Uses of stable isotopes in clinical diagnosis and research in the paediatric population

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The landmark experiments of Schoenheimer and Rittenberg in the 1930s provided the scientific foundation for the ensuing development and application of stable isotope techniques in clinical diagnosis and research.1

Stable isotopes are non-radioactive atoms of the same chemical element, which differ only in their number of neutrons.2 Many elements also have radioactive (non-stable) isotopes. Aspects of macronutrient metabolism have been investigated employing molecules labelled with ²H (D or deuterium), ¹³C, ¹⁵N, and ¹⁸O. Extensive literature is available detailing the use of ²⁶Mg, ²⁵Mg, ⁴²Ca, ⁴⁶Ca, ⁴⁸Ca, ⁵⁷Fe, ⁵⁸Fe, ⁶⁷Zn, and ⁷⁰Zn isotopes in studies of mineral metabolism. The most commonly used radioactive isotopes are ¹⁴C and ³H (tritium).3 More than 6000 stable isotope labelled compounds (tracers) are commercially available for use in metabolic studies. Examples of some of these tracers are [¹-¹³C] leucine, [¹-¹³C, ¹⁵N] leucine, [ring-D₃] phenylalanine, and [6,6]-D₂ glucose. It is currently accepted that these compounds have negligible biological side effects, which renders them ethically acceptable for use in children.⁴

Following intravenous or oral administration, the tracer is metabolically indistinguishable from the equivalent unlabelled compound of interest (tracee). The metabolic fate of the compound can be assessed qualitatively and quantitatively by measuring the relative abundance of tracer and tracee and/or their respective metabolites with time. The detectable mass difference of tracer and tracee allows the analysis of compounds, extracted from plasma, by gas chromatography–mass spectrometry (GC–MS), picogram sample size, analytical range 0.1–100 mole %, precision ±0.2 mole %).⁵

The detection limit is considerably less than 0.1 mole %, when tracers with multiple stable isotope labels (for example ring-D₃ phenylalanine) are used.⁶ Stable isotopes in breath (¹³CO₂ and ¹⁷CO₂) are analysed using an isotope ratio mass spectrometer (IRMS, microgram sample size, analytical range 0.0001–0.01 atom % excess, precision ±0.0001 atom %).⁷

Combustion IRMS has essentially the same analytical capabilities as IRMS but allows the combustion of tissue samples with subsequent analysis of gaseous isotope enrichment.⁸ Stable isotopes of minerals are typically analysed by thermal ionisation mass spectrometry (TIMS) or inductively coupled plasma mass spectrometry (ICP-MS) with high precision and sensitivity.⁹

The advantages of stable isotope labelled compounds compared with their radioactive counterparts are many. Most importantly, several different stable isotope tracers can be safely administered simultaneously to the same subject without compromising future studies. The plasma volume which is needed from one sample to analyse the isotope enrichment is small, allowing even preterm infants to be studied. On average 0.5 ml of plasma is needed for one study. The intramolecular location of one or more label(s) is determined easily, which allows the mapping of metabolic pathways.¹⁰ In contrast, no realistic radioactive tracers exist for certain chemical elements (O₃, N₂) and the handling, application, and disposal of radioactive tracers makes such studies hazardous to all participants.¹¹

This review is written for (general) paediatricians to provide a concise and up to date overview of the use of stable isotope labelled compounds in clinical diagnosis and research in the paediatric population. For more detailed information on stable isotope models and additional applications the interested reader is referred to recently published reviews.¹² ¹⁰

Breath tests

With few exceptions stable isotope breath tests are based on the ingestion of substrates that are labelled with ¹³C atoms.¹³ Following digestion and absorption, the substrates are metabolised and eventually oxidised to yield H₂O, ¹³CO₂, and ¹⁷CO₂. Increased isotope enrichment in breath (¹³CO₂) can be measured as outlined above and it is assumed that this increase directly reflects the intestinal absorption and metabolism of the substrate. The advantages are obvious. Breath tests are non-invasive and can be performed conveniently in an outpatient setting. The tracer is given orally and breath samples are subsequently collected. Breath tests do not provide metabolic information regarding pathways in which the substrate may play a role until it is finally oxidised. This may be of particular significance in patients with perturbed intermediary metabolism.

