilepsy were compared to results found in the rest of the patients. There was no difference between the two groups. The p value of the systemic data (arterial and venous lactate, levels, Ra, Rd and lactate oxidation) was 0.64 ± 0.13 (range, 0.28–0.96) and leg data (lactate uptake, lactate release, lactate oxidation, % of lactate uptake that is oxidized) was 0.32 ± 0.09 (range, 0.11–0.62). Thus, no distinction between glucose tolerant and intolerant patients has been made in the presentation of data.

Oxygen uptake and relative exertion: VO₂max was about half that found in healthy subjects (Table 1). During the constant-workload test, all subjects exercised for 30 min at the same absolute workload (60 ± 7 W; range
35–106 W), which corresponded to 66 ± 3% of VO\textsubscript{2max} in the patients and 32 ± 3% in healthy subjects. Average VO\textsubscript{2} during exercise was similar in patients and healthy subjects (14 ± 1 vs. 13 ± 1 ml min\textsuperscript{-1} kg\textsuperscript{-1}). In accordance with the higher relative workload performed by the patients, the average heart rate for the entire exercise period was higher (140 ± 9 beats per minute (BPM)) than in healthy controls (96 ± 4 BPM; p < 0.05), and so was the rate of perceived exertion (14 ± 1 vs. 8 ± 0; p < 0.05).

**Whole body and limb lactate kinetics:** At rest, the arterial lactate concentration and rate of appearance of lactate (\(R_a\)) were higher in patients vs. healthy subjects. During exercise, the arterial lactate concentration increased 3-fold in the patients, but remained unchanged in healthy controls (Fig. 1A). \(R_a\) increased almost 3-fold during exercise in the patients, but only increased (p < 0.05) very little in the healthy controls (Fig. 1B). Rate of disappearance of lactate (\(R_d\)) essentially mirrored changes in \(R_a\) in both groups (data not shown). In both patients and healthy controls, the exercise-induced increase in \(R_d\) from the circulation was directed toward lactate oxidation, which was nearly 100% in the controls (Fig. 1D). Since lactate \(R_d\) in the patients was much higher than in the controls, the absolute quantity of lactate oxidized was nearly 6-fold higher in patients (Fig. 1C). At the onset of exercise, lactate utilization due to oxidation was higher in patients vs. controls (Fig. 1C),

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**Fig. 1.** (A) Arterial lactate concentrations. (B) Rate of lactate appearance (solid line) and disappearance (dashed line). (C) Whole body lactate oxidation. (D) The fraction (%) of lactate that is removed via oxidation. *p < 0.05 vs. healthy subjects. Values at each time-point are mean ± S.E.

**Fig. 2.** (A) Unidirectional lactate release (solid line) and uptake (dashed line). (B) net lactate release. (C) Lactate oxidation. (D) Lactate oxidation as a percentage of the unidirectional lactate uptake. *p < 0.05 vs. healthy subjects. Values at each time-point are mean ± SE.
but it could not match the increased production ($R_a$), causing increased accumulation of lactate in the blood. Later in exercise, this match was better and blood lactate levels stabilized.

The changes in systemic lactate kinetics and oxidation during exercise were almost entirely caused by skeletal muscle contractions (Fig. 2). At rest, the leg net lactate exchange was negligible in both groups; however, during exercise, a substantial net lactate release was observed in the patients, which was constant during the 30 min of exercise (Fig. 2A). In contrast, the controls had only a small, but significant, net lactate release after 10 min of exercise, after which a net lactate exchange was absent (Fig. 2A). The unidirectional lactate uptake and release was similar at rest, but many fold higher during exercise in patients compared to healthy controls (data not shown).

The fraction of lactate that was taken up by the leg and oxidized was similar in patients and healthy controls (Fig. 2C), but the absolute quantity of lactate that was oxidized by the leg was 2–3-fold higher in patients (Fig. 2B).

### Table 2
Whole body and leg lactate turnover in 10 patients with mitochondrial myopathy at rest and during 66% of VO$_{2\text{max}}$ compared to results in 10 healthy subjects at rest and during 32% and 65% of VO$_{2\text{max}}$.

<table>
<thead>
<tr>
<th></th>
<th>Mitochondrial patients</th>
<th>Healthy subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rest</td>
<td>Exercise, 66% of VO$_{2\text{max}}$</td>
</tr>
<tr>
<td>Arterial lactate (mmol/l)</td>
<td>1.9 ± 0.3</td>
<td>5.4 ± 1.2</td>
</tr>
<tr>
<td>Rate of lactate appearance (μmol (min kg)$^{-1}$)</td>
<td>24.0 ± 2.4</td>
<td>75.3 ± 19.0</td>
</tr>
<tr>
<td>WB lactate oxidation (μmol (min kg)$^{-1}$)</td>
<td>13.6 ± 1.3</td>
<td>64.5 ± 9.8</td>
</tr>
<tr>
<td>Fractional lactate oxidation (%)</td>
<td>58.5 ± 5.1</td>
<td>99.1 ± 7.0</td>
</tr>
<tr>
<td>Net lactate release (mmol min$^{-1}$)</td>
<td>0.2 ± 0.1</td>
<td>4.3 ± 1.2</td>
</tr>
<tr>
<td>Limb lactate oxidation (mmol min$^{-1}$)</td>
<td>0.1 ± 0.0</td>
<td>0.6 ± 0.5</td>
</tr>
<tr>
<td>Limb lactate uptake oxidized (%)</td>
<td>17.5 ± 11.6</td>
<td>60.2 ± 18.0</td>
</tr>
</tbody>
</table>

Values are mean ± SE.

