Fat Metabolism During Exercise in Patients With Mitochondrial Disease

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Objective: To determine whether patients with defects of the respiratory chain have metabolic adaptations that promote a preferential use of fats or carbohydrates, similar to what is observed in metabolic myopathies affecting glycolysis or fat oxidation.

Design: Causation and case-control study. Fat metabolism was determined by means of indirect calorimetry and stable isotope technique in patients and healthy subjects. Patients carried various types and loads (mean [SE], 72% [5%]) of mitochondrial DNA (mtDNA) mutations in skeletal muscle. All subjects exercised at the same absolute workload (mean [SE], 65 [10] W), corresponding to 72% (in patients) and 30% (in healthy subjects) of maximum oxygen consumption.

Setting: Neuromuscular research unit.

Participants: Ten patients with mtDNA mutations and 10 sex-matched healthy subjects.

Main Outcome Measures: Fat turnover, plasma concentrations of palmitate and total free fatty acids, glucose mobilization, and total carbohydrate oxidation.

Results: Fat turnover and plasma concentrations of palmitate and total free fatty acids were similar in patients and healthy subjects at rest and during exercise. In line with the higher relative workload of the patients, glucose mobilization and total carbohydrate oxidation were higher in the patients compared with the healthy subjects.

Conclusion: During moderate-intensity exercise, the balance between fat and carbohydrate use in patients with mtDNA mutations matches that seen in healthy subjects, indicating that manipulating dietary fat and carbohydrate content is not a feasible therapeutic option to improve exercise intolerance in these disorders.

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In patients with mitochondrial DNA (mtDNA) mutations, mutated and wild-type mtDNA copies coexist in the same cell, and the ratio (mutation load) largely determines the severity of the phenotype in each tissue.12-14 Because skeletal muscle has a high prevalence of mutation load2-4 and a high oxidative capacity during exercise, exercise intolerance is the most common symptom in these conditions. At present, there is no cure for mitochondrial disease. It has been speculated that the biochemical bottleneck caused by the mutation in mtDNA could be bypassed or widened by ingesting redox compounds,5 enzyme activators,6 vitamins,8 coenzymes,10 or a fat-rich diet.11 However, none of these interventions improved exercise capacity in patients with mitochondrial myopathy. To treat the exercise intolerance with dietary interventions, it is necessary to understand the metabolism and the relative contribution of fat and carbohydrates in these conditions, which, to our knowledge, have not previously been studied.

Previous studies have shown that exercise intolerance in patients with impairment of fat oxidation or muscle glycogen breakdown is greatly improved by supplements of carbohydrates.12-14 Unlike metabolic myopathies affecting carbohydrate and lipid metabolism specifically, however, the defects in patients with mitochondrial myopathy cause a common metabolic bottleneck for all fuels, thus limiting the possibilities for bypassing the block. Oxidation of carbohydrates yields more adenosine triphosphate per oxygen (O2) molecule than does oxidation of fat; considering the oxidative defects in patients with respiratory chain disorders, it could be hypothesized that patients with mitochondrial dysfunction rely more on carbohydrates for energy to compensate for impaired respiratory chain activity.15 In this study, we used stable isotope techniques to investigate fat turnover during exercise and indirect calorimetry to measure fat and carbohydrate oxi-
ification in 10 patients with high loads of different mtDNA mutations in skeletal muscle. The findings were compared with those in 10 sex-matched, healthy subjects.

**METHODS**

**SUBJECTS**

Ten patients with different mtDNA mutation types and loads in skeletal muscle and ten healthy subjects matched for sex were included (Table). The study was approved by the ethics committee of the University of Copenhagen. All subjects were informed about the nature of the study and gave written consent to participate.

**PREEXPERIMENTAL PREPARATIONS**

To determine a workload corresponding to 65% of maximal O$_2$ uptake (V$_{O2}$max) in the patients, subjects with mitochondrial myopathy performed an incremental exercise test to exhaustion, followed by a 15-minute constant workload test after a 1-hour rest. Subjects exercised on a bicycle ergometer (CPE 2000; MedGraphics, St Paul, Minnesota), and gas exchanges and heart rate were measured on a cardiopulmonary exercise test system (CPX/D; MedGraphics) or a gas and airflow analyzer (Quark b; Cosmed, Rome, Italy).

