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 CO₂ 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 bloodstream. 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. Transferrase function of 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 \( \beta \)-oxidation spiral.

In mitochondrial fatty acid \( \beta \)-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 \( \beta \)-oxidation spiral.

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

For the pathophysiology of fatty acid \( \beta \)-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 \( \beta \)-hydroxybutyrate, which represent an essential source of energy in brain under fasting conditions.

Under fasting conditions patients with a fatty acid \( \beta \)-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, hypereosinophilia 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 \( \beta \)-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 \( \beta \)-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 \( \beta \)-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 percentiles, respectively, from those without disease from those with disease. We have observed a significant difference in fatty acids in plasma from patients with MCADD, diacylglycerol acyltransferase and leucocytosis.

In order to make a correct diagnosis of the patient is essential to determine the level of ketone bodies in the blood and urine, in plasma, and in leucocytes. In contrast to either glucose or insulin, glucagon stimulates \( \alpha \)-ketoglutarate dehydrogenase, gluconeogenic, and leucocytosis. Further in the emergence of ketogenesis in the metabolism of leucocytes there is a patient who presented with ketosis and showed a substantial increase of ketone bodies in leucocytes. This patient is a patient who is a patient with MCADD who showed a strict increase of ketone bodies in the leucocytes.

Further investigation of the metabolism of leucocytes in a patient with MCADD showed an increase of ketone bodies in the leucocytes.

Cellulose,

We developed fibroblast cell lines using rat fibroblasts (20) that can be used in the investigation of the influence of fatty acids in the study of skin fibroblasts, which can influence the metabolism of fatty acids (chapter 3). The aim of this study was that the study of these patients could improve the treatment of fatty acid oxidation disorders. Using rat fibroblasts, we have observed a strict increase of fatty acids in the leucocytes. We have further observed an increase of fatty acids in the leucocytes.

As mentioned before, the patients with MCADD showed a strict increase of fatty acids in the leucocytes. We have observed a strict increase of fatty acids in the leucocytes. We have further observed an increase of fatty acids in the leucocytes.
Transferase 2 (CPT II) is the critical component in the carnitine-acylcarnitine cycle. This enzyme is involved in the transport of long-chain fatty acyl-CoA (acyl-CoA) across the mitochondrial inner membrane. The role of CPT II is to facilitate the entry of long-chain fatty acids into the mitochondrial matrix, where they are further oxidized via β-oxidation. The activation of CPT II requires the presence of carnitine, which acts as a shuttle to transport the fatty acyl-CoA into the mitochondria. Defects in CPT II can lead to a range of metabolic disorders, including mitochondrial fatty acid oxidation defects, such as medium-chain acyl-CoA dehydrogenase deficiency (MCAD) and long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency (LCHAD).

Glucagon is a hormone that plays a critical role in the regulation of glucose homeostasis. It is secreted by the pancreas in response to hypoglycemia and acts to stimulate glycogenolysis and gluconeogenesis, leading to an increase in blood glucose levels. However, in the case of CPT II deficiency, the increased levels of glucagon may not be effective in improving glucose homeostasis due to the limited availability of long-chain fatty acids for oxidation. This can result in the accumulation of long-chain acyl-CoA derivatives in the liver and other tissues, which can interfere with the normal function of other enzymes involved in fatty acid oxidation.

In conclusion, the oxidation of long-chain fatty acids is a complex process that involves multiple steps and enzymes. Defects in any of these steps can lead to metabolic disorders with varying degrees of severity. Early recognition and diagnosis of these disorders are crucial for the management of symptoms and prevention of long-term complications.
frequency of $A_{481} \rightarrow G$ carriers in The Netherlands a study is performed in which 6195 Guthrie cards are analyzed for the presence of the $A_{481} \rightarrow G$ 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 $\beta$-oxidation defect, will add to the long term prognosis of patients suffering from this important and intriguing group of disorders.