Study of protein assimilation, using stable isotope techniques

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RESUMEN: La información existente sobre la asimilación de proteínas es escasa y contradictoria. Esto es debido especialmente a la inexistencia de una técnica de confianza y no invasiva. La reciente disponibilidad de proteínas marcadas con siótopos estables ha permitido estudiar la asimilación de proteínas usando técnicas de trazadores. Con estas técnicas se puede comprobar que la digestión de proteínas depende tanto de las características del alimento ingerido como de la digestión y absorción en el tracto intestinal superior. Este último está reducido significativamente en la enfermedad pancreática y está también comprometido por algunos fármacos utilizados a veces en la práctica clínica. Adicionalmente, confirmamos que una parte significativa de la proteína fácilmente digerible de la dieta no se asimila en el intestino delgado.

SUMMARY: Information on the efficiency and kinetics of protein assimilation in humans is scanty and moreover controversial. This is mainly due to the lack of a reliable and non-invasive measuring technique. The recent availability of stable isotope labelled protein allowed to study protein assimilation using tracer techniques. Applying these techniques, we showed that protein digestibility depends both on characteristics of the ingested meal and on the digestive and absorptive capacity of the upper gastrointstinal tract. The latter is significantly impaired in pancreatic disease but is also compromised by some drugs often used in clinical practice. We moreover confirmed that a substantial amount of even easily digestible dietary protein escapes assimilation in the small intestine.

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Palabras clave

Proteínas, asimilación, isótopos estables, humanos.

Key words

Protein, assimilation, stable isotopes, humans.

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Introduction

Protein is an important constituent of food in humans. This can be considered either from a gastrointestinal or from a nutritional point of view. The digestion of proteins involves a complex series of degradative processes which are elicited mainly by the hydrolytic enzymes originating from the stomach, pancreas and the brush border of the small intestine (1). Information about the efficiency and kinetics of the process of protein assimilation is scarce and controversial. This is largely due to the complexity of the subject (e.g. unknown contribution of endogenous and exogenous protein sources) and shortcomings of measuring techniques currently available.

We aimed to evaluate protein assimilation by means of tracer techniques. Stable isotopes are the preferred tracers in studies involving human subjects mainly because of safety. The application of tracer techniques using stable isotopes has hitherto largely been hindered by the lack of a representative substrate, i.e. labelled protein. Our laboratory recently developed a simple

and easily reproducible methodology for the production of highly enriched proteins. Large amounts of egg proteins highly enriched with either ^{15}N (1.35 AP), ^{13}C (1.34 AP), or ^{2}H were obtained by giving laying hens free access to a food, which had been enriched with isotopically labelled amino acids (L-[^{15}N(leucine-, L-[1-^{13}C(leucine, and L-[ring-$^{2}H_4$(phenylalanine, respectively) in a well defined manner (2).

The production of ^{13}C-labelled protein made it possible to study protein assimilation by means of breath test technique. Breath tests are simple, easily repeatable and non-invasive. Although breath tests provide semi-quantitative information only, they are unique because the obtained information represents a dynamic evaluation of the gastrointestinal function under consideration, rather than a static estimation. As will be illustrated in the present paper, breath test techniques are very useful for the study of dietary (e.g. food preparation) and pharmaceutical influences (e.g. gastric acid suppression) on protein assimilation as well as the impact of gastrointestinal diseases (e.g. pancreatic disease). By these studies, moreover, insight may be gained in the normal physiology of protein assimilation.

Material and methods

Subjects and study design

In order to determine 'normal' breath test derived parameters of protein assimilation, ten healthy volunteers (5 males and 5 females, age 19 to 30 y) performed the ^{13}C-egg white breath test in standard conditions (3).

Subsequently we studied the influence of gastric acid suppression on protein assimilation. For this purpose, 10 healthy volunteers (5 males and 5 females, age 18 to 27) were studied by means of the ^{13}C-egg white breath test in two different randomly applied test situations: 1) without and 2) after peroral administration of 40 mg of omeprazole during three days (4).

The influence of heat pre-treatment on egg protein assimilation was evaluated in «healthy» ileostomy patients (1 male and 4 females, age 28 to 76 y). The patients were studied in two different randomly applied test situations: 1) after ingestion of 200 g of ^{13}C- and ^{15}N-labelled egg white protein administered as a cooked test meal and 2) after ingestion of the same but raw protein meal. Protein assimilation was simultaneously evaluated by breath test technique and analysis of ileostomy effluent (5).

