Chapter 5

Experimental evidence for protein oxidative damage and altered antioxidant defense in patients with medium-chain acyl-CoA dehydrogenase deficiency

Catharina M.L. Touw[1,2#], Terry G.J. Derks[1,2,3,5*#], Graziela S. Ribas[4,6], Giovana B. Biancini[5,6], Camila S. Vanzin[5,6], Giovanna Negretto[4,6], Caroline P. Mescka[5,6], Dirk Jan Reijngoud[1,2], G. Peter A. Smit[2,3], Moacir Wajner[5,6] and Carmen R. Vargas[4,5,6]

[1]Research Laboratory of Pediatrics, Beatrix Children's Hospital, and [2]Center for Liver, Digestive and Metabolic Diseases, and [3]Section of Metabolic Diseases University Medical Center Groningen, University of Groningen, Groningen, The Netherlands
[4]Programa de Pós-Graduação em Ciências Farmacêuticas, [5]Departamento de Bioquímica, Instituto de Ciências Básicas da Saúde and [6]Serviço de Genética Médica, HCPA, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil
# These authors contributed equally to this study

Accepted for publication
J Inherit Metab Dis. 2014, March 13
PMID: 24623196

ABSTRACT

The objective of this study was to test whether macromolecule oxidative damage and altered enzymatic antioxidative defenses occur in patients with medium-chain acyl coenzyme A dehydrogenase (MCAD) deficiency. We performed a cross-sectional observational study of *in vivo* parameters of lipid and protein oxidative damage and antioxidant defenses in asymptomatic, non-stressed MCAD-deficient patients and controls. Patients were subdivided into three groups, based on therapy: patients without prescribed supplementation, patients with L-carnitine supplementation, and patients with L-carnitine plus riboflavin supplementation.

Compared to healthy controls, non-supplemented MCAD-deficient patients and patients receiving L-carnitine supplementation displayed decreased plasma sulfhydryl content (indicating protein oxidative damage). Increased erythrocyte superoxide dismutase activity in patients receiving L-carnitine supplementation probably reflects a compensatory mechanism to scavenge reactive species formation. The combination of L-carnitine plus riboflavin was not associated with oxidative damage.

These are the first indications that MCAD-deficient patients are subjected to protein oxidative damage and that combined supplementation of L-carnitine and riboflavin may prevent these biochemical alterations. The results suggest involvement of free radicals in the pathophysiology of MCAD deficiency. The underlying mechanisms behind the increased SOD activity upon L-carnitine supplementation need to be determined. Further studies are necessary to determine the clinical relevance of oxidative stress, including the possibility of antioxidant therapy.

INTRODUCTION

Medium-chain acyl coenzyme A dehydrogenase (MCAD) deficiency [OMIM 201450] is the most common inherited disorder of mitochondrial fatty acid oxidation (mFAO) [1]. Acute clinical presentation classically involves infants in the first five years of life [2]. During periods with increased metabolic stress, fasting can precipitate acute symptoms, such as drowsiness or lethargy, sometimes progressing into coma or sudden death. Pathognomonic metabolite profiles in plasma or whole blood consist of increased concentrations of medium-chain fatty acids and their carnitine derivatives [3]. Treatment consists of prevention of prolonged fasting, an emergency regimen and may include supplementation of vitamins (L-carnitine and/or riboflavin), but evidence for any treatment is limited [1]. The prognosis is very good after diagnosis, to which neonatal bloodspot screening (NBS) programs have made important contributions [4,5].

Oxidative stress can be defined as a state where the production of reactive species (RS) cannot be compensated for by the antioxidant defense, i.e. RS removal capacity, leading to macromolecule damage [6]. The intimate relationship between mFAO, electron transport and oxidative phosphorylation justifies the study of oxidative stress in human disorders of mFAO [1]. Macromolecule oxidative damage, impaired antioxidant defense and defect mitochondrial energy homeostasis have been demonstrated after incubation of rat brain, liver and skeletal muscle preparations with medium-chain fatty acid metabolites [7–10]. Additionally, evidence for oxidative stress in mFAO defects was reported in various genetic mouse models [11,12].

