In vivo kinetic studies in inborn errors of metabolism: expanding insights in (patho)physiology
Huidekoper, H.H.

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Chapter 6b

In vivo glucose and fat metabolism at rest and during moderate-intensity exercise in patients with medium-chain acyl-CoA dehydrogenase deficiency

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Submitted
Abstract

**Objective:** to study glucose and fat metabolism at rest and during moderate-intensity exercise in patients with medium-chain acyl-CoA dehydrogenase deficiency (MCADD).

**Study design:** contemporary stable isotope methodology with [U-13C]palmitate and [6,6-2H2]glucose in combination with deuterated water was applied to compare fat and glucose metabolism between four adult patients with MCADD (age: 27.3 ± 9.3 yrs, BMI: 24.6 ± 3.8 kg/m²) and four matched control subjects (age: 27 ± 4.6 yrs, BMI: 23.4 ± 3.5 kg/m²) at rest and during 1.5 hrs. of moderate-intensity cycling exercise (50% Wmax).

**Results:** no significant differences were detected between patients with MCADD and control subjects in glucose kinetics, either at rest or during exercise, or in palmitate turnover, FFA turnover, whole-body fat oxidation, carbohydrate oxidation, muscular glycogen oxidation or fat oxidation from plasma and muscle derived triglycerides during exercise. Plasma FFA oxidation was significantly lower in patients at the end of exercise. At rest, plasma FFA turnover was significantly higher in patients with MCADD, whereas plasma FFA concentrations were not significantly different. Norepinephrine at rest was significantly higher in patients with MCADD, but no significant differences in regulatory hormones were detected between patients and control subjects at the end of exercise.

**Conclusions:** whole-body fat oxidation is not impaired and gluconeogenesis is stimulated normally in adult patients with MCADD during moderate-intensity exercise. Plasma FFA turnover at rest is elevated in MCADD patients and might result in ectopic fat accumulation, eventually causing insulin resistance.

**Abbreviations**

- **CHO**: whole-body carbohydrate oxidation
- **EE**: energy expenditure
- **EGP**: endogenous glucose production
- **FAO**: fatty acid oxidation
- **FAT**: whole-body fat oxidation
- **FFA**: free fatty acids
- **GGL**: rate of glycogenolysis
- **GNG**: rate of gluconeogenesis
- **MCADD**: medium-chain acyl-CoA dehydrogenase deficiency
- **R_a**: rate of appearance
- **R_d**: rate of disappearance
- **R_ox**: rate of oxidation
- **RER**: respiratory exchange ratio
- **TG**: triglycerides
Introduction

Patients with medium-chain acyl-CoA dehydrogenase deficiency (MCADD; MIM# 201450) are unable to oxidize medium-chain fatty acids, resulting in the accumulation of fatty acid oxidation (FAO) intermediates and their CoA and carnitine esters. MCADD is probably the most common disorder of FAO (1) and is included in most neonatal screening programs. MCADD generally presents with lethargy and hypoketotic hypoglycemia, usually following an episode of prolonged fasting, especially in combination with an intercurrent infectious disease. Under normal conditions patients with MCADD are completely asymptomatic (2). Long-term sequelae have been reported in MCADD (3;4), most likely attributable to hypoglycemic episode(s) and/or the toxic effects of accumulating CoA or carnitine esters. In general, patients with MCADD are managed by avoidance of prolonged fasting. In accordance, it is suggested that prolonged exercise should better be avoided. Since both dietary fat as well as adipose tissue contain predominantly long-chain fatty acids (5), patients with MCADD should be able to partially oxidize fat, e.g. by reducing palmitic acid (C16:0) to octanoic acid (C8:0) by completing four full cycles of fatty acid oxidation resulting in the production of four acetyl-CoA units. However, whole-body fat oxidation rates have never been measured \textit{in vivo} in MCADD patients. Heales and co-workers did measure oxidation of [1-\textsuperscript{13}C]octanoate in four patients with MCADD, but failed to detect any impairments in [1-\textsuperscript{13}C]octanoate oxidation rates (6). This might be attributed to the activity of other acyl-CoA dehydrogenases with substrate specificity overlapping that of MCAD (7).

