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Validation of the ^{13}C-octanoic acid breath test for measurement of equine gastric emptying rate of solids using radioscintigraphy

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Keywords: horse; gastric emptying; radioscintigraphy; ^{13}C-octanoic acid; breath test

Summary

Reasons for performing study: **Disordered gastric motility may be a significant factor in the pathogenesis of many equine conditions. Although tests for liquid phase emptying rate have been validated in the horse, there are no effective tests for solid phase emptying measurement that can be performed routinely in the field.**

Objectives: **The objective of this study was the assessment of a novel stable isotope technique, the ^{13}C-octane acid breath test (^{13}C-OABT), for the measurement of gastric emptying of solid ingesta, by direct comparison with the optimum method of gastric scintigraphy.**

Methods: **To facilitate dual measurement of gastric emptying, a test meal was used containing baked egg yolk labelled with both ^{13}C-octanoic acid and ^{99m}technetium sulphur colloid. Simultaneous, serial lateral gastric scintigraphs and expiratory breath samples were obtained in 12 healthy horses after voluntary ingestion of the test meal. Analysis of breath $^{13}CO_2$:$^{12}CO_2$ ratio was performed by continuous flow isotope ratio mass spectrometry. Power regression was used to determine the gastric emptying coefficient, the gastric half-emptying time ($t_{1/2}$) and duration of the lag phase (t_{lag}).**

Results: **Significant correlations ($P<0.001$) were found between the 2 techniques for measurement of both $t_{1/2}$ and t_{lag}. In addition, scintigraphic left $t_{1/2}$ was correlated significantly to breath test gastric emptying coefficient ($P<0.001$).**

Conclusions: **It was concluded that the ^{13}C-octanoic acid breath test is a reliable diagnostic procedure to measure gastric emptying rate of solids in the horse.**

Potential relevance: **Being safe, noninvasive and easy to perform, this test has potential value as both sensitive diagnostic modality and humane research tool for motility studies.**

Introduction

Recent advances in stable isotope tracer production and analytical capacity have resulted in a rapid expansion in the use of stable isotope breath tests in clinical medicine for the investigation of physiological and metabolic functions. The ^{13}C-octanoic acid breath test (^{13}C-OABT) was first validated for the measurement of gastric emptying of solids in human subjects using radioscintigraphy (Ghoos *et al.* 1993). Subsequently, it has been validated by further research groups (Duan *et al.* 1995; Ziegler *et al.* 1996; Choi *et al.* 1998) and by a European multicentric trial (BIOMED 1 - Project PL93-1239; Delbende *et al.* 1998) and is now the diagnostic modality of choice for disorders of the upper gastrointestinal tract in children, pregnant women and critical care patients (Ritz *et al.* 2001).

The rationale of the technique is that, following ingestion of a ^{13}C-octanoate-labelled meal, the tracer leaves the stomach without being metabolised. It is then absorbed rapidly by the small intestine and undergoes hepatic oxidation, leading to production of $^{13}CO_2$, which is exhaled (Swart and van den Berg 1998). Since these post gastric events are constant in rate, the change in $^{13}CO_2$:$^{12}CO_2$ ratio in expiratory breath is dependent on the rate of gastric emptying of the tracer, as this is the rate-limiting step (Ghoos *et al.* 1993). Hence, the rate of appearance of $^{13}CO_2$ in the breath after ingestion of a solid phase-labelled test meal may be used to measure the rate of gastric emptying of solids.

Disordered gastric motility may be a significant factor in the pathogenesis of conditions such as gastric ulceration, equine dysautonomia, post operative ileus, gastric impaction and idiopathic recurrent colic. Previous methods of assessing solid phase gastric emptying in the horse have included transit measurements of administered plastic beads (Adams and MacHarg 1985) or polystyrene spheres (Milne *et al.* 1996), radiographic tracking of radiopaque markers (Baker and Gerring 1994) and scintigraphy following nasogastric intubation with radiolabelled solids (Sojka and Cantwell 1988; Levy and Sojka 1991; Neuwirth 1994; Ringger *et al.* 1996). The ^{13}C-OABT offers several advantages over all of these techniques; it is noninvasive, safe (nonradioactive), easy to perform, free from user interpretation and requires minimal equipment at the site of application. If validated, the ^{13}C-OABT would have potential value as both a sensitive diagnostic tool in equine gastroenterology and a humane research tool for future motility studies. In order to validate the ^{13}C-OABT in the horse for measurement of gastric emptying of solids, it was compared here with the currently accepted reference method of gastric scintigraphy (Merritt 1999).

