Assessment of the rate of solid-phase gastric emptying in ponies by means of the ^{13}C-octanoic acid breath test: a preliminary study

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Summary

The aim of this study was to assess the feasibility of applying the ^{13}C-octanoic acid breath test for assessment of gastric emptying in ponies by investigating the pattern of ^{13}C enrichment in breath following the administration of a test meal ± ^{13}C-octanoic acid. After a 14 h fast, the ponies received either no meal (*Test I*) or a standardised test meal labelled with 0 mg (*Test II*), 125 mg (*Test III*), 250 mg (*Test IV*) or 500 mg (*Test V*) ^{13}C-octanoic acid. For each test (I–V), exhaled breath samples were collected in duplicate at 1 h and immediately before ingestion of the test meal and at frequent intervals thereafter for 12 h. Breath samples were analysed by continuous flow isotope ratio mass spectrometry. Three indices of breath ^{13}C-enrichment were computed; half dose recovery time ($t^1/_2$), gastric emptying coefficient (GEC) and time to peak breath ^{13}C-enrichment t(max). For *Tests I* and *II*, the ratio of $^{13}CO_2$:$^{12}CO_2$ remained stable for the duration of the sampling period. For *Tests III, IV* and *V*, an increase in the ratio of $^{13}CO_2$:$^{12}CO_2$ was detected. The test was reproducible within individuals, and intersubject variation was low. Further validation studies of this noninvasive technique are justified.

Introduction

The analysis of exhaled breath is an investigative method utilised increasingly in human gastroenterology (Swart and van den Berg 1998). Typically, such tests involve detection of either a gas produced endogenously, or an isotope excreted following the administration of a labelled substrate. The ^{13}C-octanoic acid breath test (^{13}C-OBT) was developed recently as a noninvasive and nonradioactive technique for measurement of the rate of gastric emptying of solids in human medicine (Ghoos *et al.* 1993). The ^{13}C-OBT is based on the detection of ^{13}C enrichment in breath following the ingestion of the ^{13}C-labelled substrate, octanoic acid. Octanoic acid is a medium chain fatty acid that is absorbed rapidly and completely in the duodenum and oxidised in the liver to produce CO_2, which enters the bicarbonate pool and is excreted in the breath (Maes 1994). Gastric emptying is the rate-limiting step in the process of absorption and metabolism of octanoic acid, and the appearance of the label in the exhaled breath is a direct reflection of the rate and pattern of gastric emptying.

In the horse, the rate of gastric emptying of liquids has been assessed successfully using the acetaminophen absorption test (Doherty *et al.* 1998), impedance epigastrography (Baker and Gerring 1994a) and phenol-red dye dilution (Baker and Gerring 1994b; Sosa-León *et al.* 1997). However, delayed gastric emptying is not always detectable in the liquid phase since different mechanisms control the gastric emptying of solids and liquids (Parkman *et al.* 1995). In man, radioscintigraphy is the gold standard method for assessment of gastric emptying. Although this technique has been used successfully to assess liquid and solid phase gastric emptying in the horse (Sojka and Cantwell 1988; Levy and Sojka 1992; Neuwirth 1994; Ringger *et al.* 1996), it involves the administration of radioactive material, requires expensive imaging equipment and can be performed only at a limited number of research facilities worldwide.

The ^{13}C-OBT may be a potentially useful method for clinical assessment of disordered gastric emptying in diseased animals and, as a research tool, for investigation of the effect of gastric prokinetic drugs on the rate of gastric emptying in the horse. The aim of this study was to investigate the feasibility of applying the ^{13}C-OBT for the assessment of solid-phase gastric emptying in ponies.

Materials and methods

Animals

Four mature British native-breed ponies, age 6–15 years and 200–304 kg bwt, were used. They were stabled in individual loose-boxes and maintained on a hay diet. The ponies were allowed daily free exercise in a riding arena. All animals were stabled for at least 1 month prior to inclusion in any investigations. This study was approved by the Animal Welfare and Ethics Committee of the University of Glasgow.

