Renal response to short- and long-term exercise in very-long-chain acyl-CoA dehydrogenase-deficient (VLCAD−/−) mice

Sara Tucci1*, Antonia Krogmann2†, Diran Herebian2 and Ute Spiekerkoetter1

Abstract

Background: Deficiency of very long-chain acyl-CoA dehydrogenase (VLCAD) is the most common disorder of mitochondrial β-oxidation of long-chain fatty acids. In order to maintain glucose homeostasis, the kidney and liver as the main gluconeogenic organs play an important role under conditions of impaired fatty acid oxidation. However, little is known about how a defective fatty acid oxidation machinery affects renal metabolism and function as well as renal energy supply especially during catabolic situations.

Methods: In this study, we analyzed VLCAD−/− mice under different metabolic conditions such as after moderate (1 h) and intensive long-term (1 h twice per day over 2 weeks) physical exercise and after 24 h of fasting. We measured the oxidation rate of palmitoyl-CoA (C16-CoA) as well as the expression of genes involved in lipogenesis and renal failure. Oxidative stress was assessed by the function of antioxidant enzymes. Moreover, we quantified the content of glycogen and long-chain acylcarnitines in the kidney.

Results: We observed a significant depletion in renal glycogen with a concomitant reduction in long-chain acylcarnitines, suggesting a substrate switch for energy production and an optimal compensation of impaired fatty acid oxidation in the kidney. In fact, the mutants did not show any signs of oxidative stress or renal failure under catabolic conditions.

Conclusions: Our data demonstrate that despite Acadvl ablation, the kidney of VLCAD−/− mice fully compensates for impaired fatty acid oxidation by enhanced glycogen utilization and preserves renal energy metabolism and function.

Keywords: VLCAD deficiency; Fatty acid oxidation; Acylcarnitines; Glucose homeostasis; Oxidative stress

Background

Deficiency of the very long-chain acyl-CoA dehydrogenase (VLCAD) is the most common disorder of mitochondrial β-oxidation of long-chain fatty acids with an incidence of 1:30,000 to 1:100,000 newborns [1,2]. The clinical phenotype of VLCAD deficiency (VLCADD) is very heterogeneous and presents with different severity and age of onset [3]. The symptoms usually manifest in situations of increased energy demand such as fasting, infectious illnesses, and intensive or prolonged physical exercise when the organism relies on fatty acid β-oxidation for energy supply. Before the implementation of newborn screening programs for fatty acid oxidation defects, three different phenotypes could be identified. The most severe phenotype appeared in the first weeks and months of life and presented with hypoketotic hypoglycemia, hypertrophic cardiomyopathy, and encephalopathy. The infantile hepatic phenotype was triggered by infections and presented with hypoketotic hypoglycemia, hepatopathy, and lethargy. The milder later-onset myopathic phenotype was characterized by muscle weakness, rhabdomyolysis, and myoglobinuria in adolescents or young adults [4,5].

The VLCAD−/− mouse is a reliable model and develops symptoms during catabolic situations as occur in humans with a milder later-onset phenotype [6-12]. Indeed, although asymptomatic under resting conditions, VLCAD−/− mice present with hypoglycemia, hepatopathy, and skeletal myopathy during catabolism [9,13]. Compensatory mechanisms based on enhanced glucose oxidation are effective
and supply sufficient energy at rest [14-16]. The role of the kidneys in providing energy as a gluconeogenic organ such as the liver has not been studied yet. Therefore, we here investigated how a defective fatty acid oxidation machinery affects kidney function and kidney metabolism with special focus on glucose supply. To address this question, we measured the palmitoyl-CoA (C16-CoA) oxidation rate in the kidney of wild-type (WT) and VLCAD−/− mice in situations of normal and increased energy demand as well as the expression of other dehydrogenases with overlapping substrate specificity. Renal damage and function were characterized by the activity of antioxidant enzymes and by the expression of genes upregulated immediately prior to renal failure. Finally, renal glycogen content and acylcarnitines as markers for impaired energy production from fatty acid oxidation were quantified.

