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RESEARCH REPORT

Is L-Carnitine Supplementation Beneficial in 3-Methylcrotonyl-CoA Carboxylase Deficiency?

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Abstract *Background*: 3-Methylcrotonyl-CoA carboxylase deficiency (3-MCCd) is an autosomal recessive disorder in the catabolism of leucine. In the present study, we investigated the current and prior medical condition of patients with 3-MCCd in the Faroe Islands and their carnitine levels in blood, urine and muscle tissue with and without L-carnitine supplementation to evaluate the current treatment strategy of not recommending L-carnitine supplementation to Faroese 3-MCCd patients.

Methods: Blood and urine samples and muscle biopsies were collected from patients at inclusion and at 3 months. Eight patients received L-carnitine supplementation when recruited; five did not. Included patients who received supplementation were asked to stop L-carnitine, the others were asked to initiate L-carnitine supplementation during the study. Symptoms were determined by review of hospital medical records and questionnaires answered at baseline and after the intervention.

Results: The prevalence of 3-MCCd in the Faroe Islands was 1:2,400, the highest reported worldwide. All patients were homozygous for the *MCCC1* mutation c.1526delG. When not administered L-carnitine, the 3-MCCd patients ($n = 13$) had low plasma and muscle free carnitine levels, 6.9 (SD 1.4) μmol/L and 785 (SD 301) nmol/g wet weight, respectively. L-Carnitine supplementation increased muscle and plasma carnitine levels to a low-normal range, 25.5 (SD 10.9) μmol/L and 1,827 (SD 523) nmol/g wet weight, $p < 0.01$, respectively. Seven of the thirteen 3-MCCd subjects suffered from self-reported fatigue with some alleviation after L-carnitine supplementation.

Conclusion: 3-MCCd is common in the Faroe Islands. Some symptomatic 3-MCCd patients may benefit biochemically and clinically from L-carnitine supplementation, a more general recommendation cannot be given.

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Introduction

3-Methylcrotonyl-CoA carboxylase deficiency (3-MCCd) (OMIM 210200 and 210210) is a defect in the degradation pathway of leucine. 3-MCCd leads to abnormally high levels of 3-methylcrotonylglycine in urine and 3-hydroxyisovalerylcarnitine in the blood. Increased renal excretion of 3-hydroxyisovalerylcarnitine is a natural way of excreting toxic intermediary metabolites that interfere with normal metabolism at the cost of low plasma carnitine levels (Roschinger et al. 1995). Among organic acidurias, 3-MCCd is the most frequently diagnosed disorder at neonatal tandem mass spectrometry (MS/MS) screening (Koeberl et al. 2003; Schulze et al. 2003; Wilcken et al. 2003; Stadler et al. 2006; Lam et al. 2013). The 3-MCC enzyme consists of two subunits, α and β, encoded by the *MCCC1* and *MCCC2* genes, located at 3q25–q27 and 5q12–q13, respectively (Baumgartner et al. 2001; Gallardo

et al. 2001; Holzinger et al. 2001). Mutations in either gene can lead to 3-MCCd, and more than 130 mutations have been reported (Grunert et al. 2012). 3-MCCd in the Faroe Islands is, to the best of our knowledge, caused by homozygosity for a single mutation in the *MCCC1* gene, the c.1526delG deletion.

Patients with 3-MCCd have low blood carnitine levels (Arnold et al. 2008; Grunert et al. 2012). The most severe forms of 3-MCCd have onset in infancy and include lethal cases (Baykal et al. 2005). Some patients suffer episodes of vomiting, lethargy and muscle weakness. These episodes can lead to metabolic decompensation with seizures, coma and death (Gallardo et al. 2001; Baykal et al. 2005). A variety of other symptoms, mostly neurological, have been reported but most patients are asymptomatic. The clinical picture may show extensive intrafamiliar variation (Visser et al. 2000; Baumgartner et al. 2004; Darin et al. 2007; Dirik et al. 2008; Eminoglu et al. 2009; Grunert et al. 2012). However, most children detected through newborn screening remain asymptomatic (Stadler et al. 2006; Lam et al. 2013; Koeberl et al. 2003).

A population-based voluntary screening programme was initiated in 2009 on the Faroe Islands to detect individuals with abnormally low blood carnitine levels caused by primary carnitine deficiency (PCD) from 2009 to 2011; 26,462 participated (Rasmussen et al. 2014). The screening programme also revealed individuals with secondary carnitine deficiency, including 3-MCCd.