TABLE 1: Study design

Test No.	Meal	Substrate	No. animals
I	None	None	3
II	75 g oats 50 g bran 1 egg yolk	250 mg octanoic acid[1]	3
III	75 g oats 50 g bran 1 egg yolk	125 mg ^{13}C-octanoic acid[2]	3 (Repeated on 3 occasions)
IV	75 g oats 50 g bran 1 egg yolk	250 mg ^{13}C-octanoic acid	4 (Repeated on 6 occasions)
V	75 g oats 50 g bran 1 egg yolk	500 mg ^{13}C-octanoic acid	3

[1]Caprylic acid (n-octanoic acid)[5].
[2]Octanoic acid-1-^{13}C (minimum 99% atom % ^{13}C)[6].

Breath collection technique

The breath collection technique used in this study was validated previously as a method of breath collection for measurement of breath hydrogen in ponies (Murphy *et al.* 1998). Breath samples were collected using a plastic tube[1] (nasal tube: 40 cm long, internal diameter 12 mm) with a valve attached at one end, 2 cm from which was situated a side exit port (8FG Dog Catheter)[2] (Fig 1a). The nasal tube was inserted into the ventral nasal meatus and the animal allowed to breathe normally through the tube for a few moments (Fig 1b). The valve at the distal end of the tube served as an indicator of the period of expiration. Expiratory breath samples were taken in duplicate into 20 ml syringes (Plastipak)[3] by aspiration through the side port. The syringes were sealed with a 3 way tap[2] and samples were immediately transferred into collection tubes (Exetainer)[4] by attaching the syringe to a 19 gauge 2 inch needle[3], piercing the lid of the tube and then opening the 3 way tap to allow the breath sample to enter the evacuated tube.

Test meal

The basic test meal consisted of 75 g oats, 50 g bran and 70 ml water. The carbon substrate was added to an egg yolk which was then baked in a microwave oven before being mixed with the test meal. The octanoic acid substrate is lipophilic and very soluble in egg yolk, and baking increases retention of the substrate in the solid-phase (Maes 1994).

Study design

Following an overnight fast (12–14 h), the ratio of ^{13}C:^{12}C excretion in breath was determined over a 13 h sampling period. The ponies ingested either no test meal (*Test I*), a test meal with 250 mg octanoic acid (Caprylic acid: n-octanoic acid)[5] (*Test II*), or a test meal with 125 mg (*Test III*), 250 mg (*Test IV*) or 500 mg (*Test V*) ^{13}C-octanoic acid (Octanoic acid-1-^{13}C [minimum 99% atom %^{13}C][6]; Table 1). Breath samples were collected in duplicate at 1 h (t = -60 min) and immediately before (t = 0 min) ingestion of the test meal, and then every 15 min for 4 h, every 30 min for another 4 h and every 60 min for a further 4 h. The ^{13}C concentration in duplicate breath samples were compared to assess the accuracy of the breath sampling method. *Test IV* was repeated on 6 occasions on each pony under identical conditions of meal composition, study design and animal management.

All samples were analysed within 4 weeks of collection, and at least 1 week was allowed between tests. Animals had access to water at all times during the test, but access to food was denied until the end of the sampling period. The ponies remained at rest in their loose-boxes throughout the sampling period, and their CO_2 production was assumed to remain constant at 0.156 l/m^2 body surface area/min (Orr *et al.* 1975). Body surface area was calculated using a formula derived for use in ponies, body surface area (m^2) = 10.5 x bwt (g)$^{2/3}$/10,000 (Orr *et al.* 1975).

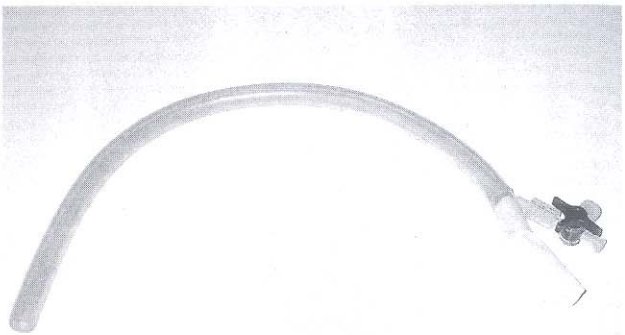

Fig 1a: The nasal tube used for breath collection in these investigations.