### Table 3
Whole body and leg carbohydrate, glucose and lactate utilization in 10 patients with mitochondrial myopathy and 10 healthy subjects at rest and during exercise.

<table>
<thead>
<tr>
<th></th>
<th>Mitochondrial patients</th>
<th>Healthy subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rest</td>
<td>Exercise</td>
</tr>
<tr>
<td>Arterial glucose, (mmol/l)</td>
<td>6.3 ± 1.6</td>
<td>5.6 ± 0.4$^b$</td>
</tr>
<tr>
<td>$R_a$ glucose (mmol min$^{-1}$)</td>
<td>17.6 ± 1.3$^a$</td>
<td>17.2 ± 1.2$^a$</td>
</tr>
<tr>
<td>WB CHO oxidation (μmol (min kg)$^{-1}$)</td>
<td>9.4 ± 2.9</td>
<td>54.6 ± 10.3$^b$</td>
</tr>
<tr>
<td>WB lactate oxidation (μmol (min kg)$^{-1}$)</td>
<td>13.6 ± 1.3</td>
<td>65.1 ± 10.0$^b$</td>
</tr>
<tr>
<td>WB Lactate/WB CHO oxidation (%)</td>
<td>61 ± 27$^a$</td>
<td>40 ± 5</td>
</tr>
<tr>
<td>Net leg glucose uptake (mmol min$^{-1}$)</td>
<td>0.2 ± 0.1</td>
<td>0.5 ± 0.4$^b$</td>
</tr>
<tr>
<td>Leg CHO oxidation (mmol min$^{-1}$)</td>
<td>0.0 ± 0.0</td>
<td>1.6 ± 0.3$^b$</td>
</tr>
<tr>
<td>Leg lactate oxidation (mmol min$^{-1}$)</td>
<td>0.0 ± 0.0</td>
<td>1.1 ± 0.3$^b$</td>
</tr>
<tr>
<td>Leg lactate/leg CHO oxidation (%)</td>
<td>26 ± 11$^a$</td>
<td>61 ± 11$^a,b$</td>
</tr>
</tbody>
</table>

Values are mean ± SE.

$^a p < 0.05$ vs. healthy subjects.

$^b p < 0.05$ vs. rest.

Whole body and limb glucose turnover, and total carbohydrate kinetics: glucose concentrations were similar at rest and during exercise in both groups, but arterial glucose concentrations decreased significantly only in the patients during exercise (Table 2). $R_a$ of glucose was higher in the patients at rest and during exercise vs. controls (Table 2). The net leg glucose uptake was similar at rest, but about 2-fold higher during exercise in patients (Table 2). This was also reflected by a higher RER in patients (Rest: 0.82; Ex: 0.93; $p < 0.05$) vs. the healthy subjects (Rest: 0.80; Ex: 0.84), and a higher whole body and leg carbohydrate (CHO) oxidation in the patients vs. controls (Table 2).

At rest, whole body and leg oxidation of lactate as a fraction of total CHO oxidation were higher in the patients. During exercise, leg lactate oxidation/leg CHO oxidation was higher in the patients vs. controls (Table 2).

Hormonal and free fatty acid (FFA) responses: at rest, norepinephrine levels were higher in patients than healthy controls, and during exercise, epinephrine and norepinephrine rose to higher levels in patients vs.
healthy controls (Table 3). At rest, insulin levels were also higher in patients vs. healthy controls, and during exercise, insulin did not change significantly in either group (Table 3).

After the first 10 min of exercise, FFA decreased in the patients (rest: 411 ± 68, 10 min ex: 280 ± 37 mmol/l; p < 0.05), but increased to the resting level during the last 30 min of exercise (Table 3). There was no change in FFA level from rest compared to the average FFA concentration throughout the 30 min of cycling in patients, while FFA increased after 30 min of exercise in the healthy controls (see Table 4).