The objective of this study was to substantiate whether macromolecule oxidative damage and altered antioxidative defense occur in patients with MCAD deficiency. We determined parameters of lipid and protein oxidative damage and enzymatic antioxidant defenses in blood samples that were obtained from MCAD-deficient patients during routine hospital visits under normal, unstressed conditions.

METHODS

Patients

The study was approved by the Ethics Committee of the University Medical Center Groningen. Written informed consent was obtained from all patients and/or their parents.

MCAD-deficient patients were recruited from the Section of Metabolic Diseases, Beatrix Children's Hospital, University Medical Center Groningen, Groningen, The Netherlands. Blood samples from 27 MCAD-deficient patients (9 males, 18 females; median age 5.3 years, age range 0-27 years) were obtained during normal hospital visits. The complete group of patients was subdivided into three groups, based on the treatment provided. In our practice, we aim to normalize of plasma free carnitine concentrations (i.e. >16 µmol/l) in case we prescribe L-carnitine (30-50 mg/kg/d). Although riboflavin supplementation is not routinely prescribed, some older patients in our cohort still use this

supplementation, which they are familiar with. In those patients, riboflavin was dosed 50-150 mg/d, according to Duran *et al.* [13].

Biological sample collection and preparation

Blood samples from patients and controls were processed and stored by the same procedure. Blood samples from 12 age- and gender-matched control subjects (6 males and 6 females; median age 10.0 years, age range 3-27 years) were recruited anonymously from the laboratory of the general practitioners' service in Groningen, The Netherlands. At the time of sampling, no blood samples from younger children were available.

After venous puncture and collection in heparinized vials, plasma and cells were separated by centrifugation at 3000 x g for 10 minutes at 4°C. Plasma was removed by aspiration and stored at -80°C until further analysis. After plasma separation, erythrocytes samples were washed 3 times with cold NaCl 0.9% in equal volumes of erythrocytes and NaCl, and centrifuged for 10 minutes at 800 x g, after each washing. Lysates were prepared by addition of 1 ml of distilled water to 100 µL of washed erythrocytes and subsequent storage at −80 °C until determination of the antioxidant enzyme activities.

Plasma analysis

Thiobarbituric acid reactive species (TBA-RS), a measure for malondialdehyde which is a parameter of lipid oxidative damage, were determined spectrophotometrically in plasma according to the method described by Esterbauer and Cheeseman [14]. TBA-RS were expressed in nmol/mg protein.

Sulfhydryl content, a parameter of protein oxidative damage, was determined in plasma spectrophotometrically based on the reduction of 5.5'dithio-bis (2-nitrobenzoic acid) by thiols, yielding a yellow derivative [15]. The sulfhydryl content is inversely correlated to oxidative damage to proteins and results were reported as µmol/L. Carbonyl content, a parameter of protein oxidative damage, was determined in plasma spectrophotometrically as previously described by Levine *et al.*[16]. Plasma carbonyl content is expressed as nmol/mg protein.

Plasma protein concentration was determined according to the Biuret method using a commercial kit (Labtest Kit®, Lagoa Santa, Minas Gerais, Brazil).

Erythrocyte antioxidant analysis

Antioxidant enzyme activities were determined spectrophotometrically in erythrocyte lysates. Superoxide dismutase (SOD) converts superoxide radicals into hydrogen peroxide. Cytosolic SOD activity was determined using a kit (RANSOD®, Randox Laboratories, Antrim, United Kingdom), and isrepresented as units/mg protein. Catalase (CAT) catalyzes the degradation of hydrogen peroxide into molecular oxygen and water. CAT activity was determined according to the method described by Aebi [17]. Glutathione peroxidase (GPx) catalyzes the conversion of glutathione (GSH) and hydrogen peroxide into glutathione disulfide and water. GPx activity was measured using a kit (RANSEL®, Randox Laboratories, Antrim, United Kingdom). Erythrocyte GSH is a parameter of the non-enzymatic antioxidant defense. GSH concentrations were measured according to Browne and Armstrong [18] and