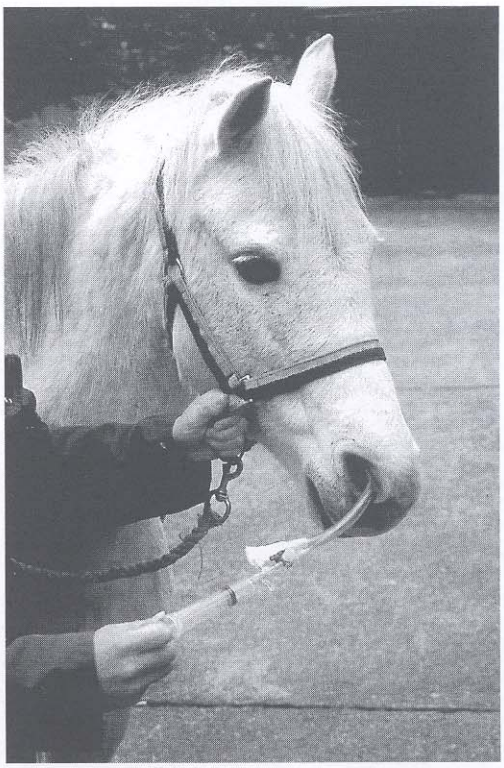

Fig 1b: The tube was inserted into the ventral nasal meatus and the pony was allowed to breathe through the tube on a number of occasions, before breath samples were taken in 20 ml syringes, through the side port at the distal end of the tube.

TABLE 2: Reference ranges of the gastric emptying breath test parameters following ingestion of 250 mg ^{13}C-octanoic acid on 6 occasions by 4 individuals. GEC, the gastric emptying coefficient, $t^1/_2$ the half dose recovery time and t(max), the time of maximal breath ^{13}C-enrichment

	GEC	$t^1/_2$ (h)	t(max) (h)
Mean	3.21	2.60	1.64
Standard deviation	0.40	0.42	0.36
Range	2.28–4.17	1.76–3.28	0.99–2.4
Percentile 75	3.30	2.94	1.91

^{13}C measurement

^{13}C-enrichment was measured with reference to a 3% CO_2 (balance N_2) gas standard that had been independently calibrated against an international standard. ^{13}C abundance is expressed in units of parts/million (ppm) and enrichment is expressed in units of ppm excess ^{13}C, having subtracted the average ^{13}C-abundance of the predose (baseline) breath samples. All samples were analysed within 4 weeks of collection by continuous flow isotope ratio mass spectrometry (IRMS) (ABCA)[7].

Data analysis

Analysis of test results utilised the mean of duplicate measurements taken at each time point. Samples with CO_2 concentrations less than 0.3% were rejected, as they were not considered to be samples of expiratory breath and, in these cases, a single replicate measurement was used. Results were expressed as either % dose administered recovered per hour, (PDR), or ppm ^{13}C-enrichment.

The $^{13}CO_2$ excretion curve (% dose recovered per hour against time), was plotted using the formula:

$$y = at^be^{-ct}$$

where y is the percentage of the ^{13}C dose recovered in breath per hour (PDR, %/hour), t is the time in hours and a, b and c are regression constants. This equation is derived from the χ^2 statistical distribution and was previously described for modelling the ^{13}C-octanoic acid gastric emptying curve in man (Maes 1994). The Solver function of a Microsoft Excel computer programme was used to calculate values for a, b and c using nonlinear regression analysis.

Three variables were calculated using the plotted ^{13}C-enrichment curve, the gastric emptying coefficient (GEC), the time to peak breath ^{13}C-enrichment (t(max)) and the half dose recovery time ($t^1/_2$). The GEC was defined as a global index of the rate of gastric emptying calculated as the natural log of a (ln a) (Maes 1994). The half dose recovery time ($t^1/_2$) was determined by analysing the area under the fitted cumulative $^{13}CO_2$ excretion curve and calculating the time at which half of the recovered ^{13}C dose has been excreted in the breath. The half dose recovery time was calculated using the Excel function Gammainv (0.5;b + 1;1/c), and t(max), was calculated as b/c (Ghoos *et al.* 1993; Maes 1994).

