Normal rates of whole-body fat oxidation and gluconeogenesis after overnight fasting and moderate-intensity exercise in patients with medium-chain acyl-CoA dehydrogenase deficiency

Hidde H. Huidekoper[1], Mariëtte T. Ackermans[2], René Koopman[4], Luc J.C. van Loon[4], Hans P. Sauerwein[3], and Frits A. Wijburg[1,*]

[1]Department of Pediatrics, [2]Department of Clinical Chemistry, Laboratory for Endocrinology and Radiochemistry, [3]Department of Endocrinology and Metabolism, Academic Medical Centre, University of Amsterdam, Amsterdam, The Netherlands; [4]Department of Human Movement Sciences, Maastricht University Medical Centre, Maastricht, The Netherlands.

[*]Correspondence:
Frits A. Wijburg, MD PhD
Department of Pediatrics (H7-270)
Academic Medical Center
University Hospital of Amsterdam
PO-box 22660
NL-1100 DD Amsterdam
The Netherlands
Email: f.a.wijburg@amc.nl
Phone: +31-20-5662567
Fax: +31-20-6917735

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Summary

Background: Impairments in gluconeogenesis have been implicated in the pathophysiology of fasting hypoglycemia in medium-chain acyl-CoA dehydrogenase deficiency. However, whole body glucose and fat metabolism have never been studied *in vivo.*

Methods: Stable isotope methodology was applied to compare fat and glucose metabolism between four adult patients with MCADD and four matched controls both at rest and during 1.5 h of moderate-intensity exercise. Additionally, intramyocellular lipid and glycogen content and intramyocellular acylcarnitines were assessed in muscle biopsies collected prior to and immediately after cessation of exercise.

Results: At rest, plasma FFA turnover was significantly higher in patients with MCADD, whereas the plasma FFA concentrations did not differ between patients and controls. Blood glucose kinetics did not differ between groups both at rest and during exercise. Palmitate and FFA turnover, total fat and carbohydrate oxidation rate, the use of muscle glycogen and muscle derived triglycerides during exercise did not differ between patients and controls. Plasma FFA oxidation rates were significantly lower in patients at the latter stages of exercise. Free carnitine levels in muscle were lower in patients, whereas no differences were detected in muscle acetylcarnitine levels.

Conclusions: Whole-body or skeletal muscle glucose and fat metabolism were not impaired in adult patients with MCADD. This implies that MCADD is not rate limiting for energy production under the conditions studied. In addition, patients with MCADD have a higher FFA turnover rate after overnight fasting, which may stimulate ectopic lipid deposition and, as such, make them more susceptible for developing insulin resistance.

Synopsis: Adult patients with MCADD exhibit normal rates of whole-body fat oxidation and gluconeogenesis after overnight fasting and during moderate-intensity exercise suggesting that MCADD is not rate limiting for energy production under the conditions studied.

Keywords: fatty acid oxidation; glucose metabolism; fasting; exercise; medium-chain acyl-CoA dehydrogenase deficiency

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

In patients with medium-chain acyl-CoA dehydrogenase deficiency (MCADD; MIM# 201450) oxidation of medium-chain fatty acids is severely hampered, 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 (Tanaka et al 1997) and is included in most newborn 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 (Roe et al 2001). Long-term sequelae, probably attributable to the initial episode(s) of hypoglycemia, have been reported in MCADD (Derks et al 2006; Saudubray et al 1999). Patients are managed by avoidance of prolonged fasting. The role of restriction of prolonged moderate-intensity exercise, which largely depends on (long-chain) fatty acids as substrate for energy production, has not been intensively studied.

Patients with MCADD should be able to use a significant portion of stored or ingested long-chain fatty acids for generation of ATP by oxidizing palmitic acid (C16:0) to octanoic acid (C8:0). However, whole-body fat oxidation rates have not been measured in MCADD patients. Heales and co-workers did measure oxidation of [1-^{13}C]octanoate in four patients with MCADD, but remarkably did not detect an impaired oxidation rate (Heales et al 1994). This might be attributed to the activity of other acyl-CoA dehydrogenases with substrate specificity overlapping that of MCAD (Wanders et al 1988).

