\[ ^{13} \text{C} \] carbohydrate breath tests

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In paediatric practice proper digestion of carbohydrates is of major importance for adequate growth and development and for prevention of intestinal complaints. Several carbohydrates can be considered in this respect: (a) lactose: the main carbohydrate in the nutrition of early life and also in later life for many white people; (b) starch: the main energy supplier in our food; (c) fructose: a constituent of fruit juices and increasingly used as a sweetener; and (d) galactose: a component of lactose. The fate of this sugar will be considered in more detail in section five, dealing with the measurement of liver function.

Lactose malabsorption by hypolactasia can be diagnosed in several ways. The non-invasive test most commonly used is the breath hydrogen test after lactose provocation. The reliability of this test, however, is disputed owing to non-H₂ producing flora, or extra intestinal influences that can disturb the test. Moreover, this test has to be done with unphysiological high doses of substrate to get a measurable hydrogen response in breath. This prompted investigators to develop diagnostic tools which permit the analysis of lactase enzyme activity non-invasively in a more direct way and therefore hopefully also in a more accurate and quantitative way. Hiele et al used naturally enriched \[^{13} \text{C} \] lactose and measured the \[^{13} \text{CO}_2 \] excretion in breath to diagnose adult hypolactasia patients.

In the BIOMED programme SIGN this principle has also been used by Koets et al (abstract 1) in paediatric patients. In 27 patients, lactase activity in jejunal biopsy specimens were compared with \[ H_2 \] and \[^{13} \text{CO}_2 \] breath tests after administration of \[^{13} \text{C} \] lactose. Combination of the \[ H_2 /^{13} \text{CO}_2 \] test results produced a higher sensitivity (85%) and a lower specificity (65%) than the outcome of both tests separately. The use of a combined \[ H_2 /^{13} \text{CO}_2 \] breath test can reduce the number of jejunal biopsies needed for diagnosis of hypolactasia, without significant loss of discriminatory power. The same group (Stellaard et al, abstract 2) further analysed the factors involved in the \[^{13} \text{CO}_2 \] excretion in breath after lactose administration. By comparing the \[^{13} \text{CO}_2 \] excretion rate after administration of \[^{13} \text{C} \] lactose, pre-digested \[^{13} \text{C} \] lactose, \[^{13} \text{C} \] glucose, and \[^{13} \text{C} \] galactose, the rate limiting step in \[^{13} \text{C} \] lactose use was investigated. Exercise increased four hours cumulative percentage dose recovered (cPDR) significantly. It was concluded that in healthy volunteers, glucose oxidation is the rate limiting step in the overall process of lactose use. In mild lactase deficient subjects, exercise shifts the rate limiting step to intestinal hydrolysis of lactose. In hypolactasic patients the rate limiting step is at the level of intestinal hydrolysis of lactose.

Fructose ingestion may lead to diarrhoea and abdominal pain in susceptible individuals. By means of a breath \[ H_2 \] test, it has been shown that the absorption capacity of fructose is limited in adults as well as in children. Interestingly, both the percentage of malabsorbers and the peak breath \[ H_2 \] increases were higher in toddlers compared with younger and older children. The relation of apple juice consumption to chronic non-specific diarrhoea (toddler’s diarrhoea) has been well established and attributed to the high fructose content of apple juice. Fructose absorption is facilitated by equimolar doses of glucose and amino acids (especially L-alanine), the mechanism underlying this effect remains unclear. To study fructose absorption in a more direct way, Hoekstra combined breath \[ H_2 \] studies with breath \[^{13} \text{CO}_2 \] studies (abstract 3). The results, however, did not clarify the mechanisms behind this stimulating effect of glucose and amino acids on intestinal fructose absorption. Variation in \[^{13} \text{CO}_2 \] output in breath can be caused by changes in oxidation rate, absorption rate or, in the case of colonic fermentation, colonic production rate. Without the knowledge of the rate limiting step in the overall utilisation process, the use of the \[^{13} \text{CO}_2 \] excretion rate in breath for mechanistic interpretation is difficult.

Starch digestion is affected by several factors. One of these is the physicochemical characteristics of the starch granules which can be influenced by pre-treatment such as cooking and heating and hydrolysis of the starch molecule by the enzyme \(\alpha\)-amylase. To monitor the digestion rate of corn starch, which is naturally enriched in \[^{13} \text{C} \], the \[^{13} \text{CO}_2 \] breath test can be used, as first described by Hiele et al. Especially in the study of the small intestinal digestion of resistant starch (Hylon VII and Novelose), this can be helpful to characterise the digestibility of the product as shown by Vonk et al (abstract 4). The six hour cPDR of raw Hylon VII and raw Novelose is 16.6 (SD 6.0) % and 15.5 (SD 6.0) % respectively. The six hour cPDR of glucose is 30.5 (SD 4.9) %. If we assume that 100% of the glucose is absorbed and the post-absorption metabolism of glucose from fructose itself or from starch is the same, then the six hour cPDR can be compared and used as an index of intestinal absorption. When these data for Hylon VII and Novelose are calculated in this way, both types of resistant starch are digested for 54% and 51% respectively in the small intestine. Colonic \[^{13} \text{CO}_2 \] excretion, which is defined as the \[^{13} \text{CO}_2 \] output in breath which parallels \( H \), excretion, starts after about eight hours, which implies that in the time period in which digestion is studied, colonic metabolism does not play a major part in \[^{13} \text{CO}_2 \] production.
(1) Detection of low intestinal lactase activity in children by use of a combined 13CO2/H2 breath test
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The use of combined 13CO2/H2 breath test allows for the detection of low intestinal lactase activity. The test involves administration of 13C lactose followed by measurement of 13CO2 in breath, and it is sometimes necessary to take care in the interpretation of the data. These problems will not be insurmountable especially if more research studies are done in which the overall metabolic pathways are studied simultaneously. For example, by combining the measurement of the appearance of 13C glucose in plasma with breath tests will provide the information needed that should ultimately allow breath tests to stand by themselves as tools for routine diagnosis.