Analysis of variance (ANOVA) was used to perform statistical analyses for significance and to estimate the reproducibility of the test. A further measure of reproducibility was provided by the calculation of the intersubject coefficients of variation (CV% = [s.d./mean] x 100).

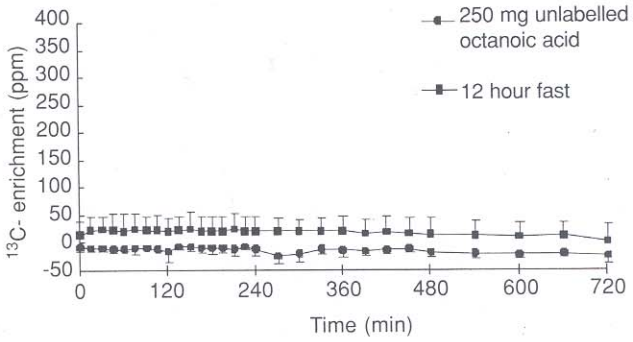

Fig 2: Mean excretion of ^{13}C (± s.d.) for all animals (n = 3) over the 12 h test period following a 12–14 h fast (Test I) *and following ingestion of a test meal and unlabelled substrate (250 mg octanoic acid)* (Test II).

The models derived using the 250 mg (*Test IV*) and 500 mg doses (*Test V*) were used to examine the effect of reducing the duration and frequency of sampling on the gastric emptying indices, by repeating mathematical curve fitting using longer sampling intervals and shorter sampling durations. The recalculated gastric emptying indices were compared with the original values (that is, where all the data for a single test was used) using a Students *t* test for paired samples. Results were expressed as the mean ± s.d. Values of $P<0.05$ were considered significant.

Results

All animals ingested the test meals within 5 min of presentation. For all tests performed, there was a good correlation (rho = 0.92) between the $^{13}CO_2$ levels of the replicate breath samples (n = 1161) taken at each time point, indicating the reliability of the collection method. The pattern of $^{13}CO_2$ excretion remained constant over the 12 h sampling period following both the fasted test (*Test I*) and the ingestion of the unlabelled test meal (*Test II*). Mean ^{13}C-enrichment over the 12 h sampling period was 20.8 ± 5.03 ppm (mean ± s.d.) in the fasted study (*Test I*) (Fig 2), and -11.44 ± 5.45 (mean ± s.d.) following ingestion of the test meal and unlabelled octanoic acid (*Test II*) (Fig 2). There was a negative shift in baseline associated with the ingestion of the unlabelled test meal (*Test II*).

In *Tests III*, *IV* and *V*, ingestion of the labelled substrate (^{13}C-octanoic acid) was associated in all cases with significant increases above baseline levels of $^{13}CO_2$ excretion, and these increases were proportional to the dose ^{13}C-octanoic acid administered (Fig 3). In 33% of cases, the ^{13}C-enrichment curve described a phasic pattern, with the recovery of ^{13}C represented by 2 or more peaks (Fig 5).

The reproducibility of the test was assessed by comparing the parameters calculated in 6 separate tests on 4 individual animals (Table 2, Fig 4). There was no significant difference between the replicate tests in each animal (intrasubject variation), P = 0.55, 0.77 and 0.93 for GEC, t(max) and $t^1/_2$, respectively. The intersubject variation was not significant, P = 0.70, 0.48 and 0.09 for GEC, t(max) and $t^1/_2$, respectively. Intrasubject variation was described using the coefficient of variation and the mean variation in the 3 gastric emptying parameters for all animals is shown in Table 3.