In order to evaluate the influence of pancreatic disease on pancreatic disease, ^{13}C-egg white breath test results obtained in 26 healthy volunteers were compared with those obtained in 16 patients with pancreatic disease. Measurement of trypsin output after maximal stimulation was moreover carried out in 7 patients with pancreatic disease and 6 healthy volunteers. These data were correlated with the breath test results (6).

Test meal

The (solid) test meal used in all studies consisted of 200 g of ^{13}C-labelled egg white, the yolk of one egg, and 200 ml water. Total caloric content of the test meal was 150 Kcal (25 g of protein, 5.56 g of fat and a negligible amount of carbohydrate). The test meal was cooked in a microwave oven before ingestion, unless otherwise stated. The test meal had to be consumed in less than 15 minutes. Sodium chloride as an appetiser was allowed. No extra food was allowed during the test. From 3 hours after the start of the test on, drinking of water was permitted.

^{13}C-egg white breath test

All subjects were studied after an overnight fast of at least 12 h. Breath samples were taken before ingestion of the test meal and each 15 minutes thereafter during 6 hours, and analysed for $^{13}CO_2$ concentration by isotope ratio mass spectrometry (ABCA, Europa Scientific, Crewe, UK). The data were expressed in percentage of administered dose of ^{13}C, recovered per hour (% ^{13}C dose/h) and in cumulative percentage administered dose of ^{13}C, recovered over time (% ^{13}C dose cum) according to calculations described in detail by Ghoos et al. (7).

Analysis of ileal effluents

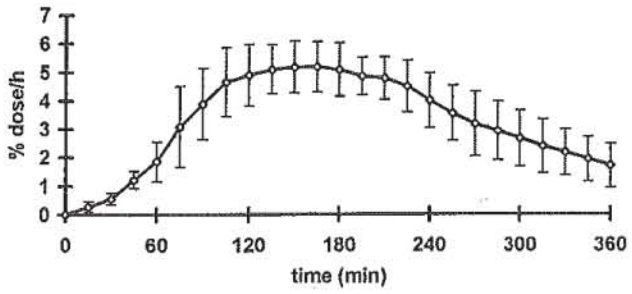
Ileal effluents were collected for 24 hours after ingestion of the test meal. The exogenous (i.e. dietary) nitrogen in the ileal effluents was calculated from total nitrogen and 15N-enrichment of both the ileal effluent and the test meal. Results were expressed in percentage of ingested protein, recovered in the ileal effluent over 24 h (% Nex24h).

Results

Protein assimilation in healthy volunteers

Figure 1 shows the mean $^{13}CO_2$ excretion in expired breath, expressed in percent of administered dose per hour, in 10 normal volunteers. The mean (±SEM) maximal percentage excretion of administered dose per hour was 5.75±0.27 and was reached 168±15 min after the protein meal was ingested. The mean (±SEM)

Figure 1



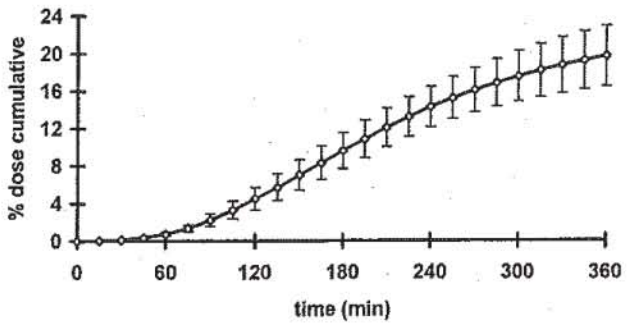
$^{13}CO_2$ excretion curve, expressed in percentage of administered dose, recovered per hour (% ^{13}C dose/h), in 10 normal volunteers after ingestion of a test meal consisting of 200 g of ^{13}C-labelled egg white. Values are means±SD, n = 10.

cumulative percentage recovery of administered dose of ^{13}C after 6 hours (figure 2) was 19.71±1.0. (3).