expressed as nmol/mg protein. Total antioxidant status (TAS) represents the quantity of tissue antioxidants and was measured using a kit (Randox, Antrim, United Kingdom). The results are expressed in mmol/l. Protein content of erythrocyte lysates was determined by the method of Lowry. The final enzymatic activities are presented as percentage of control values.

Statistical analysis

Statistical analysis was performed using GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego California USA, www.graphpad.com) and SPSS version 20 (International Business Machines Corp., Armonk New York, USA). Data were tested for statistically significant outliers using the Grubbs' test with a conservative approach (alpha = 0.01) (http://graphpad.com/quickcalcs/Grubbs1.cfm). Normality of the data was assessed using the Shapiro-Wilk normality test. Differences between the complete group of patients and controls were tested using either the two-tailed unpaired Student's t-test or Mann-Whitney test. Differences between patient subgroups and the control group were tested by the Kruskal-Wallis test and followed by the Dunn's multiple comparison test when the *p*-value was significant. Correlations were analyzed with either Pearson's analysis or Spearman's analysis. In case of a correlation between outcome parameter and age, multiple linear regression was performed. Non-parametric data were log-transformed before linear regression. Differences were considered statistically significant at $p<0.05$.

RESULTS

Table 1 summarizes the patient characteristics. All patients had an *ACADM* genotype that has been associated with clinical symptoms. In all patients, plasma acylcarnitine profiles and urinary organic acid analysis were severely abnormal, demonstrating the classical biomarkers of the disease at the time of the study (data not shown).

Parameters of macromolecule oxidative damage and antioxidant defense in controls and MCAD-deficient patients were not influenced by the *ACADM* genotype (Table 2). Plasma TBA-RS values appeared significantly increased in MCAD-deficient patients, and correlated negatively to age in the patient group only (r^2 0.25, $p<0.05$). Subdivision of the patient group according to treatment showed no significant differences in TBA-RS concentrations between the different groups.

We observed that plasma sulfhydryl content, which is inversely related to protein oxidative damage, was significantly decreased in MCAD-deficient patients when compared to controls ($p < 0.05$, Table 2). After correction for age, data in both the patient group without supplementation (median 541.4 µmol/L, range 445.9-641.9 µmol/L, $p<0.05$), and the patient group with L-carnitine supplementation (median 536.5 µmol/L, range 531.6-565.9 µmol/L, $p<0.05$) indicated oxidation of sulfhydryl groups (Figure 1). Addition of riboflavin to L-carnitine supplementation appeared to be effective in preventing protein oxidative damage. In contrast, carbonyl levels, which are products of protein oxidation, were similar in MCAD-deficient patients and controls.