Methods

Animals

VLCAD−/− mice have been generated as described in detail by Exil et al. [13]. Experiments were performed on fourth- to fifth-generation intercrosses of C57BL6+129sv VLCAD genotypes. Littermates served as controls, and genotyping of mice was performed as described previously [13]. Groups consisting of 5 to 6 mice of both genders, at the age of 12 to 13 weeks, were investigated under resting and different catabolic conditions. The mice were sacrificed at the end of the experiment by CO2 asphyxiation. The kidneys were rapidly removed and immediately frozen in liquid nitrogen. The experiments have been performed twice. All animal studies were performed with the approval of the University Institutional Animal Care and Use Committee and in accordance with the Committees' (LANUV) guidelines.

Diet composition, exercise protocols, and fasting

After weaning, the mice of each genotype were fed a normal purified mouse diet containing 5% crude fat in form of LCT, corresponding to 12% of metabolizable energy as calculated with Atwater factors (ssniff® EF R/M Control, ssniff Spezialdiäten GmbH, Soest, Germany). All mice groups received water ad libitum. The mice were analyzed under well-fed conditions or after 24 h of fasting.

As mice are nocturnal animals, treadmill running was performed during the dark cycle. To analyze the effects of different exercise bouts, one group of WT and VLCAD−/− mice was exercised for 60 min once. The mice were placed in an exercise chamber, and after an adaptation period of 15 min, initial belt speed was set to 4 m/min and increased every 5 min by 2 m/min to a maximum of 16 m/min. The mice were exercised until they displayed signs of exhaustion (>2 s spent on the shocker plate without attempting to reengage the treadmill) or the exercise was terminated after 60 min. The second group of mice was subjected to a long-term exercise stress. The animals had to run 45 min twice a day for 2 weeks (5 days per week) at 0° inclination. The long-term exercise protocol started with a running speed of 10 m/min. At day 5, the running speed was reduced to 8 m/min until day 10 and further reduced to 6 m/min at day 11 until the end of the experiment. Both experiments were conducted on a Columbus Instruments Simplex II metabolic rodent treadmill (Columbus, OH, USA) consisting of four individual lanes without inclination and a shock plate incentive (3 Hz, 200 ms, 160 V, 1.5 mA).

Tissue homogenates and protein expression

The tissues were homogenized in Cellytic MT Buffer (Sigma-Aldrich, Steinheim, Germany) in the presence of 1 mg/ml protease inhibitors and centrifuged at 4°C and 16,000 × g for 10 min to pelletize any cell debris. The clear supernatant was immediately used for the enzyme assays or stored at −80°C. The protein concentration of tissue homogenates was determined using the BSA method as described previously [17].

Oxidation rate and analysis of palmitoyl-CoA by LC-MS/MS

The oxidation rate of C16-CoA was measured as reported previously [15]. C16-CoA, was purchased from Sigma (Steinheim, Germany). Reaction products were detected by LC-ESI-MS/MS in positive ionization mode according to the published literature [15].

Enzyme activities

Catalase, glutathione peroxidase (GPX), and NAD(P)H: quinone oxidoreductase were measured to determine the development and cellular localization of oxidative stress. The peroxisomal catalase activity was measured fluorometrically by the production of the highly fluorescent oxidation product resorufin [18]. The mitochondrial glutathione peroxidase activity was determined by calculating the oxidation rate of nicotinamide adenine dinucleotide phosphate (NADPH) to NADP+ spectrophotometrically at 340 nm for 4 min as previously described [19,20]. The NAD(P)H: quinone oxidoreductase, marker for cytosolic oxidative stress was measured as described by Milder et al. [21]. Briefly, the reactions contained 25 mM Tris-HCl (pH 7.4), 0.7 mg/ml bovine serum albumin, and 0.2 mM NADH. The reactions were started by the addition of 40 μM 2,6-dichlorophenolindophenol. The reactions were performed in the absence and presence of 20 μM dicumaral. NQO1 activity is defined as the dicumarol-inhibitable reduction of 2,6-dichlorophenolindophenol measured at 600 nm at 30°C.

Analysis of acylcarnitines

The analysis of long-chain acylcarnitines was performed in the kidney as previously described [22,23]. Briefly, the acylcarnitines were extracted from the tissues with acetonitrile/water (80%/20% v/v) in the presence of [2H3] free
carnitine, \([^{2}H_{3}]\) octanoyl-carnitine, and \([^{2}H_{3}]\) palmitoyl-carnitine as internal standard. The extracted supernatant was dried, and the butylated acylcarnitines were analyzed by electron spray ionization tandem mass spectrometry (ESI-MS/MS). All even-chain C14-C18 acylcarnitines (saturated and unsaturated) were measured.