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Materials and methods

Subjects

Twelve mature horses from the Texas A&M University research herd were used in accordance with Animal Use Protocol 2000-112 approved by the University Laboratory Animal Care Committee. These animals had no known history or physical evidence of gastrointestinal disease. Potential subjects were rejected if biochemical or total blood cell parameters were found to lie outside the reference range. The horses (4 mares, 8 geldings) were of mixed breed (7 Quarter Horses, 3 Quarter Horse cross, one Thoroughbred, one Arabian), median age 8 years (mean 11.1, range 2–25 years) and median bodyweight 467.2 kg (mean 469.4, range 347.0–563.4 kg). Ivermectin anthelmintic[1] was administered orally at least 2 weeks prior to the procedure and all subjects were maintained on *ad libitum* alfalfa hay only for at least 2 weeks prior to each experiment, having previously grazed Coastal Bermuda grass at pasture.

Test meal composition

The test meal consisted of 150 g crimped oats, 100 g bran, 200 ml water and 2 dual-labelled egg yolks. For each test, approximately 1 mg/kg bwt ^{13}C-octanoic acid (Octanoic acid-1-^{13}C, minimum 99 atom % ^{13}C)[2] and 14–19 mCi ^{99m}technetium sulphur colloid (in-house preparation)[3] was added to 2 egg yolks, baked in a microwave oven until firm and mixed thoroughly into the test meal.

Study design

Following an overnight fast (14 h) to ensure an empty stomach, the test meal was ingested voluntarily in the nuclear medicine room. Gastric radioscintigraphy and expired breath collection for isotope analysis were performed concurrently and the horse was restrained manually until the protocols for both procedures were completed. For all experiments, the protocol, equipment and personnel were the same. Each horse was measured once only.

^{13}C-octanoic acid breath test

Breath collection: This was performed using a modified Aeromask[4] fitted with a 250 ml aluminium coated polyethylene bag[5]. The horse was allowed to breathe several times through the mask before the bag (fitted with a unidirectional valve) was attached. Duplicate expiratory samples were transferred from this bag to 10 ml Exetainer[6] tubes, prior to stable isotope analysis. Three basal breath samples were collected 60, 15 and 0 mins before test meal ingestion (-60, -15, 0 mins) and thereafter at 15 min intervals for 6 h, then 30 min intervals for a further 4 h.

^{13}C abundance: The ^{13}C:^{12}C ratio of each breath sample was determined by automated continuous flow isotope ratio mass spectrometry (ABCA analysis)[7] and expressed relative to an international standard. This ratio was converted to parts per million (ppm) ^{13}C and expressed as ppm excess ^{13}C, after subtraction of the average ^{13}C-abundance of the 3 baseline (predose) breath samples. The percentage dose recovery (PDR) of the administered isotope in the breath was also calculated and plotted against time, as described by Wyse *et al.* (2001).

Gastric radioscintigraphy

Serial left and right lateral 30 secs gastric scintigraphs were obtained at 15 min intervals, until the radioactive counts per 30 sec interval in the region of interest had decreased to less than 10% of that at time zero. Each region of interest was drawn freehand around the gastric activity and the total counts in the region were obtained using Nuclear Mac software[8]. Since ^{99m}technetium has a short $t_{1/2}$, counts were corrected for radioactive decay of the isotope using the formula:

$$A = A_0 e^{-\lambda t}$$

where A = corrected count at time t, A_0 = initial count, λ = decay constant ($0.693/t_{0.5}$ with $t_{0.5}$ = half-life of isotope) and t = image acquisition time in minutes after time 0. The left- and right-side counts and their geometric mean (square root of the product of left and right) were calculated at each time point.

Effect of diet on basal ^{13}C expiratory output

In a preliminary experiment to assess the stability of basal metabolic ^{13}C production under test conditions, this parameter was measured in 4 of the 12 horses maintained on the test diet (alfalfa hay alone). To determine dietary influence on basal ^{13}C output, the results were compared with those in 5 separate horses maintained on a diet of Coastal Bermuda grass (*Cynodon dactylon*). The latter is a C_4 plant and relatively enriched with ^{13}C (Boutton 1991). The same test protocol and test meal as described above were used but without the addition of either isotope to the meal. Breath samples were collected and analysed according to the given protocol and ^{13}C abundance expressed as ppm ^{13}C against time.

