Computational analysis of carbohydrate metabolism
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CHAPTER 11

Summary
To be able to understand the (patho) physiology of carbohydrate metabolism and etiology of inborn and acquired metabolic diseases, such as GSD and type II diabetes, it is of crucial importance to have insight in relevant metabolic fluxes. For instance: is high blood glucose due to increased glucose production or is it caused by impaired peripheral uptake? This thesis is focused on quantitative assessment of changes in carbohydrate metabolism induced by different interventions. Presented and discussed are a number of calculation algorithms to evaluate and quantify carbohydrate metabolism in conscious small laboratory animals, particular in (genetically-modified) mice.

Hepatic and peripheral carbohydrate metabolism and their regulation are described in Chapter 2. Specific processes and mechanisms that are of importance for this thesis are reviewed and discussed, in detail.

All novel procedures developed for use in mice that are presented in this thesis involve dilution and/or isotope incorporation algorithms. The available tracers for these kinds of studies and argumentation to use stable isotopes are discussed in Chapter 3. Additionally, the instrumentation used for the analyses is presented and discussed.

In Chapter 4, the different kinetic models are discussed. Firstly, a model for blood glucose metabolism is presented. Shown are the sites or compartments where tracers were introduced and where samples were taken during the experiments, i.e., labeling and sampling sites. Next, the principles of isotope dilution and isotope incorporation techniques are discussed. Detailed explanations are given of algorithms for the proposed mathematical model of hepatic carbohydrate metabolism. Furthermore, algorithms are discussed for the extended hyperinsulinaemic euglycaemic clamp technique, for the single pool, first-order kinetic model, and for metabolic non-steady-state models.

Glycogen storage disease type I is a well-known inherited disease of glucose metabolism. The underlying cause is the absence of glucose-6-phosphatase activity resulting in fasting-induced hypoglycemia, hyperlactacidemia, and hyperlipidemia. However, the effects of this disease on hepatic carbohydrate metabolism were unknown. In Chapter 5 an animal model is described in which glucose-6-phosphate translocase was pharmacologically inhibited to induce an acute model of glycogen storage disease type Ib. A partial block of glucose-6-phosphate translocase with S4048 resulted in decreased blood glucose levels, a decreased glucose production, and elevated hepatocellular G6P and glycogen levels. Using the mathematical model for hepatic carbohydrate metabolism, we were able to show that the gluconeogenic flux, i.e., the flux from fructose-1,6-bisphosphate to glucose-6-phosphate, was not affected. However, the conversion of glucose-6-phosphate changed from mainly glucose to mainly glycogen. Furthermore, this experiment showed that for estimations of de novo synthesis rates of
glucose-6-phosphate, fluxes have to be measured from fructose-1,6-bisphosphate to all its metabolites. In addition, a very poor relationship appears to exist between changes in mRNA expression of so called rate controlling enzymes and the calculated carbohydrate flux through these enzymes, underlining the necessity to quantify metabolic fluxes.

For decades, rats have been used in medical research. But in the nineteen-nineties, mice became more and more the rodent species of choice because of the possibilities to apply genetic engineering in these animals. However, to be able to perform the methodologies in mice that were used for rats, a number of physical and analytical problems had to be tackled. Chapter 6 describes a number of improvements in experimental and analytical techniques that addresses these issues. Infusion rates and tracer concentrations were adapted to take the smaller body size into account and to allow assessment of the higher glucose turnover rates in mice. It is demonstrated that very small sample sizes can be taken multiple times during experiments when blood and urine samples are collected on special filter paper. Furthermore, analytical methods were adjusted to these small sample sizes without reductions in accuracy and reproducibility of the measurements. Finally, the method was validated in three groups of conscious freely-moving mice that were fasted for varying periods of time.

The “gold standard” to test insulin sensitivity is the hyperinsulinemic euglycemic clamp technique (HIEC). The procedure was adapted for use in mice, in relation to the protocol presented in chapter 6. An example of the HIEC in mice is presented in Chapter 7. A study is presented that was designed to asses insulin sensitivity in conscious, freely-moving lean and ob/ob mice that were treated with the LXR agonist GW3965. It is demonstrated that a metabolic steady-state was reached after three hours of insulin infusion and that this could be maintained for another three hours. The steady-state period was used to calculate kinetic parameters like hepatic glucose production and metabolic clearance rate. It was shown that the anti-diabetic effects of the LXR agonist seen in ob/ob mice is related to an enhanced metabolic clearance of blood glucose (especially to adipose tissue) and, surprisingly, that the glucose production by the liver remained unchanged.

The bile acid-activated Farnesoid X Receptor (FXR) plays a prominent role in control of bile acid synthesis and bile acid transport and is also involved in regulation of lipid and lipoprotein metabolism. Additionally, it was recently shown that there is a link between FXR and maintenance of glucose homeostasis, particular during the fasting-feeding transition. Chapter 8 describes a study in which glucose absorption was quantified in Fxr−/− and control mice. The study shows that, compared to wild-type mice, Fxr−/− mice have a delayed glucose absorption due to an enhanced intracellular glucose/glucose-6-phosphate cycling within the enterocytes. To prove this, we used
different mathematical models. Firstly, an OGTT with labeled glucose was used to estimate the fractional glucose absorption rate, which was confirmed in a second experiment, i.e., assessment of the non-steady-state model according to Steele. Thirdly, the single pool first-order kinetic model was used to estimate the fractional contribution of administrate glucose in blood. Finally, a compartmental model was constructed in which we were able to support the observation that the delayed absorption is related to enhanced glucose/glucose-6-phosphate cycling in $Fxr^{-/}$ mice.

The HOMA-index is widely used to estimate insulin resistance in humans and, in multiple cases, also in laboratory animals. This index is calculated as the product of fasting glucose and insulin concentrations relative to a population considered to have normal glucose and insulin levels. For more detailed examinations a number of tests have been developed that were mostly performed under different metabolic conditions compared to the HOMA-index. In Chapter 9 a protocol is introduced that is performed under identical metabolic conditions as the HOMA-index. A small amount of stably-labeled glucose was administered to fasted conscious and freely-moving mice. Using the single-pool, first order kinetic model, it was possible to determine blood glucose kinetics and the HOMA-index in the same experimental setting. The test was validated and compared to constant infusion protocols in a well-established animal model of reduced insulin sensitivity, i.e., the high-fat fed mouse model. The reduced insulin sensitivity calculated in the high-fat group could be linked to a reduced metabolic clearance rate of blood glucose caused by a reduced apparent volume of distribution. Surprisingly, there were no differences in turnover rates and pool sizes. The parameters retrieved from the single-pool, first order kinetic model were identical to those retrieved from constant infusion protocols.

Finally, in Chapter 10 the novel protocols are critically reviewed and compared to other methods. The mathematical model to calculate hepatic carbohydrate metabolism is compared to three other often used models. Advantages and disadvantages are discussed for all methodologies.