Breath analysis—a key to understanding intestinal function

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Summary

For many years clinicians have used odour of patients' breath as a key to understanding metabolic processes and the dysfunction thereof. In recent times, breath as an end product of metabolic processes has been largely ignored. This is, in part, due to the difficulty in analysing specific components in the breath or the need to use radioactive markers. The development of affordable mass spectrometers and gas chromatographs that have greater sensitivity and improved through–put, coupled with an increasing array of substrates labelled with stable nucleides, has opened the door to using breath as an expired metabolic product to monitor disease and nutrient assimilation. A major but simple innovation has been the development of analytical kits that can be used at remote centres and then posted back to a central laboratory. These will allow measures of nutrient digestion and gastrointestinal health to be made in animals 'on farm'. Breath tests should prove to be a powerful analytical tool for nutrition research and veterinary diagnostics if they are used against a background of nutritional and clinical observations.

Introduction

The investigation of digestive processes and absorption of food has relied, and still relies, on nutrient balance studies. However, many dietary supplements or novel ingredients have subtle effects on intestinal function resulting in alterations in digesta pH, microbial populations, barrier function and absorptive surface area. All of these, either individually or combined, affect feed digestion and nutrition, and hence susceptibility to disease. The effects of incomplete digestion of carbohydrates and its consequences in pigs, poultry and horses have been reviewed recently (Pluske *et al.* 1997). However, carbohydrates may have beneficial effects on intestinal health. Indeed, fructo–, gluco– and mannan–oligosaccharides have been reported to be protective against pathogen colonisation and their subsequent translocation as well as reducing the anti–nutritive effects of lectins, as reviewed by Iji and Tivey (1998). Understanding the action(s) on the intestinal tract for any given ingredient usually involves invasive processes, including fistulation, endoscopy, or the collection of intestinal samples either by biopsy or post–mortem (Butler 1996). These techniques may introduce stress factors that are known to affect digestive physiology or represent investigations of pathologies.

The difficulties in studies on animals are compounded when we investigate similar problems in humans. This is particularly true for paediatrics, when the application of invasive techniques is limited to individuals with serious gastrointestinal disease. Therefore, one of the goals in gastroenterology has been the development of non–invasive protocols to assess and monitor digestive tract physiology and patho–physiology with minimum impact to the individual. This has driven the development of Breath Tests (Butler 1996; Swart and van den Berg 1998). Acceptance of breath test analysis is increasing, for example breath hydrogen is now considered the 'gold standard' for diagnosis of lactose intolerance in the paediatric population (Robb and Davidson 1987). Such tests rely on specific substrates being ingested that, on metabolism, produce gases which are freely diffusible and can be carried by the blood stream to the lungs for excretion.

Adaptation of such technology to nutrition research in man and animals as well as veterinary diagnostics is in its infancy, though there are indications of the potential of breath analysis in cattle (Mcleary *et al.* 1997), horses (Bracher *et al.* 1995), pigs (Zentek 1992) and companion animals (Papasouliotis 1998). Our aim in this paper is to review the current breath test technologies, their potential application to understanding digestive physiology, and future developments.

Determinants of digestion and assimilation of nutrients

Digestion of nutrients and maintenance of a healthy gastrointestinal tract require co–ordination of digestive processes (inclusive of microbial populations), mucosal integrity and mucosal immunity. This concept is based on the Kyberplus system for diagnosis of gastro–intestinal diseases that cause maldigestion or malabsorption (Schutz *et al.* 1998). In this model, digestive capacity is an integration of residence time, enzyme secretion, absorptive mechanisms, microbial activity, surface area and barrier function (Figure 1). Such variables change with the animal age, and for pigs have been reviewed by Cranwell (1995) and Le Dividich *et al.* (1998); they are also influenced by type and quantity of nutrients or anti–nutrients in the diet. For example, pathological reactions against gluten or soy protein in man, cattle and pigs result in enteropathies (Kilshaw and Slade 1982; Li *et al.* 1990; Trier 1993). The impact of these is a failure to thrive (Figure 1).