Table 1: Characteristics of the MCAD-deficient patients and control group

Patient	Gender	Age	NBS	Supplementation	Allele 1	Allele 2
(no.)	*(m / f)*	*(years)*	*(yes / no)*	*(no / C / C+R)*		
1	m	5.6	yes	no	c.985A>G	c.985A>G
2	f	1.5	yes	no	c.985A>G	c.985A>G
3	f	2.1	yes	no	c.233T>C	c.233T>C
4	m	18.7	no	no	c.985A>G	c.157C>T
5	f	5.7	yes	no	c.985A>G	c.985A>G
6	f	3.6	yes	no	c.985A>G	c.985A>G
7	f	1.5	yes	no	c.985A>G	c.233T>G
8	f	0.0	yes	no	c.985A>G	c.985A>G
9	f	4.3	yes	no	c.985A>G	c.985A>G
10	f	1.7	yes	no	c.985A>G	c.985A>G
11	m	3.1	yes	no	c.985A>G	c.985A>G
12	m	2.9	yes	no	c.985A>G	c.789A>C
13	m	0.8	yes	no	c.985A>G	c.789A>C
14	m	5.5	yes	no	c.985A>G	c.789A>C
15	f	2.9	yes	C	c.985A>G	c.985A>G
16	f	3.4	yes	C	c.985A>G	c.985A>G
17	f	26.0	no	C	c.985A>G	c.985A>G
18	f	11.3	no	C	c.985A>G	c.985A>G
19	f	4.9	yes	C	c.985A>G	c.985A>G
20	m	5.9	yes	C	c.985A>G	c.985A>G
21	m	5.3	yes	C	c.985A>G	c.985A>G
22	m	8.4	no	C + R	c.985A>G	c.985A>G
23	f	8.9	no	C + R	c.985A>G	c.985A>G
24	f	12.7	no	C + R	c.985A>G	c.985A>G
25	f	18.3	no	C + R	c.985A>G	c.985A>G
26	f	21.4	no	C + R	c.985A>G	c.985A>G
27	f	23.6	no	C + R	c.985A>G	c.985A>G

Legend: No, no supplementation; C, carnitine supplementation; C+R, carnitine and riboflavin supplementation; NBS, identified by the Dutch population NBS program. Median age and range are depicted for the control group.

Plasma TAS was significantly increased in MCAD-deficient patients when compared to controls (Table 2). Subdivision according to treatment showed that this effect was mostly attributable to the non-supplemented patient group (p=0.08) however, treatment groups did not differ significantly from the control group. Erythrocyte activities of SOD and CAT were significantly increased in the complete group of MCAD-deficient patients, compared to controls (Table 2). L-carnitine supplementation was associated with significantly higher SOD activities (median 325%, range 126-540%, p<0.05, Figure 2), and plasma free carnitine concentrations correlated with SOD activities in MCAD-deficient patients

(Pearson r 0.398; $p<0.05$). Riboflavin supplementation had no additive effect (Figure 2). Associations between supplementation and CAT activities were not found. The anti-oxidative parameters did not correlate with age.

Table 2. Parameters of oxidative damage and antioxidant defense in plasma and erythrocyte lysates from controls and MCAD-deficient patients.

Parameter	Controls			Patients			*p* value
	median	*range*	*n*	*median*	*range*	*n*	
TBA-RS (nmol/mg protein)	0.018	0.007-0.071	12	0.037	0.010-0.092	26	0.040#
Sulfhydryl (µmol/L)	613.7	526.7-654.1	12	548.8	445.9-641.9	27	0.000*
Carbonyl (nmol/mg protein)	0.22	0.12-0.26	12	0.21	0.11-0.28	27	0.737
SOD (%)	91.7	9.3-194.7	11	194.2	22.7-540.0	26	0.015*
CAT (%)	94.5	79.7-136.6	12	129.8	68.6-353.6	25	0.014#
GPx (%)	98.0	64.5-164.9	12	107.5	20.0-200.0	26	0.376
GSH (nmol/mg protein)	3.46	2.05-6.56	12	3.30	0.23-6.20	25	0.224
TAS (mmol/l)	0.50	0.39-1.12	12	0.65	0.46-1.11	27	0.046#

Legend: No, no supplementation; C, carnitine supplementation; C+R, carnitine and riboflavin supplementation. TBA-RS: Thiobarbituric acid reactive species; SOD: superoxide dismutase; CAT: catalase activity: GPx: glutathione peroxidase; GSH: glutathione; TAS: total antioxidant status. *, tested by Student's t-test; #, tested by Mann-Whitney test.

