Mitochondrial betha-oxidation

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SUMMARY

The mitochondrion is the powerhouse of the cell, where energy is generated. Energy can be generated from the oxidation of proteins, polysaccharides and fats supplied by consuming food. Sugars, fatty acids and several amino acids are converted into the acetyl unit of acetyl-CoA. Acetyl-CoA is donating its acetyl units to the citric acid cycle, where oxidation to CO_2 is taking place. Adenosine triphosphate (ATP) is generated as electrons flow to oxygen upon oxidative phosphorylation.

Thus, fatty acids are primarily available as supply from food. Secondary, fatty acids are available as a supply created from intracellular stores. After a period of fasting the glycogen reservoir is consumed first. When fasting lasts longer than one day, fats/fatty acids will be released from adipose tissue into the blood stream. Fatty acids with a chain length of 12 to 20 carbon atoms with no ('saturated') or up to two double bonds ('unsaturated') are mostly used as fuel.

Uptake of fatty acids takes place very efficiently in the liver. Fatty acid binding proteins (FABPs) are presumed to play a role in uptake of fatty acids. Their proposed functions can be summarized as follows. In the β-oxidation FABPs act as carriers for fatty acids between the plasma membrane and the outer mitochondrial membrane and peroxisomal membrane, while they are also involved in the regulation of the cellular concentration of fatty acids and their CoA esters.

Before fatty acids can be used by the cell, they will be combined first with coenzyme A to form a highly polar thioester. This reaction is catalyzed by fatty acyl-CoA synthetases. When the fatty acids, specifically long-chain fatty acids, are activated to long-chain fatty acyl-CoA they can cross the mitochondrial outer membrane. Short and medium chain fatty acids can readily penetrate mitochondria without carrier mediated transport. They are activated to their CoA esters in the mitochondrial matrix and subsequently degraded in the fatty acid β-oxidation. Before long-chain fatty acyl-CoA can undergo fatty acid β-oxidation they have to be transported across the inner mitochondrial membrane. Carnitine has an essential physiological function in this process.

Carnitine plays a physiological role in a number of other metabolic processes, including buffering of the mitochondrial acyl-CoA/CoA couple; scavenger system for acyl groups; peroxisomal fatty acid oxidation; intracellular communication by means of storage and transport of metabolic energy; facilitating the oxidation of keto acids, branched-chain amino acids and medium-chain fatty acids (activated in cytosol or cell and not in the matrix of the mitochondrion) and membrane stabilization.

As mentioned before carnitine plays a physiological role in facilitating the oxidation of long chain fatty acids. After activation of a long-chain fatty acid, such as palmitate, the palmitoyl-CoA ester is transferred through the mitochondrial outer membrane into the intermembrane space without the use of the carnitine system. Transesterification to palmitoylcarnitine via a reaction catalyzed by carnitine palmitoyltransferase 1 (CPT 1) takes place, because the inner membrane of the mitochondrion is virtually impermeable to CoA and its derivatives. CPT 1 is localized on the inner aspect of the outer mitochondrial membrane.

The palmitoylcarnitine is transported across the mitochondrial inner membrane via a carnitine acylcarnitine translocase. Carnitine acylcarnitine translocase is an antiport system whereby palmitoylcarnitine is transported into the matrix in exchange for carnitine.

The matrix palmitoylcarnitine is a substrate for carnitine palmitoyltransferase 2 (CPT 2) using matrix CoASH to form palmitoyl-CoA in the matrix, thus releasing carnitine. This process makes palmitoyl-CoA available for entering the mitochondrial fatty acid β-oxidation spiral.

In mitochondrial fatty acid β-oxidation stepwise degradation of fatty acids of various chain lengths takes place, generating the needed energy. After four sequential steps of dehydrogenation (plus simultaneous transfer of electrons to electron transfer flavoprotein [ETF]), hydration, again dehydrogenation and thiolytic cleavage, the resulting acyl-CoA ester now shortened by 2 carbon atoms, can reenter the β-oxidation spiral.

Fatty acid β-oxidation is regulated by a mechanism in which interaction of CPT 1 and malonyl-CoA plays a central role. Hormonal control of fatty acid β-oxidation is exerted at the level of substrate mobilization from adipose tissue and by indirect effects on CPT 1. Of relevance are the inhibiting effect of insulin and the indirect stimulation of glucagon on β-oxidation.

For the pathophysiology of fatty acid β-oxidation disorders it is essential to appreciate the central role that fatty acids play in times of fasting. Beyond a day of fasting, glycogen reserves are depleted and alternative means are activated: gluconeogenesis, ketogenesis and fatty acid oxidation. Oxidation of fatty acids results in the end-product, acetyl-CoA, which can be the precursor of the ketone bodies, i.e. acetoacetate and β-hydroxybutyrate, which represent an essential source of energy in brain under fasting conditions.

Under fasting conditions patients with a fatty acid β-oxidation disorder develop hypoketotic hypoglycemia due to an inability to generate acetyl-CoA resulting in low levels of ketone bodies and an inability to generate sufficient glucose. Apart from that presenting symptoms including lethargy leading to coma, Reye-like syndrome, acute life threatening event (ALTE), muscle weakness, cardiac problems, hepatomegaly and abnormal laboratory findings (hyperuricemia, hyperammonemia, dicarboxylic aciduria, acidosis, increased activity of alanine and aspartate aminotransferases and of creatine kinase and alterations in plasma or tissue carnitine concentration) can appear. However, fatty acid oxidation defects only produce abnormalities intermittently. So during an acute attack only the urinary organic acid profile and blood carnitine levels can give an indication for inherited fatty acid oxidation disorders. The study described in this thesis was undertaken to try to shed more light upon this group of disorders.