The objective of the present study was to investigate the prior and current health status of Faroese 3-MCCd patients and to quantify blood, urine and muscle levels of carnitine and to investigate the effect of L-carnitine supplementation. We know of no prior studies performed to determine the level of carnitine in muscle tissue in patients with 3-MCCd, nor studies that determine the effect of L-carnitine supplementation in this group of patients.

Currently there are no uniform treatment recommendations for 3-MCCd patients – including whether or not to give L-carnitine supplementation (Arnold et al. 2008). L-Carnitine supplementation of patients with 3-MCCd in the Faroe Islands has generally not been recommended, the rationale being that although the patients have low blood carnitine levels, only few seem to suffer symptoms or are at an increased risk of severe medical complications.

Methods

All seventeen registered adult Faroese 3-MCCd patients were invited to participate. A total of thirteen patients were enrolled (see flowchart in Fig. 1). Ten patients were diagnosed with 3-MCCd in the population screening programme. P3 and P8 were diagnosed as parents to heterozygous children detected through newborn screening. P6, a sibling of P3, was diagnosed through family testing of P3's direct relatives.

The design of the study included baseline evaluation, an intervention and an end of study evaluation after 3 months. Blood, urine and muscle tissue were collected at inclusion and at 3 months. Included participants were divided into two groups – depending on whether they received L-carnitine supplementation. Eight patients were on L-carnitine supplementation when included. They were asked to stop L-carnitine intake after the baseline samples had been collected. These patients had received L-carnitine supplementation for at least 2 years, doses ranging from 1.33 to 6 g daily corresponding to 19–87 mg/kg/daily. A second set of samples were obtained after 3 months without L-carnitine supplementation.

Five participants did not receive L-carnitine at baseline evaluation. These L-carnitine-naive 3-MCCd patients received a fixed oral L-carnitine dosage of 1 g three times daily for 3 months, doses ranging from 33 to 46 mg/kg/daily, which was the time we estimated it would take to reach steady-state levels of carnitine in muscle tissue. New samples were collected at 3 months.

Patients 1, 4, 8 and 9 refused to discontinue L-carnitine supplementation, patient 9 due to pregnancy, and patients 1, 4 and 8 were concerned about negative health consequences. Patient number 11 stopped L-carnitine supplementation before we were able to obtain new samples from him due to miscommunication. Patients 2, 3, 5, 6, 7, 10, 12 and 13 are represented in both groups.

Diagnosis was confirmed by DNA analysis performed in the Centre for Inherited Metabolic Diseases (CIMD), Department of Clinical Genetics, Rigshospitalet, Copenhagen (Denmark). Subjects were also genetically analysed for PCD.

All available hospital medical records were systematically reviewed for admissions and outpatient contacts, and the reasons for referral were grouped into the following groups: symptoms from the central nervous system, cardiopulmonary, gastrointestinal, urogenital and endocrine systems as well as gynaecological, obstetrical and orthopaedic complaints.

All patients answered during baseline evaluation a questionnaire. The questionnaire had six main sections: (1) perceived prior and current health status, (2) possible 3-MCCd symptoms (more often ill than others and/or more sick than others when ill, tendency to vomit when ill with a fever, unexplained fainting spells or cardiovascular symptoms), (3) physical capability (feeling reduced physical capability compared to peers, participation in strenuous physical activities, and participation in strenuous organised sport on a national level), (4) dietary habits (craving for meat), (5) L-carnitine supplementation (dosage, effects and adverse effects), and (6) other medications (dosage and