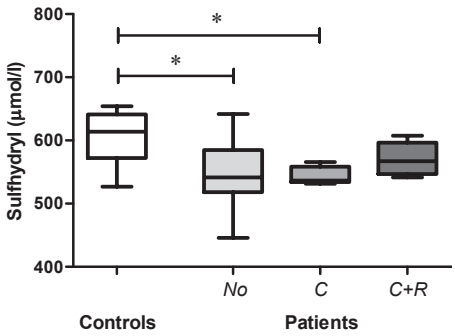

Figure 1. Plasma sulfhydryl content in controls and MCAD-deficient patients, according to their treatment.
Legend: No, no supplementation; C, carnitine supplementation; C+R, carnitine and riboflavin supplementation. * $p <$ 0.05. Boxes represent interquartile ranges with median, whiskers indicate ranges.

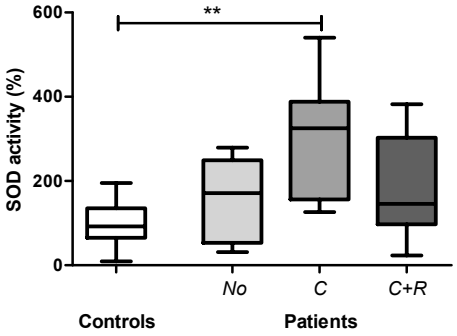

Figure 2. SOD activities in controls and MCAD-deficient patients, according to their treatment.
Legend: No, no supplementation; C, carnitine supplementation; C+R, carnitine and riboflavin supplementation. * $p <$ 0.05. Boxes represent interquartile ranges with median, whiskers indicate ranges.

DISCUSSION

Macromolecule oxidative damage and impaired antioxidant defenses have been reported to play a role in the pathophysiology of several inherited metabolic disorders and the parameters have been suggested as biomarkers for follow up [19,20]. Previous experimental studies in rat tissue preparations incubated with medium-chain fatty acid metabolites [7–10] and genetic mouse models [11,12] demonstrated a relationship between macromolecule oxidative damage, impaired antioxidant defense and defective mFAO. MCAD-deficient patients display abnormal medium-chain fatty acid metabolite profiles in plasma and urine under normal, unstressed circumstances, even though they are clinically asymptomatic. Therefore, this study evaluated lipid and protein oxidative damage and the antioxidant status in MCAD-deficient patients under these normal, unstressed circumstances.

This study demonstrates decreased sulfhydryl contents and altered antioxidant defense in MCAD-deficient patients. Decreased sulfhydryl indicates protein oxidative damage. Two thirds of the sulfhydryl groups are protein bound and the remaining is a component of small molecules, such as GSH, a well-known effective antioxidant [15]. Sulfhydryl groups also act as free radical scavengers preventing lipid oxidative damage [15]. Several *in vitro* studies reported mitochondrial dysfunction and oxidative damage after incubation with metabolites accumulating in MCAD deficiency [7–10]. In rat brain, octanoic and decanoic acid caused uncoupling of mitochondrial oxidative phosphorylation, mitochondrial cytochrome c release, increased lipid and protein oxidation damage, and decreased GSH levels [8]. Scaini *et al.* recently demonstrated similar effects in preparations of rat liver and skeletal muscle cells, where TBA-RS (lipid peroxidation) and carbonyl content (protein oxidation) were increased, and muscle GSH was decreased [9].

The theoretical rationale *in favor of* L-carnitine supplementation in MCAD deficiency is to reduce the number and severity of metabolic decompensations by (*a*) correcting the secondary carnitine deficiency, (*b*) removing, conjugating and increasing urinary excretion of toxic metabolites, (*c*) restoring

intramitochondrial acyl-CoA/CoA ratios, and (*d*) the antioxidant effects of carnitine [21]. Nevertheless, there is no systematic clinical study supporting *routine* L-carnitine supplementation in most inherited metabolic diseases, including MCAD deficiency [22]. The role of L-carnitine supplementation in MCAD deficiency therefore remains a matter of debate.