Calculation of gastric-emptying indices

Radioscintigraphic test data: The counts in the gastric region of interest from the left and right series and the geometric mean were plotted separately against time as the percentage of retained radioactivity after decay correction. Siegel's formula (Siegel *et al.* 1988) was used to fit a modelled curve to the data, from which indices of gastric emptying were calculated:

$$y(t) = 1 - 1(1-e^{-kt})^{\beta}$$

where y(t) is the fractional meal retention at time t, k is the gastric emptying rate/min, t is the time interval in minutes and β is a rate constant. The best fit curves and hence the unknown constants were calculated by a nonlinear least squares regression analysis programmed in a Microsoft Excel Solver[8] function. The scintigraphic gastric half-emptying time ($t_{1/2s}$) is equivalent to that time at which the area under the modelled curve demonstrates loss of half of the administered radioactive dose and was calculated from the equation:

$$t_{1/2s} = -\ln[1 - 2^{-1/\beta}]/k$$

In addition, the scintigraphic lag phase prior to gastric emptying, t_{lags}, was calculated. This is equivalent to $\ln\beta/k$, where $\beta>1$ indicates an initial lag period prior to emptying and $\beta<1$ indicates rapid early emptying.

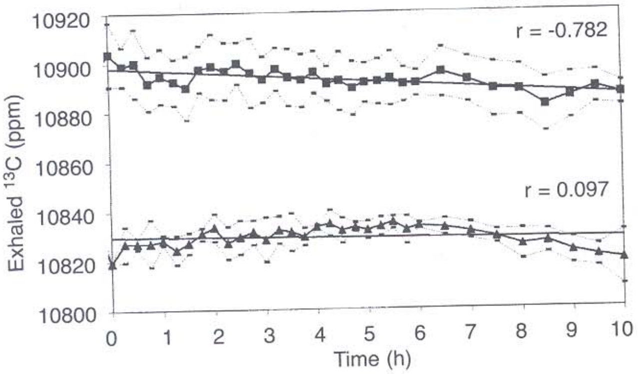


Fig 1: Comparison of mean ± s.d. basal ^{13}C output in horses maintained on Coastal Bermuda grass (■), n = 5, with those maintained on alfalfa hay (▲), n = 4. Mean expiratory ^{13}C values are plotted against time over the 10 h period after ingestion of the test meal at time 0. ^{13}C ppm is significantly higher at each timepoint for the Bermuda grass group ($P \leq 0.0025$). The r values represent the Pearson correlation coefficient with time.

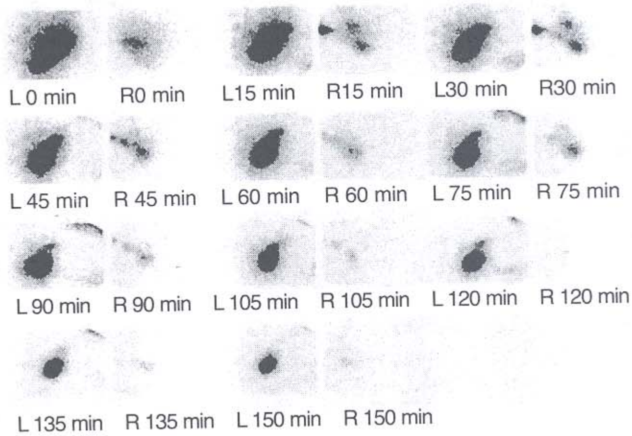


Fig 2: Paired gastric scintigraphic images (L = left; R = right) from horse with rapid gastric emptying rate (scintigraphic half-emptying time [$Lt_{1/2s}$]= 0.70 h). Serial images are illustrated, each collected for 30 sec at 15 min intervals. The right-side region of interest loses definition at 105 min post ingestion of the radioisotope in this subject.

^{13}C-octanoic acid breath test data: All samples containing less than 0.5% CO_2 were rejected to minimise analytical inaccuracies. Data were plotted against time as either the ppm excess ^{13}C, or the percentage of the isotopic dose recovered per hour (PDR/hour). The formula of Siegel *et al.* (1988) was modified to fit the breath test data as follows:

$$y(t) = m(1 - e^{-kt})^{\beta}$$

where y(t) is the cumulative percentage of ^{13}C excretion in breath at time t and m is the total cumulative ^{13}C recovery when time is infinite. The gastric emptying coefficient (GEC), which reflects the gradient of the emptying curve, was also calculated from the breath test data, using the formula described by Ghoos *et al.* (1993).