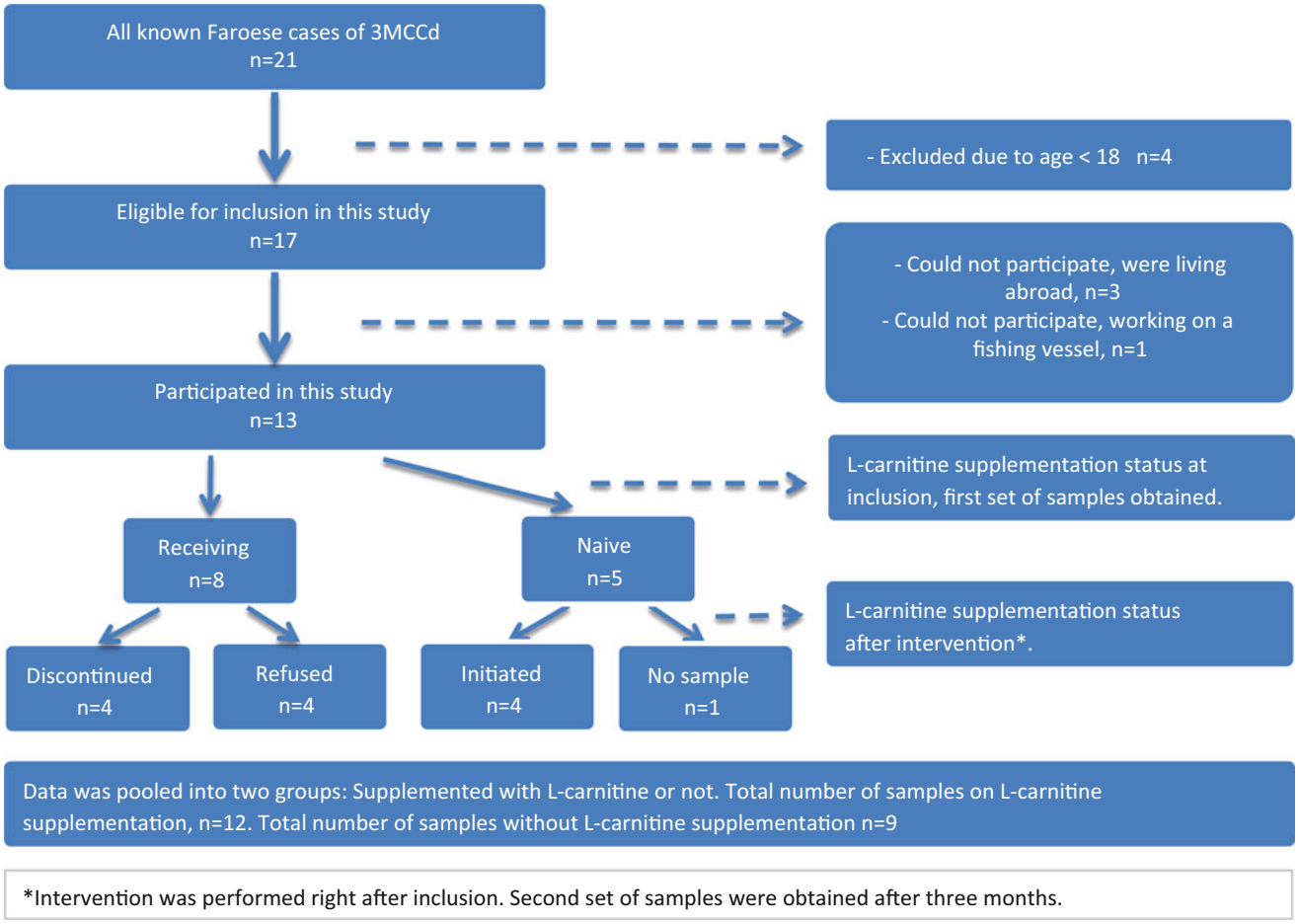

Fig. 1 Inclusion of patients presented in a flowchart

indication stated in free text field). Following the intervention, another questionnaire was answered, which focused on the perceived physical effect of initiation or discontinuation of L-carnitine supplementation. Available answer options were yes, no and unknown except for other medications. Questionnaires were completed by the patients. Symptoms at the time of diagnosis were collected from medical records.

Routine blood samples were analysed in the National Hospital (Faroe Islands) (haemoglobin, mean cell volume, mean cell haemoglobin concentration, leucocytes and leucocyte differential count, thrombocytes, sodium, potassium, creatinine, urea, uric acid, lactate dehydrogenase, creatine kinase, creatine kinase MB isoenzyme, alanine aminotransferase, total cholesterol, high-density lipoprotein, low-density lipoprotein, c-reactive protein), while plasma/urine acylcarnitines and urine 3-hydroxyisovaleric acid were determined at CIMD, Denmark. Electrocardiograms (ECG) were analysed using the Minnesota ECG criteria (Prineas 1982). Echocardiography was performed initially in all patients according to current guidelines (Lang et al. 2005).

Muscle biopsies were taken from the medial part of the m. vastus lateralis using the Bergstrom technique (Bergstrom 1975). The procedure was performed under controlled conditions by a trained biopter. Biopsies were taken with local anaesthesia from the thigh. One elderly male subject on carnitine supplementation had highly atrophic thigh muscles probably due to old age. The subject was biopted thrice, but the samples contained only fat and connective tissue, which is not an uncommon finding in biopsies at old age. The samples were analysed at CIMD, Denmark, to determine the muscle content of carnitine.