**PROTOCOL**

Experiments started at 9 AM. The subjects were instructed to eat a carbohydrate-rich diet for 3 days before the study to ensure high levels of hepatic and muscle glycogen stores. A catheter was inserted in each arm, one in the cubital vein for stable isotope infusion and one in the distal cephalic vein for blood sampling. A heating pad was used to arterialized the venous blood samples.

A primed, constant-rate infusion of palmitate labeled with uniformly $^{13}$C enriched (U-$^{13}$C) (0.0026 mg/kg/min, primed by a 0.085-mg/kg sodium bicarbonate [NaH$^{13}$CO$_3$] bolus) was delivered by a pump (Gemini PC2; IMED, San Diego, California). Two hours after the start of the isotope infusion, all subjects cycled for 30 minutes. Patients cycled at an intensity of 65% of V$_{O2}$max and healthy subjects exercised at the same absolute workload as patients. Heart rate and rate of perceived exertion (using the Borg scale) were monitored every other minute during exercise. Blood and expired gases for isotope measurements were collected at 10-minute intervals at rest and during exercise. Expired air was collected in a 15-L Douglas bag, and 10-mL air samples from the bags were injected into venipuncture test tubes (Vacutainer tubes; Becton Dickinson, Mountain View, California) for analyses of $^{13}$CO$_2$ enrichment.

During exercise, a proportion of the labeled $^{13}$CO$_2$ that is produced during fat oxidation will be stored in tissues instead of being excreted via the lungs. Furthermore, some of the labeled palmitate will be fixed in other metabolites via isotopic exchange reactions, mainly in the tricarboxylic acid cycle. Owing to this loss of tracer, there is a need to correct the oxidation rate, which can be performed by conducting an acetate correction trial. On a separate day, one of the patients with mitochondrial myopathy (patient 7 in the Table) and one of the healthy subjects completed such an acetate correction trial. Thus, the acetate correction factor found in this patient is used to represent the rest of the patients. The study protocol was identical to that described, except that the infused tracer was [1,2-$^{13}$C]-acetate.

**ANALYSES OF BLOOD SAMPLES AND EXPIRED $^{13}$CO$_2$**

The acetate and bicarbonate tracers (99% enriched; Cambridge Isotope Laboratories, Andover, Massachusetts) were dissolved in 0.9% saline solution. The palmitate tracer (98% enriched; Cambridge Isotope Laboratories) was dissolved in heated, sterile water, passed through a 0.2-µm filter, and mixed with 5% human serum albumin. Tracer calculations were performed as described perviously.

<table>
<thead>
<tr>
<th>Patient No./Sex/Age, y</th>
<th>mtDNA Mutation</th>
<th>mtDNA Mutation Load in Muscle, %</th>
<th>Clinical Symptoms/Findings</th>
<th>Height, cm</th>
<th>Weight, kg</th>
<th>V$_{O2}$max, mL/kg/min</th>
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<tbody>
<tr>
<td>1/F/51</td>
<td>12 113-14 422 deletion$^a$</td>
<td>50</td>
<td>CPEO, EI</td>
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<tr>
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<td>65</td>
<td>14</td>
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<td>76</td>
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<td>75</td>
<td>EI, SS, HI, DM, Enc</td>
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<td>EI, ME, Ataxia</td>
<td>178</td>
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<td>EI</td>
<td>170</td>
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<tr>
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<td>93</td>
<td>EI</td>
<td>169</td>
<td>70</td>
<td>17</td>
</tr>
</tbody>
</table>

All patients, mean (SE)$^c$ . . . 72 (5) . . . 169 (4)$^d$ 63 (5)$^d$ 21 (2)$^d$

Healthy subjects, mean (SE)$^e$ . . . . . . . . . 172 (3) 73 (4) 41 (2)

Abbreviations: CPEO, chronic progressive external ophthalmoplegia; DM, diabetes mellitus; EI, exercise intolerance; Enc, encephalopathy; GI, glucose intolerance; HI, hearing impairment; ME, myoclonic epilepsy; mt, mitochondrial; SS, short stature (defined as height being <2 SD of normal); V$_{O2}$max, maximum oxygen consumption; ellipses, not applicable.