**Extraction and analysis of renal glycogen**

The glycogen concentrations of the kidney were quantified as duplicates by using an enzymatic kit (EnzyChrom™ Glycogen Assay Kit, BioTrend, Cologne, Germany) on an Infinite M200 Tecan (Crailsheim, Germany) plate reader. The assays were performed following the manufacturer’s instructions.

**Real-time PCR analysis**

Total renal RNA was isolated with the RNeasy mini kit (Qiagen, Hilden, Germany). Forward and reverse primers for β-actin, acyl-CoA oxidase (AOX), lipocalin 2 (Ngal), kidney injury molecule 1 (KIM1), heme oxygenase 1 (Ho1), stearoyl element binding protein-1c (SREBP-1c), fatty acid synthase (FASN), and acetyl-CoA carboxylase 1α (ACC1α) were designed with the FastPCR program (R. Kalendar, Institute of Biotechnology, Helsinki, Finland). Gene function and primer sequences are reported in Table 1. Real-time PCR was performed in a single-step procedure with the QuantiTect SYBR Green™ RT-PCR (Qiagen, Hilden, Germany) on an Applied Biosystems 7500 Sequence Detection System in Micro Amp 96-well optical reaction plates capped with MicroAmp optical caps (Applied Biosystems, Foster City, CA, USA). The values in all samples were normalized to the expression level of β-actin as internal standard.

**Statistical analysis**

All data are presented as mean ± standard error of the mean (SEM). All data were tested with the Kolmogorov-Smirnov test and the Levene’s test to investigate the Gaussian distribution and the homoscedasticity. Statistical analysis of differences between two means was assessed by unpaired Student’s t test to correct for multiple comparisons using the Holm-Sidak method. Multiple means were compared by a two-way analysis of the variance (ANOVA) to test the effects of genotype and applied stress (GraphPad Prism 6.0, San Diego, CA, USA). A probability level of \(p < 0.05\) was regarded as significant.

**Results**

**Turnover rate of palmitoyl-CoA oxidation and gene expression of different dehydrogenases**

To test whether the oxidation rate in the kidney of VLCAD\(^{-/-}\) mice is influenced by increased energy demand or fasting, we measured the turnover rate of C16-CoA in mice during different conditions. As shown in Figure 1A, under resting condition, the turnover rate in VLCAD\(^{-/-}\) mice was significantly reduced by 20% in mutants as compared to the littermates. Interestingly, 1 h on the treadmill did not affect the oxidation capacity of the VLCAD\(^{-/-}\) mice, whereas a training protocol over 2 weeks significantly reduced the turnover rate of C16-CoA in the VLCAD\(^{-/-}\) mice as compared to sedentary mutants (9 ± 0.49 vs. 13.1 ± 0.54 mU/mg). A similar significant reduction of C16-CoA oxidation capacity was also observed after 24 h of fasting in both genotypes, as shown in Figure 1. Fasting, therefore, did not result in a genotype-specific effect in contrast to long-term physical exercise. We also did not observe a genotype-specific effect on the gene expression of other dehydrogenases with partly

### Table 1 Gene function and primer sequences

| Probe          | Forward 5′ → 3′        | Reverse 5′ → 3′        | GenBank          |
|----------------|------------------------|------------------------|------------------|
| β-actin        | TAGGCACCCAGGGTGATGG    | CTCCATGTCGTCAGTAGG     | NM_009606.2      |
| β-oxidation    |                         |                        |                  |
| MCAD           | GAAAGTTGGGATGAGTGG     | AAGCACACATCAATGGCTGC   | NM_007382.4      |
| LCAD           | GGGAAAGCAAGCCCTCGCAG   | TCTGCTCATGGCTATGGCACC  | NM_007381.3      |
| AOX            | TGCCCAGGTGGAGAAGCCTAGC | TCAGACTGCGGCGCTCCAGGC | NM_015729.2      |
| Renal failure  |                         |                        |                  |
| Ngal           | GTGTTGCTGTGCTGGGCGCTG  | TGGCCAGCGCGTGGCCTTG    | NM_008491        |
| KIM1           | GTCAGCATCTCAAGCGCTGG   | GCCAGAAGGTCCCTCAAGAG   | NM_001166631     |
| Ho1            | ATGGAGCTGTTGGAGGAGCTG  | CACTGCCACTGTTGGCAACAGGC| NM_010442.2      |
| Lipogenesis    |                         |                        |                  |
| SREBP-1c       | CAGCTCAGAGCCCTGATG     | TTGATAGAAGGCAAGGCATAGG| NM_01148         |
| ACC1α          | TCAACAGCTGAGCTCCACACG  | ACTGTCATCGATATCGCAGCG  | NM_133360        |
| FASN           | TCTGGAATTCCGCAAGGGCTACC| TTCGGGGTTGCCCTGCAAGG   | NM_007988.3      |
overlapping substrate specificity to VLCAD, the mitochondrial MCAD and LCAD as well as the peroxisomal AOX. However, we observed a remarkable upregulation of AOX in both genotypes as adaptive response to fasting [24] (Figure 1D). Of particular interest was the significant reduction of C16-CoA oxidation rate in VLCAD−/− mice after long-term physical exercise as compared to mutants under resting condition, suggesting enhanced energy production from glucose.