Analyses of acylcarnitines and carnitine in plasma, muscle and urine were performed using stable-isotope dilution combined with ultra-performance liquid chromatography–tandem mass spectrometry, using a Quattro Micro triple quadrupole mass spectrometer (Waters, Milford, Massachusetts). d_3-Carnitine, d_3-acetylcarnitine, d_3-propionylcarnitine, d_3-butyrylcarnitine, d_9-isovalerylcarnitine, d_3-octanoylcarnitine, d_3-tetradecanoylcarnitine and d_3-hexadecanoylcarnitine (Hermen ten Brink, Vrije Universiteit, Amsterdam, the Netherlands) were added to samples before extraction/homogenisation. Carnitine and acylcarnitines in all three matrices

Table 1 Blood, plasma, urine and muscle data

Sample site	Sample	Unit	Carnitine supplementation Mean (SD)	No carnitine Mean (SD)	Stud. *t*-test[a] *p*-values
Blood	Haemoglobin	mmol/L	8.1 (0.8)	8.5 (0.6)	0.19
	Potassium	mmol/L	3.8 (0.3)	3.7 (0.2)	0.41
	Creatinine	μmol/L	71.8 (10.9)	78.2 (19)	0.34
	Urea	mmol/L	4.3 (1.1)	4.1 (1.2)	0.73
	ALAT	U/I	22.2 (9.7)	24.2 (17)	0.34
	Random blood glucose	mmol/L	4.9 (0.8)	5.2 (0.5)	0.84
	LDL	mmol/L	3.58 (1.2)	3.5 (1.2)	0.74
Plasma	Free carnitine	μmol/L	25.5 (10.9)	6.9 (1.4)	<0.002
	Acetylcarnitine	μmol/L	5.3 (3.8)	0.8 (0.2)	<0.002
	3-HIV-carnitine	μmol/L	17.3 (3.8)	10.1 (4.8)	<0.002
	Total carnitine	μmol/L	49.6 (15.9)	20.1 (9.4)	<0.002
	Free/total	%	49.6 (8.9)	41.7 (5.4)	0.029
Urine	Creatinine	mmol/L	12.7 (7.4)	14.7 (6.2)	0.52
	Free carnitine	μmol/mmol creatinine	24.2 (32)	2.3 (0.8)	0.056
	Acetylcarnitine	μmol/mmol creatinine	5.3 (7.5)	0.1 (0.03)	0.053
	3-HIV-carnitine	μmol/mmol creatinine	155.5 (68.5)	52.2 (14.7)	<0.002
	3-HIVA	mmol/mmol creatinine	2.2 (0.9)	1.9 (0.5)	0.37
	Total carnitine	μmol/mmol creatinine	184.7 (81)	57.1 (13.7)	<0.002
	Free/total	%	7.8 (8.9)	4.4 (2.2)	0.29
Muscle[b]	Free carnitine	nmol/g wet weight	1,827 (523)	785 (301)	<0.002
	Acetylcarnitine	nmol/g wet weight	195 (55)	86 (26)	<0.002
	3-HIV-carnitine	nmol/g wet weight	2,112 (746)	1,152 (593)	<0.002
	Total carnitine	nmol/g wet weight	4,288 (1,161)	2,117 (800)	<0.002
	Free/total	%	43 (8)	38 (9)	0.18

[a] Student's *t*-test. At $p < 0.05$, difference between groups was significant
ALAT alanine aminotransferase, *LDL* low-density lipoprotein, *3-HIVA* 3-hydroxyisovaleric acid, *3-HIV-carnitine* 3-hydroxyisovalerylcarnitine
Carnitine supplementation group, all patients except P11; [b] no relevant muscle biopsy obtained from P1. No supplementation group: P2, P3, P5, P6, P7, P10, P11, P12 and P13

were quantified using external spiked plasma calibration curves.

Quantitative urine analysis for 3-hydroxyisovaleric acid was performed by stable-isotope dilution combined with GC–MS (HP 6890 GC coupled to a HP5973 mass selective detector) using a d6-3-hydroxyisovaleric acid (Hermen ten Brink, Vrije Universiteit, Amsterdam, the Netherlands).

Statistics and Analysis

Data are presented as mean and standard deviation in parenthesis. Two-tailed Student's *t*-test was used when analysing differences in mean values of routine blood samples and plasma, urine and muscle carnitine values in two groups, with ($n = 9$) and without ($n = 12$) L-carnitine supplementation. Level of significance was $p < 0.05$. The method of Bonferroni was used to correct for repeated testing, the Bonferroni critical value was $0.05/24 = 0.0021$, corrected *p*-value, $p < 0.002$. Correlation between plasma and muscle free carnitine values was calculated with Pearson's test for correlation. Results were computed with Statistical Package for the Social Sciences (SPSS).

Informed consent was obtained from all patients for being included in the study and the study was approved by the Faroese Ethical Committee.