$^a$ Indicates the deletion sites in mtDNA.

$^b$ These novel mutations are reported by Anitori et al$^{[16]}}$ and Schwartz and Vissing.$^{[17]}

$^c$ Mean (SE) age was 39 (3) years.

$^d$ Indicates different from healthy subjects ($P<.05$).

$^e$ Indicates 10 subjects matched for age (mean [SE], 40 [4] years) and sex (5 female and 5 male).
diately spun at 4°C and examined for lactate and glucose on a commercially available analyzer (model 2300 STAT plus; YSI, Yellow Springs, Ohio). Insulin and epinephrine levels were analyzed as previously described.22 Free fatty acids were examined on an automatic analyzer (Cobas Fara; Roche, Basel, Switzerland). Isotope enrichments of the used tracers were determined by gas chromatography mass spectrometry (Finnigan MAT, Bremen, Germany) as previously described.21

CALCULATIONS

The following equations were used to calculate whole-body palmitate in patients with mitochondrial myopathy and healthy subjects.

Whole-body palmitate rates of appearance (Ra) and rates of disappearance (Rd) were calculated using the non–steady state equations of Steele,23 adapted for stable isotopes,24 as follows:

\[ Ra = \frac{F - V(C_2 - C_1)}{((E_2 + E_1)/(t_2 + t_1))} \]

\[ Rd = Ra - V(C_2 - C_1)\frac{t_2 - t_1}{t_2 + t_1} \]

where F is the infusion rate (in micromoles per kilograms per minute); V = 0.145 and is the distribution volume for the tracer; C1 and C2 are the trace concentrations (in millimoles per liter) at times t1 and t2; and E1 and E2 are the plasma enrichments at t1 and t2.

Breath enrichments are expressed as the tracer/tracee ratio (TTR) as follows:

\[ TTR_{breath} = \frac{(13C/12C)_{sample} - (13C/12C)_{background}}{16} \]

where sa indicates sample and back, the background value.

Whole-body plasma palmitate oxidation was calculated by considering pulmonary \(^{13}C\)O\(_2\) production25 and applying an acetate correction factor (rest = 20; exercise = 75) determined under similar exercise intensities.26

Palmitate oxidation (Ox, in micromoles per minute) was calculated as follows:

\[ Ox_{palmitate} = \frac{(VCO_2 \times TTR_{breath}/16)}{(TTR_{palmitate} \times Acetate \ Correction \ Factor/100)} \]

and carbohydrate oxidation was calculated as follows:

\[ Ox_{CHO} = (4.858VCO_2 - 3.226V_2) \]

STATISTICS

Values are expressed as mean (SE). P < .05 (2-tailed testing) was considered significant. Differences between the patients and the healthy subjects were assessed by an unpaired t test. Differences between rest and exercise in the individual were assessed by a paired t test. Whenever appropriate, 2-way analysis of variance was used to test for changes in time.

RESULTS

O\(_2\) CONSUMPTION

The VO\(_2\)max in patients was almost half of that found in healthy subjects (Table). All subjects exercised for 30 minutes at the same absolute workload (65 [10] W; range, 33-134 W), which corresponded to 72% (4%) of VO\(_2\)max in the patients and 30% (1%) in healthy subjects. Average VO\(_2\) during exercise tended to be higher in the patients (15 [1] mL/kg/min) vs healthy subjects (12 [0] mL/kg/min) (P = .06) (Figure 1A). In accordance with the higher relative workload performed by the patients with mtDNA mutations, the average heart rate and rate of perceived exertion were higher during exercise in patients (140 [9] W and 14 [1] beats/min, respectively) (P < .001) vs healthy subjects (96 [4] W and 8 [0] beats/min, respectively).