The etiology of fasting hypoglycemia in FAO disorders still remains to be completely elucidated. Impaired gluconeogenesis, due to insufficient activation of pyruvate carboxylase (EC 6.4.1.1) by low levels of acetyl-CoA or trapping of CoA in acyl-CoA esters (Griffith et al

1989), and an increased acyl-CoA:CoA ratio, decreasing the activity of other CoA-dependent enzymes involved in gluconeogenesis (Kean et al 1979), have been implicated.

In the present study we assessed whole-body and skeletal muscle glucose and fat metabolism in adult patients with MCADD in comparison to matched control subjects using *in vivo* stable isotope techniques, in combination with extensive metabolic studies in plasma and muscle biopsies, during rest and moderate intensity cycling exercise.

Materials and Methods

Subjects

Four adult patients with enzymatically confirmed MCADD, all homozygous for the common 985A>G mutation (Wang et al 1999), were studied. All patients were in good general health. Four adult healthy volunteers, with normal acylcarnitine profiling, matched for sex, age and body composition (Table 1), were studied as controls. Written informed consent was obtained from all subjects prior to the experiments. All studies were approved by the Institutional Review Board of the University Hospital of Amsterdam.

Prior to the study

One to three weeks 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 ($Vo_{2\ max}$) and maximal workload capacity (Kuipers et al 1985). All subjects refrained from exhaustive exercise and maintained a carbohydrate-rich diet 3 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 p.m. on the evening prior to each experiment.

Patients were studied without carnitine supplementation and patients who used carnitine (patients 1 and 2) stopped this medication 2 weeks prior to the experiments.

Preparation of tracers

All tracers were obtained from Cambridge Isotope Laboratories, Cambridge, MA and all were 99% pure. All tracer infusion fluids were prepared by the Department of Clinical Pharmacy of

the Academic Medical Center, Amsterdam, The Netherlands. [6,6-$^{2}H_2$]glucose, [1,2-^{13}C]-sodium-acetate and [^{13}C]sodium-bicarbonate were all dissolved in 0.9% saline. [U-^{13}C]-potassium-palmitate was dissolved in heated (60 °C) sterile water and passed through a 0.2 μm filter into a preheated pasteurized plasma solution, after which it was immediately used.

Study protocols (Figure 1)

Study 1 (plasma palmitate and glucose kinetics): on the evening prior to the study subjects were given deuterium enriched water ($^{2}H_2O$) 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 (Ackermans et al 2001). A blood sample was collected before administration of the first dose to check for background enrichment of deuterium in plasma water. Thereafter, subjects were only allowed to drink 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 in both arms. One catheter was used for infusion of [U-^{13}C]-potassium-palmitate and [6,6-$^{2}H_2$]glucose and the other for venous blood sampling. At baseline, a blood and breath sample were collected to determine background plasma tracer enrichment and to determine background $^{13}CO_2$ enrichment in the expired breath. Thereafter, a muscle biopsy was taken from the *vastus lateralis* muscle using the Bergström technique (Bergstrom 1975), which was immediately frozen in liquid nitrogen. At 9.30 h a primed continuous infusion of [6,6-$^{2}H_2$]glucose (99% pure; Cambridge Isotope Laboratories, Cambridge, MA) was started (bolus: 8.8 μmol/kg; continuous infusion: 0.11 μmol/kg·min) and at 10.00 h a continuous infusion of [U-^{13}C]potassium-palmitate was started (0.01 μmol/kg·min) after administration of a bolus of [^{13}C]sodium-bicarbonate to prime the bicarbonate pool (0.74 μmol/kg). At 11.30, 4 blood samples were drawn at 5 min intervals to determine plasma enrichment of [6,6-$^{2}H_2$]glucose and [U-^{13}C]palmitate under basal resting