Pharmacokinetic data on $^{13}CO_2$ appearance in breath

In order to determine the pharmacokinetics of ^{13}C-octanoic acid absorption and metabolism, the dynamics of $^{13}CO_2$ appearance in the breath after direct intraduodenal administration of ^{13}C-octanoic acid were also determined in 3 of the horses used for the validation study. Food was withheld for 14 h prior to sedation with xylazine (0.5 mg/kg bwt). After gastroscopic location (Olympus)[9] of the pyloric antrum, a polythene cannula was passed into the duodenum. Octanoic acid-1-^{13}C (1 mg/kg bwt) was instilled through this cannula, using 10 ml vegetable oil of low natural ^{13}C abundance (canola oil) as a carrier. Breath samples were collected immediately before instillation and afterwards at 5 min intervals for the first hour, 10 min intervals for the second hour and then 15 min intervals for a further 4 h. Each horse was maintained in stocks throughout and allowed access to water but not to feed.

Statistical evaluation of results

Effect of diet on basal ^{13}C expiratory output: Two-tailed *t* tests were used to compare the mean basal output of ^{13}C at each time for the 2 groups of horses maintained on different diets. Relationship of this parameter with time was studied by linear regression analysis.

Validation of ^{13}C-OABT vs. scintigraphy: The relationships between the gastric emptying indices obtained by scintigraphy ($t_{1/2s}$, t_{lags}) and the data gained from the breath test analyses ($t_{1/2b}$, t_{lagb} and GEC) were evaluated by Pearson correlation and linear regression. Bland Altman statistics were used to demonstrate the effect of measurement magnitude on the correlation between the 2 different methods (Bland and Altman 1995, 1999) and 95% confidence limits were established. The relationship between the 3 scintigraphic data sets (left, right and geometric mean) was also evaluated by correlation and linear regression.

Univariate analysis was performed on each of the scintigraphic and breath test parameters in the 12 subjects to establish a reference range for each parameter. In addition, the coefficient of variation (s.d./mean x 100%) was calculated for each variable.

Results

Basal ^{13}C production studies showed that the horses maintained under test conditions (on an alfalfa hay diet) prior to assessing basal ^{13}C output had a very stable ^{13}C:^{12}C expiratory ratio in the measured period after unlabelled test meal consumption. The collated mean ± s.d. ppm excess ^{13}C in this group after test meal consumption was small (1.54 ± 6.21 ppm excess ^{13}C). In contrast, the horses maintained on coastal grass had a significant reduction in basal ^{13}C output over time (Fig 1) with a Pearson correlation coefficient of -0.782. The coastal grass group also produced a significantly higher mean basal ^{13}C output than the alfalfa test subjects (10893.05 ± 10.21 vs. 10829.14 ± 6.17 ppm ^{13}C; $P<0.0005$) over the 10 h test period.

Scintigraphic measurement of gastric emptying and determination of the gastric region of interest was most easily and accurately performed from the left-sided gamma camera images. Radioactive counts/30 s interval were on average 6.86 times greater for the left region of interest than the right side at time zero (s.d. 1.72, range 4.30–9.50). This ratio was not correlated to either body mass or body surface area. In addition to the low counts, accurate measurement of the right side region of interest was often made more difficult by superimposition of radioactive small

