13C protein breath tests

Y Ghoos, B Beaufrère

Tracer techniques are attractive for the in vivo study of different aspects of nutrient assimilation and metabolism. Stable isotopes are the preferred tracers in studies involving human subjects, mainly because of safety reasons. Their widespread use in recent years has been stimulated by improvements both in the increased availability and diversity of stable isotope labelled compounds and in analytical (that is, mass spectrometric) methods for their quantitative analysis.1

Labelled amino acids have been used to study protein metabolism in vivo in a large number of studies mainly directed towards the measurement of protein synthesis and breakdown rates. Generally speaking, constant infusions of labelled amino acids such as L-[1-13C]-leucine are used.2,3 To study protein assimilation or protein metabolism during feeding, however, a representative oral tracer—that is, labelled protein, is needed.

Only a few techniques have hitherto been described for the production of stable isotope labelled proteins. Limitations and drawbacks inherent to those techniques have prevented their widespread application (small yield, low enrichment level of protein, inadequate labelling pattern), but now two proteins common in the normal diet are available in labelled forms—that is, milk and egg proteins labelled with L-[1-13C]-leucine.

These proteins have been recently produced by “Laboratoire de Nutrition Humaine” (Clermont-Ferrand, France) and by “Laboratoire Digestive-Absorption” (Leuven, Belgium) (abstract 1).4,5 In both cases the labelling technique is well described so that it can be reproduced by other scientists. Each technique has advantages for different reasons: production of egg proteins has a high tracer recovery, and infusion of lactating cows produces two types of proteins (whey protein and casein) with different effects in vivo. Labelled proteins can be used to evaluate protein assimilation in various diseases in adults and children (pancreatic disease, abstract 2),6 to monitor the beneficial or detrimental effect of pharmaceuticals on protein assimilation (abstract 3),7 and to study the influence of other macronutrients (carbohydrates, lipids) on the assimilation process of protein. Kinetic protein metabolism studies during feeding to evaluate whole body protein synthesis, oxidation, and breakdown are the most appropriate tool to determine the optimal conditions for the use of dietary protein in humans. These studies require the use of an intravenous tracer together with oral administration of labelled protein.8

Although the following abstracts describe the use of a protein test meal, which is unphysiological, they detail important first steps in this work, just as in the first pilot breath tests on lipid digestion a high fat test meal was used. In future studies, however, it is planned to incorporate the labelled protein into formula food, as has already been achieved with whey protein (Professor B Beaufrère, personal communication). The aim is to develop a standard physiological meal, well accepted by all age groups of the population.


(1) The production of milk and egg proteins, enriched with stable isotopes, for the in vivo study of protein assimilation and metabolism during feeding: an European collaborative study

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Introduction: Recently, methods have been developed for the production of milk and egg proteins, both labelled with L-[1-13C]-leucine. With the aid of these substrate proteins, protein assimilation and protein metabolism during feeding can be studied in an accurate way. Methods: Twenty-eight volunteers participated in the breath test studies (12 women, 16 men; mean age 43 years, range 18 to 71). None of the subjects had a history of gastrointestinal or metabolic disease or previous surgery (apart from appendectomy), nor was anyone taking medication. The volunteers were either evaluated after ingestion of egg white protein (n=10) or whey protein (n=18). The solid egg white protein test meal consisted of 200 g of egg white (containing 22 g of egg white protein), half of which (that is, 11 g) was labelled with L-[1-13C] leucine, and 200 ml water. Total caloric content of the test meal was 367 kJ. The (solid) test meal had to be consumed within 15 minutes. The liquid whey protein test meal consisted of 30 g of whey protein, being a mixture of labelled and unlabelled protein. 13C leucine labelled whey proteins were obtained by infusing lactating cows. Total caloric content of the test meal was 502 kJ. The protein was dissolved in 250 ml water and ingested as a
The figure shows the 13CO2 excretion curve, expressed in percentage of administered dose recovered per hour obtained after ingestion of either 22 g of egg protein (n=10) or 30 g of whey protein (n=18), both intrinsically labelled with L-[1-13C]leucine. Values are means (SEM).

Abstract 1, Table 1 Values of protein assimilation

<table>
<thead>
<tr>
<th></th>
<th>Whey protein (SD)</th>
<th>Egg white protein (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Max %/hour</td>
<td>9.02 (0.41)</td>
<td>5.75 (0.27)</td>
</tr>
<tr>
<td>tmax (minutes)</td>
<td>137 (4)</td>
<td>168 (15)</td>
</tr>
<tr>
<td>% dose cum six hours</td>
<td>27.99 (1.11)</td>
<td>19.71 (1.03)</td>
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(3) Evaluation of the role of gastric digestion in overall protein assimilation by a combined 13C egg white - 14C-octanoic acid breath test

The role of gastric digestion in normal protein assimilation is generally thought to be minimal. The aim of our study was to investigate whether inhibition of gastric acid secretion affects overall protein assimilation. Ten healthy volunteers were studied using a combination of a newly developed 13C leucine egg white and 14C octanoic acid breath test. The test meal consisted of 22 g of 13C labelled egg white protein, the yolk of one egg doped with 74 kBq of 14C octanoic acid, and 200 ml of water. The yolk and egg white were baked separately, but given together. Breath samples were taken before ingestion of the meal and at 15 minute intervals thereafter for six hours, and analysed for 13CO2 and 14CO2 enrichment. Each subject was studied in two different test situations, in random order: (a) without and (b) after peroral administration of 40 mg of omeprazole over three days. Gastric emptying values and values of protein assimilation were paired-wise compared with the values obtained in the control study using the Mann-Whitney-Wilcoxon test.

No difference in gastric emptying rate could be detected with or without omeprazole (half emptying time: p=1.00). Major differences were shown in protein assimilation kinetics. The 13C excretion curve was significantly flattened (13CO2 peak excretion: p<0.01) and the 14C peak excretion time significantly delayed (p<0.05) as compared with the control study.

Inhibition of gastric acid secretion has a major influence on protein assimilation kinetics, most probably attributable to impaired gastric digestion. Simultaneous measurement of gastric emptying rate excluded altered gastric emptying rate as a possible explanation of the observed differences.
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