Results

Plasma and muscle free carnitine levels increased significantly when oral L-carnitine was given, $p < 0.01$. Mean plasma free carnitine increased from 6.9 (1.4) to 25.5 (10.9) μmol/L and mean muscle free carnitine increased from 785 (301) to 1,827 (523) nmol/g wet weight after L-carnitine supplementation (Table 1).

There was a significant positive correlation between plasma and muscle free carnitine irrespective of L-carnitine supplementation, $R^2 = 0.657$, $p < 0.01$ (Fig. 2).

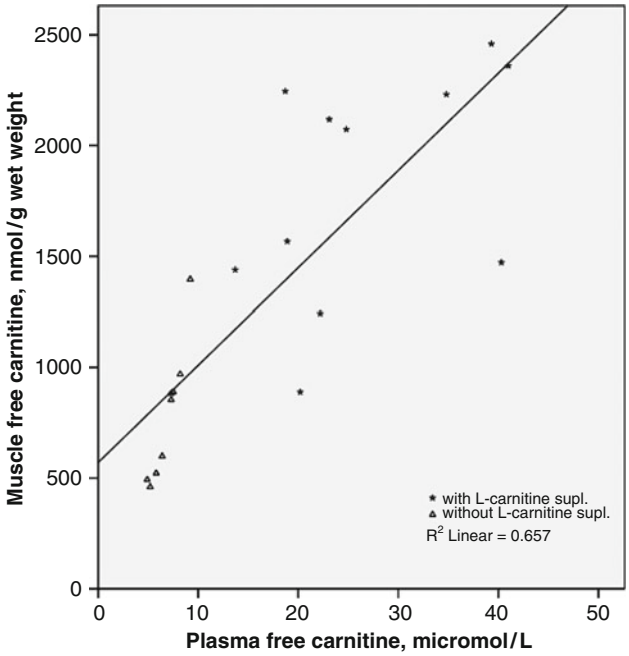

Fig. 2 Plasma free carnitine plotted against muscle free carnitine irrespective of L-carnitine supplementation. There was a significant positive linear correlation, $R^2 = 0.657$, $p < 0.01$

Changes in levels of urine free carnitine and acetylcarnitine demonstrated a tendency towards significance, $p = 0.056$ and $p = 0.053$, respectively, while levels of 3-hydroxyisovalerylcarnitine and total carnitine increased on oral L-carnitine supplementation, $p < 0.01$ (Table 1). Routine blood samples did not differ before and after L-carnitine supplementation (Table 1). Quantitative urine analysis demonstrated no difference between groups regarding levels of 3-hydroxyisovaleric acid, $p = 0.37$ (Table 1).

Review of hospital medical records revealed largely unremarkable medical histories, including 18 live births, three spontaneous abortions and admissions with vertigo, pneumonia, pulmonary embolism, heart palpitations and an extrauterine pregnancy (Table 2). Furthermore, P1 is followed regularly for non-insulin-dependent diabetes mellitus and gout. Baseline characteristics including blood pressure, heart rate, routine blood samples, ECG (electrocardiogram) and transthoracic echocardiography were unremarkable (Tables 1 and 2). Symptoms at the time of diagnosis ranged from none to fatigue and palpitations (Table 2). Mean BMI (body mass index) was 23 and 22 kg/cm^2 and mean age was 49 and 38.3 in male and female 3-MCCd patients, respectively (Table 2).

Six of 13 patients reported chronic fatigue that was alleviated by L-carnitine supplementation. One patient, who did not perceive fatigue at baseline, reported feeling more fit when given L-carnitine. Three patients reported fatigue from childhood. Four patients reported heart palpitations. Two patients stated a tendency to vomit when ill with a fever both in childhood and as adults. Six patients reported participation in strenuous physical activities on a regular basis. Four patients reported a stronger than normal craving for meat. Few side effects of L-carnitine supplementation were reported – one though experienced weight gain and developed an unpleasant body odour (Table 2).

A total of 21 patients, including children and adults, have been diagnosed with 3-MCCd in the Faroe Islands. All were homozygous for a single deletion, c.1526delG, in the *MCCC1* gene. On January 1, 2014, the population of the Faroe Islands was 48,308 (Faroese Board of Public Health 2010). In the screening period from 2009 to 2011, 11 3-MCCd patients were diagnosed from the 26,462 samples collected. Thus, the prevalence of 3-MCCd is 1:2,400. A total of 34,000 live births were recorded in the Faroe Islands from January 1970 until December 2012. During this period, 13 patients with 3-MCCd were born – giving an incidence of 1:2,615 live births.

Subject number 13 was found to be a carrier for the PCD-related c.95A>G (p.N32S) mutation in the *SLC22A5* gene.