The influence of gastric acid suppression

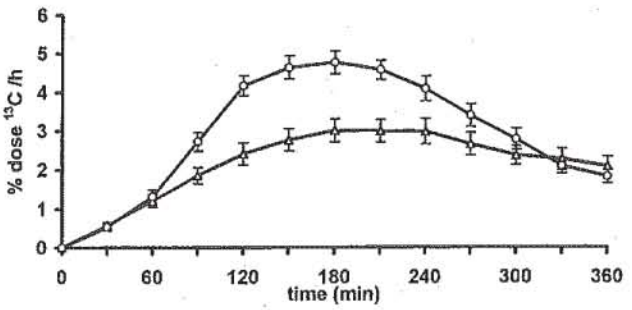
Omeprazole pre-treatment obviously affected protein assimilation kinetics in healthy volunteers as is shown in figure 3. The $^{13}CO_2$ excretion curve was significantly flattened (maximal % ^{13}C dose/h: $p < 0.01$, paired Wilcoxon) and the maximal $^{13}CO_2$

Figure 2



Cumulative $^{13}CO_2$ excretion curve, expressed as cumulative percentage administered dose of ^{13}C, recovered over time (% dose ^{13}C cum), in 10 normal volunteers after ingestion of a test meal consisting of 200 g of ^{13}C-labelled egg white. Values are means±SD, n = 10.

Figure 3



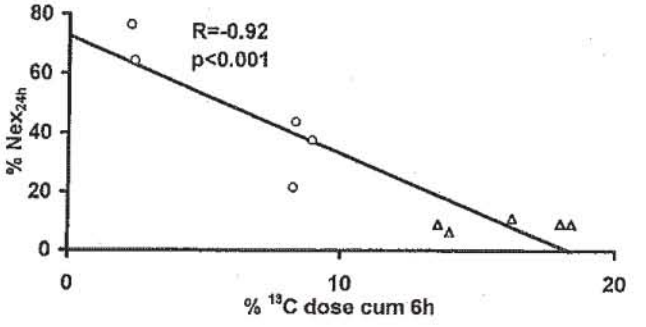
$^{13}CO_2$ excretion curve, expressed in percentage of administered dose, recovered per hour (%dose ^{13}C/h), in 10 normal volunteers before (circles) and after (triangles) omeprazole treatment. Values are means±SEM, n = 10.

excretion time significantly delayed as compared to the control study (4).

The influence of heat pre-treatment on egg protein assimilation

As demonstrated in figure 4, raw egg protein was

Figure 4



Correlation between the cumulative amount of exogenous nitrogen, recovered in the ileal effluents over 24 h ($N_{exo\ 24\ h}$ cum) and the cumulative percentage of administered dose of ^{13}C, recovered in breath over 6 h (% dose ^{13}C cum 6h). (trangles: individual values obtained after ingestion of 25 g of raw egg protein; circles :individual values obtained after ingestion of 25 g of cooked egg protein). 25 g of protein corresponds to 4 g of nitrogen.

significantly more resistant to digestion than heat-treated egg protein. An excellent (negative) correlation was found between % ^{13}C dose cum 6h and $\%Nex_{24h}$ (5).

The influence of pancreatic disease

The % ^{13}C dose cum 6h was significantly lower in patients with pancreatic disease as compared to the control group ($p < 0.0001$, non-paired t-test). An excellent correlation was observed between % ^{13}C dose cum 6h and the tryspin activity after maximal pancreatic stimulation (figure 5) (6).

Discussion

Information about the efficiency and the kinetics of protein assimilation *in vivo* is scarce and moreover controversial. This is mainly due the lack of a safe, reliable and non-invasive measuring technique. Stable isotopes may be very helpful in solving this problem, as they can be used as non-radioactive tracers in biological studies.

^{13}C-breath tests are simple, easily repeatable and non-invasive. Hence, they are very suitable to perform studies on a large scale both in normal volunteers and in patients. Although breath tests only provide semi-quantitative information (i.e. they reveal adequacy of function compared to a normal standard), they are very interesting as a functional test because the obtained information represents a dynamic evaluation rather than a static estimation. Breath tests have already been used in the past to study the assimilation of fat and carbohydrates (8, 9). The recent availability of large amounts of protein, highly enriched with ^{13}C, allowed breath test technology to be adapted for the study of protein assimilation as well. The protein substrate used is specifically labelled with L-[1-13C]leucine. Owing to the small size of the plasma and intracellular leucine pool and owing to the high turnover rates of leucine in these pools, metabolism of leucine is very fast (10). The appearance of $^{13}CO_2$ in breath after ingestion of protein labelled with L-[1-^{13}C]leucine therefore reflects protein assimilation kinetics rather than metabolic dynamics.