Interestingly, our study may support the rationale *against* L-carnitine supplementation, as sulfhydryl content was decreased and SOD activity was increased in patients with MCAD deficiency receiving L-carnitine. L-carnitine supplementation increases (acyl)carnitine concentrations in blood and also stimulates the carnitine cycle, transporting acyl-CoA from the cytosol into the mitochondrial matrix, thereby increasing intramitochondrial acyl-CoA concentrations [1]. Tonin *et al.* recently reported the effect of incubating cerebral cortical cells from developing rats with high concentrations of medium-chain acylcarnitines (hexanoylcarnitine, octanoylcarnitine, decanoylcarnitine and cis-4-decenoylcarnitine, 0.01 - 1.0 mM) [10]. In particular, decanoylcarnitine was responsible for lipid and protein oxidative damage. Sauer *et al.* found no negative effect of medium-chain acylcarnitines (with chain-lengths until octanoylcarnitine) on mitochondrial respiratory chain enzyme activities in bovine heart mitochondria [23]. Nevertheless, octanoyl-CoA was found to inhibit complex III, unlike any other acyl-CoA ester. Octanoyl-CoA is the most important substrate of MCAD and hence, although speculative, may accumulate even more in case of L-carnitine supplementation in combination with MCAD deficiency. Koeth *et al.* recently demonstrated in studies in humans and mice that free carnitine concentrations can promote artherosclerosis, via the formation of trimethylamine-*N*-oxide from carnitine by intestinal microbiota [24].

Riboflavin is the precursor of flavin adenine dinucleotide (FAD), an essential cofactor of many intramitochondrial enzymes, including MCAD. Duran *et al.* studied the *in vitro* MCAD enzyme activity in lymphocytes from five MCAD-deficient patients before and after three weeks of riboflavin supplementation at a dose of 50-150 mg/day [13]. Octanoyl-CoA dehydrogenase activity at least doubled in these patients after supplementation. Although this study did not discriminate between a *specific* increase of residual MCAD enzyme activity, a *general* increase of octanoyl-CoA dehydrogenase activity, or a combination, this has been the theoretical basis behind riboflavin supplementation in a subset of our older MCAD-deficient patients. In our study, patients receiving L-carnitine and riboflavin displayed normal sulfhydryl concentrations and SOD activities in contrast to patients with only L-carnitine supplementation. Our observations suggest an antioxidant role of riboflavin as described by Depeint *et al.*[25], which could be explained by the chaperone function enhancing folding efficiency of MCAD protein in patients carrying a missense mutation in the *ACADM* gene [26].

Several methodological aspects of this study need to be discussed. First, it was difficult to obtain age- and gender-matched control samples, especially for the younger group of patients. Second, this is an observational cross-sectional study in samples that were obtained during normal hospital visits, which only enables us to speculate about causal relations. Third, the studied parameters showed large ranges, which can be partially explained by the inter-assay variability. In addition, we studied parameters in biological samples (blood) from our patients. It is important to realize that blood carnitine concentrations do not necessarily reflect tissue homeostasis, and blood oxidative stress

parameters may thus be no reflection of oxidative stress in the liver [27]. The MCAD-deficient mouse model is characterized incompletely [28,29] and would facilitate mechanistic studies under acute and chronic conditions, discriminating contributions of different oxygen-consuming organs such as the brain, liver, heart and muscle.

Summarizing, we demonstrate oxidative damage and altered antioxidant status in asymptomatic MCAD-deficient patients. Population NBS programs for the disorder identify increasing numbers of patients, whose metabolite profiles never normalize. Although *in vitro* studies suggest that these metabolites may contribute to oxidative damage, their increased concentrations have never been associated with any clinical phenotype. Interesting experimental data originate from animal studies. Ibdah *et al.* studied ageing heterozygotes of a knock-out mouse model of long-chain mFAO, in which hepatic steatosis and insulin resistance were associated with reduced liver GSH and increased antioxidant enzyme activities [11]. These observations suggest that oxidative damage, hepatic steatosis and insulin resistance are intimately related under circumstances of perturbed mFAO. Data from large follow-up cohort studies of MCAD-deficient patients until adulthood are not yet available.