4. Discussion

High plasma lactate levels in patients with impaired mitochondrial function have usually been ascribed to enhanced production of lactate in skeletal muscle [8–10], because anaerobic breakdown of glucose is favored under conditions of impaired respiratory chain function. Although lactate production is undoubtedly enhanced in these conditions, the dynamics between production and removal, and the site of removal (liver or muscle), is unknown, because lactate turnover has never been studied in these conditions. In this study, we investigated the lactate turnover in patients with severely impaired mitochondrial function due to mtDNA mutations by measuring lactate release, uptake and oxidation using stable isotope technique combined with arterio-venous sampling of blood from the lower limb before and during isolated quadriceps exercise in these conditions. The principal new findings are that in skeletal muscle with impaired mitochondrial function, there is a mismatch between lactate release and uptake initially during exercise, leading to the high plasma lactate levels that are seen in patients with mtDNA mutations. However, during continued exercise, lactate is oxidized in muscle of the patients to the same extent as that in healthy control subjects, and to such a degree that plasma lactate concentration levels off after 20 min of exercise. These findings suggest that even in muscle carrying deleterious mtDNA mutations, lactate is an important fuel for oxidative metabolism.

Patients with mitochondrial dysfunction typically complain about premature fatigue [17]. In this study, four out of 10 patients experienced fatigue after 30 min of exercise at a workload of only 66% of VO2max. At this workload, sedentary healthy subjects are able to exercise for at least an hour [18]. There is no general agreement on whether lactate is implicated in the pathogenesis of fatigue, or if it is simply a clinical marker of anaerobic metabolism [19]. Studies have indicated that lactate through H+ accumulation per se may induce fatigue [19] and that exaggerated lactate-induced increase in H+ levels could cause the premature fatigue seen in patients with mitochondrial myopathy [8]. Considering the possible role of lactic acidosis for exercise intolerance in patients with mtDNA mutations, Dichloroacetate, which reduces blood lactate concentration at rest and during exercise in healthy subjects [20], has been used as an approach to treat patients with mtDNA mutations. Although two open trial studies showed significant effect on exercise capacity in patients with mtDNA mutations, two placebo-controlled studies were not able to verify these findings [2,3]. These findings indicate that the premature fatigue seen in these patients is not related to build-up of lactate [2,3]. In contrast, the present study shows that lactate is an important fuel during exercise in these conditions.

In order to produce sufficient ATP in the respiratory chain, all five complexes must be intact and all substances fueling the complexes must be available. Many mutations in mtDNA result in impaired activity of one or more complexes. It has been speculated if “over fueling” an intact complex would help bypass or widen impaired complex capacity [21–24]. Since fat yields less ATP per O2 molecule compared to carbohydrate, it could be hypothesized that patients with defects with of the respiratory chain would benefit from down-regulation of fat oxidation. The present study, however, shows that the higher RER and whole body and leg carbohydrate turnover was caused by a relative higher workload in the patients compared to healthy subjects rather than a preferential carbohydrate oxidation in patients with severely impaired mitochondrial function.

In this study we chose to compare patients with mitochondrial myopathy to healthy controls exercising at the same absolute workload. Taken the nature of the disease in the patients with mtDNA mutations into account, it is impossible to determine an optimal
workload at which patients should be compared to healthy control subjects. This is so, since the maximal workload capacity of patients with severely impaired mitochondrial function due to mtDNA mutations is less than half of that found in age-matched healthy controls. Since energy requirement relates to workload, patients and healthy control subjects were investigated performing the same absolute workload. Correspondingly, the two groups had the same oxygen uptake, and thus the same ATP turnover. Despite similar ATP turnover, patients had not only a much higher lactate release, but also lactate utilization to such an extend that release-and utilization balanced to a steady-state level after 20 min of exercise. When comparing the data from healthy control subjects exercising at the same relative workload (65% of VO2max) lactate turnover in patients with impaired mitochondrial function due to mtDNA mutations was comparable to healthy subjects after 20 min of exercise, despite that the workload was twice as high in the patients (Table 2) [25]. The data from the healthy subjects in our and other studies [25,26] points toward that patients with severely impaired oxidative capacity due to mitochondrial dysfunction has an overall intact capacity to oxidize lactate.

The reason to a higher plasma lactate in patients with mitochondrial myopathy due to mtDNA mutations is most likely cased by a different muscle lactate release/uptake pattern compared to healthy control subjects rather than an impaired ability to utilize lactate in skeletal muscle. In healthy control subjects, the arterial concentration and net muscle lactate release reaches a peak within 5 min of exercise [6,18,27–31] and decreases thereafter (Figs. 1A and 2A). This phenomenon is thought to be related to a delay in oxygen delivery in the initial phase of exercise. This results in a transient exaggerated glycolytic ATP production [32,33] and thus either a small net muscle lactate release or a net lactate uptake [7,34]. In contrast, for still unknown reason, lactate release did not decrease after the initial 5 min of exercise in the patients with impaired mitochondrial function, leading to a higher plasma lactate level in the patient than healthy control subjects.

5. Conclusion

In this study where arterial-venous lactate turnover was studied at rest and during exercise in patients with severely impaired mitochondrial function and in healthy control subjects performing exercise at the same absolute workload, despite a higher plasma lactate level, patients were able to oxidize lactate to the same extent as that seen in healthy control subjects. Moreover, our data indicate that the higher plasma lactate level is caused by a different lactate turnover pattern in the initial phase of exercise, rather than an impaired capacity to utilization lactate. Thus, the premature fatigue seen in patients with mtDNA mutations is not induced by lactate accumulation per se.

References


