In vivo kinetic studies in inborn errors of metabolism: expanding insights in (patho)physiology
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Summary
At present, several hundreds of different inborn errors of metabolism have been characterized. Many of these disorders have a severe clinical phenotype. In the last decades, knowledge about the pathophysiology of metabolic disorders has rapidly progressed, resulting in improved treatment strategies and better quality of life for patients. This has also lead to the inclusion of numerous metabolic disorders in newborn screening programs worldwide.

However, the pathophysiological mechanism(s) in many metabolic disorders still remain unresolved. Until now most research in the field of inborn errors of metabolism has focused exclusively on the enzymatic defect and the subsequently raised or diminished concentrations of metabolites in the affected pathway(s). Although this approach has resulted in essential knowledge, this 'static' form of research does not provide information on the actual biochemical flux through the metabolic pathway \textit{in vivo}, thereby limiting the interpretation of the data obtained with respect to the functional consequences of an enzymatic defect.

The aim of this thesis was to study the dynamics of affected metabolic pathways in different inborn errors of metabolism \textit{in vivo} with use of contemporary stable isotope methodology, in order to expand knowledge on the pathophysiology in these disorders. In addition, new insights in normal physiology were also obtained. In chapter 1 the different stable isotope techniques used in this thesis are described as well as an outline of the studies performed.

In chapter 2 an age-dependent regression model for endogenous glucose production (EGP) in humans from birth to late adulthood is described. This model was based on data of the rate of appearance (Ra) of glucose in plasma, as determined with the \([6,6\text{-}^{2}\text{H}_2]\)-glucose dilution method, obtained in children and adults in studies performed by our group over the last decade. Furthermore, the relation between EGP and estimated brain weight is studied, also after normalisation for body composition since this changes significantly from childhood to adulthood. It is concluded that the established regression model accurately estimates minimal glucose requirement after an overnight fast during resting conditions in healthy subjects and may be used for designing evidence based glucose supplementation protocols for children as well as for interpretation of data from studies on EGP in children with various disorders, including inborn errors of metabolism. In addition, it is suggested that cerebral glucose uptake per gram estimated brain weight remains more or less constant throughout all ages.

Patients with glycogen storage disease type 1 (GSD-1) have been shown in previous studies to still produce glucose up to 50% of normal despite the absolute deficiency of glucose-6-phosphatase, the final enzyme in the pathway of EGP. This enigma remained to be resolved. In chapter 3 we demonstrate glucose production from both glycogenolysis (GGL) and gluconeogenesis (GNG) in a patient with GSD-1a during a short fasting period. In addition, glucose kinetics were also quantified in a patient with fructose-1,6-
bisphosphatase deficiency, an absolute defect of hepatic and renal gluconeogenesis, demonstrating glucose production via GNG up to 20% of normal. Based on these data, and the fact that recently a second glucose-6-phosphatase was functionally characterized in muscle, it is suggested that muscle may contribute to EGP via both GGL and GNG during fasting.

Idiopathic ketotic hypoglycemia (KH) is the most common cause of hypoglycemia in childhood. The etiology of this disorder is still poorly understood. Therefore, in chapter 4, EGP, GGL, GNG and glucose uptake (GU) were quantified during a standardized fasting test in twelve children, aged 2.5 to 11.5 yrs, with documented KH. We demonstrate that the five youngest subjects became hypoglycemic during fasting due to the inability to sustain an adequate EGP from both GGL and GNG. GNG was not increased when GGL became decreased during fasting, which may have resulted from a limitation in the supply of alanine, an important gluconeogenic amino acid. However, since glucose requirement per kg body weight is much higher in young children, because of their higher brain-to-body mass ratio, it is suggested that KH is not a disease entity but merely represents the lower tail of the Gaussian distribution of fasting tolerance in children.