Lipid accumulation and oxidative stress
Because catabolism stimulates lipolysis resulting in marked lipid accumulation in different organs of VLCAD−/− mice [11], we measured the renal lipogenesis in all groups. We therefore tested the expression at mRNA level of genes involved in this pathway, such as the transcription factor SREBP-1c, which regulates lipid homeostasis, fatty acid biosynthesis, and glucose metabolism [25], as well as of its target genes ACC1α and FASN, responsible for de novo biosynthesis and elongation of fatty acids as they directly correlate with the triglyceride accumulation in the kidney [26]. As shown in Figure 2A,B,C, in contrast to results previously obtained from the liver [11,12], the expression of these genes was strongly downregulated in all groups independently of the applied stress.

To appoint the development of oxidative stress due to catabolism as occur in VLCAD−/− mice [11], we measured the specific activity of NADPH:quinone oxidoreductase, GPX, and catalase, which are antioxidant enzymes neutralizing oxidative stress of cytosolic, mitochondrial, and peroxisomal origin, respectively. However, neither the activity of NADPH:quinone oxidoreductase nor of catalase was affected by fasting or exercise, (Figure 3A,B), with the exception of GPX activity, which was significantly reduced in the VLCAD−/− mice as compared to WT after the exercise of about 2 weeks (8.51 ± 0.45 vs. 16.97 ± 3.88 U/mg; Figure 3C). These data are in line with the reduced C16-CoA oxidation rate. Moreover, the expression of genes massively upregulated immediately prior to kidney failure, namely, Ngal, KIM1, and Ho1, was unaffected under all applied stress conditions (Figure 4A,B,C).

Effect of different stressors on glycogen content and long-chain acylcarnitines in the kidney
In a situation of increased energy demand such as during physical exercise, both glucose and glycogen strongly contribute to the energy supply. We therefore measured the glycogen content in the kidney under resting conditions, after physical exercise, and after 24 h of fasting. Under resting conditions, the glycogen content did not differ.

Figure 1 Oxidation rate of palmitoyl-CoA (C16-CoA) (A) and gene expression of fatty acid dehydrogenases [MCAD (B), LCAD (C) and AOX (D)]. White and black bars represent WT and VLCAD-/- mice, respectively. Values are represented as mean ± SEM (n = 5-6). * indicates significant differences between WT and VLCAD-/- mice within an experimental set. # indicates significant differences between WT or VLCAD-/- mice under different stress conditions as compared to resting mice. * and # values were considered significant if p < 0.05 (Two way ANOVA with Bonferroni correction and Student’s t-test).
between the WT and VLCAD−/− mice. However, after physical exercise, glycogen was more than twofold reduced in the VLCAD−/− mice as shown in Figure 5A. After fasting, the glycogen content was also reduced in both genotypes although significantly only in the mutants.

Acylcarnitines reflect the efficiency of mitochondrial β-oxidation. The analysis of long-chain acylcarnitines in
kidney tissue revealed that under resting conditions, the content of these metabolites was more than twofold higher in the VLCAD−/− mice as compared to the littermates (12.8 ± 0.13 vs. 5.7 ± 0.07 μmol/mg; Figure 5B). Of interest was the significant reduction of acylcarnitine content in the VLCAD−/− mice after 24 h of fasting, suggesting that under this condition the kidney must rely on a different energy source more likely glucose.