CONCLUSIONS

MCAD-deficient patients display oxidative damage and altered antioxidant defense. Due to the intimate relationships between mFAO, electron transport and oxidative phosphorylation, RS may be generated by different simultaneous actions. Patients receiving both L-carnitine and riboflavin did not display oxidative damage. L-carnitine alone was associated with protein oxidative damage, and altered antioxidant defense. Patients receiving L-carnitine showed similar results to non-supplemented patients with regards to oxidative stress. Future mechanistic studies on the role of oxidative stress in MCAD deficiency are required.

ACKNOWLEDGEMENTS AND FUNDING

The authors thank the patients and parents for their participation. This work was supported in parts by the UMC Groningen (Mandema Stipend to TGJD), CAPES, CNPq and FIPE/HCPA-Brazil (the latter three to MW and CRV).

REFERENCES

1. Roe CR, Ding J. Chapter 101: Mitochondrial Fatty Acid Oxidation Disorders. In: Scriver CR, Beaudet AL, Sly WS, Valle D, editors. Metabolic and Molecular Bases of Inherited Disease. 8th ed. Columbus, OH: McGraw-Hill; 2005.
2. Derks TG, Reijngoud DJ, Waterham HR, Gerver WJ, van den Berg MP, Sauer PJ, Smit GP. The natural history of medium-chain acyl CoA dehydrogenase deficiency in the Netherlands: clinical presentation and outcome. *J Pediatr* 2006; **148**(5): 665-70.
3. Vianey-Liaud C, Divry P, Gregersen N, Mathieu M. The inborn errors of mitochondrial fatty acid oxidation. *J Inherit Metab Dis* 1987; **10 Suppl 1**: 159-200.
4. Wilcken B, Haas M, Joy P, et al. Outcome of neonatal screening for medium-chain acyl-CoA dehydrogenase deficiency in Australia: a cohort study. *Lancet* 2007; **369**(9555): 37-42.
5. Touw CM, Smit GP, de Vries M, et al. Risk stratification by residual enzyme activity after newborn screening for medium-chain acyl-CoA dehyrogenase deficiency: Data from a cohort study. *Orphanet J Rare Dis* 2012; **7**(1): 30.
6. Halliwell B. Biochemistry of oxidative stress. *Biochem Soc Trans* 2007; **35**(Pt 5): 1147-50.
7. Schuck PF, Ceolato PC, Ferreira GC, et al. Oxidative stress induction by cis-4-decenoic acid: relevance for MCAD deficiency. *Free Radic Res* 2007; **41**(11): 1261-72.
8. Schuck PF, Ferreira Gda C, Tonin AM, et al. Evidence that the major metabolites accumulating in medium-chain acyl-CoA dehydrogenase deficiency disturb mitochondrial energy homeostasis in rat brain. *Brain Res* 2009; **1296**: 117-26.
9. Scaini G, Simon KR, Tonin AM, et al. Toxicity of octanoate and decanoate in rat peripheral tissues: evidence of bioenergetic dysfunction and oxidative damage induction in liver and skeletal muscle. *Mol Cell Biochem* 2012; **361**(1-2): 329-35.
10. Tonin AM, Grings M, Knebel LA, et al. Disruption of redox homeostasis in cerebral cortex of developing rats by acylcarnitines accumulating in medium-chain acyl-CoA dehydrogenase deficiency. *Int J Dev Neurosci* 2012; **30**(5): 383-90.
11. Ibdah JA, Perlegas P, Zhao Y, et al. Mice heterozygous for a defect in mitochondrial trifunctional protein develop hepatic steatosis and insulin resistance. *Gastroenterology* 2005; **128**(5): 1381-90.
12. Tucci S, Primassin S, Spiekerkoetter U. Fasting-induced oxidative stress in very long chain acyl-CoA dehydrogenase-deficient mice. *FEBS J* 2010; **277**(22): 4699-708.
13. Duran M, Cleutjens CB, Ketting D, Dorland L, de Klerk JB, van Sprang FJ, Berger R. Diagnosis of medium-chain acyl-CoA dehydrogenase deficiency in lymphocytes and liver by a gas chromatographic method: the effect of oral riboflavin supplementation. *Pediatr Res* 1992; **31**(1): 39-42.
14. Esterbauer H, Cheeseman KH. Determination of aldehydic lipid peroxidation products: malonaldehyde and 4-hydroxynonenal. *Methods Enzymol* 1990; **186**: 407-21.
15. Aksenov MY, Markesbery WR. Changes in thiol content and expression of glutathione redox system genes in the hippocampus and cerebellum in Alzheimer's disease. *Neurosci Lett* 2001; **302**(2-3): 141-5.
16. Levine RL, Garland D, Oliver CN, et al. Determination of carbonyl content in oxidatively modified proteins. *Methods Enzymol* 1990; **186**: 464-78.
17. Aebi H. Catalase in vitro. *Methods Enzymol* 1984; **105**: 121-6.
18. Browne RW, Armstrong D. Reduced glutathione and glutathione disulfide. *Methods Mol Biol* 1998; **108**: 347-52.
19. Wajner M, Latini A, Wyse AT, Dutra-Filho CS. The role of oxidative damage in the neuropathology of organic acidurias: insights from animal studies. *J Inherit Metab Dis* 2004; **27**(4): 427-48.

