¹³C protein breath tests

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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 “Laboratorium Digestie-Absorptie” (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 physicochemical properties. 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.

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(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, 11g) 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

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Figure 1 $^{13}\text{CO}_2$ 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- ^{13}C]-leucine. Values are means (SEM).

Abstract 1, Table 1 Values of protein assimilation

	Whey protein (SD)	Egg white protein (SD)
Max %/hour	9.02 (0.41)	5.75 (0.27)
t_{max}	127 (4)	168 (15)
% dose cum six hours	27.99 (1.11)	19.71 (1.03)

liquid meal in less than five minutes. Breath tests were carried out after an overnight fast of at least 12 hours. Breath samples were collected twice immediately before the meal and at regular intervals for a period of six hours after the meal. All breath samples were analysed for $^{13}\text{CO}_2$ concentration by means of an isotope ratio mass spectrometer (IRMS). The results were expressed as the percentage of ^{13}C -recovery/hour and as cumulative values over six hours assuming a CO_2 production of 300 mmol/m^2 body surface area/hour. **Results:** Figure 1 shows the $^{13}\text{CO}_2$ excretion rate in expired breath in the two test conditions. Differences in $^{13}\text{CO}_2$ excretion rate are obvious. After ingestion of labelled whey protein a rapid increase of the $^{13}\text{CO}_2$ rate in breath is observed, reaching a maximum after about 130 minutes. The slope of the descending part of the curve was equally steep. The $^{13}\text{CO}_2$ excretion obtained after ingestion of labelled egg white protein is delayed and flattened compared with whey protein. However, the curve is still characterised by an ascending part, a maximal excretion, and a descending part. Three values of protein assimilation were derived: maximum percentage excretion of administered dose per hour (max %/hour), the time when this maximum is reached (t_{max}), and the cumulative percentage recovery of administered dose of ^{13}C after six hours (% dose cum six hours) and these are represented in table 1. **Conclusion:** Large amounts of highly enriched milk and egg proteins can be obtained in a reproducible manner. These proteins can be used as tracers in studies on protein assimilation and protein metabolism during feeding. Apparent differences in the assimilation kinetics of egg and milk proteins were shown by means of breath test technique. The nutritional and metabolic implications of this finding have to be explored further. For this purpose, kinetic protein metabolism studies using ^{13}C -labelled protein as oral tracer are undoubtedly the most appropriate instrument.

(2) Protein assimilation in normal subjects and patients with pancreatic disease, studied with a ^{13}C egg white breath test

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Background: Exocrine pancreatic insufficiency is a principal cause of impaired assimilation of dietary proteins. **Aims:** The present study aimed (a) to evaluate protein assimilation by a recently developed ^{13}C egg white breath test in patients with pancreatic disease and (b) to correlate the excretion of $^{13}\text{CO}_2$ in breath with the trypsin output in the duodenum after maximal stimulation of the pancreas. **Methods:** 48 healthy volunteers and 30 patients were studied after ingestion of a test meal consisting of 22 g of ^{13}C labelled egg protein. Breath samples were taken before and after ingestion of the meal for a six hour period and analysed for $^{13}\text{CO}_2$ by on line gas chromatographic purification-IRMS (ABCA, Europa Scientific, Crewe, UK). Moreover, pancreatic trypsin output after maximal stimulation was measured in 13 patients and in nine healthy volunteers. **Results:** The six hour cumulative $^{13}\text{CO}_2$ excretion measured in breath was significantly lower in patients than in the control group (mean (SEM): $6.23 (0.82) \% v 19.16 (0.58) \%$, $p < 0.0001$). An excellent correlation was found between the six hour cumulative $^{13}\text{CO}_2$ excretion and the trypsin activity after maximal pancreatic stimulation.

Conclusions: The ^{13}C egg white breath test allows detection of malassimilation of protein in a non-invasive manner and proved to be promising as an indirect pancreatic function test.

(3) Evaluation of the role of gastric digestion in overall protein assimilation by a combined ^{13}C egg white - ^{14}C -octanoic acid breath test

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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 normal protein assimilation. Ten healthy volunteers were studied using a combination of a newly developed ^{13}C leucine egg white and ^{14}C octanoic acid breath test. The test meal consisted of 22 g of ^{13}C labelled egg white protein, the yolk of one egg doped with 74 kBq of ^{14}C 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 $^{13}\text{CO}_2$ and $^{14}\text{CO}_2$ 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 $^{13}\text{CO}_2$ excretion curve was significantly flattened ($^{13}\text{CO}_2$ peak excretion: $p<0.01$) and the $^{13}\text{CO}_2$ 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.