The patient cohort includes three sib pairs, P3 and P6, P4 and P8 and P7 and P11.

Discussion

The incidence of 1:2,615 and prevalence of 1:2,400 of 3-MCCd in the Faroe Islands are far greater than reported elsewhere: 1:41,676 in California (Schulze et al. 2003; Wilcken et al. 2003; Lam et al. 2013), 1:64,000 in North Carolina (Koeberl et al. 2003) and 1:84,700 in Bavaria in South Germany

Table 2 Baseline characteristics including ECG and TTE

ID	Age	Gender	Genetic status c.1526delG	fC0[a] µmol/L	BMI kg/m2	Sys./dia. BP mmHg	ECG Changes	TTE		At diagnosis Symp.[b]	Effect of L-carnitine[b]	Medical history Previous admissions
								LVMI (g/m2)	LVEF %			
P1	69	Male	+/+	8	23	157/82	None	83.9	58	Fatigue	Relieved fatigue	None
P2	20	Female	+/+	7	22	123/70	None	68.6	59	Fatigue	Relieved fatigue	None
P3	39	Female	+/+	5.3	24	106/58	None	62.9	59	Fatigue	Relieved fatigue	Ectopic pregnancy
P4	46	Male	+/+	6	26	133/96	None	70.9	55	Palpitations	None	Vertigo, chest pain
P5	46	Female	+/+	4	21	105/62	None	83.5	54	Palpitations	None	Palpitations
P6	49	Female	+/+	5	23	135/83	None	55.1	58	Fatigue	Relieved fatigue	None
P7	43	Female	+/+	8.2	21	116/76	None	63.3	53	Fatigue, often ill	Relieved fatigue	Gallstone
P8	35	Female	+/+	4.3	20	117/69	None	58	62	Fatigue, palpitations	Relieved fatigue, less sick	Spontaneous abortion
P9	22	Female	+/+	4	22	127/69	None	71.7	56	Palpitations	Relieved fatigue	Spontaneous abortion
P10	18	Male	+/+	4	19	141/76	None	82.7	56	Vomiting when sick	None	None
P11	64	Male	+/+	6.4	23	147/85	None	74.2	56	None	None	Pneumonia, PE
P12	52	Female	+/+	5.8	20	121/82	None	69.5	53	None	None	Spontaneous abortion
P13	48	Male	+/+	5.2	25	113/63	None	109.7	58	Vomiting when sick	None	None
Group mean values for numeric values												
Male	49	–	–	5.9	23	138/80	–	84.3	57	–	–	–
Female	38.3	–	–	5.5	21	119/71	–	66.6	57	–	–	–
All	42.4	–	–	5.6	22	126/75	–	73.4	57	–	–	–

[a] Measured pretreatment with L-carnitine when diagnosed during the population screening
[b] Based on medical journal review and questionnaires
fC0 free plasma carnitine, *BMI* body mass index, *Sys.* systolic, *dia.* diastolic, *BP* blood pressure, *ECG* electrocardiogram, *TTE* transthoracic echocardiography, *LVMI* left ventricle mass index (normal value = <115 for males and <95 for females), *LVEF* left ventricular ejection fraction (normal value= > 55), *Symp.* symptoms, *PE* pulmonary embolism

(Stadler et al. 2006). The listed prevalences and incidences are conservative estimates, because we only report on carnitine-deficient 3-MCCd cases and not everyone in the Faroe Islands participated in the voluntary screening programme. The Faroese population stems from a genetic isolate and expanded rapidly during the last three centuries with an almost tenfold increase, from 5,000 to 48,308 individuals, making a founder effect a probable cause of the high prevalence of 3-MCCd in the population (Jorgensen et al. 2002).

We found that all Faroese 3-MCCd patients were carnitine depleted when not treated with L-carnitine, with values of free carnitine in plasma ranging from 4 to 8.2 μmol/L. Free carnitine in plasma increased, $p < 0.01$, when 3-MCCd patients were supplemented with oral L-carnitine, indicating that oral L-carnitine supplementation of 3-MCCd patients can restore levels of free carnitine in plasma to lower range normal values.