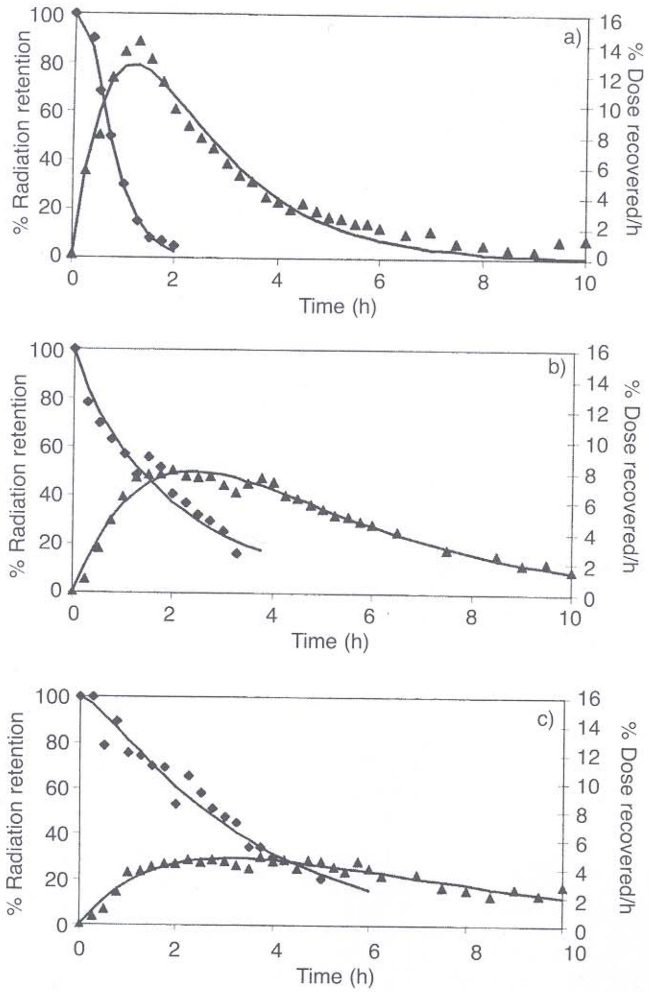


Fig 3: Results of simultaneous ^{13}C-octanoic acid breath test and gastric radioscintigraphy in 3 typical cases. (A) Rapid gastric emptying rate ($t_{1/2s}$ = 0.73 h); (B) Normal gastric emptying curve ($t_{1/2s}$ = 1.34 h); (C) Slow gastric emptying pattern ($t_{1/2s}$ = 2.62 h). Scintigraphic data (◆) on the left y-axis and breath test data (▲) on the right y- axis are plotted against time. The continuous lines represent the modelled data, calculated using the formula of Siegel et al. *(1988).*

intestine or ascending colon during the test (Fig 2). The correlation between gastric half-emptying times measured from the left and right regions of interest was relatively low (r = 0.714, P<0.05). Therefore, gastric emptying indices calculated from the left side were used for validation of the ^{13}C-OABT data.

Figure 3 illustrates solid phase gastric emptying as measured by simultaneous radioscintigraphy and ^{13}C-octanoic acid breath test. Three typical examples are shown, ranging from rapid (Fig 3a) to typical (Fig 3b) to slow (Fig 3c) gastric emptying rate. The mean gastric emptying indices in the 12 subjects, as measured by the 2 different techniques, are shown in Table 1. With both scintigraphic and ^{13}C-OABT assessment, there was considerable range in individual $t_{1/2}$ values (0.56–4.21 and 1.99–6.74 h, respectively). This parameter was not correlated to gender, age of animal, bodyweight or body surface area. The coefficient of interindividual variation was, therefore, relatively high for both scintigraphic $t_{1/2}$ ($Lt_{1/2s}$) and breath test $t_{1/2}$ ($t_{1/2b}$) and higher for the established measurement technique of scintigraphy (69.23 and 40.37%, respectively).

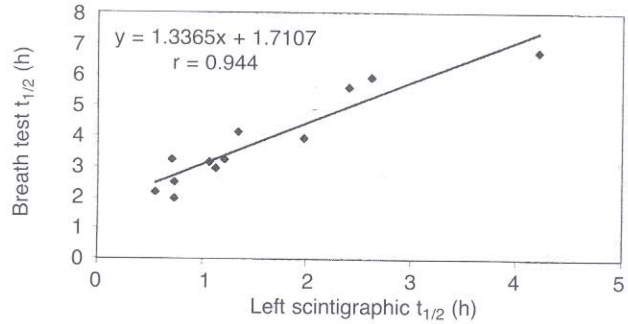


Fig 4: Comparison of gastric half-emptying times determined by ^{13}C-octanoic acid breath test ($t_{1/2b}$) with those determined by scintigraphy ($Lt_{1/2s}$) in all individuals (n = 12). Best regression model is shown.

TABLE 1: Univariate analysis showing mean, s.d., range and CV% (coefficient of interindividual variation) for parameters of gastric emptying derived by radioscintigraphy and ^{13}C-octanoic acid breath test in 12 healthy horses