New data indicate that one in every 300 Europeans suffers from either symptomatic or latent gluten–sensitivity (Pruessner 1998). Sub–clinical situations produce continuing health concerns due to increased intestinal permeability, reduced digestive capacity, and hence impaired nutritional balance. Such conditions tend to make the individual susceptible to bacterial infections and possibly increase sensitivity to dietary antigens (Ferguson 1994). Similar situations probably occur in the animal kingdom, possibly accounting in part for the observed variability in energy digestion in poultry (Hughes and Choct 1997) or a transient hypersensitivity to diet at weaning (Li *et al.* 1990). A.G. Cummins (pers. comm.) has demonstrated in rats that weaning leads to a physiological inflammation resulting in the expansion of the intra–epithelial lymphocyte population, and an activation of the mucosal immune system as judged by interleukin 2 receptor expression (IL2R) (Masjedi *et al.* 1999). This results in a controlled remodelling of the villus–crypt axis, but an inappropriate activation may lead to structural changes that lie between the normal and the total villus atrophy seen in gluten– or soy–sensitive enteropathies (Figure 2). The effects that such changes have on digestive processes has been difficult to quantify due to the lack of experimental or diagnostic tools that can detect altered intestinal function. Breath tests now provide a possible way forward to assess such situations. Initially this will be confined to experimental work but, once proven, will be adaptable to on–farm assessments, allowing routine monitoring of animal health or responses to dietary changes which reduce performance and that would otherwise go undetected until slaughter.

Principle of breath analysis

Analytical methods to determine breath hydrogen (H_2), methane (CH_4) and carbon dioxide (CO_2) to monitor gastrointestinal function were first introduced in 1960s (Butler 1996). H_2, CH_4 and CO_2 tests differ fundamentally in the origin of the gas, with the former two being a monitor of bacterial fermentation, whereas CO_2 is predominantly, although not exclusively, produced by the animal's metabolism. The use of ^{13}C stable isotope