The etiology of fasting hypoglycemia in MCADD as well as in other disorders of FAO remains to be elucidated. Impaired gluconeogenesis due to either insufficient activation of pyruvate carboxylase, a key enzyme in gluconeogenesis, caused by low levels of acetyl-CoA or trapping of CoA in acyl-CoA esters (8) and/or by an increased acyl-CoA:CoA ratio, decreasing the activity of other CoA-dependent enzymes involved in gluconeogenesis (9), may be implicated. The latter hypothesis is supported by previous observations of reduced concentrations of metabolites in the gluconeogenic pathway in mice deficient for very-long-chain acyl-CoA dehydrogenase (VLCADD; MIM# 201475) under stressed conditions (10).

In the present study we quantified the major fluxes of both fat and glucose metabolism using contemporary stable isotope methodology in adult patients with MCADD at rest and during moderate-intensity exercise in comparison to matched control subjects. Our goals were to establish the capacity of FAO in MCADD and to determine if gluconeogenesis is indeed impaired \textit{in vivo} in MCADD.
Materials and Methods

Subjects
Four adult patients with enzymatically confirmed MCADD, three males and one female, were studied. All were homozygous for the common 985A>G mutation (11). All patients were in good general health. Four adult healthy volunteers were included as control subjects and were matched for sex, age and body composition (Table 1). Written informed consent was obtained from all subjects prior to the experiments. All studies were approved by the Institutional Review Board.

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Sex</th>
<th>Age (y)</th>
<th>Height (m)</th>
<th>Weight (kg)</th>
<th>Body fat (%)</th>
<th>Fat-free mass (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 1</td>
<td>M</td>
<td>41</td>
<td>1.78</td>
<td>65.8</td>
<td>17.6</td>
<td>54.2</td>
</tr>
<tr>
<td>Patient 2</td>
<td>M</td>
<td>21</td>
<td>1.97</td>
<td>86.2</td>
<td>22.4</td>
<td>66.9</td>
</tr>
<tr>
<td>Patient 3</td>
<td>F</td>
<td>22</td>
<td>1.68</td>
<td>73.4</td>
<td>32.8</td>
<td>49.3</td>
</tr>
<tr>
<td>Patient 4</td>
<td>M</td>
<td>25</td>
<td>1.85</td>
<td>100.0</td>
<td>31.3</td>
<td>68.7</td>
</tr>
<tr>
<td>Control 1</td>
<td>M</td>
<td>32</td>
<td>1.76</td>
<td>70.3</td>
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<td>55.8</td>
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<tr>
<td>Control 2</td>
<td>M</td>
<td>21</td>
<td>1.96</td>
<td>76.4</td>
<td>13.6</td>
<td>66.0</td>
</tr>
<tr>
<td>Control 3</td>
<td>F</td>
<td>27</td>
<td>1.77</td>
<td>71.0</td>
<td>30.3</td>
<td>49.5</td>
</tr>
<tr>
<td>Control 4</td>
<td>M</td>
<td>28</td>
<td>1.83</td>
<td>94.5</td>
<td>30.8</td>
<td>65.4</td>
</tr>
</tbody>
</table>

Prior to the study
At least one week prior to the experimental trials, an incremental exhaustive exercise test was performed on an electrically braked cycle ergometer (Ergometrics er900L, Ergoline, Bitz, Germany) to determine individual maximal oxygen uptake ($\text{VO}_2\text{ max}$) and maximal workload capacity (12). All subjects refrained from exhaustive exercise and maintained a carbohydrate-rich diet three days prior to the experiment. On the evening prior to the experiments, subjects consumed a standardized meal containing 54 Energy % (En%) carbohydrate, 29 En% fat and 17 En% protein. All subjects remained fasted from 8 pm on the evening prior to each experiment.

Patients with MCADD were studied without carnitine supplementation and those who were using carnitine supplementation discontinued supplementation two weeks prior to the experiments.