The possibility of simplifying the test protocol by reducing the sampling frequency was examined by comparing the gastric emptying indices derived using progressively increasing intervals between samples in the first 4 h of the test. Reducing

TABLE 3: Coefficients of variation % ([s.d./mean] x 100) of the gastric emptying breath test parameters in healthy ponies - GEC, the gastric emptying coefficient, $t^1/_2$ the half dose recovery time and t(max), the time of maximal breath ^{13}C-enrichment (250 mg ^{13}C-octanoic acid ingested by 4 ponies on 6 occasions)

	GEC	$t^1/_2$ (h)	t (max) (h)
Pony No. 101	19.6%	12.4%	20.6%
Pony No. 102	13.9%	25.3%	33.8%
Pony No. 103	5.1%	6.6%	8.5%
Pony No. 104	8.3%	10.3%	20.8%
Mean	11.7%	13.6%	20.9%

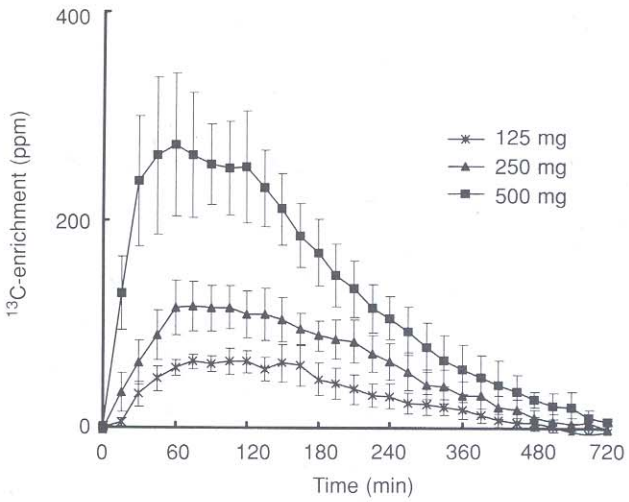

*Fig 3: Mean excretion of ^{13}C (± s.d.) for all animals over the 12 h test period, following ingestion of a test meal and 125 mg (*Test III, *n = 3), 250 mg (*Test IV, *n = 4) and 500 mg (*Test V, *n = 3) labelled substrate (^{13}C-octanoic acid).*

sampling frequency from every 15 min to every 30 or 60 min resulted in significant aberration of the test results (probability values are shown in Table 4). The possibility for reducing the test duration from 12 h was also examined in the same way, by recalculating the gastric emptying parameters while progressively shortening the test duration. Reducing the test duration from 12 to 6 h did not have a significant effect on the parameters of gastric emptying (Table 5).

Discussion

The results of this present study confirmed that ^{13}C-OBT is a safe, simple and reproducible technique which may prove useful for the assessment of gastric emptying in ponies.

Because $^{13}CO_2$ breath tests are carried out against a natural isotopic abundance of approximately 1.1% ^{13}C in exhaled CO_2, it was necessary to establish baseline levels of $^{13}CO_2$ excretion in the pony, before attempting to enrich ^{13}C excretion by administration of an exogenous ^{13}C-substrate. The natural variation in ^{13}C-abundance in breath is a reflection of the source of endogenous fuel, which is affected by the metabolic activity (Schoeller *et al.* 1980). In order to minimise the natural variation in the isotopic abundance of ^{13}C in breath, the ponies in this study were kept at rest during the test protocol. When $^{13}CO_2$ was monitored over 12 h in ponies kept under these conditions and fasted, there was no significant fluctuation in the natural fasting isotope ratio of breath CO_2. This finding verified that, under the conditions in this study, the possible effect of background variation in $^{13}CO_2$:$^{12}CO_2$ on the ^{13}C-octanoic acid breath test is negligible (Maes 1994), and confirmed the potential for enrichment of breath $^{13}CO_2$ in ponies.

The test meal functioned essentially as a carrier for administration of the ^{13}C-octanoic acid substrate and it was important that the test meal did not, in itself, provide a source of ^{13}C that could interfere with the signal produced by the

TABLE 4: Comparison of gastric emptying parameters calculated using different sampling intervals over the first 4 h. The mean difference between the 2 sampling intervals, s.d. and P value for each test are shown

	GEC			$t^1/_2$			t(max)		
Minutes	Mean difference	s.d.	P	Mean difference	s.d.	P	Mean difference	s.d.	P
15–30	0.62	0.85	0.0001	0.39	0.49	0.16	0.93	0.82	0.01
15–60	1.91	0.27	0.0001	1.21	0.48	0.0001	2.23	0.78	0.001

GEC = gastric emptying coefficient; $t^1/_2$ = half dose recovery time (h); t(max) = time of maximal breath ^{13}C-enrichment (h); P = probability.