Discussion
In this study, we demonstrate that the kidney in the VLCAD−/− mice fully compensates for a defective fatty acid oxidation most likely enhancing glucose oxidation. In contrast to the liver, the main gluconeogenic organ, fasting does not result in an increased renal lipid accumulation and lipogenesis is not induced. In fact, neither signs of oxidative stress nor renal failure are observed during catabolic situations. Importantly, especially long-term exercise promotes a substrate switch so that the kidneys fully rely on glycogen as main source for energy production, while the contribution of fatty acid oxidation is rather minor.

As under catabolic conditions the mice develop hepatic and muscular symptoms [10-12], we hypothesized the same for the kidneys despite the existence of an enzyme with overlapping substrate specificity, the long-chain acyl-CoA dehydrogenase (LCAD) [6,15]. Surprisingly, the C16-CoA oxidation rate was significantly reduced after exercise or fasting, suggesting that the kidney does not rely on fatty acid oxidation during catabolism. Because we expected a renal lipid accumulation as we previously observed in the

![Figure 4](http://www.molcellped.com/content/1/1/5)

**Figure 4** Expression of genes upregulated during renal failure. (A) Ngal, lipocalin; (B) KIM1, kidney injury molecule 1; (C) Hoo1, heme oxygenase 1. White and black bars represent WT and VLCAD−/− mice, respectively. Values are represented as mean ± SEM (n=5 to 6). Asterisk indicates significant differences between WT and VLCAD−/− mice within an experimental set. Number sign indicates significant differences between WT or VLCAD−/− mice under different stress conditions as compared to resting mice. Asterisk and number sign denote that values were considered significant if p<0.05 (two-way ANOVA with Bonferroni correction and Student’s t test).

![Figure 5](http://www.molcellped.com/content/1/1/5)

**Figure 5** Renal metabolite content. (A) Glycogen content and (B) long-chain acylcarnitine accumulation in the kidney of WT and VLCAD−/− mice under different stress conditions. White and black bars represent WT and VLCAD−/− mice, respectively. Values are represented as mean ± SEM (n=5 to 6). Asterisk indicates significant differences between WT and VLCAD−/− mice within an experimental set. Number sign indicates significant differences between WT or VLCAD−/− mice under different stress conditions as compared to resting mice. Asterisk and number sign denote that values were considered significant if p<0.05 (two-way ANOVA with Bonferroni correction and Student’s t test).
Kidneys play a distinctive role in glucose homeostasis through glucose filtration, reabsorption, consumption, and gluconeogenesis. In particular, the last process contributes up to 40% to the whole-body glycogen biosynthesis, leaving the kidney a gluconeogenic organ in vivo as important as the liver [29,30]. Especially during fasting when glycogen store are depleted and gluconeogenesis becomes the most important process for sustaining the supply of glucose, the kidneys increase their net contribution to glucose release [31]. Although we did not measure glycogen biosynthesis, we observed that during catabolic situations, the glycogen content in VLCAD<sup>−/−</sup> mice dramatically reduced probably to maintain whole-body glucose homeostasis. These data strongly correlate with the accumulation of long-chain acylcarnitines in many organs which reflect the induced mitochondrial β-oxidation during catabolism but an ineffective oxidation of fatty acids. Very surprisingly, we observed an important correlation between glycogen content and long-chain acylcarnitine accumulation in contrast to other organs [10,11], indicating that during situations of increased energy demand, the kidney mostly likely relies on glucose oxidation. Comparable results have been already described for the skeletal muscle of 1-year-old VLCAD<sup>−/−</sup> mice under resting conditions [15]. Here, the enhanced glucose oxidation as a compensatory mechanism for a defective FAO machinery was associated with a strongly reduced content of long-chain acylcarnitine.

**Conclusions**

Our study shows that under different stress situations, the potential of renal gluconeogenesis can fully compensate the increased energy demand protecting the organ from toxic accumulation of acylcarnitines. The acute renal failure as described in single-case reports appears to be secondary to rhabdomyolysis and myoglobinuria.

**Abbreviations**

ESI-MS/MS: electrospray ionization tandem mass spectrometry; FAO: fatty acid oxidation; VLCAD: very long-chain acyl-CoA dehydrogenase; VLCAD<sup>−/−</sup> mice: very-long-chain acyl-CoA dehydrogenase-deficient mice; WT: wild type.

**Competing interests**

The authors declare that they have no competing interests.