Preparation of tracers
All tracers were obtained from Cambridge Isotope Laboratories, Cambridge, MA and all were at least 99% pure. All tracer infusion fluids were prepared by the Department of Clinical Pharmacy of the Academic Medical Center, Amsterdam, The Netherlands.
[6,6-2H2]glucose, [1,2-13C]sodium-acetate and [13C]sodium-bicarbonate were all dissolved in 0.9% saline. [U-13C]potassium-palmitate was dissolved in heated (60 °C) sterile water and passed through a 0.2 μm filter into preheated pasteurized plasma solution, after which it was immediately used in the trial of day 1.

Study protocols (figure 1)

Day 1 (palmitate and glucose kinetics): on the previous evening subjects were given deuterium enriched water (2H2O) at a dose of 5 g per kg body water divided in 5 doses within 120 min, to reach 0.5% of deuterium enrichment in body water [13]. A blood sample was collected before administration of the first dose to check for background enrichment of deuterium in plasma water. Hereafter, the subjects were only allowed to drink tap water enriched with deuterated water up to 0.5% until completion of the test. The next morning, subjects were put in the supine position and intravenous catheters were inserted into an antecubital vein of both arms. One catheter was used for infusion of [U-13C]potassium-palmitate and [6,6-2H2]glucose and the other for blood sampling. At baseline, a blood and breath sample was collected to determine background enrichment of tracers in plasma and to determine background enrichment of 13CO2 in expired air. At 9.30 h a primed continuous infusion of [6,6-2H2]glucose was started (bolus: 8.8 μmol/kg; continuous infusion: 0.11 μmol/kg·min) and at 10.00 h a continuous infusion of [U-13C]potassium-palmitate was started (0.01 μmol/kg·min) after administration of a bolus of

![Figure 1. Study protocols of day 1 (palmitate and glucose kinetics) and day 2 (acetate recovery factor). The shaded bars represent doubled rates of tracer infusion during exercise.](image-url)
At 11.30 four blood samples were drawn at 5 minute intervals to determine plasma enrichment of [6,6-²H₂]glucose and [U-¹³C]palmitate under basal resting conditions. Additionally, two blood samples were drawn at 11.30 h and 11.45 h to determine fractional gluconeogenesis under basal resting conditions. Hereafter, subjects started to exercise at a workload of 50% of the $W_{max}$ as determined in the incremental exhaustive exercise test. In order to maintain plasma enrichment of [6,6-²H₂]glucose and [U-¹³C]palmitate during exercise approximately constant, the infusion rates were doubled to 0.22 μmol/kg·min and 0.02 μmol/kg·min, respectively. During exercise, carbon dioxide production ($V_{\text{CO}_2}$) and oxygen consumption ($V_{\text{O}_2}$) were measured every last 10 min of every 15 min of exercise using an Oxycon Pro system in mixed chamber mode (Jaeger, Wuerzburg, Germany) and blood and breath samples were drawn every 15 min until the end of the test. Blood samples were immediately centrifuged at 3000 rpm for 10 min, after which plasma was collected and stored at -20°C. Blood samples for determination of fractional gluconeogenesis were immediately deproteinized after collection by adding an equal amount of 10% perchloric acid. These samples were centrifuged at 4000 rpm for 20 min, after which the supernatant was collected and stored at -20°C. The test was terminated after 1.5 h of exercise, after which subjects were given a carbohydrate-rich drink and a meal to replenish losses. All subjects remained fasted during studies.

Day 2 (acetate recovery factor): in order to correct for $^{13}$C label trapping in the side pathways of the citric acid cycle (14), the acetate recovery factor was determined in all subject individually under the exact same conditions as used in study day 1 (15). Only one intravenous catheter was needed in order to infuse [1,2-¹³C]sodium-acetate. During rest the infusion rate of [1,2-¹³C]sodium-acetate was 0.08 μmol/kg·min and during exercise this rate was increased to 0.16 μmol/kg·min. Breath samples were collected every 15 min during exercise until the end of the test. Study day 2 was done at least 7 days apart from study 1 in each subject.