We have demonstrated that mean muscle free carnitine increased from 785 to 1,827 nmol/g wet weight of muscle tissue when the patients were supplemented with L-carnitine. Reference mean levels in normal subjects were reported by Opalka et al. (2001) and Madsen et al. (2013) to be 2,400 (800) and 2,914 (249) nmol/g wet weight of muscle tissue, respectively. The difference in reported mean values by Opalka et al. and Madsen et al. might have been caused by an age difference between the cohorts – as mean muscle free carnitine levels reportedly decrease with age (Opalka et al. 2001). The levels of mean muscle carnitine in 3-MCCd patients treated with L-carnitine are in the lower range of the normal reference interval reported by Opalka et al., but below that found by Madsen et al. Mean muscle carnitine reported by Madsen et al. was quantified by the CIMD as in the present study, but on a younger study population (age 19–31), which may explain the lower values in our older cohort compared to the reference interval. We conclude that a significant increase in muscle free carnitine to a low to low-normal level can be obtained by administration of L-carnitine when given for 3 months or more.

We have demonstrated that rising plasma values of free carnitine are an indicator of rising intramuscular free carnitine levels in 3-MCCd patients (Fig. 2) as there was a significant positive correlation between increasing plasma carnitine levels and an increase in the level of carnitine in muscle – the principal store of carnitine in the body. Patients with a secondary carnitine deficiency such as 3-MCCd have a normally functioning carnitine transporter and are thus able to transport more carnitine into the intramuscular compartment when plasma carnitine levels increase. We conclude that plasma levels of free carnitine reflect the intramuscular levels and can be used as a marker for intramuscular levels of free carnitine.

Review of hospital medical records and questionnaires revealed unremarkable present and past medical histories concerning serious illnesses or diseases. Furthermore, routine blood samples did not differ before and after L-carnitine supplementation. Baseline ECGs, blood pressures and transthoracic echocardiograms were unremarkable as well. The reported clinical picture in the literature is heterogeneous, ranging from fatal cases (Gallardo et al. 2001; Baykal et al. 2005) to asymptomatic adult cases (Gallardo et al. 2001) including mothers diagnosed due to abnormal newborn screening results in their infants (Grunert et al. 2012). Clinical presentation varies even within families (Visser et al. 2000; Eminoglu et al. 2009). Neurological abnormalities have been reported (Baykal et al. 2005) including one case of multiple sclerosis (Darin et al. 2007). The mother of siblings P4 and P8 suffered multiple sclerosis – however, the patients and their brother did not present or complain of neurological deficits.

Carnitine depletion in PCD has been linked to sudden death (Rasmussen et al. 2012). In PCD, the organic cation transporter 2 (OCTN2) is deficient and leads to intracellular carnitine depletion in contrast to the situation in patients with 3-MCCd who have a normal OCTN2 activity that will probably secure a sufficient intracellular carnitine level and prevent sudden death.

Though their medical histories were unremarkable, some patients reported mild but long-lasting symptoms. Fatigue was the main symptom reported by 54% of the patients. Given the small sample size and the difficulties in grading fatigue even in large populations, the research group chose not to include a formal grading of the self-reported symptoms of fatigue. Grunert et al. (Grunert et al. 2012) reported that among 88 patients, only one patient (#87) or 1.1% reported suffering from chronic tiredness – patient #87 was Faroese and also included in our study as P8. The prevalence of fatigue was thus much higher in our cohort. Reasons for the difference might include different interview/reporting procedures between the studies, cultural differences in feeling fatigued and reporting it as a diagnosis and a possible association between the Faroese *MCCC1* mutation c.1526delG and fatigue.

Two patients described consistent vomiting when ill with a fever, which could indicate a tendency to slight metabolic decompensation. Metabolic decompensation with vomiting during illness is well described in cases of organic aciduria (Pasquali et al. 2006).

3-MCCd patients seem slender; mean BMI was 23 and 22 kg/cm^2 for males and females, respectively. A Faroese population health survey from 2010 showed that mean BMI for men and women aged 40 to 70 was 28 and 26, respectively (Statistics Faroe Islands 2014). Thus, the 3-MCCd patients seem to have a normal BMI, though lower than the national average. Our sample size is small and only 8 of 13 patients fall into the sampled age group (Table 2). Lower than national average BMI has not been reported for 3-MCCd by other research groups, but the reason for the finding is unclear and might be due to chance.

The enzymatic activity of 3-methylcrotonyl-CoA carboxylase was not quantified in the present study. It has been reported previously by Grunert et al. (2012) for patient P3, labelled patient #73c, and patient P8, labelled patient #87, as being severely reduced. All cases are homozygous for the *MCCC1* mutation c.1526delG and therefore we expect reduced enzymatic activity in all subjects.

The patients in this study are enzymatically and molecularly homogenous concerning 3-MCCd, which may be a strength when evaluating the clinical consequences of the disease. However, though generally mild, the phenotype varies between the patients, which has also been demonstrated previously within families (Eminoglu et al. 2009), making general conclusions and treatment recommendations less viable and might instead call for future individualised treatment strategies.