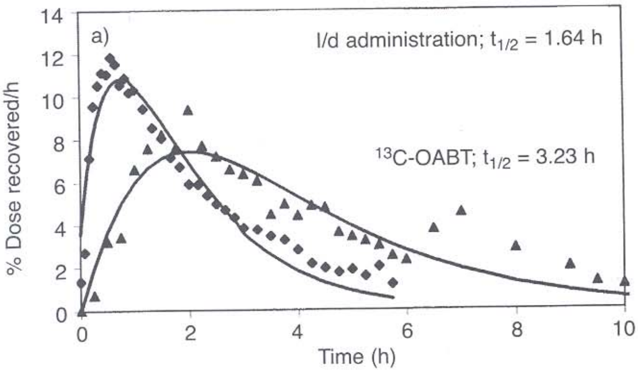
Measurements	Mean ± s.d.	Range	CV%
Scintigraphy			
Left side $t_{1/2}$ (h)	1.56 ± 1.08	0.56–4.21	69.23
Right side $t_{1/2}$ (h)	1.16 ± 0.69	0.18 –2.19	60.09
Geo. mean $t_{1/2}$ (h)	1.27 ± 0.76	0.43–2.74	59.84
Left side t_{lag} (h)	0.36 ± 1.37	-2.24–3.86	380.56
^{13}C-OABT			
$t_{1/2}$(h)	3.79 ± 1.53	1.99–6.74	40.37
t_{lag} (h)	2.32 ± 1.14	1.16–5.52	49.14
GEC*	2.09 ± 1.27	-1.50–3.35	60.48

$t_{1/2}$ = gastric half-emptying time (h); t_{lag} = duration of lag phase (h), equivalent to lnβ/k, negative if β<1, i.e. when early rapid emptying occurs; GEC = gastric emptying coefficient. *Siegel *et al.* (1988).

There was a strong positive correlation between the gastric half-emptying times, as determined by scintigraphy and ^{13}C-OABT (Fig 4) with a Pearson correlation coefficient of 0.944 (P<0.001). A strong negative correlation was also found between the breath test gastric emptying coefficient and $Lt_{1/2s}$ (n = 12, r = -0.930, P<0.001). Further significant correlations were found between $t_{1/2b}$ and scintigraphic geometric mean (GM) $t_{1/2s}$ (n = 12, r = 0.890, P< 0.001) and between GEC and $GMt_{1/2s}$ (n = 12, r = -0.810, P< 0.001). The correlation between the 2 techniques for determination of lag phase duration was significant only when those scintigraphic curves (3/12) with β<1 (indicating rapid early gastric emptying) were excluded (n = 9, r = 0.932, P<0.001). The numerical relationship between mean $Lt_{1/2}$s and $t_{1/2}$b was best defined by equation 1:

$$y = 1.3365x + 1.7107$$

where y = breath test $t_{1/2b}$ and x = scintigraphic $Lt_{1/2s}$. The effect of bypassing the gastric emptying stage in the production of $^{13}CO_2$, by intraduodenal gastroscopic administration of ^{13}C-octanoic acid, is shown in Figure 5. The half-time for absorption and metabolism of the isotope is similar in each case, despite the large difference in $t_{1/2b}$ measured in each subject (mean ± s.d. 1.70 ± 0.10 h). In the third gastroscopic subject, $t_{1/2}$ for recovery of the isotope was more prolonged ($t_{1/2}$ = 2.28 h), but the recovery curve described a gastric emptying pattern, suggesting misplacement or retrogradal movement of the isotope. The given $t_{1/2}$ for post gastric absorption and metabolism of the isotope was



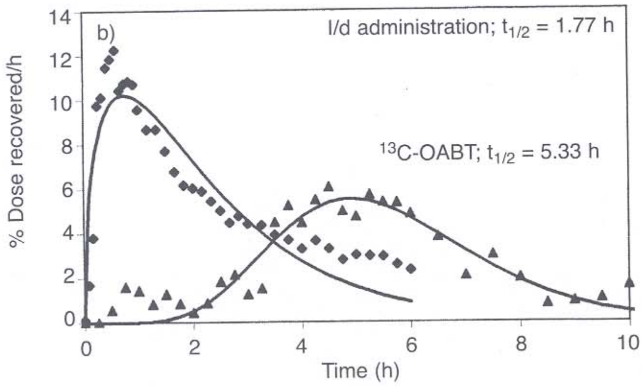


Fig 5: Intraduodenal (I/d) and ^{13}C-OABT $t_{1/2}$ in the same subjects, one with typical gastric emptying rate (a), and one with a slow rate of emptying (b). The half-time for absorption and metabolism of the isotope following intraduodenal administration is very similar in each subject. Intraduodenal (◆) and breath test (▲) stable isotope dose recoveries are plotted over the 10 h test duration. The continuous lines represent the modelled data, calculated using the formula of Siegel et al. *(1988).*