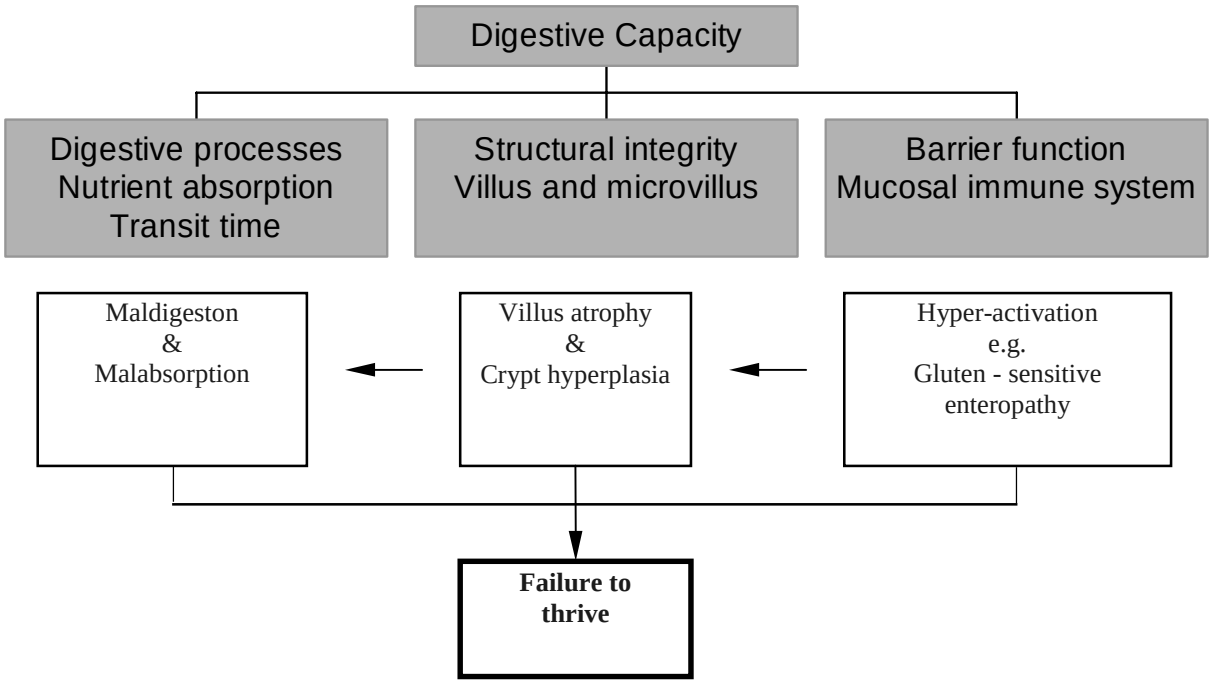

Figure 1 Adaptation of the Kyberplus concept (Schutz *et al.* 1998) for diagnosis of gastrointestinal diseases to assess digestive processes (shaded boxes). Hypersensitivity to food which alters gastrointestinal function, resulting in a failure to thrive, is shown in the open boxes.

tests for either tissue or bacterial tests is illustrated in Figure 3, typical examples of such tests being for fat absorption (using, for example, ^{13}C–triolein) and for upper intestinal infections like *Helicobacter pylori* (e.g. ^{13}C–urea), respectively. Resultant enrichment curves show clear time dependence of $^{13}CO_2$ appearance in the breath. Either a reduction or an enhancement may be observed, depending on the level of nutrient digestion by the animal or the number of bacteria present in the intestinal tract. Therefore, timing and level of gas production are important parameters allowing digestive site and capacity to be estimated. However, if assessing functional capacity is the primary goal, then the cumulative gas production for a given period is the usual analysis applied. This allows greater discrimination in the determination of normal as compared with compromised intestinal function.

Following the first reports of breath tests, whether based on H_2, CH_4 or CO_2, a wide range of tests has been subsequently developed targeting specific features of intestinal and liver function (Table 1). This listing is not exhaustive. For CO_2 Breath Tests the development of new tests has been possible following the observation that an individual at rest produces CO_2 at a uniform rate, thereby allowing interval breath collection for the assessment of absorption and metabolism of the test substrate. Presently, the urease test for *Helicobactor pylori* infections and breath H_2/CH_4 for lactose intolerance are increasingly being used. Adoption of breath tests has been limited due to either the lack of awareness or the lack of equipment. The latter is particularly true for ^{13}C based tests. In addition, there is also the perception that breath tests (BT) do not allow investigations of physiological processes in a mechanistic manner i.e. the 'black box' problem as described by Rating and Langhans (1997). Using ^{13}C–glucose BT as an example, they show that a reduced $^{13}CO_2$ accumulation in the breath may result from either reduced absorption from the intestinal tract or decreased glucose metabolism in diabetes. However, breath testing using an appropriate substrate should be performed against a background of clinical or experimental observations (including physiological status, activity level and recent food history) to prevent the possibility of misinterpretation.