conditions. Additionally, blood samples were collected at 11.30 and 11.45 h to determine fractional gluconeogenesis under basal resting conditions. Thereafter, subjects started to exercise at a workload of 50% W_{max} as previously determined by the incremental exhaustive exercise test. To maintain plasma [6,6-$^{2}H_2$]glucose and [U-^{13}C]palmitate enrichment levels during exercise, the infusion rates were doubled to 0.22 µmol/kg·min and 0.02 µmol/kg·min, respectively. During exercise, carbon dioxide production (Vco_2) and oxygen consumption (Vo_2) were assessed during the latter 10 min of each 15 min period throughout the exercise bout using an Oxycon Pro system in mixed chamber mode (Jaeger, Wuerzburg, Germany). Blood and expired breath samples were collected every 15 min until the end of the test. Blood samples were immediately centrifuged at 1000 *g* for 10 min, after which plasma was collected and stored at -20ºC. 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 1300 *g* for 20 min, after which the supernatant was collected and stored at -20ºC. The test was terminated after 1.5 h of exercise or at exhaustion, after which a second muscle biopsy from the *vastus lateralis* muscle was immediately taken and frozen in liquid nitrogen. Hereafter, subjects were given a carbohydrate-rich drink and a meal.

Study 2 (acetate recovery factor): to correct for temporary ^{13}C label entrapment in the side pathways of the tricarboxylic acid cycle (Sidossis et al 1995), the acetate recovery factor was determined in all subjects individually under the exact same conditions as in study 1 (van Loon et al 2003). Only one intravenous catheter was needed to infuse [1,2-^{13}C]-sodium-acetate. During rest the infusion rate of [1,2-^{13}C]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 2 was done at least 7 days apart from study 1 in each subject.

Termination of exercise in patient 2: patient 2 was unable to continue exercise for more than 45 min on study 1 because of fatigue and lightheadedness. He did not experience any adverse clinical symptoms such as muscle pain, muscle cramps or nausea and recovered quickly after cessation of exercise. His plasma glucose was within normal range during the entire 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: plasma glucose concentrations were analyzed on a Beckman glucose analyzer (Beckman Coulter B.V., Mijdrecht, The Netherlands). Plasma 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. Plasma glucagon concentrations were determined by RIA (Linco Research, St. Charles, MO, USA). Plasma norepinephrine and epinephrine concentrations were measured by an in-house HPLC method. Plasma free fatty acid (FFA) levels were measured by an enzymatic method (NEFA-C; Wako Chemicals GmbH, Neuss, Germany). Plasma acylcarnitines were measured using electrospray tandem-mass spectrometry (van Vlies et al 2005). Acetoacetic acid, 3-hydroxybutyric acid and lactate were measured using a enzymatic/spectrophotometric Cobas FARA assay (Roche Diagnostics B.V, Almere, the Netherlands).

Plasma [6,6-$^{2}H_{2}$]glucose enrichment: Plasma glucose enrichments were determined as described previously (Ackermans et al 2001). Briefly, plasma was deproteinized with methanol and evaporated to dryness. The extract was derivatized with hydroxylamine and acetic anhydride (Reinauer et al 1990). 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_f 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 [6,6-$^2H_2$]glucose enrichment.

Deuterium enrichment in glucose at position C5 and in plasma water: glucose was converted to a hexamethylene tetra-amine (Ackermans et al 2001; Landau et al 1996). 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_f 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 (Previs et al 1996).

Plasma palmitate concentration and [U-^{13}C]palmitate enrichment: plasma free palmitate concentrations were measured as described by Ruiter et al (Ruiter et al 2001). 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_f 0.2 µm, Varian, Palo Alto, CA, USA).

$^{13}CO_2$ enrichment in expired air: $^{13}CO_2$ enrichment in expired air was determined by gas chromatography continuous-flow, isotope-ratio mass spectrometry (GC-IRMS; BreathMAT^{plus}, Finnigan-MAT, Bremen, Germany).

Muscle biopsy analysis: immunofluorescence assays were performed to assess intramyocellular lipid content (Oil red O-staining (Koopman et al 2001)) and intramyocellular

glycogen content (PAS staining (Schaart et al 2004)) as described previously. Acylcarnitine profiling was performed according to the method of van Vlies et al (van Vlies et al 2005).

Calculations and statistical analysis

R_a and R_d of glucose, palmitate and FFA: the glucose and palmitate appearance (R_a) and disappearance (R_d) rates were calculated with Steele's non-steady-state equations (Steele 1959), corrected for the contribution of the tracer to total compound concentration (Rosenblatt et al 1988). The volume of distribution (pV) was estimated at 160 ml/kg for glucose and at 40 ml/kg for palmitate (van Loon et al 2001). The plasma FFA R_a and R_d were calculated by multiplying the plasma palmitate R_a and R_d, respectively, by the ratio of plasma FFA concentration over plasma palmitate concentration. As these parameters were calculated from differences between subsequent blood samples, data are expressed at a time point between the actual time points during which blood samples were collected.