Termination of exercise in patient 2: patient 2 did not finish the exercise test on study day 1 and stopped the test after 45 minutes of cycling because of fatigue and lightheadedness. He did not experience any adverse clinical symptoms like muscle pain, muscle cramps or nausea and recovered quickly after termination of the test. His plasma glucose was within normal range during the whole test. Because of this, data from three patients with MCADD were compared to data of the control subjects during the second half of exercise.

Analytical methods

Plasma parameters: glucose levels were analyzed on a Beckman glucose analyzer (Beckman Coulter B.V., Mijdrecht, The Netherlands). Insulin and cortisol concentrations were determined on an Immulite 2000 system (Diagnostic Products Corporation, LA, USA). Insulin was measured with a chemiluminescent immunometric assay, cortisol was measured with a chemiluminescent immuno assay. Glucagon was determined by RIA.
Norepinephrine and epinephrine were measured by an in-house HPLC method. Plasma free fatty acid (FFA) levels were measured by an enzymatic method (NEFAC; Wako Chemicals GmbH, Neuss, Germany). Acylcarnitines were measured using electrospray tandem-mass spectrometry. Lactate was determined using an enzymatic Cobas FARA assay (Roche Diagnostics B.V., Almere, the Netherlands).

Plasma \([6,6-2H_2]\)glucose enrichment: glucose enrichments were determined as described previously (13). Briefly, plasma was deproteinized with methanol and evaporated to dryness. The extract was derivatized with hydroxylamine and acetic anhydride (16). The aldonitrile pentaacetate derivative of glucose was extracted into methylene chloride and evaporated to dryness. The extract was reconstituted in ethylacetate and injected into a gas chromatograph/mass spectrometer (HP 6890 series GC system and 5973 Mass Selective Detector, Agilent Technologies, Palo Alto, CA, USA). Separation was achieved on a J&W DB17 column (30 m x 0.25 mm, d, 0.25 μm; J&W Scientific, Folsom, CA). Glucose ions were monitored at \(m/z\) 187, 188 and 189. The isotopic enrichment of glucose was determined by dividing the peak area of \(m/z\) 189 by the peak area of \(m/z\) 187, after correction for background enrichment of \([6,6-2H_2]\)glucose.

Deuterium enrichment in glucose at position C5 and in plasma water: glucose was converted to a hexamethylene tetra-amine (13;17). Hexamethylene tetra-amine was injected into a gas chromatograph/mass spectrometer (HP 6890 series GC system and 5973 Mass Selective Detector, Agilent Technologies, Palo Alto, CA, USA). Separation was achieved on an AT-amine column (30 m x 0.25 mm, d, 0.25 μm; Alltech Associates Inc, Deerfield, IL, USA). Hexamethylene tetra-amine ions were monitored at \(m/z\) 140 and 141. Deuterium enrichment in plasma was determined by a method adapted from Previs et al (18).

Plasma palmitate concentration and \([U-13C]\)palmitate enrichment: plasma free palmitate concentration was measured as described by Ruiter et al (19). The carbon enrichment of palmitate was measured on a GC/C/IRMS system (HP 6890 GC Agilent technologies, Palo Alto, CA, USA, Delta Plus IRMS system Thermo Finningan, Bremen, Germany) Separation was achieved on a CP Sil 19 CB column (25m x 0.32 mm, d, 0.2 μm, Varian, Palo Alto, CA, USA).

\(^{13}\)CO\(_2\) enrichment in expired air: enrichment of \(^{13}\)CO\(_2\) in expired air was determined by gas chromatography continuous-flow, isotope-ratio mass spectrometry (GC-IRMS; BreathMAT\(^\text{Plus}\), Finnigan-MAT, Bremen, Germany).