Potential of breath tests

Motility of the gastro–intestinal tract

The rate at which food moves through the intestinal tract is critical to the digestive processes. For humans, motility tests for gastric emptying and oral–caecal transit time employ techniques like gamma scintography (Arganyi 1995) or magnetic resonance (Kunz 1998). These are sensitive tests that give results with high resolution, but they require individuals to be tested where the imaging equipment is located and to ingest radioactive substrates. In addition, determining the fluid and solid phase emptying has been problematic. Breath tests utilising ^{13}C labelled substrates, e.g. octanoic acid, have been successfully used to estimate gastric emptying for liquid meals in mice (Figure 4, adapted from Symond *et al.* 1998). Once in the duodenum, ^{13}C–octanoic acid is rapidly absorbed and oxidised to liberate $^{13}CO_2$. The use of this substrate as a marker of gastric emptying of solid food has provide difficult in mice (data not shown). This is most likely due to incomplete incorporation of the label into the solid phase of the diet. However, this disadvantage can be overcome by selecting another marker. Figure 4, also depicts the estimation gastric emptying for a solid meal using a feed pellet containing 18% casein, components of the diet having naturally elevated levels of ^{13}C. This carbon is released through digestive processes in the small intestine. In these cases it is the time dependence of the $^{13}CO_2$ appearance in the breath that is being monitored, rather than the enrichment with $^{13}CO_2$. Estimates of half–times for emptying were increased six fold with solid

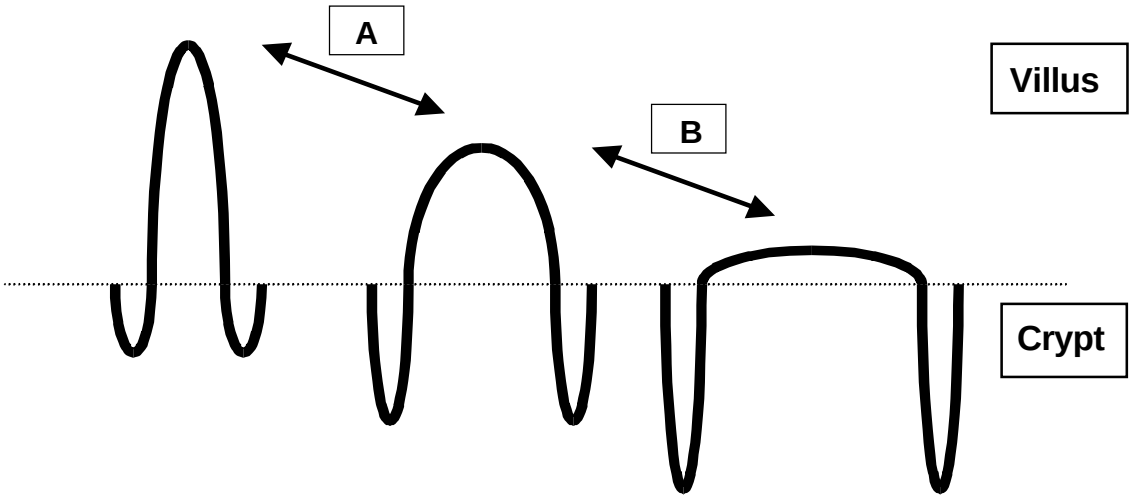

Figure 2 Representation of how the crypt–villus axis is influenced by either an appropriate (A) or hyper–activation (B) of the mucosal immune system upon the introduction of solid foods at weaning. Dotted line represents the crypt–villus junction.

foods compared to fluid diets: 148 and 25.5 minutes respectively (Symond *et al.* 1998). Measures of oro–caecal transit time can be made by using non–absorbable but fermentable substrates, e.g. lactulose or lacto–ureide (Table1). Thus breath test technology allows estimates of gastric emptying and oro–caecal transit times to be made either between animals on a given diet or intra–animal with varying diets. Such methods allow rapid determination of compartmental emptying of the gastrointestinal tract that cannot be made from marker retrieval in faeces, which takes 5–10 days in animals.

Assessment of small intestinal and absorptive processes

Investigation of intestinal nutrient absorption in humans involves faecal collections over extended periods which are often poorly tolerated by patients. Similarly, nutrient balance studies with animals require faecal collection over several days. In addition, for energy balance studies, it is difficult to separate the contribution of processes in the small intestine from those in the large intestine.