A complete description of all breath tests applied in the paediatric population is clearly beyond the scope of this review. A recently published Gut supplement presents an extensive series of papers reporting a wide range of diagnostic breath tests in children.¹¹ However, we will exemplify the usefulness and clinical impact of stable isotope breath tests in several relevant clinical areas.

Depending on the type of tracer, the isotope enrichment in breath is an indirect reflection of gastrointestinal and/or liver function. The most commonly used substrates in this context are trioctanoin, triolein, octanoic acid, aminopyrine, and caffeine respectively.¹² ¹³

The ¹³C urea breath test exemplifies a stable isotope breath test which is used for the diagnosis and management of Helicobacter pylori infection and for monitoring the effectiveness of therapy.²⁰ ²¹
**Stable isotopes in clinical diagnosis and research**

In a clinical standpoint, accurate measurement of total body water can provide indirect information relating to whole body composition and energy balance. The mechanisms of energy balance control are unclear and the study of this balance is complicated by the fact that alterations in body weight may reflect a change in energy stores, an alteration in total body water, or both.

Total body water can be calculated by employing standard isotope dilution techniques. Knowledge of the “fixed” water content of the fat free mass permits calculation of this body compartment. Subtraction of this mass from body weight provides indirectly the mass of body fat. Both $^2\text{H}_2\text{O}$ and $^1\text{H}_2\text{O}$ have been used to estimate total body water in renal insufficiency, cystic fibrosis, malnutrition, and in endocrine disorders. 15-19

The doubly labelled water method (employing $^2\text{H}_2\text{O}$ and $^1\text{H}_2\text{O}$) has become established, over the past 20 years, as the only method currently available for measuring total energy expenditure in completely free living subjects. 20 Following isotope administration and equilibration, isotopically labelled hydrogen will leave the body both as water and as carbon dioxide (action of carbonic anhydrase). Monitoring the individual enrichment decay curves for these two isotopes over 7–14 days will result in different slopes. The difference in slopes is directly proportional to total body carbon dioxide production. This direct estimate of metabolic rate can then be converted to units of heat production by incorporating knowledge or estimates of the chemical composition of the foodstuffs being oxidised. This influences the energy equivalence of each litre of carbon dioxide produced.

The method has been validated in infants. 21,22 Application of the method to determine energy expenditure in small for gestational age infants, and growth and development in normal infancy and in malnourished children have been reported. 23–25 Published data relating to energy expenditure in short and normal stature Guatemalan children and obese and non-obese adolescents (12–18 years) are available. 26,27

**Body composition and energy expenditure**

Inborn errors of metabolism are ideal study areas for the use of stable isotopes. Many biochemical pathways have intermediary compounds of which stable isotopes are available, representing different enzymatic activities. Consequently, children with inborn errors of metabolism were among the first to be studied with stable isotopes. 28,29

Stable isotope breath tests were used in inborn errors of metabolism for non-invasive diagnosis and reliable semiquantitative estimation of whole body enzyme capacity in subjects with galactosaemia and maple syrup urine disease. 30–32 Response to therapeutic intervention and genotype-phenotype correlations were assessed in children with thiamine responsive maple syrup urine disease, 33 and galactosaemia, respectively. 34

Stable isotope studies involving infusions of tracer and blood sampling have been undertaken in many different inborn errors of metabolism, including glycogen storage diseases, 35–37 galactosaemia, 38 phenylketonuria (PKU), 39–40 organic acidurias, 41 and urea cycle defects. 42 Thompson et al have shown that phenylalanine or propionate are metabolised in children with PKU or certain organic acidurias (methylmalonic aciduria and propionic aciduria), respectively. 43

Significant phenylalanine hydroxylation in children with classical PKU was recently contradicted by van Spronsen et al who showed clearly reduced (whole body) enzyme activities when compared with normal healthy control subjects. 44 They also concluded that dietary phenylalanine tolerance did not correlate with in vivo hydroxylation of phenylalanine, an important finding which increases the significance of adequate substrate disposal (for protein synthesis).