Calculations and statistical analysis

Ra and Rd of glucose, palmitate and FFA: the rates of appearance (Ra) and disappearance (Rd) of glucose and palmitate were calculated with Steele’s non-steady-state equations (20), corrected for the contribution of the tracer to total compound concentration (21). The volume of distribution (pV) was estimated at 160 ml/kg for glucose and at 40 ml/kg for palmitate (22). The Ra and Rd FFA were calculated by multiplying the Ra and Rd of palmitate, respectively, by the ratio of plasma FFA concentration over plasma palmitate concentration. As these parameters were calculated from differences between
subsequent blood sample data are expressed at the mean time point between the actual time points at which the blood samples were drawn.

**Absolute gluconeogenesis and glycogenolysis:** absolute gluconeogenesis (GNG) was calculated by multiplying \( R_a \) glucose by the fractional gluconeogenesis. Fractional gluconeogenesis (in %) was calculated as the ratio of deuterium enrichment at position C5 in glucose over deuterium enrichment in plasma water (17). Absolute glycogenolysis (GGL) was calculated by subtracting absolute GNG from \( R_a \) glucose.

**Substrate oxidation:** as indirect calorimetry could only be reliably assessed during exercise conditions, since the Oxycon Pro system was calibrated for exercise and not for resting conditions, whole-body fat (FAT) and whole-body carbohydrate oxidation (CHO) rates were only calculated during exercise using the non-respiratory quotient (23). The rate of palmitate oxidation (\( R_{ox} \) palmitate) was calculated from indirect calorimetry, plasma palmitate \( R_a \) and the appearance rate of \(^{13}\)CO\(_2\) in the expired air. The latter was corrected for carbon label retention using the acetate recovery factor as described previously (22). It has been shown that plasma glucose oxidation (\( R_{ox} \) glucose) equals \( R_a \) glucose during moderate-intensity exercise (24). Plasma FFA oxidation (\( R_{ox} \) FFA) was calculated by multiplying \( R_{ox} \) palmitate with the ratio of plasma FFA concentration over plasma palmitate concentration. The contributions of other fat sources, e.g. plasma and muscle derived triglyceride oxidation (\( R_{ox} \) TG), and muscle derived glycogen oxidation (\( R_{ox} \) glycogen) to whole-body fat and carbohydrate oxidation could be calculated by subtracting \( R_{ox} \) FFA or \( R_{ox} \) glucose from FAT or CHO, respectively. For the former it was assumed that every TG molecule contains three fatty acids and that the molecular mass of TG averaged 861 g/mol (25).

**Statistical analysis:** all data are expressed as median (range). The Mann-Whitney \( U \) test was used to determine significant differences between patients with MCADD and control subjects. A \( P \)-value of <0.05 was considered to be statistically significant. All statistical analyses were done with SPSS software (SPSS Inc., Chicago, Illinois).

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<p>| Table 2. Glucose kinetics at rest and during exercise |</p>
<table>
<thead>
<tr>
<th>Parameter</th>
<th>MCADD patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exercise (min)</td>
<td>rest</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>5.1 (4.8-5.3)</td>
</tr>
<tr>
<td>EGP (μmol/kg·min)</td>
<td>12.7 (9.0-13.5)</td>
</tr>
<tr>
<td>( R_a ) glucose (μmol/kg·min)</td>
<td>12.4 (9.3-13.8)</td>
</tr>
<tr>
<td>GGL (μmol/kg·min)</td>
<td>5.3 (3.7-7.8)</td>
</tr>
<tr>
<td>GNG (μmol/kg·min)</td>
<td>6.7 (4.7-8.0)</td>
</tr>
</tbody>
</table>

* \( n = 3 \)
Results

Maximal exhaustive exercise test

\( \text{Vo}_2 \text{ max} \) was 4.08 (2.89-4.23) l/min in patients with MCADD and 3.51 (3.0-3.91) l/min in control subjects. \( W_{\text{max}} \) was 308 (225-335) watts in patients and 283 (255-345) watts in control subjects. Both were not significantly different between patients and control subjects.

Plasma parameters

All subjects remained normoglycemic during the studies (Table 2). Plasma FFA concentrations increased in all subjects during exercise (Table 3), as did plasma octanoylcarnitine in patients with MCADD (Table 4), indicating activation of FAO during exercise. No significant differences were detected during either rest or exercise in plasma concentrations of palmitate, FFA, insulin, glucagon, cortisol and epinephrine between patients with MCADD and control subjects (Tables 3 and 4). Norepinephrine levels were significantly higher in patients with MCADD at rest (\( P = 0.021 \); Table 4).