(2) Rate limiting step in lactose use in humans. Consequences for the diagnosis of mild lactase deficiency
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In the present study, the steps involved in the use of lactose—that is, digestion, monosaccharide absorption, galactose metabolism, and glucose oxidation—were studied in healthy adults by analysing breath 13CO2 excretion after consumption of 40 g 13C lactose, predigested lactose, and fructose. The effect of increased glucose oxidation was measured under exercise conditions (bicycling, 50 Watt) applying 80 g 13C lactose. No difference was observed in the 13CO2 excretion curves after administration of glucose, predigested lactose, and lactose. The four hour cumulative percentage doses recovered in breath were 20.3 (SD 4.5) %, 19.2 (SD 5.5) %, and 19.9 (SD 4.9) % for glucose, predigested lactose, and lactose, respectively. Exercise increased four hour cPDR significantly: 66.1 (SD 6.2) % v 19.6 (SD 3.9) %. One subject had a mild non-symptomatic lactose malabsorption indicated by a positive H2 breath test at rest and during exercise. The 13CO2 breath test of subject A at rest was indistinguishable from that of the others (four hours cPDR 16.6 v 19.6 (SD 3.9) %), whereas the test was clearly positive during exercise (four hours cPDR 20.5 v 66.1 (SD 6.2) %).

Conclusion: In healthy volunteers, glucose oxidation is the rate limiting step in the overall process of lactose use. In mild lactase deficient subjects, exercise shifts the rate limiting step to intestinal hydrolysis of lactose.

(3) Use of 13C breath test in fructose malabsorption
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Fructose ingestion may lead to diarrhoea and abdominal pain in susceptible individuals. Fructose absorption is facilitated by equimolar doses of glucose or amino acids (L-alanine): the mechanism underlying this effect remains unclear. To study intestinal fructose absorption in a more direct way we combined breath H2 studies with 13CO2 studies.
Gastric emptying was studied using L-glycine-1-\(^{13}\)C in children, aged 12.1–16.0 years. After 25 g of fructose and 27.5 g of glucose, when given together, gastric emptying was significantly (p < 0.05) slower than with either sugar alone. In a second series of experiments five children, aged 12.0–15.9 years, were tested with 25 g of fructose, alone and with equimolar doses of glucose and L-alanine. Four younger children, aged 3.1–6.1 years, were tested with 2 g/kg (maximum 37.5) of fructose, alone or with an equimolar dose of L-alanine. All fructose solutions were enriched with 15 mg of D-fructose-\(^{13}\)C-6. In all nine children, fructose was malabsorbed as judged by breath H\(_2\) increases 20 ppm, and the addition of glucose or L-alanine resulted in significantly lower breath H\(_2\) increases (p < 0.005 for glucose, p<0.001 for alanine). In contrast, the addition of alanine or glucose did not change the pattern of breath \(^{13}\)CO\(_2\) excretion in the five older children, whereas in the four younger children (with relatively higher doses) L-alanine addition resulted in significantly lower increases in breath \(^{13}\)CO\(_2\). In the group with four younger children, for each time point breath H\(_2\) and \(^{13}\)CO\(_2\) concentrations after fructose were compared with those after fructose plus L-alanine; in 20 out of 24 points both H\(_2\) and \(^{13}\)CO\(_2\) were higher after fructose. These results suggest that \(^{13}\)CO\(_2\) not only originated from the oxidation of absorbed substrate but also, at least in part, from colonic bacterial metabolism.

(4) Digestion of resistant starch

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To increase the colonic availability of short chain fatty acids, resistant starch can be used. Part of this resistant starch is digested in the small intestine, the residue spills over into the colon. To develop a monitoring system of starch digestion, we compared the metabolic fate of three types of starch: custard (25% amylose, 75% amylopectin), Hylon VII (62% amylose, 38% amylopectin) and Novelose (62% amylose, 38% amylopectin).

The three types of starch are of corn origin and therefore naturally enriched in \(^{13}\)C. After administration of 40 g of starch to seven healthy volunteers, CO\(_2\) and \(^{13}\)CO\(_2\) excretion rate in breath were analysed each 30 minutes during six hours. Administration of 40 g of \(^{13}\)C glucose and analysing \(^{13}\)CO\(_2\) response was done as a control and reference experiment. In three healthy volunteers \(^{13}\)CO\(_2\) and H\(_2\) response were measured during 26 hours after Hylon VII consumption. \(^{13}\)CO\(_2\) was measured by isotope ratio mass spectrometry (IRMS). After administration of 40 g of the (raw) substrate the six hours cPDR was 30.5 (SD 4.9) % (glucose), 32.8 (SD 2.7) % (custard), 16.6 (SD 6.0) % (Hylon VII) and 15.5 (SD 5.9) % (Novelose). In the \(^{13}\)CO\(_2\) response roughly two phases can be distinguished: <six hours, which is due to intestinal absorption, and >eight hours, which parallels H\(_2\) production and is presumably of colonic origin.

Assuming that glucose is totally absorbed by the small intestine, the \(^{13}\)CO\(_2\) response in breath after glucose consumption can be used as a reference for starch digestion. Comparing these data it can be calculated that starch is digested in the small intestine completely (custard), or partly: 54% (Hylon VII) and 51% (Novelose). These data show that the \(^{13}\)CO\(_2\) response in breath can be used as a monitoring system for starch digestion of \(^{13}\)C enriched starch.
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