**Authors’ contributions**

ST designed and conducted the research and wrote the manuscript. AK conducted the research. DH performed LC-ESI-MS/MS analysis. US drafted the manuscript and had primary responsibility for the final content. All authors read and approved the final manuscript.

**Acknowledgements**

The study was financially supported by grants from the Deutsche Forschungsgemeinschaft (DFG): SFB 575 and SFB 612 of Heinrich Heine University Dusseldorf, Germany.

**Author details**

1. Department of General Pediatrics, Center for Pediatrics and Adolescent Medicine, University Hospital Freiburg, Mathildenstrasse 1, Freiburg 79106, Germany.
2. Department of General Pediatrics, Neonatology and Children’s Cardiology, University Children’s Hospital, Dusseldorf 40225, Germany.

**Received:** 23 March 2014 **Accepted:** 22 July 2014

**Published online:** 02 October 2014

**References**

1. Arnold GL, Van Hove J, Freedenberg D, Strauss A, Longo N, Burton B, Garganta C, Ficicioglu C, Cederbaum S, Harding C, Boles RG, Matem D, Chakraborty P, Feigenbaum A (2009) A Delphi clinical practice protocol for the management of very long chain acyl-CoA dehydrogenase deficiency. Mol Genet Metab 98:85–90
2. Spiekerkoetter U, Sun B, Zykovich T, Wanders R, Strauss AW, Wendel U (2003) MS/MS-based newborn and family screening detects asymptomatic patients with very-long-chain acyl-CoA dehydrogenase deficiency. J Pediatr 143:335–342
3. Strauss AW, Powell CK, Hale DE, Anderson WM, Ahuja A, Brackett JC, Sims HF (1995) Molecular basis of human mitochondrial very-long-chain acyl-CoA dehydrogenase deficiency causing cardiomyopathy and sudden death in childhood. Proc Natl Acad Sci USA 92:10496–10500
4. Gregersen N, Andresen BS, Cordonoy MJ, Cordonoy TJ, Olsen RK, Bolund L, Bross P (2001) Mutation analysis in mitochondrial fatty acid oxidation defects: exemplified by acyl-CoA dehydrogenase deficiencies, with special focus on genotype-phenotype relationships. Hum Mutat 18:169–189
5. Kompore M, Rizzo WB (2008) Mitochondrial fatty-acid oxidation disorders. Semin Pediatr Neurol 15:140–149
6. Chegany M, Brinke H, Ruther JP, Wijburg FA, Stoll MS, Miniker PE, van Weeghel M, Schulz H, Hoppel CL, Wanders RJ, Houten SM (2009) Mitochondrial long chain fatty acid beta-oxidation in man and mouse. Biochim Biophys Acta 1791:806–815
7. Exil VJ, Gardner CD, Rottman JN, Sims H, Bartels B, Khuchua Z, Sindhal R, Ni G, Strauss AW (2006) Abnormal mitochondrial bioenergetics and heart rate
8. Goetzman ES, Tian L, Wood PA (2005) Differential induction of genes in liver and brown adipose tissue regulated by peroxisome proliferator-activated receptor-alpha during fasting and cold exposure in acyl-CoA dehydrogenase-deficient mice. Mol Genet Metab 84:39–47

9. Spielkerkoetter U, Rütt J, Tokunaga C, Wendel U, Mayatepek E, Wilburg FA, Strauss AW, Wanders RJ (2006) Evidence for impaired gluconeogenesis in very long-chain acyl-CoA dehydrogenase-deficient mice. Horm Metab Res 38:625–630

10. Spielkerkoetter U, Tokunaga C, Wendel U, Mayatepek E, Ilijst L, Vaz FM, van Vliet H, Overmans H, Duran M, Wilburg FA, Wanders RJ, Strauss AW (2005) Tissue carnitine homeostasis in very-long-chain acyl-CoA dehydrogenase-deficient mice. Pediatr Res 57:760–764

11. Tucci S, Primassin S, Spielkerkoetter U (2010) Fasting-inducing oxidative stress in very long chain acyl-CoA dehydrogenase-deficient mice. FEBS J 277:4699–4708

12. Tucci S, Primassin S, Ter Veld F, Spielkerkoetter U (2010) Medium-chain triglycerides impair lipid metabolism and induce hepatic steatosis in very long-chain acyl-CoA dehydrogenase (VLCAD)-deficient mice. Mol Genet Metab 101:40–47