For fat absorption studies in humans, breath tests using ^{13}C–labelled substrates offer an attractive alternative to three day faecal collections because they provide a simple test that can be performed in a single day with the minimum of discomfort to the individual. Reliability of such tests requires establishing appropriate doses of substrate and meal type. Davidson and co–workers (1998) investigated these factors in an open cross–over study in 15 patients with a mean age of 10.75 years. The preferred substrates for fat absorption are triolein and mixed triglyceride (MTG). However, data from this study (Figure 5) indicate that triolein showed considerably more ($P<0.05$) variability when given with a solid diet than did MTG. Further, the assessment of absorption using MTG was meal independent. This may relate to differences in digestion of these fats and the absorption of resulting fatty acids or metabolism of the fatty acids by the individual, and illustrates the need for careful selection of substrate. However, this study does show the potential of the ^{13}C–lipid breath test when using both substrates to assess the influence of meal composition on the digestion and absorption fats of varying composition.

Absorption tests are not limited to fats and have been extended to proteins and carbohydrates (Table 1). Evenepoel *et al.* (1998) have demonstrated the application of ^{13}C labelled substrates to protein digestion and absorption in humans. Processing of the protein influences the ^{13}C recovered in the breath; raw protein yielded significantly lower levels than cooked

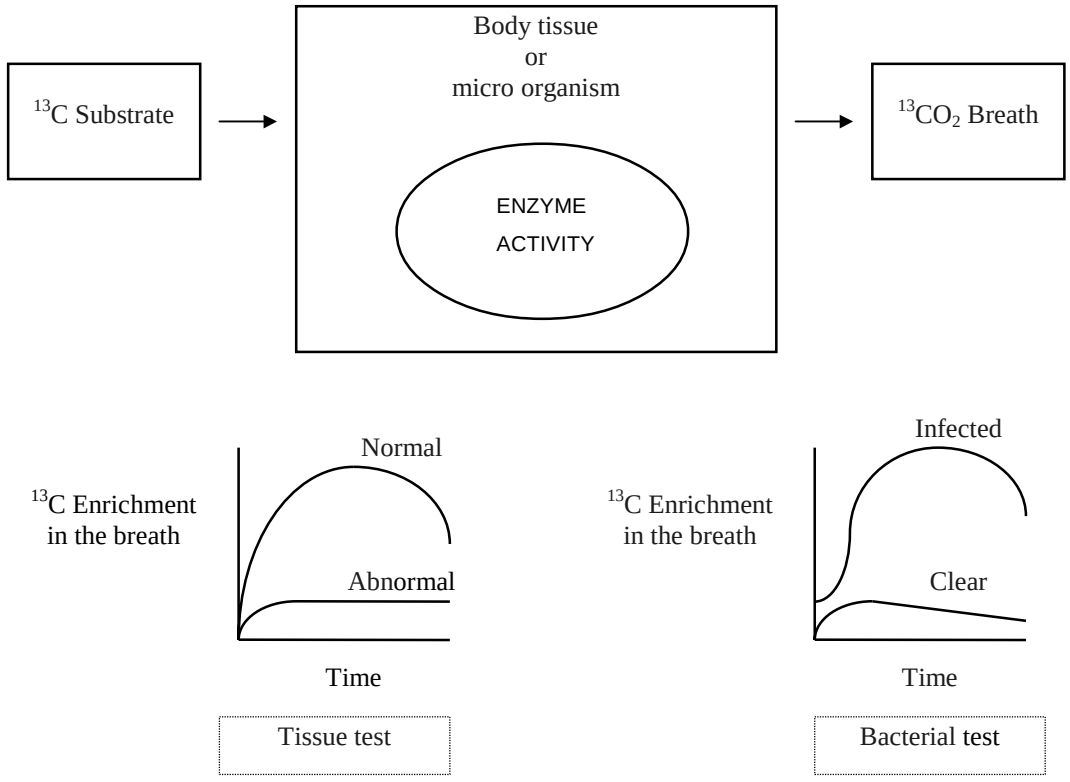

Figure 3 Principles of $^{13}CO_2$ Breath Tests; theoretical ^{13}C enrichment curves. Animals are given a single oral dose of ^{13}C–substrate which is labelled at a target bond. Breath samples are collected at timed intervals and ^{13}C enrichment is measured using an Isotope Ratio Mass Spectrometer (IRMS). Ideal excretion curves are illustrated for a tissue test (assessment of animal function) and a bacterial test (assessment of infection or overgrowth).