Absolute gluconeogenesis and glycogenolysis: absolute gluconeogenesis (GNG) was calculated by multiplying glucose R_a 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 (Landau et al 1996). 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 carbohydrate (CHO) oxidation rates were only calculated during exercise using the non-protein respiratory quotient (Peronnet et al 1991). The palmitate oxidation rate (R_{ox}) was calculated from indirect calorimetry, plasma palmitate R_d 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 (van Loon et al 2001).

It has been shown that plasma glucose oxidation (R_{ox} glucose) equals glucose R_d glucose during moderate-intensity exercise (Jeukendrup et al 1999). Plasma FFA oxidation (R_{ox} FFA) was calculated by multiplying palmitate R_{ox} 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 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 (Frayn 1983).

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 IBM SPSS Statistics v19 software (2010 SPSS Inc./IBM).

Results

Exercise performance

$Vo_{2\ max}$ and W_{max} were assessed at 4.08 (2.89-4.23) L/min and 308 (225-335) W in patients and 3.51 (3.0-3.91) L/min and 283 (255-345) W in control subjects respectively. Differences between groups were not significant.

Plasma parameters

All subjects remained normoglycemic during all experiments (Table 2). Plasma FFA concentrations increased in all subjects during exercise (Table 3), as did plasma octanoylcarnitine levels in MCADD patients (Table 4). No significant differences were detected in plasma palmitate, FFA, acetoacetic acid, 3-hydroxybutyric acid , insulin, glucagon, cortisol and epinephrine concentrations between MCADD patients and controls at rest or during exercise (Tables 3 and 4). At rest, plasma norepinephrine levels were higher in patients with MCADD patients when compared with the matched controls ($P = 0.021$; Table 4).

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

In all subjects, a decline 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 with a median value of 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. No significant differences were observed in EE between patients and control subjects.

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 at rest or during 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).

Palmitate, FFA and fat kinetics and oxidation

Plasma palmitate and FFA kinetics are described in Table 3. During rest, both R_a FFA and R_d FFA were significantly higher in patients with MCADD ($P = 0.043$). No significant differences could be detected in R_a palmitate, R_a FFA, R_d palmitate, R_d FFA, FAT and R_{ox} TG during exercise between patients and control subjects. At the end of exercise, R_{ox} palmitate and R_{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_{ox} palmitate and R_{ox} FFA (Figure 3; Table 4). R_{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).

Muscle biopsy analysis

No significant differences were detected between patients and controls in intramyocellular lipid content and intramycocellular glycogen content in muscle biopsies, both before or after

exercise (*data not shown*). Acylcarnitines in muscle biopsies are described in Table 5. Octanoylcarnitine was significantly higher in patients with MCADD both before ($P = 0.028$) and after ($P = 0.021$) exercise, as was hexanoylcarnitine (*before:* $P = 0.042$; *after:* $P = 0.020$). Free carnitine was significantly lower in patients with MCADD both before ($P = 0.043$) and after ($P = 0.021$) exercise. Acetylcarnitine was not significantly different between patients and control subjects, either before ($P = 0.564$) or after ($P = 0.083$) exercise.

Discussion

We report on glucose and fat metabolism in otherwise healthy adult patients with MCADD at rest and during moderate-intensity exercise following an overnight fast and compare these data with those obtained in matched healthy control subjects. Remarkably, whole-body fat oxidation in patients was not significantly impaired during exercise and gluconeogenesis was not inhibited. However, basal plasma FFA turnover at rest after an overnight fast was significantly higher in patients than in controls.