For this purpose we have developed sensitive and reliable enzyme assays for diagnosis of several fatty acid β−oxidation deficiencies including short-chain, medium-chain, and (very) long-chain acyl-CoA dehydrogenase (see chapters 2-4). The concentration of the 3-hydroxy fatty acid acyl-CoA, formed from the substrate fatty acid acyl-CoA during the enzyme assay, could be measured after hydrolysis as its 3-hydroxy fatty acid. For quantification we have used stable isotopes and gas chromatography/mass spectrometry (GC-MS).

As we have mentioned before, fatty acid oxidation defects only produce intermittent abnormalities. Especially when a patient, suspected to suffer from a defect in the β-oxidation, is in an asymptomatic phase of the disease the diagnosis of this patient can be very difficult as is the case in medium-chain acyl-CoA dehydrogenase (MCAD) deficiency. MCAD deficiency is the most common defect of β-oxidation. Thus we have evaluated our 21 patients with MCAD deficiency with respect to their clinical symptoms at presentation, laboratory findings, diagnostic and therapeutic approach (see chapter 5). Prompt diagnosis and initiation of treatment will improve long-term prognosis of a MCAD deficient (MCADD) patient. We have found that due to our family screening program in which first degree family members of MCADD patients are screened for MCAD, 30% of these asymptomatic family members (brothers and sisters) were MCADD. Thus making

the percentage of ALTEs in our population lower compared to literature, 5% versus 21%, respectively. For genetic counselling carrier detection takes place, in which leucocytes from the parents of a MCADD patient are investigated. Definitive evaluation of a patient suspected to suffer from a MCAD deficiency should include GC-MS of urinary organic acids in order to look for specific metabolites (glycin derivatives, 5-hydroxy hexanoic acid, dicarboxylic acids), determination of the carnitine status, and specific enzyme assays in leucocytes or cultured skin fibroblasts.

In order to give an impression of the diverging presentation of different disorders of β-oxidation we describe two patients, each with a specific defect (see chapter 6). The first patient is the fourth case described in literature with an unique β-oxidation defect at the level of the carnitine acylcarnitine carrier. The presenting clinical symptoms were cardiorespiratory insufficiency, subcomatose state and bradycardia with decreased peripheral circulation. The combination of extreme hypoglycemia, hyperammonaemia and the observed pattern of urinary organic acids rose suspicion to consider defects in either gluconeogenesis, urea cycle or fatty acid β-oxidation. Defects in either gluconeogenesis or urea cycle were ruled out. Measurement of the overall β-oxidation in leucocytes revealed a complete absence of long-chain fatty acid (palmitate) β-oxidation. Further investigations in cultured skin fibroblasts on the level of long-chain fatty acid metabolism showed a deficiency of carnitine acylcarnitine translocase. The second patient is a patient with a succinyl-CoA acetoacetate transferase deficiency, a defect of ketolysis, who presented early in life with recurrent ketoacidotic attacks and persistent ketonuria. He showed a normal development later in life up to 9 years of age. Although this defect is not strictly a defect in mitochondrial β-oxidation, there are some similarities between a defect of ketolysis and mitochondrial fatty acid β-oxidation. In succinyl-CoA acetoacetate transferase deficiency ketone body production is adequate but utilization is disturbed in contrast to a mitochondrial β-oxidation defect, in which the production of ketone bodies is disturbed. In both mitochondrial β-oxidation and ketolysis defects consequences for the brain under fasting conditions are identical.

Cultured skin fibroblasts are a highly useful source for elucidating inherited defects. We developed a screening procedure to detect β-oxidation defects in cultured skin fibroblasts using the fluorescent dye 2-[4-(dimethylamino)styryl]-1-methylpyridinium iodide (DASPMI) (chapter 7). This fluorescence microscopical approach is used to investigate whether it might provide insight as to how β-oxidation related enzyme defects can influence the mitochondrial redox status, which presumably arises from abberations in the structure of mitochondria. However, no abberations could be detected in cultured skin fibroblasts from MCADD patients. Apart from this approach, we have also studied the influence of β-oxidation related enzyme defects on the mitochondrial redox status (chapter 8). We have used cultured skin fibroblasts from MCADD patients. Our hypothesis was that a presumed perturbation of mitochondrial structure in tissue from MCADD patients on increased concentration of octanoate could be the basis of the sometimes mild lactic acidosis in MCADD patients. From literature we have learned that in experiments using rabbits that were treated with pathological concentrations of octanoate showed perturbation of mitochondrial structure in the brains were found. We were expecting to find increased ratios of lactate/pyruvate, which provide insight in the mitochondrial redox status. We have challenged cultured skin fibroblasts from MCADD patients with increasing amounts of octanoic acid and measured the lactate/pyruvate ratios. However, unfortunately our hypothesis has not been affirmed by our standarized experiments.

As mentioned before MCAD deficiency is the most common defect of β-oxidation. The $A_{985} \rightarrow G$ transition is the most common mutation in MCAD deficiency. This mutation is present in 90% of the patients with a MCAD deficiency. In order to determine the

frequency of $A_{985} \rightarrow G$ carriers in The Netherlands a study is performed in which 6195 Guthrie cards are analyzed for the presence of the $A_{985} \rightarrow G$ mutation (see chapter 9). These cards were obtained from the 5 PKU screening laboratories in The Netherlands. The estimated prevalence of newborns with MCAD deficiency is 1: 12,500 in The Netherlands. Information concerning the prevalence together with its high morbidity and mortality may provide the background to consider an extended screening for this common MCAD mutation in newborns from The Netherlands.

We hope that the results of the investigations presented in this thesis and the description of clinical and biochemical features together with the approach to the patient suspected for a mitochondrial β-oxidation defect, will add to the long term prognosis of patients suffering from this important and intriguing group of disorders.