Fatty acid oxidation in health and disease
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Summary

In mammalian cells energy homeostasis is maintained by the oxidation of carbohydrates, amino acids and fatty acids. Mitochondrial fatty acid β-oxidation (FAO) is the primary pathway for the degradation of fatty acids in mammals. FAO is catalyzed by a series of enzymes that degrade an acyl-CoA in a cycle of 4 enzymatic steps to acetyl-CoA units. Inborn errors have been described for most of these enzymes in humans. The most common disease presentation in FAO deficiencies is hypoketotic hypoglycemia, which is often provoked by fasting in combination with an illness. This clinical feature can lead to coma or even sudden death, but can be easily prevented by the avoidance of fasting or intravenous glucose administration. Because of this, the Dutch expanded neonatal screening includes medium-chain acyl-CoA dehydrogenase (MCAD), very long-chain acyl-CoA dehydrogenase (VLCAD) and long-chain hydroxyacyl-CoA dehydrogenase (LCHAD) deficiency (since January 1, 2007). Other symptoms that occur in FAO disorders are cardiomyopathy and rhabdomyolysis. The pathophysiology of all symptoms is still poorly understood. Unfortunately, interventions that prevent hypoglycemia, do not seem to prevent the development of cardiomyopathy and rhabdomyolysis. Although neonatal screening will prevent mortality associated with hypoglycemia, it will also identify patients who are at risk to develop cardiomyopathy and rhabdomyolysis. It is therefore of great importance to gain more knowledge on the pathophysiology of FAO disorders in order to develop rational therapeutic strategies to prevent or treat cardiomyopathy and rhabdomyolysis. Therefore, the overall aim of this thesis was to understand the specific functions of FAO enzymes in physiology. Using this knowledge we also addressed the pathophysiology of FAO deficiencies.

Auxiliary enzymes in FAO are crucial for the oxidation of unsaturated fatty acids. There are three different types of auxiliary enzymes involved, including 2,4-dienoyl-CoA reductases (DECR), Δ3,5,Δ2,4-dienoyl-CoA isomerases and Δ3,Δ2-enoyl-CoA isomerases (ECI). At this moment, there are no deficiencies described for the isomerases. To obtain more insight in the function of auxiliary enzymes in FAO and to explore a potential presentation of a human deficiency, we characterized a mouse model deficient in Eci1 in chapter 2. Eci1 is one of the enzymes converting cis-3 or trans-3 enoyl-CoAs to trans-2 enoyl-CoAs, which is crucial for further oxidation by the regular FAO enzymes. We found that Eci1 deficient mice present with a mild phenotype. Upon fasting, Eci1 mice displayed a trend to ketotic hypoglycemia with elevated levels of C12:1- and C14:1-acylcarnitine. We provide evidence that Eci2, a second Eci expressed in mitochondria, can (partially) take over the function of Eci1. Therefore, the mild phenotype is explained by functional redundancy between Eci1 and Eci2. To identify potential patients with Eci1 deficiency, we suggest screening patients with ketotic hypoglycemia for an accumulation of C12:1-acylcarnitine.

In mammalian cells there are three Ecis described, Eci1 expressed in mitochondria, Eci2 expressed in mitochondria and peroxisomes, and Eci as an integral part of EHHADH expressed in peroxisomes. In chapter 3 we described the molecular and functional characterization of Eci3, a novel peroxisomal Eci. Eci3 is rodent-specific and arose after duplication of the Eci2 gene. We show that mouse Eci3 is selectively expressed in kidney. In contrast, Eci3 expression in the rat is ubiquitous. In silico gene co-expression analysis for Eci3 in kidney did not yield (lipid) metabolism as enriched biological process. Moreover, the co-expressed genes for rat and mouse Eci3 had little overlap suggesting that the molecular evolution of Eci3...
function is still ongoing and its primary role may not be related to FAO.

To study the reciprocal relationship between glucose metabolism and FAO in heart and skeletal muscle and the role of FAO in the development of insulin resistance, we generated and studied a mouse model harboring a point mutation in the Cpt1b gene as described in chapter 4. This E3A point mutation renders the Cpt1b enzyme insensitive to feedback inhibition by malonyl-CoA. We hypothesized that Cpt1b<sup>E3A</sup> mice exhibit increased FAO flux in heart and skeletal muscle due to decreased malonyl-CoA sensitivity. Indeed, CPT1b<sup>E3A</sup> mice have less malonyl-CoA sensitivity, but based on acylcarnitine profiling only the heart showed signs of increased FAO flux. Interestingly, in the heart, skeletal muscle and BAT of the Cpt1b<sup>E3A</sup> mice, the total Cpt1b activity is decreased due to lower Cpt1b protein levels with normal Cpt1b mRNA levels. The protein levels of Cpt1b could be increased under conditions that promote protein folding such as low temperature suggesting reduced stability of the mutant protein. Furthermore, Cpt1b<sup>E3A</sup> mice had deficient adaptive thermogenesis, similar to well-established FAO deficiency models. Despite the overall mild phenotypic presentation of Cpt1b<sup>E3A</sup> mice, our data suggest an important role for Cpt1b in the regulation of FAO.

The long-chain acyl-CoA dehydrogenase (LCAD) KO mouse model is a good model for human VLCAD deficiency. In chapter 5, we studied the fasting-induced changes in cardiac morphology, function, and triglyceride (TG) storage in the LCAD KO mouse in a noninvasive fashion using magnetic resonance imaging (MRI) and proton magnetic resonance spectroscopy (<sup>1</sup>H-MRS). The left ventricular (LV) mass was higher in LCAD KO mice indicating LV myocardial hypertrophy. TG content at baseline was already higher in the LCAD KO mice and increased further in fasted LCAD KO mice. Furthermore, the LV ejection fraction and diastolic filling rate decreased after fasting. Ex vivo analysis revealed increased myocardial ceramide content in fasted LCAD KO mice. Taken together, this study revealed accumulation of myocardial TG in LCAD KO mice. The accumulating lipid metabolites such as ceramide could be responsible for the observed fasting-induced impairment of cardiac function.

Unfortunately, the LCAD KO mouse has a relatively mild phenotypic presentation. It recapitulates human VLCAD deficiency with respect to the hypoketotic hypoglycemia and some cardiac abnormalities. However, myopathy and rhabdomyolysis, which are very common in VLCAD deficiency are absent in the LCAD KO mouse. In chapter 6, we described an attempt to generate a better model. Because the LCAD/VLCAD double KO mouse is reported to be lethal, we combined the LCAD and VLCAD KO mouse models by generating mice that lack one VLCAD allele on the LCAD KO background. We hypothesized that these LCAD/VLCAD-deficient mice would have a more severe phenotype including myopathy and rhabdomyolysis. We identified in the LCAD/VLCAD-deficient mice a significant increase in the C14:1/C2 acylcarnitine ratio when compared with all other genotypes tested. Furthermore, energy expenditure in the LCAD/VLCAD deficient mice was decreased. However, plasma levels of creatine kinase, a marker for rhabdomyolysis, were unchanged in all genotypes tested. These data suggest that despite a lower FAO capacity, the LCAD/VLCAD deficient mice do not develop myopathy and rhabdomyolysis.