Respiratory exchange ratio (RER) and energy expenditure (EE)

In all subjects, a decrease in RER was observed during exercise. RER was 0.90 (0.84-0.97) in patients with MCADD and 0.87 (0.81-0.88) in control subjects after 38 min of exercise, and 0.84 (0.83-0.89) in patients and 0.83 (0.80-0.86) in control subjects after 83 min of exercise. No significant differences were detected in RER between patients and control subjects during exercise.

EE remained approximately constant during exercise in all subjects as it was 0.53 (0.43-0.58) kJ/kg-min in patients with MCADD and 0.51 (0.46-0.62) kJ/kg-min in control subjects after 38 min of exercise, and 0.45 (0.44-0.63) kJ/kg-min in patients with MCADD and 0.52 (0.48-0.61) kJ/kg-min after 83 min of exercise. Differences in EE between patients and control subjects were not significant.
Glucose kinetics and oxidation

Glucose kinetics in all subjects during rest and exercise are reported in Table 2. No significant differences were detected between patients and control subjects during either rest or exercise, neither in EGP, \( R_d \) glucose, GGL and GNG (Table 2) nor in CHO, \( R_{ox} \) glucose and \( R_{ox} \) glycogen (Figure 2). CHO decreased during exercise in all subjects as did \( R_{ox} \) glycogen, whereas \( R_{ox} \) glucose increased during exercise in all subjects (Figure 2).

### Table 3. Palmitate and FFA kinetics at rest and during exercise

<table>
<thead>
<tr>
<th>Parameter</th>
<th>MCADD patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exercise (min)</td>
<td>rest</td>
</tr>
<tr>
<td>Palmitate (mmol/L)</td>
<td>0.16 (0.12-0.19)</td>
</tr>
<tr>
<td>( R_a ) palmitate (μmol/kg-min)</td>
<td>2.87 (1.72-3.89)</td>
</tr>
<tr>
<td>( R_d ) palmitate (μmol/kg-min)</td>
<td>2.87 (1.79-3.95)</td>
</tr>
<tr>
<td>( R_{ox} ) palmitate (μmol/kg-min)</td>
<td>ND</td>
</tr>
<tr>
<td>FFA (mmol/L)</td>
<td>0.78 (0.51-0.95)</td>
</tr>
<tr>
<td>( R_a ) FFA (μmol/kg-min)</td>
<td>12.55 (8.74-19.96) †</td>
</tr>
<tr>
<td>( R_d ) FFA (μmol/kg-min)</td>
<td>12.57 (9.08-20.29) †</td>
</tr>
<tr>
<td>( R_{ox} ) FFA (μmol/kg-min)</td>
<td>ND</td>
</tr>
</tbody>
</table>

* \( n = 3 \)
† \( P <0.05 \) as compared to control subjects
ND = not determined

### Table 4. Plasma hormones, octanoylcarnitine and ketone body concentrations

<table>
<thead>
<tr>
<th>Parameter</th>
<th>MCADD patients</th>
<th>Control subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>rest</td>
<td>end exercise*</td>
</tr>
<tr>
<td>Insulin (pmol/L)</td>
<td>61 (45-103)</td>
<td>28 (23-46)</td>
</tr>
<tr>
<td>Glucagon (ng/L)</td>
<td>52 (35-63)</td>
<td>79 (58-86)</td>
</tr>
<tr>
<td>Cortisol (nmol/L)</td>
<td>294 (251-532)</td>
<td>371 (287-438)</td>
</tr>
<tr>
<td>Epinephrine (nmol/L)</td>
<td>0.15 (0.08-0.28)</td>
<td>0.58 (0.45-1.82)</td>
</tr>
<tr>
<td>Norepinephrine (nmol/L)</td>
<td>1.46 (0.89-1.92) †</td>
<td>6.80 (4.55-8.24)</td>
</tr>
<tr>
<td>Octanoylcarnitine (μmol/L)</td>
<td>2.41 (1.63-4.30) †</td>
<td>10.58 (9.56-13.58) †</td>
</tr>
<tr>
<td>Free carnitine (μmol/L)</td>
<td>11.32 (8.73-19.23) †</td>
<td>20.43 (12.75-29.26)</td>
</tr>
<tr>
<td>Lactate (mmol/L)</td>
<td>0.8 (0.5-0.9)</td>
<td>0.9 (0.9-1.0) †</td>
</tr>
</tbody>
</table>