TABLE 5: Comparison of gastric emptying parameters calculated using different test durations. The mean difference between the 2 test durations, s.d. and P value for each test are shown

	GEC			$t^1/_2$			t(max)		
Hours	Mean difference	s.d.	P	Mean difference	s.d.	P	Mean difference	s.d.	P
12–10	0.02	0.08	0.86	0	0	1.0	0	0	0.99
12–8	0.01	0.01	0.96	0	0	1.0	0.02	0.02	0.92
12–6	0.09	0.06	0.47	0.01	0.02	0.94	0.13	0.10	0.39
12–4	0.36	0.17	0.01	0.06	0.08	0.64	0.38	0.23	0.01

GEC = gastric emptying coefficient; $t^1/_2$ = half dose recovery time (h); t(max) = time of maximal breath ^{13}C-enrichment (h); P = probability.

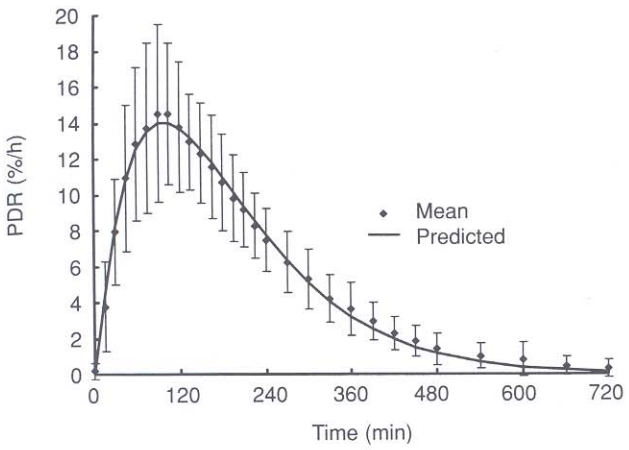

*Fig 4: Breath excretion of ^{13}C over a 12 h test period, following ingestion of a test meal labelled with 250 mg ^{13}C-octanoic acid (*Test IV*), on 6 separate occasions by 4 ponies. Each data point represents the mean PDR/h (percentage dose recovered/h) ± s.d. of 24 individual tests at each time point.*

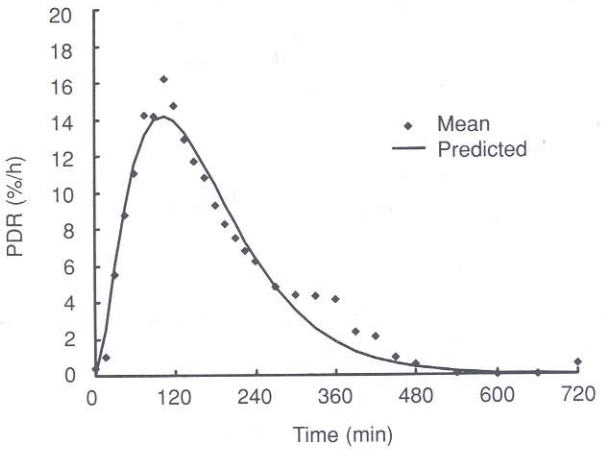

*Fig 5: Sample ^{13}C-excretion curve illustrating typical dual phase pattern seen in 8 of 24 gastric emptying curves analysed. (*Test III, *pony No. 101, Replicate 1). The percentage of the ^{13}C dose recovered (percentage dose recovered, PDR) is plotted over the 12 h test duration.*

metabolism of the ^{13}C-octanoic acid tracer. The metabolism of a test meal produces a shift in background ^{13}C-abundance, that is a reflection of the enrichment above or below natural abundance of the carbon contained in the meal (Schoeller *et al.* 1980). There was no significant change in $^{13}CO_2$ excretion in the 12 h following ingestion of an unlabelled test meal in any of the ponies in this study. Although there was a slight negative shift in baseline $^{13}CO_2$ associated with ingestion and metabolism of the test meal (indicating enrichment below natural atomic abundance (Schoeller *et al.* 1980), this change was unlikely to affect the detection of the test signal. This finding confirmed that the test meal was an appropriate vehicle for administration of the labelled substrate. It was concluded that neither the ingestion of the test meal, nor the fasting protocol, induced a change in the rate or pattern of $^{13}CO_2$ excretion that could have deleterious effects on the detection of the signal provided by the labelled substrate.