Three out of four patients with MCADD were able to complete the 1.5 h of moderate intensity exercise twice without any apparent clinical or biochemical adverse affects. Patient 2 stopped to exercise after 45 min. during study 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 whole-body fat oxidation (FAT) was observed in all subjects during exercise, mainly due to an increase in plasma fatty acid oxidation (Figure 2). Whole-body carbohydrate oxidation (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 (Romijn et al 1993; van Loon et al 2001). Both FAT and CHO did not differ significantly between patients and control subjects during exercise, although plasma FFA oxidation was significantly lower in patients at the end of the test. Comparable studies in patients with defects in long-chain FAO, very-long-chain acyl-CoA dehydrogenase deficiency (VLCADD; MIM# 201475) and carnitine palmitoyltransferase II deficiency (CPT II; MIM# 600650), 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 (Orngreen et al 2004; Orngreen et al 2005). However, plasma palmitate oxidation was likely underestimated in these studies as an individual acetate recovery factor, correcting for temporary ^{13}C label fixation in the side pathways of the tricarboxylic acid cycle during studies (Sidossis et al 1995), was not applied. RER did not differ significantly between patients and control subjects in our study suggesting that FAT and CHO likely contributed equally to energy production during exercise. This is supported by the observation that no significant differences could be detected between patients and controls in intramyocellular lipid and glycogen content. We therefore conclude that whole-body fat oxidation is not significantly impaired in patients with MCADD during moderate-intensity exercise. As in our study 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, despite the observation that free carnitine levels in muscle were significantly lower in patients than in control subjects. This is supported by previously published data on fasting and exercise tolerance in MCADD (Derks et al 2007; Huidekoper et al 2006).

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 (Boden 2004). During exercise, both GGL and GNG increased resulting in an almost threefold increase in EGP in line with previously reported data on EGP in healthy subjects at an exercise workload of 50% of W_{max} (van Loon et al 2001). Thus, in our study we did not find any evidence of impaired gluconeogenesis in MCADD patients, despite significant accumulation of FAO intermediates (Table 4/5), demonstrating that inactivation of

pyruvate carboxylase or decreased activity of CoA-dependent enzymes involved in gluconeogenesis did not play a role after overnight fasting and prolonged moderate-intensity exercise in MCADD.

We detected a significantly higher plasma R_a FFA after an overnight fast in patients with MCADD compared to control subjects (Table 3), demonstrating a higher rate of lipolysis in patients. This was also reported in a patient with the disorder of long-chain FAO, long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency (LCHADD; MIM# 609016) (Halldin et al 2007). This was considered to be a compensatory mechanism for failing energy production, by increasing fatty acid availability for oxidation by other acyl-CoA dehydrogenases (Halldin et al 2007). However, the mechanism that caused the increased lipolysis was not understood, as no differences were detected in levels of regulatory hormones. We show 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 (Duncan et al 2007) the increased rate of lipolysis can be explained by the high norepinephrine concentration.

Not only the R_a FFA but also the R_d FFA was significantly higher in patients after an overnight fast. A prominent feature in patients with MCADD and other FAO disorders is accumulation of intracellular fat in liver and muscle after a metabolic derangement (Roe et al 2001). Although no significant differences could be detected in intramyocellular lipid content at baseline in this study, the higher R_d FFA in patients with MCADD suggests a higher influx of fatty acids in tissues, which may eventually contribute to intracellular fat accumulation even in the absence of a metabolic derangement with hypoglycemia. This might result in an increased risk for insulin resistance as ectopic fat accumulation in liver and muscle has been implicated in the pathophysiology of insulin resistance (Petersen et al 2006). Indeed, hepatic insulin resistance could be demonstrated in LCAD -/- mice, a model for a defective long-

chain FAO (Zhang et al 2007). It may therefore be of interest to study insulin resistance in older patients with FAO disorders.

In conclusion, moderate-intensity exercise after an overnight fast without carnitine supplementation is well tolerated in otherwise healthy adult patients with MCADD, and is not accompanied by a significant decrease in whole-body fat oxidation or an altered substrate selection for energy production as compared to control subjects. The physiological increase in gluconeogenesis during exercise is not impaired in MCADD. Therefore, there is no need to discourage normal, moderately intense, exercise in healthy MCADD patients. However, patients with MCADD have higher FFA turnover rate with an increased rate of disappearance of fatty-acids at fasting during rest, which may make them prone to develop insulin resistance. Avoiding prolonged fasting periods may therefore still be important in MCADD patients, even in the absence of an intercurrent illness.

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Legends

Figure 1 Study protocols of study 1 (palmitate and glucose kinetics) and study 2 (acetate recovery factor). The shaded bars represent doubled rates of tracer infusion during exercise.