* \( n = 3 \)
† \( P <0.05 \) as compared to control subjects
Palmitate, FFA and fat kinetics and oxidation

Palmitate and FFA kinetics are described in Table 3. During rest, both R\textsubscript{a} FFA and R\textsubscript{d} FFA were significantly higher in patients with MCADD (P = 0.043). No significant differences could be detected in R\textsubscript{a} palmitate, R\textsubscript{a} FFA, R\textsubscript{d} palmitate, R\textsubscript{d} FFA, FAT and R\textsubscript{ox} TG during exercise between patients and control subjects. At the end of exercise, R\textsubscript{ox} palmitate and R\textsubscript{ox} FFA were significantly lower in patients with MCADD (P = 0.034; Table 3 / Figure 2).

FAT increased in all subjects during exercise, as did R\textsubscript{ox} palmitate and R\textsubscript{ox} FFA (Figure 3; Table 4). R\textsubscript{ox} TG remained essentially the same in patients with MCADD, whereas it first increased in control subjects during exercise and then decreased during the second part of the exercise test (Figure 2).

Discussion

We report on glucose and fat metabolism in patients with MCADD deficiency during rest and moderate-intensity exercise after an overnight fast compared to matched healthy control subjects. Our data suggest that whole-body fat oxidation is not significantly impaired and gluconeogenesis is not inhibited in patients with MCADD under the conditions studied. However, basal plasma FFA turnover at rest after an overnight fast was significantly higher in patients with MCADD than in control subjects.

Three out of four patients with MCADD were able to complete the 1.5 hrs. of moderate-intensity exercise twice without any apparent clinical or biochemical adverse affects. Patient 2 did stop to exercise after 45 min. on study day 1 because of fatigue and lightheadedness. His biochemical parameters did not differ from the other patients. In addition, as he experienced anxiety for the first test, and as he did not experience the same symptoms on study day 2, failure to complete the test on day 1 was likely not related to the MCADD.
An increase in FAT was observed in all subjects during exercise, mainly due to an increase in plasma fatty acid oxidation (Figure 2). CHO decreased in all subjects as a result of decreased muscle glycogen oxidation, which was only partially balanced by an increase in plasma glucose oxidation (Figure 2). This corresponds to previously published data on substrate utilization during moderate-intensity exercise obtained in healthy subjects (22;26). Both FAT and CHO did not differ significantly between patients with MCADD and control subjects during exercise, although plasma FFA oxidation was significantly lower in patients at the test. Comparable studies in patients with defects in long-chain FAO, VLCADD and carnitine palmitoyltransferase II deficiency (CPT II; MIM# 600650),

**Figure 2.** Fat and carbohydrate oxidation during moderate-intensity exercise in patients with MCADD (●) and control subjects (○) (mean ± SEM). The left panel shows whole-body fat oxidation (FAT), plasma free fatty acid oxidation (Rox FFA) and fat oxidation from plasma and muscle derived triglycerides (Rox TG). The right panel shows whole-body carbohydrate oxidation (CHO), plasma glucose oxidation (Rox glucose) and muscle derived glycogen oxidation (Rox glycogen). 'a' means n=3.
indeed demonstrated an impaired FAT during 1 h of moderate-intensity exercise, which was fully compensated by CHO as reflected by a higher RER during exercise in patients compared to control subjects (27,28). However, as in our study RER did not differ significantly between patients and control subjects, FAT and CHO likely contributed equally to energy production during exercise. We conclude that whole-body fat oxidation was not significantly impaired in patients with MCADD during moderate-intensity exercise. As FAO was stimulated through both fasting and exercise we suggest that adult patients with MCADD not only have a good tolerance towards overnight fasting but also to prolonged moderate-intensity exercise without carnitine supplementation. This is supported by previously published data on fasting and exercise tolerance in MCADD (29,30).