protein. The authors of this study also demonstrated a highly significant negative correlation between breath $^{13}CO_2$ and the recovery of exogenous protein in ileal effluent, indicating the potential of the breath test to estimate true ileal digestibility of protein

Carbohydrate tests have been limited due to their dependence on hind gut fermentation processes. As such, they are a malabsorption rather than absorption test. There are two main disadvantages: first, the individual may not be a hydrogen or methane producer and hence a false positive to carbohydrate absorption may result; second, the level of gas production does not correlate to the degree of malabsorption (Rating and Langhans 1997), a positive colonic gas production indicating only that malabsorption has occurred. The advent of ^{13}C–labelled carbohydrates, both simple and complexed, allows the degree of absorption to be assessed and hence provides a measure of intestinal capacity for uptake of this group of nutrients as well as a method for assessing the impact of diet manipulations such as enzyme supplements (Weaver 1995).

Large intestinal function and microbial activity

The resident population of colonic microflora ferments carbohydrates and produces a mixture of gases (H_2,CH_4, H_2S) and organic acids, the profile reflecting the type of bacteria present. Such metabolic activity provides a valuable salvage process for energy as seen in patients with short bowel (Nordgaard 1994). King *et al.* (1998) hypothesised that the fermentation profile may be involved in the pathogenesis of irritable bowel syndrome (IBS). This author reported that IBS sufferers produce significantly more hydrogen than non–sufferers and that both symptoms and hydrogen production were reduced when 'exclusion diets' were consumed by patients. This reduction may be associated with a change in hydrogen–utilising bacteria. These reports serve to highlight the importance of colonic processes in both health and disease. Monitoring colonic gas production after challenge with a fermentable carbohydrate, e.g. lactulose (Table 1), may provide a powerful method to assess the impact of dietary change on metabolic processes in the hindgut. Such changes may be the challenge of weaning or the introduction of a new type of grain into the diet. The effect of weaning is illustrated in Figure 6 (unpublished data, pers. comm. R. Templeman). The gas production shifts with age from being predominately methane to mainly hydrogen. The significance of such change is

Table 1 Breath tests currently used in Gastroenterological and Hepatological research and diagnosis. Adapted from Rating and Langhans (1997) and Swart and van den Berg (1998).

Functional Test	Substrate
Motility	
Gastric emptying	^{13}C – Octanoate, Bicarbonate
Oro–caecal	*Lactulose
Digestion and absorption	
Lipase	^{13}C – Mixed Triglyceride, ^{13}C – Triolein
Amylase	^{13}C – Starch
Lactase	^{13}C – Lactose
Sucrase	^{13}C – Sucrose
Protein	^{13}C – L–leucine enriched proteins
Carbohydrate	^{13}C – Fructose, Glucose, Galactose
Lipid	^{13}C – Oleic acid
Malabsorption	
Carbohydrate	*Simple and complex carbohydrates
Protein	^{13}C – Labelled protein
Lipids	^{13}C – Lipids
Hepatic	
Liver glycogen storage	^{13}C – Carbohydrate
Hepatic cytochrome P450	^{13}C – Aminopyrine
Hepatic cytochrome P448	^{13}C – Caffeine
Infection	
Helicobacter Pylori	^{13}Urea
Small Intestinal Bacterial Overgrowth	*lactulose, ^{13}C – xylose, ^{13}C – glycoholic acid

*Unlabeled substrates are used to monitor bacterial fermentation as detected by H_2 and/or CH_4 production.

still to be elucidated. However, the lower gas volume when hydrogen is bound in methane as compared with it remaining as molecular hydrogen is significant, potentially protecting the pre–weaned/weaned animal from colic due to carbohydrate escaping digestion in the small intestine.