Grunert et al. (2012) described 88 3-MCCd patients with varying mutations in either *MCCC1* or *MCCC2*. They found no indication for dietetic treatment (except for maybe an emergency regimen during intercurrent illness) and recommended supplementation with L-carnitine if free carnitine levels are low or patients are symptomatic. Arnold et al. (2008) recommend L-carnitine supplementation for carnitine-deficient cases regardless of symptomatology. Faroese 3-MCCd patients were all carnitine depleted at diagnosis and some had minor symptoms. Based on the treatment strategies proposed by Grunert et al. and Arnold et al. and our finding that some patients experience alleviation of fatigue when treated with L-carnitine, it may be discussed whether L-carnitine supplementation should be recommended to Faroese 3-MCCd patients. However, the symptoms experienced by these patients are not severe – rather these patients have been feeling well for extended periods. Data from other patient groups with secondary carnitine depletion (as opposed to those with PCD) are not conclusive concerning a beneficial effect of L-carnitine supplementation, e.g. as in MCADD (medium-chain acyl-CoA dehydrogenase deficiency) patients (Madsen et al. 2013). Supplementation in patients with LCHADD (long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency) on the other hand may cause increased production of toxic 3-hydroxyacylcarnitines (Spiekerkoetter et al. 2009). Possible consequences of long-term L-carnitine supplementation are not to date sufficiently documented, and some data may raise concerns – Koeth et al. suggest a linkage between ingestion of L-carnitine supplementation and cardiovascular disease (Koeth et al. 2013). Furthermore, levels of 3-hydroxyisovaleric acid in urine did not differ between those on and those without L-carnitine supplementation, $p = 0.37$, arguing against an effect of detoxification of the L-carnitine given. It may be reasonable to supplement symptomatic patients, but it seems premature at present to give any more general recommendations.

Limitations

L-Carnitine was administered in a fixed dosage of 1 g three times daily, when provided by the research group. One could argue that doses based on body weight would render a more true reflection of the effect of L-carnitine on intramuscular carnitine levels. Fatigue, the dominating symptom reported in questionnaires, is difficult to score and represents a subjective feeling experienced by patients – however self-reported fatigue that alters quality of life must be taken into consideration and treated as a genuine symptom. In the examined patient category, the patients have had a lifelong state of secondary carnitine depletion. Quantification of fatigue in the presence of this baseline state is inherently difficult, as demonstrated in this study by the fact that a patient upon L-carnitine supplementation reported alleviation of fatigue, which the person had not been conscious of and had not reported previously.

Conclusion

3-MCCd due to the *MCCC1* c.1526delG mutation is common in the Faroe Islands compared to the rest of the world because of a probable founder effect. Levels of free carnitine in muscle tissue and blood were low in patients without L-carnitine supplementation and increased significantly to low–low-normal levels upon L-carnitine supplementation. Plasma levels of free carnitine and intramuscular levels are highly correlated regardless of carnitine level; therefore plasma carnitine levels can be used as an indicator for intramuscular levels. Seven of 13 investigated patients presented with a subjective feeling of fatigue that was alleviated after L-carnitine supplementation. Taking into consideration the newly raised concern regarding the safety of L-carnitine supplementation, the absence of dangerous symptoms and the significant but mild subjective feeling of fatigue reported by some patients, a general recommendation about supplementing all 3-MCCd patients with L-carnitine cannot be given. However, since L-carnitine supplementation can alleviate fatigue suffered by some patients, one could argue for an L-carnitine supplementation trial for these selected patients.

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Synopsis

Intramuscular levels of free carnitine correlate with plasma levels of free carnitine and can be restored to near-normal levels with oral L-carnitine supplementation in 3-MCCd patients.

Compliance with Ethics Guidelines

Conflict of Interest

Jákup Andreas Thomsen, Allan Meldgaard Lund, Jess Have Olesen, Magni Mohr and Jan Rasmussen declare that they have no conflict of interest.

Informed Consent

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000 (5). Informed consent was obtained from all patients for being included in the study.

Details of the Contributions of Individual Authors

Corresponding author Jákup Andreas Thomsen was involved in all aspects of the work and is the guarantor and wrote the article.

Jan Rasmussen has been involved in the conception, design, analysis and interpretation of data and revised the article critically for important intellectual content.

Magni Mohr has been involved in the conception, design and interpretation of data and revised the article critically for important intellectual content.

Jess Have Olesen has been involved in the conception, design, analysis and interpretation of data and revised the article critically for important intellectual content.

Allan Meldgaard Lund has been involved in the conception, design, analysis and interpretation of data and revised the article critically for important intellectual content.

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