No significant differences were detected in EGP, GNG and GGL between patients with MCADD and control subjects during both rest and exercise (Table 2). In all subjects, GNG and GGL contributed approximately 50% to EGP after an overnight fast during resting conditions, in line with previously published data on glucose kinetics after an overnight fast in healthy adults (31). During exercise, both GGL and GNG increased resulting in an almost threefold increase in EGP in concordance with previously reported data on EGP in healthy subjects at an exercise workload of 50% of $W_{\text{max}}$ (22). It has been suggested that hypoglycemia in MCADD may be caused by inactivation of pyruvate carboxylase due limited availability of acetyl-CoA, resulting in impaired gluconeogenesis (8). In addition, gluconeogenesis may also be hampered by sequestration of CoA due to by accumulating acyl-CoA esters, inactivating CoA dependent enzymes involved in gluconeogenesis (9). In our study we did not find any evidence of impaired gluconeogenesis in MCADD, as GNG during exercise increased to the same extent in both patients and control subjects, despite accumulation of FAO intermediates in patients (Table 4). However, skeletal muscle will be the predominant site of FAO during exercise and not the liver. Therefore, it is highly likely that during exercise in patients with MCADD FAO in hepatocytes still produces sufficient acetyl-CoA to stimulate pyruvate carboxylase, without significant sequestration of CoA, resulting in a normal upregulation of gluconeogenesis. Impaired gluconeogenesis may still play a role in the pathophysiology of fasting hypoglycemia under conditions where FAO is upregulated in order to produce ketone bodies.

An unexpected finding was a significantly higher plasma R₃FFA after an overnight fast in patients with MCADD compared to control subjects (Table 3), demonstrating a higher rate of lipolysis in patients. Recently, this was also reported in a patient with 3-hydroxyacyl-CoA dehydrogenase deficiency (LCHADD; MIM# 609016), a disorder of long-chain FAO. This was suggested to be a compensatory mechanism for failing energy production in an attempt to increase fatty acid availability for oxidation by other acyl-CoA dehydrogenases (32). However, the mechanism causing increased lipolysis was not elucidated as no differences were detected in hormones involved in regulating lipolysis. In contrast, we showed a significantly higher norepinephrine concentration in plasma of patients with MCADD after an overnight fast (Table 4). As catecholamines are the
primary activators of lipolysis during fasting (33) the increased rate of lipolysis may be explained by the high norepinephrine concentration.

Not only $R_a$ FFA but also $R_d$ FFA was significantly higher in patients. A prominent feature in MCADD and other FAO disorders is accumulation of intracellular fat in liver and muscle after metabolic derangement (34). The higher $R_d$ FFA as detected in our study in patients with MCADD suggests that a higher influx of fatty acids may also contribute to intracellular fat accumulation in FAO disorders, already after an overnight fast without any signs of metabolic derangement. This might result in an increased risk for insulin resistance in patients with FAO disorders as ectopic fat accumulation in liver and muscle tissue has been implicated in the pathophysiology of insulin resistance (35). Indeed, hepatic insulin resistance was recently reported in LCAD -/- mice, a model for a defective long-chain FAO (36). It may therefore be of interest to study insulin resistance in older patients with FAO disorders.

In conclusion, we demonstrate that moderate-intensity exercise after an overnight fast is well tolerated in adult patients with MCADD as a result of sufficient muscular energy production from both FAT and CHO. In addition, gluconeogenesis during exercise is increased to the same extent in patients as in control subjects. Finally, the higher FFA turnover after an overnight fast may contribute to intracellular fat accumulation, making patients with a FAO disorder prone to insulin resistance.

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References