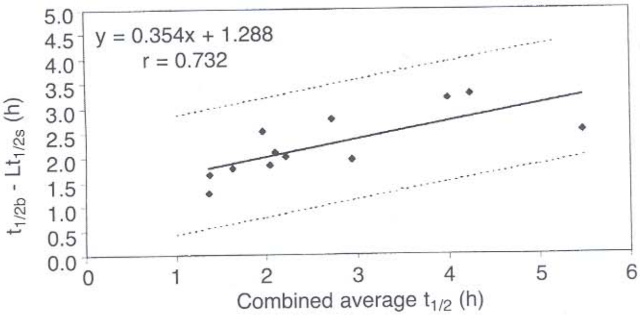


Fig 6: Bland Altman plot showing effect of measurement amplitude on mean bias (mean breath test $t_{1/2}$ ($t_{1/2b}$) - mean scintigraphic $t_{1/2}$ [$Lt_{1/2s}$]). Dotted lines represent regression-based 95% upper and lower limits of agreement. Mean difference is shown to increase with increasing measurement amplitude.

therefore similar to the point of intersection of the y axis presented in Figure 4.

Bland Altman statistics were used to investigate the observed trend in the difference in $t_{1/2}$ between the 2 methods with increasing measurement magnitude. Mean difference was plotted against average value for $t_{1/2b}$ and $Lt_{1/2s}$, which demonstrated (Fig 6) that the bias had a significant positive association with increasing magnitude. The 95% limits of agreement for this mean difference were calculated as follows:

$$D = 1.288 + 0.354A \pm 1.96 \times s.d.$$

where D = difference, A = average and s.d. = standard deviation of the mean difference (± 0.621).

Discussion

In this study, concurrent radioscintigraphic and ^{13}C-OABT measurement of gastric emptying rate in 12 healthy equine subjects revealed a strong positive correlation between the 2 techniques for the calculation of several parameters of solid phase gastric emptying. The correlation between the gastric half-emptying times derived from the 2 modalities was linear in nature, with a positive bias in the breath test that increased in size with increasing amplitude of measurement. This mean positive bias was similar in size to the empirical $t_{1/2}$ observed for absorption and metabolism of ^{13}C-octanoic acid after intraduodenal administration. Hence, in this present study, the rate-limiting step in equine $^{13}CO_2$ production after ingestion of the ^{13}C-labelled test meal appeared to be gastric emptying of the solid phase label into the small intestine. Therefore, the ^{13}C-OABT was shown to be a suitable diagnostic tool for the measurement of gastric emptying rate in healthy horses.

Before using the ^{13}C-octanoic acid tracer to measure gastric emptying rate, it was necessary to determine the basal metabolic production of $^{13}CO_2$ in the test animals after ingestion of the unlabelled test meal. The natural isotopic abundance of ^{13}C is approximately 1.1% (Boutton 1991) and endogenous production of $^{13}CO_2$ may be affected by both diet (Morrison *et al.* 2000) and metabolic fuel source (Schoeller *et al.* 1984). As predicted, the group of horses maintained on alfalfa hay (test conditions) had a constant basal production of $^{13}CO_2$ after ingestion of the unlabelled test meal, which confirmed the potential use of dietary ^{13}C enrichment. However, the horses maintained on Bermuda grass (*Cynodon dactylon*) had a higher mean basal $^{13}CO_2$ output than the alfalfa group, which decreased slightly but significantly in the 10 h period after ingestion of the test meal. Bermuda grass, being a C_4 plant, has a relatively high natural abundance of ^{13}C (Boutton 1991), higher than that of the selected test meal. Therefore, metabolism of the test meal itself was probably responsible for the shift in background ^{13}C abundance in the Bermuda grass group (Schoeller *et al.* 1980). It is recommended that when using the ^{13}C-OABT in horses, foods high in ^{13}C should be avoided when possible and a test meal of equivalent $^{13}C{:}^{12}C$ ratio as maintenance diet should be used to minimise fluctuations in basal $^{13}CO_2$ production. Should this not be practical, then a higher tracer dose of up to 1.5 mg/kg bwt ^{13}C-octanoic acid should be used in the test meal to minimise any error in calculation of gastric emptying indices.

Gastroscintigraphy is widely regarded as the optimum standard for measurement of equine gastric emptying. However, in the few scintigraphic studies reported, liquid phase emptying (Lohmann *et al.* 2000), or a combination of liquid and solid phase emptying after gastric intubation (Sojka and Cantwell 1988; Neuwirth 1994; Ringger *et al.* 1996), has been performed and a standard protocol for measurement of solid phase gastric emptying after voluntary ingestion of a standard test meal is not well described. Ringger *et al.* (1996) used geometric mean measurements to calculate scintigraphic half-emptying time and this is usually the case in small animal (Goggin *et al.* 1998) and human (Siegel *et al.* 1989; Ghoos *et al.* 1993) studies. However, in this present study, imaging of the equine right gastric region was often difficult and considered to be inaccurate, as reported previously by Lohmann *et al.* (2000). Therefore, scintigraphic gastric emptying indices calculated from the left region of interest were used preferentially for comparison with ^{13}C-OABT data.