All animals exhibited significant increases above baseline levels of $^{13}CO_2$ in exhaled breath following ingestion of a test meal incorporating either 125 mg, 250 mg or 500 mg of the labelled substrate, ^{13}C-octanoic acid. The pattern of excretion was similar to that reported in human subjects, and the mathematical model derived by Maes (1994) to describe the human gastric emptying curve was able to fit the pony data obtained in this present study. The concentration of ^{13}C-enrichment above baseline in this study, was proportional to the concentration of labelled substrate administered, an indication of the complete and rapid absorption and oxidation of octanoic acid in the pony.

In this study, mathematical analysis of the ^{13}C-excretion curves from ponies allowed the calculation of various parameters that describe the rate and pattern of gastric emptying. These indices have correlated well with parameters describing gastric emptying derived using standard radioscintigraphic methods in human studies (Ghoos *et al.* 1993). In human studies, a correction factor (given the value 66 min) has been derived that allows direct comparison of results of the ^{13}C-octanoic acid breath test with quantitative methods of assessing gastric emptying, such as radioscintigraphy (Maes 1994). It was not thought appropriate to use this factor for studies in this study; however, calculation of such a correction factor by correlating the gastric emptying breath test with scintigraphy in horses would allow direct comparison of equine breath test and scintigraphic data.

The mean gastric emptying coefficient recorded for the ponies in this study was within the reference range reported for human subjects (Ghoos *et al.* 1993); the overall rate of gastric emptying as determined by the $t^1/_2$ values was faster in ponies than in man (Ghoos *et al.* 1993). However, differences in the calorific densities and volumes of test meal administered limit direct comparisons between equine and human data. The calculation of the gastric emptying parameters, $t^1/_2$, t(max) and the gastric emptying coefficient (GEC) permitted an analysis of the repeatability of the ^{13}C-octanoic acid breath test among the ponies in this study. There was no significant difference in the gastric emptying parameters within individuals, and the mean intrasubject coefficient of variation was low, 11.7% for the GEC and 13.6% for $t^1/_2$. Similar intrasubject coefficients of variation for the same indices have been obtained using the ^{13}C-octanoic acid breath test in human subjects (n = 16), 10.82% for the GEC and 26.71% for $t^1/_2$ (Ghoos *et al.* 1993). There was no significant difference between subjects in this study, but some studies report significant intersubject variation in gastric emptying parameters when gastric emptying was assessed in man, using the ^{13}C-octanoic acid breath test (Choi *et al.* 1997a,b), and using radioscintigraphy (Brophy *et al.* 1986). The high level of reproducibility of this test between the individual ponies in this study may be a reflection of the uniform diet and exercise regimens under which the animals were maintained, conditions that would be difficult to reproduce in a human trial.

One disadvantage of the use of the ^{13}C-octanoic acid breath test in ponies was that the initial protocol followed in this study was very time-consuming (13 h), and would be a major impediment to the use of the test as a clinical diagnostic tool. An investigation of the feasibility of reducing the sampling frequency and length of the test revealed that, in the 4 healthy ponies in this study, the test period could be reduced from 12 to 6 h without significantly affecting the gastric emptying parameters. However, the initial 4 h period of sampling at 15 min intervals could not be reduced without significantly affecting the results of the test; and this finding was consistent

with studies of sampling intervals of the ^{13}C-octanoic acid breath test in man (Maes 1994; Choi *et al.* 1997b). The results of the ^{13}C-octanoic acid breath test are a reflection of the dynamic process of gastric emptying that is, by definition, best described by interval assessment over a long test period. In the present study, the least time consuming and most accurate protocol was found to consist of 4 h of sampling at 15 min intervals, followed by 2 h of sampling at 30 min intervals. Reducing the sampling frequency and test length in this way makes the test less labour-intensive, as well as reducing substantially the costs associated with sample analysis, but further study is necessary to establish an appropriate protocol for application of the breath test in animals with delayed gastric emptying.