20. Mc Guire PJ, Parikh A, Diaz GA. Profiling of oxidative stress in patients with inborn errors of metabolism. *Mol Genet Metab* 2009; **98**(1-2): 173-80.
21. Ribas GS, Biancini GB, Mescka C, Wayhs CY, Sitta A, Wajner M, Vargas CR. Oxidative stress parameters in urine from patients with disorders of propionate metabolism: a beneficial effect of L: -carnitine supplementation. *Cell Mol Neurobiol* 2012; **32**(1): 77-82.
22. Nasser M, Javaheri H, Fedorowicz Z, Noorani Z. Carnitine supplementation for inborn errors of metabolism. *Cochrane Database Syst Rev* 2012; **2**: CD006659.
23. Sauer SW, Okun JG, Hoffmann GF, Koelker S, Morath MA. Impact of short- and medium-chain organic acids, acylcarnitines, and acyl-CoAs on mitochondrial energy metabolism. *Biochim Biophys Acta* 2008; **1777**(10): 1276-82.
24. Koeth RA, Wang Z, Levison BS, et al. Intestinal microbiota metabolism of L-carnitine, a nutrient in red meat, promotes atherosclerosis. *Nat Med* 2013; **19**(5): 576-85.
25. Depeint F, Bruce WR, Shangari N, Mehta R, O'Brien PJ. Mitochondrial function and toxicity: role of the B vitamin family on mitochondrial energy metabolism. *Chem Biol Interact* 2006; **163**(1-2): 94-112.
26. Lucas TG, Henriques BJ, Rodrigues JV, Bross P, Gregersen N, Gomes CM. Cofactors and metabolites as potential stabilizers of mitochondrial acyl-CoA dehydrogenases. *Biochim Biophys Acta* 2011; **1812**(12): 1658-63.
27. Spiekerkoetter U, Tokunaga C, Wendel U, et al. Tissue carnitine homeostasis in very-long-chain acyl-CoA dehydrogenase-deficient mice. *Pediatr Res* 2005; **57**(6): 760-4.
28. Tolwani RJ, Hamm DA, Tian L, et al. Medium-chain acyl-CoA dehydrogenase deficiency in gene-targeted mice. *PLoS Genet* 2005; **1**(2): e23.
29. Herrema H, Derks TG, van Dijk TH, et al. Disturbed hepatic carbohydrate management during high metabolic demand in medium-chain acyl-CoA dehydrogenase (MCAD)-deficient mice. *Hepatology* 2008; **47**(6): 1894-904.