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 (R_{ox} FFA) and fat oxidation from plasma and muscle derived triglycerides (R_{ox} TG). The right panel shows whole-body carbohydrate oxidation (CHO), plasma glucose oxidation (R_{ox} glucose) and muscle derived glycogen oxidation (R_{ox} glycogen). 'a' means n=3.

Table 1. Subjects' characteristics

Subjects	Sex	Age (y)	Height (m)	Weight (kg)	Body fat (%)	Fat-free mass (kg)
Patient 1	M	41	1.78	65.8	17.6	54.2
Patient 2	M	21	1.97	86.2	22.4	66.9
Patient 3	F	22	1.68	73.4	32.8	49.3
Patient 4	M	25	1.85	100.0	31.3	68.7
Control 1	M	32	1.76	70.3	20.6	55.8
Control 2	M	21	1.96	76.4	13.6	66.0
Control 3	F	27	1.77	71.0	30.3	49.5
Control 4	M	28	1.83	94.5	30.8	65.4

Table 2 Glucose kinetics at rest and during exercise

Parameter	**MCADD patients**			**Control subjects**	
Exercise (min)	rest	38	83*****	rest	38
Glucose (mmol/L)	5.1 (4.8-5.3)	5.3 (4.7-6.1)	4.2 (4.0-5.0)	5.2 (4.8-5.2)	4.6 (4.4-5.5)
EGP (μmol/kg·min)	12.7 (9.0-13.5)	18.7 (16.5-23.0)	29.7 (21.42-56.7)	11.1 (10.1-12.3)	23.3 (17.9-30
R_d glucose (μmol/kg·min)	12.4 (9.3-13.8)	20.2 (11.3-24.1)	33.6 (20.0-54.2)	12.3 (10.1-13.1)	24.3 (20.1-31
GGL (μmol/kg·min)	5.3 (3.7-7.8)	9.4 (6.3-10.3)	14.1 (10.1-27.5)	4.7 (2.6-6.9)	9.6 (8.2-16.0
GNG (μmol/kg·min)	6.7 (4.7-8.0)	10.7 (6.2-13.9)	15.5 (11.3-29.2)	6.3 (5.4-7.6)	11.2 (9.0-19.

***** n = 3

Table 3 Palmitate and FFA kinetics at rest and during exercise

Parameter	**MCADD patients**			**Control subjects**
Exercise (min)	rest	38	83*****	rest
Palmitate (mmol/L)	0.16 (0.12-0.19)	0.16 (0.10-0.26)	0.28 (0.17-0.38)	0.14 (0.10-0.20)
R_a palmitate (μmol/kg·min)	2.87 (1.72-3.89)	3.33 (2.08-4.27)	4.98 (3.81-5.44)	1.71 (1.01-2.20)
R_d palmitate (μmol/kg·min)	2.87 (1.79-3.95)	3.30 (2.06-4.04)	4.76 (3.61-5.39)	1.72 (1.03-2.31)
R_{ox} palmitate (μmol/kg·min)	ND	1.84 (0.57-2.18)	2.71 (2.24-3.09) †	ND
FFA (mmol/L)	0.78 (0.51-0.95)	0.81 (0.39-1.30)	1.59 (0.75-2.02)	0.53 (0.45-0.80)
R_a FFA (μmol/kg·min)	12.55 (8.74-19.96) †	15.75 (9.73-21.87)	27.96 (17.03-28.89)	6.65 (4.41-8.86)
R_d FFA (μmol/kg·min)	12.57 (9.08-20.29) †	15.10 (9.67-21.69)	26.73 (16.11-28.65)	6.67 (4.50-9.33)
R_{ox} FFA (μmol/kg·min)	ND	8.48 (2.68-10.74)	13.79 (12.59-14.38) †	ND