Future developments

The future of breath testing for the assessment of gut function is promising. Many of the advances in recent times have resulted from the need to assess individuals using techniques that are either non–invasive or have minimal impact. This has created an interesting challenge which has been addressed most vigorously in medicine, especially in Paediatric Gastroenterology. The next step is to increase the application of this powerful technology, as well as extending what we can measure in the breath. An immediate application is nutrition research and assessment of nutrient interactions. This can be achieved with technology that we currently have at hand. Future developments to extend analytic capability on the types of components measurable and on the quantities detectable is within reach. Mass spectrometry utilising 'soft – chemical ionisation' originally designed to measure organic material in interstellar dust is being applied to the analysis of breath (Spanel and Smith 1996). Selective ion flow tube technology (SIFT), as it is known, will

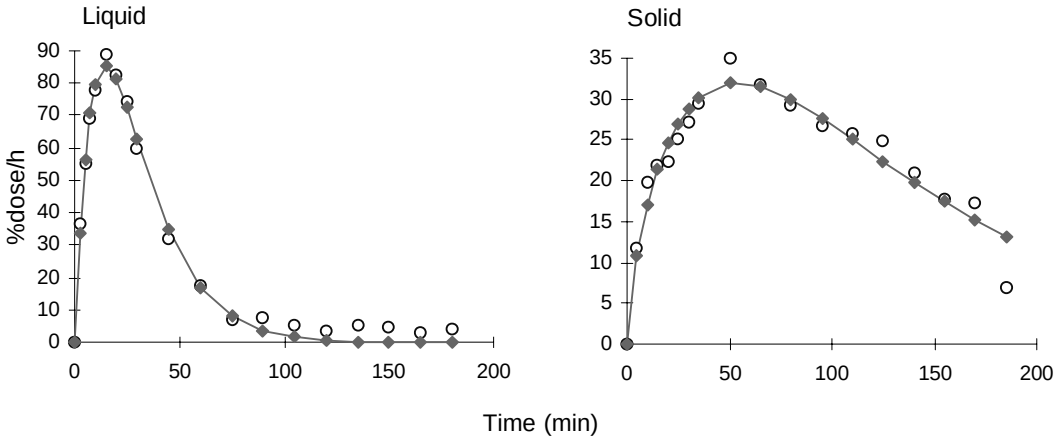

Figure 4 Assessment of gastric emptying. Typical excretion curves for $^{13}CO_2$ derived from either octanoic acid when presented in a liquid meal (a) or from a meal of 18% casein pellets (b) Open circles represent measured data while solid diamonds represent the fitted data. Correlation coefficients calculated between measured and fitted data ranged for liquid meals (a) from 0.984 – 0.997 (n = 7) and for solid diets (b) from 0.721 – 0.943 (n = 4). Data adapted from Symond *et al.* (1998).