Equine scintigraphic solid phase gastric $t_{1/2}$ has been reported to vary from mean 0.82 h (Neuwirth 1994) to 1.50 h (Levy and Sojka 1991; Ringger *et al.* 1996). The mean value in this study (1.56 ± 1.08 h, range 0.56–4.21) was comparable to that of the latter two research groups. However, direct comparison cannot be made due to the different nature of the test meal and conditions. For production of future reference values, the adoption of a standard protocol and test meal is essential. The coefficient of variation reported here for $t_{1/2s}$ (69.23%) is higher than for most human studies, but comparable to that of Levy and Sojka (1991) who gained a CV% of 53.92% in horses using a similar test meal. Interindividual CV%s for $t_{1/2b}$ (40.37%) and GEC (60.48%) were also higher than the first reported equine values derived from ponies (Wyse 1999) and most reported human values (Ghoos *et al.* 1993; Duan *et al.* 1995; Delbende *et al.* 1998). Given the range in age, weight and type of horse used here, the wide variation in gastric $t_{1/2}$ was not unexpected. However, not one of these individual factors was found to correlate significantly with gastric $t_{1/2}$ in this study. This is in contrast to human studies, where both bodyweight and body surface area have been reported to have an inverse linear relationship with gastric emptying rate (Lavigne *et al.* 1978). Further information is required on basic aspects such as the relation of equine gastric volume itself to body mass before such compounding factors can be considered further.

One further interesting phenomenon observed during the scintigraphic studies in 58% (7/12) of subjects was an occasional increase in the total radioactive counts in the left region of interest at successive time points. This was thought to have been caused by retrogradal peristaltic flow of radioactive material from the small intestine into the stomach and provides further evidence that reflux of duodenal contents into the stomach occurs regularly in the healthy horse (Kitchen *et al.* 2000).

The ^{13}C-octanoic acid breath test has been validated against radioscintigraphy for the measurement of solid phase gastric emptying in man and is now in regular clinical use for the investigation of upper gastrointestinal tract disorders (Ghoos *et al.* 1993; Choi *et al.* 1997, 1998; Delbende *et al.* 1998; Perri *et al.* 1998). In one of the first papers to report its validation for use in man, Ghoos *et al.* (1993) found a very similar relation between breath test data and gastric scintigraphy as found in this present study. To allow conversion of breath test $t_{1/2b}$ to scintigraphic $t_{1/2s}$, these researchers introduced a 'correction factor' of 1.10 h which was shown to be similar to the observed $t_{1/2}$ for absorption and metabolism of ^{13}C-octanoic acid after intraduodenal administration in 8 healthy individuals (mean = 1.03 h). Using the equine data generated here, a correction factor of 1.71 h could be used to convert breath test $t_{1/2}$ to scintigraphic $t_{1/2}$ if required. However, establishment of a standard protocol and reference range for equine ^{13}C-OABT gastric indices is in progress, which will allow this diagnostic modality to be used in isolation.

In this present study, a significant correlation ($P < 0.001$) was found between radioscintigraphy and the ^{13}C-OABT for the measurement of solid phase gastric emptying in healthy animals. Before routine clinical use of the ^{13}C-OABT in equine medicine, further validation of the test is required in horses with delayed gastric emptying, or with changes in motility induced by pharmacological manipulation. In fact, recent data (Sutton *et al.* 2002) have suggested that the ^{13}C-OABT is also useful for accurate measurement of equine delayed gastric emptying. It is therefore hoped that, in the future, this novel test will be applied to the investigation of conditions such as gastroduodenal ulceration, post operative ileus, chronic grass sickness and duodenitis/proximal jejunitis. Because the ^{13}C-OABT is noninvasive and safe, it may be used to perform serial gastric emptying studies in an individual, or to investigate the gastrointestinal effects of specific drugs in a humane manner. Sample tubes of breath may also be stored for several weeks before being sent for analysis (Schoeller *et al.* 1977) so that there is minimal equipment requirement, making this an ideal technique for field investigations.

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