The pattern of gastric emptying in the ponies in this study often (33%) described a phasic pattern with the passage of the substrate out of the stomach represented by 2 or more peaks. This apparent phasic pattern could have been produced by intragastric separation of the meal components (oats and bran), possibly due to their respective particle size or caloric density. It is possible that reflux of the labelled octanoic acid marker from the duodenum to the stomach could explain the phasic pattern of gastric emptying observed in this study. The gastric pH of the foal has been shown to undergo periods of 'spontaneous alkalisation', thought to result from reflux of ingesta and bile from the duodenum into the stomach (Baker 1992). Experimental evidence to support this theory was provided by the demonstration that peaks of duodenal activity coincided with gastric motor quiescence and an increased gastric pH (Gerring 1991). It is also possible that the phasic pattern may reflect the pattern of the migrating motor complex (MMC). The MMC is not interrupted by feeding in the horse (Ross *et al.* 1990) and the second peak seen in these studies may have been caused by the onset of phase III activity. The pattern of solid-phase gastric emptying in dogs was reported in one study to be phasic, with peaks of output in the 1st, 3rd, 4th or 5th postprandial hour, and this was thought to represent reappearance of the interdigestive MMC (Becker and Kelly 1983). Similar double peak patterns were found in the plasma cimetidine curves of dogs following oral administration of cimetidine (Oberle and Amidon 1987). The double peak in plasma cimetidine was thought to represent the cyclic gastric motility of the interdigestive MMC in the dog, since cimetidine is poorly absorbed in the stomach and has a short plasma elimination half-life (Oberle and Amidon 1987). Since the interdigestive MMC persists in the fed state in the horse (Ross *et al.* 1990), it is conceivable that the phase of the MMC during which a tracer for assessing gastric emptying is administered could affect the rate and pattern of the derived gastric emptying curve.

The advantage of the ^{13}C-octanoic acid breath test over other methods for the assessment of gastric emptying in the horse is primarily that it is a method for assessment of solid-phase gastric emptying, that is noninvasive, nonradioactive and could be carried out away from the analytical centre. The carbon isotope ratio in breath samples remained stable in sealed tubes for 60 days (Schoeller *et al.* 1977) and samples can be mailed to the laboratory for analysis. Because there is no radiation burden or toxic effect associated with the test substrate or protocol, serial tests can be performed in one individual, to monitor progress during a disease, or the effects of a drug. Furthermore, the test meal was identical to normal food, and was ingested voluntarily by the ponies in this study. Interpretation of the results of the ^{13}C-octanoic acid breath test can be completely automated, unlike radioscintigraphy, where the necessity for operator interpretation confers an inevitable degree of subjectivity on the test.

Although the results of the ^{13}C-octanoic acid breath test have correlated well with reference methods in human studies, comparison of radioscintigraphy and the ^{13}C-octanoic acid breath test should be carried out in the horse. This comparison would establish that there is no limiting step in the absorption of octanoic acid other than the rate of gastric emptying, such as might occur if octanoic acid could be absorbed through the equine stomach. The nonradioactive and noninvasive nature of the ^{13}C-OBT make it an attractive method for clinical application. However, the number of animals used in this study was small (n = 4) and establishment of reference ranges in a larger group of animals will be necessary before clinical utilisation is possible. The rate of solid-phase gastric emptying was detected with good repeatability between and within animals, suggesting that this method may have useful applications in research.

This preliminary study produced very promising data, that suggest that the ^{13}C-octanoic acid breath test is a simple method for the assessment of solid phase gastric emptying in the horse. Further validation of this method is now justified.

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Manufacturers' addresses

[1]Arnolds, Shropshire, UK.
[2]Rocket of London, Watford, UK.
[3]Becton Dickinson UK Limited, Oxford, UK.
[4]Labco Ltd., Bucks, UK.
[5]Sigma Chemicals, St. Louis, Missouri, USA.
[6]Isotec Inc., CK Gas Products Ltd., Wockingham, Berkshire, UK.
[7]Europa Scientific, Crewe, UK.

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