In vivo kinetic studies in inborn errors of metabolism: expanding insights in (patho)physiology
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Chapter 9

General discussion, future directions and conclusion
General discussion

In this final chapter the advantages and disadvantages of the use of stable isotope techniques in vivo are summarized as well as a number of factors that should be taken into account when undertaking tracer studies. Finally, general conclusions are presented on the use of in vivo stable isotope techniques in the field of inborn errors of metabolism and future directions for in vivo metabolic research in this field are suggested.

Advantages of stable isotopes in in vivo metabolic research

Several characteristics of stable isotope tracers contribute to their applicability in metabolic research in vivo (1). Foremost, stable isotope tracers do not emit any radiation, which is why they can be safely administered to human subjects and handled by researchers and analysts without any special precautions. Therefore, they can be used in the same subject in consecutive studies without any harm. Another important advantage of stable isotope tracers over radioactive tracers is that several different tracers can be administered at the same time without interfering with one another, as different labelled compounds can be analyzed separately using chromatography in combination with mass spectrometry. Hence, the functionality of several metabolic pathways can be assessed in one study, thereby limiting the number of subjects needed to answer different research or clinical questions. Additionally, no realistic radioactive isotopes exist for certain chemical elements, whereas stable isotopes for these elements are available (e.g. for nitrogen and oxygen). Because of these characteristics stable isotope tracers are ideally suited for use in humans and especially in the pediatric population as they can be safely applied in children for both metabolic research as well as for routine clinical applications (2;3). An example of the latter is the assessment of fat or carbohydrate malabsorption in children by quantification of 13CO2 production from orally administered 13C labelled fat or carbohydrates (4;5).

In this thesis, the potential value of stable isotope infusion techniques for in vivo metabolic research is demonstrated for normal physiology as well as for unravelling pathophysiological mechanisms in different inborn errors of metabolism:

- expanding insight in normal (fasting) physiology in humans
- obtaining reference values for human biochemical functionality at various ages
- unravelling pathophysiological mechanisms in inborn errors of metabolism
- functional assessment in vivo of (the) complete metabolic pathway(s) affected by an inborn error of metabolism instead of focusing on kinetic properties of the affected enzyme(s) in vitro
- monitoring (therapeutic) interventions in patients with an inborn errors of metabolism

Although potentially a very powerful tool in both research and clinical practice several factors and limitations have to be taken into account when using stable isotope techniques. These will be outlined below.
Considerations for using stable isotope techniques in vivo

A major assumption used in all stable isotopes studies is that a tracer will behave biochemically exactly the same as its unlabeled endogenous counterpart. However, it has been shown that this assumption is not always valid. This especially holds true for deuterium as the mass of deuterium ($^2$H) is twice as high as of hydrogen ($^1$H). Several of these isotopic effects of deuterium have been established, including reduction of protein and nucleic acid synthesis, changes in enzyme reaction rates, disturbance of the division rate of cells and morphological changes of cells (3). From animal studies it is known that these toxic effects of deuterium only occur at a body water enrichment of 10% to 20%, whereas deuterium enrichment of 30% to 40% has been shown potentially lethal (6;7). This toxic threshold, however, is far in excess of the percentage of deuterium enrichment in body water used in human metabolic research (8) which rarely exceeds 1.5%. Still, even at 0.5% deuterium enrichment, a transient vertigo can occur which is likely to be caused a gravitational effect of deuterium in vestibular fluid (9). Until now, no side-effects of other stable isotopes like $^{13}$C, $^{15}$N and $^{18}$O have been detected, possibly because the mass difference of these isotopes with their naturally most abundant counterpart is too small to cause any biological significant effects (10). Therefore, the use of deuterium at a low dosage and of other stable isotopes at much higher dosages is deemed safe in human metabolic research.

Secondly, thorough modelling of the possible metabolic pathways and their fluxes in which the tracer may be involved is crucial for the successful interpretation of the data obtained. If not modelled correctly, misinterpretation of metabolic fluxes will result in erroneous conclusions which may have far-reaching consequences. In most stable isotope dilution studies one single homogenous pool is assumed to quantify substrate kinetics. A prerequisite for this non-compartmental model is that the metabolite of interest only enters and exits via the pool in which the tracer is administered and from which samples for tracer analysis are collected (e.g. the plasma pool) (11). Although this prerequisite is certainly met for some substrates (e.g. glucose during fasted conditions (12)), this model will not be accurate for substrates which are metabolized in intracellular compartments during the time period of the tracer experiment. Although it is sometimes possible to quantify the metabolism of a tracer from an intracellular pool indirectly (e.g. intracellular degradation of $[^{1-13}$C]leucine can be assessed by quantifying plasma $[^{1-13}$C]alpha-ketosocaproate (KIC) enrichment (13)), it is in most cases not feasible to sample the different intracellular pools in vivo. For these circumstances a multi-compartmental model has to be devised in order to quantify substrate kinetics as accurately as possible. These models are based on assumptions on the substrates’ exchange rates between the different metabolic pools, which are estimated from the decay in enrichment of a single tracer bolus administered in the sampling pool (14). Because of these necessary assumptions multi-compartmental models are more difficult to use and in addition more prone to error.
Thirdly, when using a $^{13}$C labelled tracer in a stable isotope dilution method to quantify the rate of appearance ($R_a$) of a substrate, one should be aware of the possibility of the tracer recycling back into the sampling pool, as this will underestimate the actual $R_a$ of the substrate. This mechanism has been well established with $^{13}$C labelled glucose tracers (15).

Fourthly, the application of stable isotope techniques in human research not only requires extensive knowledge about metabolic pathways but also theoretical knowledge on, and practical experience with, the complex analytical methodology. Designing (new) tracer studies has been shown both time-consuming and costly as high investments have to be made in analytical equipment and personnel in order to be able to execute these studies. Therefore, tracer studies should only be undertaken in (collaboration with) specialised centres with the necessary expertise and logistics.

Finally, in vivo stable isotope studies are both invasive as well as time-consuming for the patient, his/her family and the nursing and medical staff. These studies generally involve drawing of numerous blood samples and collection of urine and/or breath samples, generally to be followed by several weeks up to even months for all analytical tests and data analysis to be completed. Therefore, their use in everyday clinical practise will remain limited, and these studies will be mostly used in research settings.

Future directions of research

Many research and clinical questions still remain to be answered in the field of inborn errors of metabolism. Clinical important questions that need to be resolved and may probably best be answered by the use of well designed tracer studies include:

- Optimizing natural protein content in protein restricted diets according to age in patients with disorders of branched-chain amino acid oxidation, including methylmalonic aciduria (MMA), propionic aciduria (PA) and maple syrup urine disease (MSUD).
- Assessing carbohydrate and fat metabolism in patients with various disorders of energy metabolism, both in rest and during normal daily activity, in order to optimize therapeutic strategies and quality of life in these patients.

General conclusion

In this thesis, the potentials and limitations of in vivo stable isotope methodology in the field of inborn errors of metabolism are illustrated in different studies conducted in patients with various metabolic disorders. The fact that with a limited amount of study subjects research and clinical questions can be answered satisfactory using tracer techniques makes them a very powerful tool in the field of inborn errors of metabolism, especially for translational research. However, it should be noted that, although numerous research
and clinical applications for in vivo stable isotope studies are of interest, the limitations of these techniques should be thoroughly taken into account before undertaking tracer studies, since these can result in problems that may prove to be insurmountable. Therefore, these studies should only be conducted in (collaboration with) centres with vast expertise in in vivo stable isotope methodology.

References