SUBSTRATE TURNOVER

The rates of appearance and disappearance of palmitate were similar in the patients and healthy subjects at rest and during exercise (Figure 2B and C). In line with this, palmitate oxidation did not differ between the 2 groups (Figure 2D). Total oxidation of fat was also similar in patients and healthy subjects (Figure 1D). The respiratory exchange rate was higher in the patients than in healthy subjects because of a higher total carbohydrate oxidation in the patients (Figure 1B). Acetate turnover was the same in the patients as in the healthy subjects, indicat-
ing that patients with mitochondrial dysfunction have the same acetate turnover during rest and exercise as healthy subjects. Thus, the same acetate correction factors (20% at rest and 75% during exercise) were used in both subject groups (data not shown).

PLASMA METABOLITE AND HORMONE LEVELS

Plasma palmitate and total free fatty acid levels were similar at rest in the 2 groups. After 30 minutes of exercise, plasma palmitate and free fatty acid levels were increased in healthy subjects, whereas there was no real change in the patients, although the plasma palmitate level increased from the 10th to the 20th minute and from the 20th to the 30th minute of exercise (Figure 2A and Figure 3C). The plasma glucose level was similar at rest and decreased during exercise to the same extent in both groups (Figure 3B). The plasma lactate level was higher in the patient group at rest and during exercise (Figure 3A). At rest, insulin levels were higher in the patients than in healthy subjects (Figure 4A). After 30 minutes of exercise, insulin levels decreased in the healthy subjects only (P = .002) (Figure 4). At rest, the norepinephrine level was higher in the patients than in the healthy subjects; during exercise, norepinephrine and epinephrine levels rose to a higher level in the patients (Figure 4B and C).

COMMENT

In this study, we investigated potential metabolic adaptations during exercise in patients with severe oxidative defects of skeletal muscle. The study showed that patients with mitochondrial myopathy have a normal balance between fat and carbohydrate oxidation during moderate-intensity exercise. The higher energy expenditure during exercise in the patients was caused by lower work efficiency at a higher relative consumption and, in accordance with the relatively higher work intensity, this extra energy was covered by carbohydrate oxidation (Figure 1). Our findings indicate that, when the final common pathway of fuel oxidation is impaired, there is no specific fuel preference, unlike the adaptations seen in other metabolic myopathies affected in earlier enzymatic steps of fuel breakdown. Thus, therapy directed at enhancing combustion of one fuel from another, which is used successfully in other metabolic myopathies affected in earlier enzymatic steps of fuel breakdown, probably will have little or no effect in mitochondrial myopathies.

Figure 2. Plasma palmitate concentration (A), rate of palmitate appearance (lipolysis) (B), rate of palmitate disappearance (palmitate use) (C), and palmitate oxidation (D) at rest and during 30 minutes of exercise at a workload of 70 W in 10 patients with mitochondrial myopathy and 10 healthy subjects. *Different from rest (P < .05). †Different from the 10th minute of exercise (P = .01).

Figure 3. Concentrations of plasma lactate (A), plasma glucose (B), and free fatty acid (FFA) (C) in 10 patients with mitochondrial myopathy and 10 healthy subjects at rest and during 30 minutes of exercise at a workload of 70 W. *Different from healthy subjects (P < .05). †Different from rest (P < .05). ‡Different from the 10th minute of exercise (P = .01). To convert glucose to milligrams per deciliter, divide by 0.0555; and lactate to milligrams per deciliter, divide by 0.111.
Patients with selectively compromised oxidation of fats have exercise intolerance but are able to compensate partly for their impaired fat oxidation by increasing carbohydrate combustion—particularly muscle glycogenolysis—during exercise.29 The exercise intolerance of these patients is also greatly improved by supplements that bypass the metabolic block (ie, a glucose infusion or a carbohydrate-rich diet).12,13,27 Similarly, in McArdle disease, in which muscle glycogenolysis is blocked, patients are able to mobilize and burn more hepatic glucose and fat from adipose tissue during exercise to compensate for the blocked muscle glycogenolysis.13,14,30 and the exercise intolerance is improved by oral or intravenous carbohydrate supplementation during exercise or by a carbohydrate-rich diet.14,15,28,31 In agreement with these findings, it has recently been shown that the glucose transporter protein, GLUT4, and the rate-limiting enzyme of glycogenesis, phosphofructokinase, are upregulated in the muscle of patients with McArdle disease.32