In chapter 5 glucose kinetics during fasting were quantified in two siblings with HMG-CoA lyase deficiency, a disorder of both ketogenesis and leucine degradation. Fasting hypoglycemia in this disorder was suggested to result from either the inability to produce ketones and/or inhibition of gluconeogenesis via accumulation of HMG-CoA metabolites. We show that hypoglycemia in the youngest patient resulted from a mismatch between EGP and GU, due to the lack of the glucose sparing of effect of ketones. GNG was not inhibited despite accumulation of HMG-CoA metabolites. This demonstrates the importance of ketogenesis for glycemic control during fasting.

Patients with medium-chain acyl-CoA dehydrogenase deficiency (MCADD), the most common disorder of fatty acid oxidation (FAO), are unable to oxidize medium-chain fatty acids, resulting in the accumulation of medium-chain FAO intermediates. It has been suggested that this may lead to intramitochondrial carnitine depletion, further hampering FAO. The role of L-carnitine supplementation in MCADD, however, remains debated. In chapter 6a five MCADD patients with and without L-carnitine supplementation were studied in comparison to three healthy control subjects during fasting and moderate-intensity exercise. All subjects were able to complete the two hour exercise test without any clinical or biochemical adverse effects, also without L-carnitine supplementation. In patients with MCADD without L-carnitine supplementation an increase in free carnitine and its precursor was seen during exercise, suggesting upregulation of carnitine biosynthesis to compensate for carnitine losses. The results of this non-isotopic study were used to safely design a follow-up study (chapter 6b).
Energy metabolism in adult patients with MCADD during moderate-intensity exercise after an overnight was studied in chapter 6b in comparison to control subjects matched for sex, age, and body composition. Our objective was to determine to what extent FAO is hampered under stressed conditions in MCADD. In addition, fasting hypoglycemia, which is a prominent clinical feature in MCADD and all other disorders of FAO, has been suggested to be the result of inhibition of gluconeogenesis. Therefore, glucose kinetics were also studied. No significant differences were detected between patients with MCADD and control subjects in either whole body fat and carbohydrate oxidation or in EGP, GNG and GGL. Therefore, FAO does not seem impaired after an overnight fast and during prolonged moderate-intensity exercise in adult patients with MCADD. Patients were able to increase gluconeogenesis to the same amount as control subjects. However, FFA turnover at rest was significantly higher in patients, which could result in ectopic fat accumulation. This would make patients with a disorder of FAO more prone to insulin resistance.

Patients with classical galactosemia have to maintain a galactose restricted diet in order to prevent accumulation of toxic galactose-1-phosphate. However, it has been shown that patients exhibit a significant endogenous galactose production, leading to a process of auto-intoxication. If this endogenous galactose production is suppressed when dietary galactose content is increased, thereby maintaining the net galactose balance, dietary restrictions for patients with classical galactosemia could be relaxed improving their quality of life. In chapter 7 the possibility of such a feedback mechanism is studied. However, as endogenous galactose production remained essentially the same during step-up galactose infusion, it is concluded that endogenous galactose production is not suppressed by exogenous galactose supplementation.

In primary hyperoxaluria type 1 (PH1) an excess hepatic production of oxalate, a non-functional end-metabolite that aggregates with calcium, causes renal insufficiency and eventually results in end-stage renal failure. This leads to tissue accumulation of oxalate. Because of this, plasma oxalate concentration and urinary oxalate excretion remain elevated after a combined liver and kidney transplantation. Therefore, plasma oxalate concentration is not a reliable parameter to monitor therapeutic interventions in patients with PH1 as is urinary oxalate excretion in patients with PH1 and renal insufficiency. In chapter 8 a stable isotope dilution method is presented in order to quantify $R_\alpha$ oxalate. This parameter could be used to monitor therapeutic interventions in patients with PH1 and renal insufficiency.

Finally, in chapter 9 a general discussion is presented outlining the advantages and the limitations of in vivo stable isotope techniques in the field of inborn errors of metabolism. In addition, future directions for in vivo stable isotope research in metabolic disorders are suggested. It is concluded that stable isotope methodology offers a potentially very powerful research tool. However, extensive biochemical and analytical expertise as well
as sufficient financial means are required to conduct these studies. Therefore, these studies have limited applicability in daily clinical practice and should only be performed in (collaboration with) specialized centres.