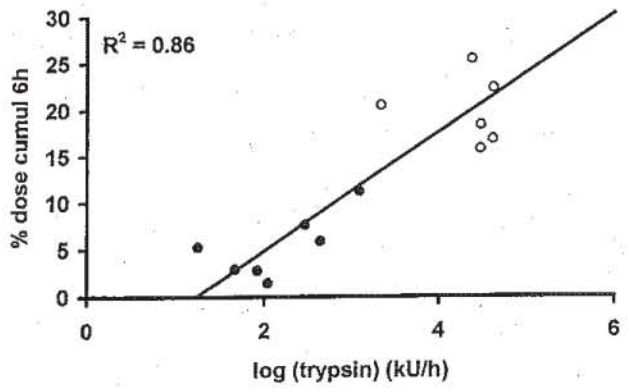
In this paper a compendium is given of the data on protein assimilation that hitherto have been obtained at our laboratory using the $^{13}CO_2$ breath test.

We demonstrated that inhibition of gastric acid secretion by omeprazole (a proton pump inhibitor) significantly hampers protein assimilation. Since this effect is most probably due to impaired gastric digestion by pH sensitive pepsins, it can be assumed that gastric digestion does play a substantial role in normal protein assimilation.

Food processing may change the digestibility of proteins either negatively or positively. We showed both by breath test technique and analysis of ileal effluents that raw egg protein is much less digestible than heat pre-treated egg protein. A significant negative correlation was found between the amount of exogenous protein escaping assimilation in the small intestine and results of the ^{13}C-egg white breath test. This finding validates the ^{13}C-egg protein breath test as an accurate alternative for the evaluation of protein digestibility. The ileostomy study also demonstrated that the assimilation of (even easily digestible) protein is not complete in subjects with an intact upper gastrointestinal tract (11).

We also used the ^{13}C-egg white breath test to evaluate protein assimilation in patients with documented pancreatic disease. Significantly impaired protein assimilation was shown in these patients as compared to healthy volunteers. An excellent correlation between the 6-h cumulative $^{13}CO_2$ excretion in breath and the duodenal trypsin output after maximal pancreatic stimulation was demonstrated. This finding is in accordance with the generally accepted view of

Figure 5



Relation between pancreatic trypsin output after stimulation and the assimilation of egg white protein, measured by the 6-h cumulative percentage of $^{13}CO_2$. (normal subjects: open circles; patients: closed circles). T = Trypsin activity (kU/h); %dc6h = percentage of administered dose of ^{13}C, recovered in breath over 6h.

pancreatic digestion being the rate liming step in overall protein assimilation. The highly significant correlation therefore represents the best validation of the ^{13}C-egg white breath test.

In the future, many other applications of the dynamic ^{13}C-protein breath test in nutritional and clinical medicine are possible. Since the utilisation of dietary protein is affected by the kinetics of intestinal delivery of amino acids to the organism, the study of the influence of other macronutrients on protein assimilation kinetics might be interesting. In this regard, the assimilation kinetics of different protein sources (egg and milk proteins) might equally worthwhile to be investigated. The ^{13}C-protein breath test can furthermore be used to evaluate protein assimilation in several digestive diseases (celiac sprue, radioenteritis, Crohn's disease, exocrine pancreatic insufficiency in cystic fibrosis, short bowel...). Apart from being a diagnostic tool, the ^{13}C-protein breath test could be valuable to monitor therapy and to evaluate the necessity of parenteral nutrition. Whether (and if so, to what extent) nutritional enteral support with protein hydrolysates is more favourable than feeding native protein is still controversial. This issue could be elucidated by comparing results of breath tests in which either native ^{13}C-egg protein or its hydrolysate is used as substrate.

In conclusion, the availability of large amounts egg proteins highly enriched with either ^{15}N, ^{13}C, or ^{2}H offers justified promises for a better understanding of the process of protein assimilation. Preliminary studies at our laboratory indicate that stable isotope labelled protein might be useful for the in vivo study of protein fermentation as well. Finally, labelled protein equally might be very helpful as an oral tracer in protein metabolism kinetic studies.

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