***** n = 3
† P <0.05 as compared to control subjects

Table 4 Plasma parameters

Parameter	**MCADD patients**		**Control subjects**	
	Rest	end exercise*****	rest	end exercise
Insulin (pmol/L)	61 (45-103)	28 (23-46)	50 (27-77)	25 (<15-34)
Glucagon (ng/L)	52 (35-63)	79 (58-86)	44 (40-66)	70 (49-88)
Cortisol (nmol/L)	294 (251-532)	371 (287-438)	326 (279-378)	391 (279-544)
Epinephrine (nmol/L)	0.15 (0.08-0.28)	0.58 (0.45-1.82)	0.29 (0.16-0.40)	1.24 (0.79-2.7
Norepinephrine (nmol/L)	1.46 (0.89-1.92) †	6.80 (4.55-8.24)	0.63 (0.57-0.75)	3.34 (3.09-5.8
Octanoylcarnitine (μmol/L)	2.41 (1.63-4.30) †	10.58 (9.56-13.58) †	0.07 (0.06-0.19)	0.30 (0.23-0.5
Free carnitine (μmol/L)	11.32 (8.73-19.23) †	20.43 (12.75-29.26)	32.21 (25.58-48.93)	32.47 (22.11-4
Acetoacetic acid (mmol/L)	0.05 (<0.05-0.15)	0.11 (<0.05-0.16)	0.05 (<0.05-0.06)	0.07 (0.06-0.1
3-Hydroxybutyric acid (mmol/L)	<0.1 (<0.1-0.1)	<0.1 (<0.1-0.1)	<0.1 (<0.1- <0.1)	0.1 (<0.1-0.2)
Lactate (mmol/L)	0.8 (0.5-0.9)	0.9 (0.9-1.0) †	0.8 (0.5-0.9)	2.0 (1.5-2.4)

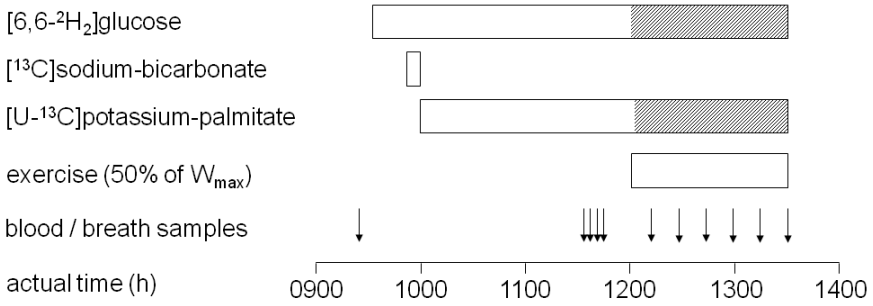
***** n = 3
† *P* <0.05 as compared to control subjects

Table 5 Acylcarnitines in muscle biopsies

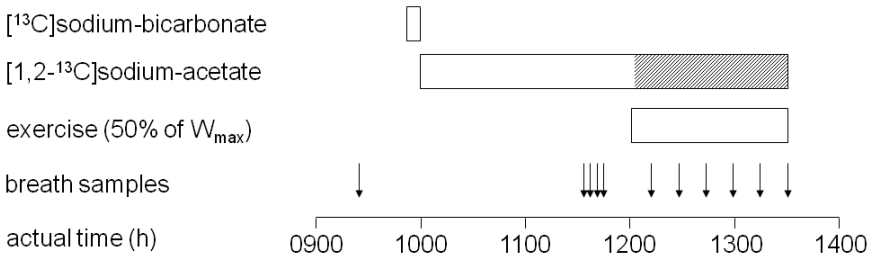
Acylcarnitine	**MCADD patients**		**Control subjects**	
(pmol/mg protein)	Rest	end exercise	rest	end exercise
Octanoylcarnitine (C8:0)	29.8 (1.6-58.0) †	61.2 (2.6-183.7) †	0.45 (0.3-1.6)	0.95 (0.7-1.1)
Hexanoylcarnitine (C6:0)	41.1 (3.8-92) †	202 (3.7-222.3) †	1.3 (1.2-5.8)	2.6 (2.5-3.6)
Acetylcarnitine (C2:0)	705 (166-1210)	489 (306-1799)	872 (591-1747)	1663 (1294-4055)
Free carnitine (C0)	1721 (1210-3711) †	945 (549-1396) †	3943 (2440-4286)	1857 (1462-2552)

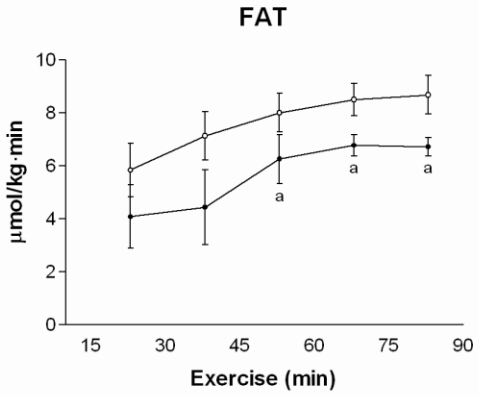
† $P < 0.05$ as compared to control subject