In line with findings in patients with blocked fat and glycogen metabolism, it has been speculated whether the biochemical bottleneck in patients with mitochondrial dysfunction due to mtDNA mutations could be widened by ingesting cofactors of the respiratory chain or by changing the diet toward fat or carbohydrate.8-11,33 One study suggests that excess free fatty acids in blood can bypass the biochemical bottleneck in patients with isolated complex I deficiency.32 The theory behind this suggestion is that increased triglyceride levels will induce an increase in levels of the reduced form of flavin adenine dinucleotide, which is used in complex II of the respiratory chain, thus bypassing complex I. The authors found that 3 of 4 patients with complex I deficiency had a higher exercise endurance than those with complex I deficiency vs carbohydrate infusion,33 but increased triglyceride levels in blood through a fat-rich diet in these conditions did not improve exercise capacity.31 Thus, from a practical standpoint, enhancing fat metabolism as a treatment for mitochondrial dysfunction seems ineffective. In other defects of the respiratory chain, downregulation of fat oxidation could theoretically be beneficial because fat yields less adenosine triphosphate per O2 molecule compared with carbohydrates. Although many of the patients we studied had compensatory increases in levels of mitochondrial enzymes in response to the primary metabolic defect, our findings indicate that there is no preferential oxidation of carbohydrate vs fat. This also indicates that changing the composition of carbohydrates and fats in the diet will not improve exercise capacity in patients with mitochondrial myopathy.

Patients with mitochondrial myopathy have a low V̇O2max compared with healthy subjects. In this study, V̇O2max in the patient group was half of that found in healthy subjects, although the healthy subjects included were sedentary. Thus, it is impossible to find healthy subjects who have a V̇O2max that matches that in patients with mitochondrial myopathy. During absolute workload, subjects use the same amount of fuel, irrespective of V̇O2max, whereas variability in V̇O2max between subjects will result in differences in absolute energy consumption when exercising at the same relative workload.34 Thus, to compare the mix between fat and carbohydrate consumption between patients with mitochondrial myopathy and healthy subjects, all subjects exercised at the same absolute workload.

In health and disease, there is a linear correlation between workload and O2 uptake, but in this study, patients with mitochondrial myopathy had a higher O2 uptake than did the healthy subjects at the same absolute workload (approximately 65 W). A similar pattern of O2 consumption has been demonstrated in patients with circulatory dysfunction and other neuromuscular disorders.35 The cause of the higher O2 uptake relative to workload is a lower work efficiency in patients vs healthy subjects, which relates to a higher use of auxiliary muscles and the exaggerated cardiac and ventilatory response to exercise.37

In healthy subjects, plasma levels of catecholamines increase and those of insulin decrease with incremental exercise intensities.38 In this study, insulin levels at rest were higher in the patients and did not decrease with exercise as seen in the healthy subjects, although the relative work intensity and sympathoadrenal response were higher in the patient group. Insulin resistance has been demonstrated in patients with the common 3243A > G point mutation,39 but insulin sensitivity in other mitochondrial disorders has not been studied. Six of the 10 patients included in this study had mtDNA mutations other than the common 3243A > G mutation, and these patients also had high resting insulin and glucose levels, with insulin levels unresponsive to exercise, indicat-
ing insulin resistance. Thus, insulin resistance may be a common finding in patients with various mtDNA mutations. Despite high insulin levels in these patients, they had normal fat oxidation. The high catecholamine levels seen in the patient group (Figure 4) likely counteracted the inhibitory effect of insulin on lipolysis.

In conclusion, this study shows that, although many of the mitochondria in skeletal muscle of patients with mutations in the mtDNA are dysfunctional, resulting in an impaired maximal exercise capacity and O2 uptake, these patients have a normal mix of fat and carbohydrate oxidation during moderate-intensity exercise. The findings suggest that manipulating dietary fat and carbohydrate content is not a feasible therapeutic treatment for these disorders.

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