13. Exil VJ, Roberts RL, Sims H, McLaughlin JE, Malkin RA, Gardner CD, Ni G, Rottman JW, Strauss AW (2003) Very-long-chain acyl-coenzyme a dehydrogenase deficiency in mice. Circ Res 93:448–455

14. Tucci S, Flogel U, Hermann S, Sturm M, Schafers M, Spielkerkoetter U (2014) Development and pathomechanisms of cardiomyopathy in very long-chain acyl-CoA dehydrogenase deficient (VLCAD) mice. Biochimica et Biophysica Acta 1842(5):677–685

15. Tucci S, Herebian D, Sturm M, Seibt A, Spielkerkoetter U (2012) Tissue-specific strategies of the very-long-chain acyl-CoA dehydrogenase-deficient (VLCAD−/−) mouse to compensate a defective fatty acid beta-oxidation. PloS One 7:e45429

16. Tucci S, Pearson S, Herebian D, Spielkerkoetter U (1832) Long-term dietary effects on substrate selection and muscle fiber type in very-long-chain acyl-CoA dehydrogenase deficient (VLCAD−/−) mice. Biochim Biophys Acta 2013:509–516

17. Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254

18. Mohanty JG, Jaffe JS, Schulman ES, Rabie DG (1997) A highly-sensitive fluorescent micro-assay of H2O2 release from activated human leukocytes using a dihydroxyphenoxazine derivative. J Immunol Meth 202:133–141

19. Lawrence RA, Burk RF (1976) Glutathione peroxidase activity in selenium-deficient rat liver. Biochem Biophys Res Comm 71:952–958

20. Mantha SV, Prasad M, Kalra J, Prasad K (1993) Antioxidant enzymes in hypercholesterolemia and effects of vitamin E in rabbits. Atherosclerosis 101:135–144

21. Milder JB, Liang LP, Patel M (2010) Acute oxidative stress and systemic NrF2 activation by the ketogenic diet. Neurobiol Dis 40:238–244

22. Primassin S, Ter Veld F, Mayatepek E, Spielkerkoetter U (2008) Carnitine supplementation induces acylcarnitine production in tissues of very-long-chain acyl-CoA dehydrogenase-deficient mice, without replenishing low free carnitine. Pediatr Res 63:632–637

23. Primassin S, Tucci S, Herebian D, Seibt A, Hoffmann L, Ter Veld F, Spielkerkoetter U (2010) Pre-exercise medium-chain triglyceride application prevents acylcarnitine accumulation in skeletal muscle from very-long-chain acyl-CoA-dehydrogenase-deficient mice. J Inherit Metab Dis 33:237–246

24. Kersten S, Seydoux J, Peters JM, Gonzalez FJ, Desvergne B, Wahli W (1999) Peroxisome proliferator-activated receptor alpha mediates the adaptive response to fasting. J Clin Investig 103:1489–1498

25. Strable MS, Ntambi JM (2010) Genetic control of de novo lipogenesis: role in diet-induced obesity. Crit Rev Biochem Mol Biol 45:199–214

26. Jiang T, Wang Z, Proctor G, Moskwitz S, Liebman SE, Rogers T, Lucia MS, Li J, Levi M (2005) Diet-induced obesity in C57BL/6J mice causes increased renal lipid accumulation and glomerulosclerosis via a sterol regulatory element-binding protein-1c-dependent pathway. J Biol Chem 280:32317–32325

27. Peacock WFt, Maisel A, Kim J, Ronco C (2013) Neutrophil gelatinase associated lipocalin in acute kidney injury. Postgrad Med 125:82–93

28. Cairns AP, O’Donoghue PM, Patterson VH, Brown JH (2000) Very-long-chain acyl-coenzyme A dehydrogenase deficiency—a new cause of myoglobinuric acute renal failure. Nephrol Dial Transplant 15:1232–1234

29. Krebs HA (1963) Renal gluconeogenesis. Adv Enzyme Regul 1:385–400

30. Meyer C, Stumvoll M, Dostou J, Welle S, Haymond M, Gerich J (2002) Renal substrate exchange and gluconeogenesis in normal postabsorptive humans. Am J Physiol Endocrinol Metab 282:E428–434

31. Ekberg K, Landau BR, Wängnot A, Chandramouli V, Efendic S, Brunengraber H, Wahren J (1999) Contributions by kidney and liver to glucose production in the postabsorptive state and after 60 h of fasting. Diabetes 48:292–298