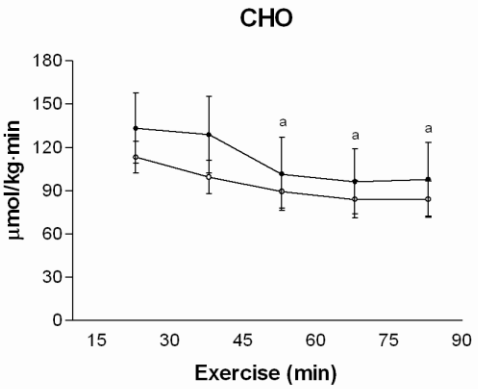
Study 1 (palmitate & glucose kinetics)

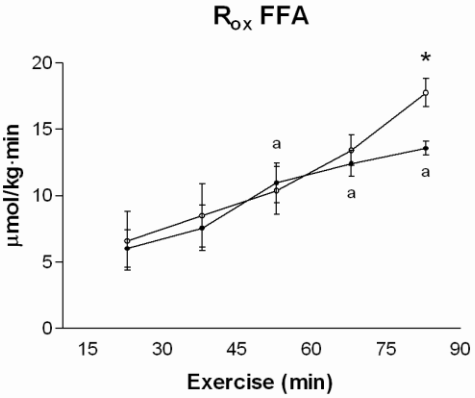


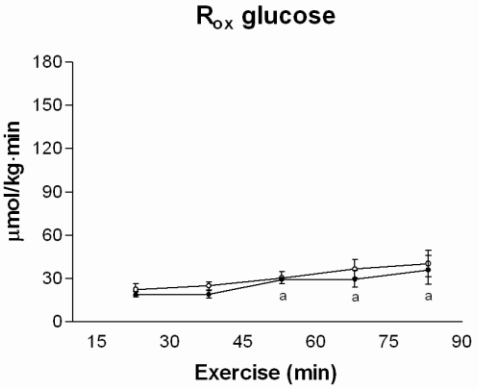
Study 2 (acetate recovery)

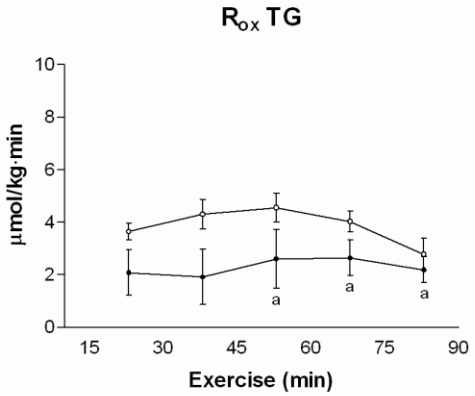


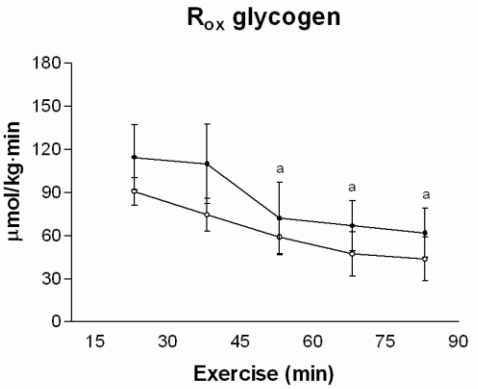

FAT
µmol/kg·min
Exercise (min)
a
a
a


CHO
µmol/kg·min
Exercise (min)
a
a
a


Rox FFA
µmol/kg·min
Exercise (min)
*
a
a
a


Rox glucose
µmol/kg·min
Exercise (min)
a
a
a


Rox TG
µmol/kg·min
Exercise (min)
a
a
a


Rox glycogen
µmol/kg·min
Exercise (min)
a
a
a

Author/s:
Huidekoper, HH; Ackermans, MT; Koopman, R; van Loon, LJC; Sauerwein, HP; Wijburg, FA

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