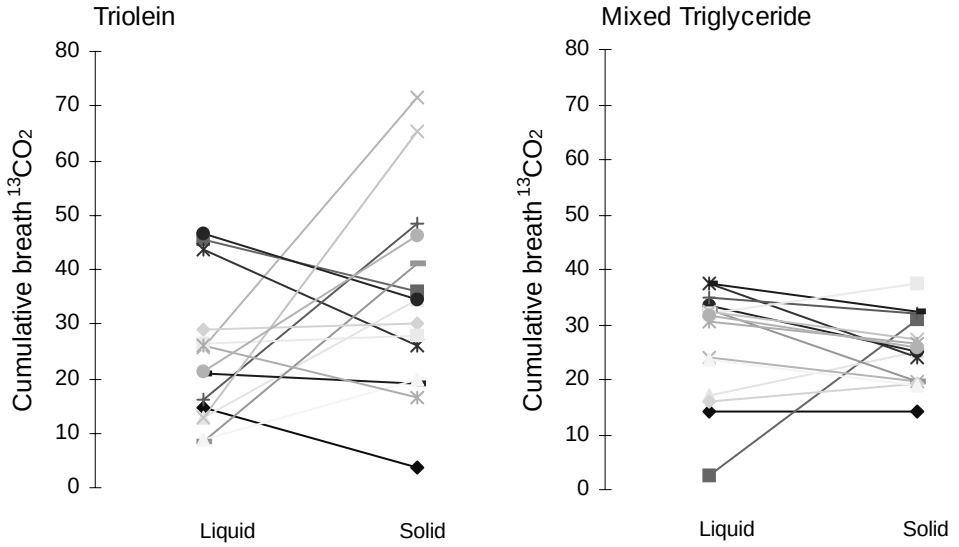

Figure 5 Cumulative breath $^{13}CO_2$ production over 4h after ingestion of ^{13}C–Trolein or ^{13}C–mixed triglyceride in either a liquid or solid meal. Subjects had a mean age of 10.75 ± 2.83 (± SD, n = 15) years and received 400 mg of labelled substrate in their meals. Data adapted from Davidson *et al* (1998).

allow complete analysis of breath composition in a single sample and at sensitivities hitherto thought impracticable. Profiles of trace gases may be indicative of particular conditions or metabolic processes. To date, studies employing such technology have been limited, and confined to medical diagnostics. Application of such technology to animal research in conjunction with present day tests should prove very powerful in elucidating real–time responses to any given dietary or medical intervention.

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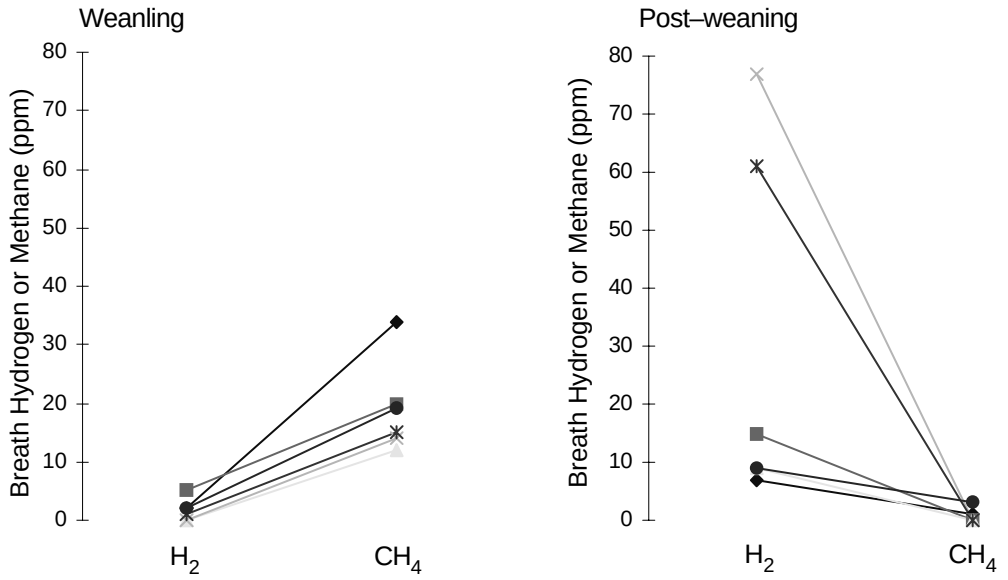

Figure 6 Hydrogen and methane excretion in the breath of weanling (21 d old) and weaned (35 d old) rats. All rats were challenged with 50 ml of a 5% lactose solution/gram body weight. Breath samples were taken on 15 occasions over 4 hours. Values represent the cumulative gas production, and data linked by solid lines are those for an individual animal. All animals were tested at both ages.

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