**Macronutrient metabolism**

Protein, lipid, and glucose kinetics in infants and children have been measured using a range of labelled substrates: $[^{1-13}\text{C}]$ leucine and $[^{13}\text{C}]$ glucose respectively. van Goudoever et al studied protein kinetics in two groups of preterm infants. 42 The first group was supplemented with amino acids while the second group received only glucose infusions. Nitrogen and leucine balance improved significantly in the first group, secondary to increased protein synthesis and decreased protein breakdown, a clear indication of the benefits of early administration of amino acids in preterm infants. These findings confirmed earlier published results. 45,46

More recently Clark et al investigated the effects of parenteral nutrition on leucine and phenylalanine kinetics in preterm infants and showed an acute increase in whole body protein synthesis as well as a reduction in protein breakdown. 47 A comparison of whole body protein turnover in appropriate for gestational age and small for gestational age infants using two labelled amino acids gave encouragingly similar quantitative results. 48

Nitrogen balance and protein turnover have been investigated during growth failure in low birthweight infants, 49 and in sick premature neonates during the first few days of life. 50
Phenylalanine hydroxylase status was investigated in sick preterm neonates with respiratory distress syndrome. The results obtained in this study did not support the hypothesis that the enzyme activity was low in these preterm infants.

Albumin synthetic rate has been measured in premature infants and in oedematous and non-œdematous protein energy malnourished children. In the first study it was found that both the fractional and absolute synthetic rate for albumin in the infants were much higher than rates found in young adults. The results from the latter study suggested that repletion of the albumin pool of children with protein energy malnutrition was not mediated by changes in the fractional synthetic rate. Additionally it was shown that the oedema of malnutrition was not solely a result of the hyperalbuninaemia.

Whole body protein metabolism has been measured in children with cancer using both labelled leucine and phenylalanine. Both tracers gave similar results which indicated that both the rates of synthesis and breakdown were higher than in normal children. The effects of feeding on protein turnover in children with cystic fibrosis and healthy controls have been compared. From their results the authors suggest that feeding may affect protein turnover differently in children with cystic fibrosis when compared with control children. It was suggested that the differences might be independent of plasma insulin concentration.

Studies of plasma arginine and leucine kinetics in paediatric burns patients suggested that the former amino acid was conditionally indispensable in terms of maintaining protein homeostasis in severely burned children. In healthy children recovering from burn injury, rates of whole body protein synthesis in relation to basal energy expenditure have been compared. From these studies it was suggested that feeding may affect protein turnover differently in children with cystic fibrosis when compared with control children. It was suggested that the differences might be independent of plasma insulin concentration.

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expensive in that the isotope is simply mixed with the food before consumption. In this instance it has to be assumed that the extrinsic label equilibrates completely with the native intrinsic inorganic element in the diet, and is absorbed and metabolised in an identical fashion. Plant and animal foods can be labelled intrinsically by the incorporation of the chosen isotope biosynthetically into the growing food. Several procedures to achieve this end have been described. When calcium and magnesium metabolism was assessed in prepubertal and pubertal girls and boys, Abrams et al showed that total calcium absorption from milk was increased in pubertal compared to prepubertal children. Almost 50% of the children studied had negative magnesium balances despite magnesium intakes based on the recommended dietary allowance for magnesium. A more detailed review relating to stable isotopes and mineral metabolism in children was recently published.

Conclusion
Stable isotopes are ideal “tools” for the dynamic assessment of in vivo metabolism in the paediatric population. Not only are these tracers safe and therefore ethically justifiable, but in addition they may be particularly important for the validation of new treatment modalities, such as novel drug treatment or gene therapy. Many more exciting studies are currently underway to enhance our knowledge of paediatric metabolism and (patho)physiology, an important factor for the continued reduction of paediatric morbidity and mortality.
