Use of the ^{13}C-octanoic acid breath test for assessment of solid-phase gastric emptying in dogs

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Objective—To assess the ^{13}C-octanoic acid breath test for determining gastric emptying in dogs.

Animals—6 healthy adult dogs.

Procedure—Food was withheld for 12 hours before each test. Expired air was collected 30 minutes and immediately before each test and at frequent intervals thereafter for 6 hours. Concentration of $^{13}CO_2$ in expired air was determined by use of continuous-flow isotope-ratio mass spectrometry. Basal concentration of $^{13}CO_2$ was measured in dogs that were not fed a test meal. Effects of the standard unlabeled test meal on basal concentration of $^{13}CO_2$ were then assessed. The optimum dose of substrate was determined by measuring $^{13}CO_2$ concentration after ingestion of the standard test meal containing 50 or 100 mg of ^{13}C-octanoic acid, whereas effect of energy density of the test meal on gastric emptying was determined after ingestion of the standard or high-energy labeled test meal. Gastric emptying coefficient (GEC), time to peak $^{13}CO_2$ concentration (t_{max}), and half-dose recovery time ($t_{½}$) were calculated.

Results—Basal concentration of $^{13}CO_2$ in expired air was not significantly affected by ingestion of the unlabeled test meal. However, $^{13}CO_2$ concentration significantly increased in a dose-dependent manner after ingestion of the labeled meal. Gastric emptying coefficient, t_{max}, and $t_{½}$ were significantly different between dogs fed the standard and high-energy test meals, indicating that ingestion of a high-energy meal delays gastric emptying.

Conclusions and Clinical Relevance—The ^{13}C-octanoic acid breath test may be a useful noninvasive and nonradioactive method for assessment of gastric emptying in dogs. (*Am J Vet Res* 2001;62:1939–1944)

Disordered gastric emptying of solids is a feature of several pathologic conditions in dogs, including hypertrophic pyloric gastropathy,[1,2] pyloric stenosis,[3] gastric neoplasia,[4,5] and dysautonomia.[6] Disordered solid-phase gastric emptying has also been implicated as a primary factor in the etiopathogenesis of gastric dilatation-volvulus in dogs.[7-9]

Dogs are often used as an experimental model for investigation of gastrointestinal motility. Radioscintigraphy has been used extensively to evaluate gastric emptying of a radiolabeled test meal[10] in dogs, either by use of external scintigraphy[8,11-15] or by measuring radioactivity in gastric outflow collected via a duodenal fistula.[16-19] Radioscintigraphy is considered the gold standard for assessment of gastric emptying, but its use in veterinary medicine is limited by the requirement of specialized equipment and the radiation hazard of the radionuclide tracer. Positioning of dogs in front of the gamma camera has also presented a problem in some studies, because dogs may struggle[8] or require sedation[14] or restraint in a Pavlov sling,[13,20] each of which may affect the rate of gastric emptying.

Fluoroscopy and radiography have also been used to assess solid-phase gastric emptying in dogs. These techniques monitor gastric transit of radiopaque markers,[21] barium-impregnated polyethylene spheres,[22] or liquid barium mixed with food.[23] However, transit time of radiopaque markers may not be an appropriate indicator of transit time of solid foods, because these markers cannot be broken down by the grinding forces of the stomach. Consequently, large markers (diameter, > 2 mm) are retropelled into the proximal portion of the stomach and only empty during phase III of the migrating motor complex, whereas small markers (diameter, < 1.5 mm) empty from the stomach quickly, probably during the liquid-phase of gastric emptying. Intermediate size markers (diameter, 1.5 to 2 mm) may empty with solid food, but this varies extensively between and within dogs.[17,24] However, the use of radiopaque markers is a convenient method for detection of abnormal gastric emptying in clinical practice.[25] Assessment of solid-phase gastric emptying by monitoring the transit of liquid barium mixed with food has generated data that varies between individual animals and studies,[23,26] possibly because of separation of the liquid barium from the solid-phase.

The **^{13}C-octanoic acid breath test** (^{13}C-OBT) has been validated for assessment of solid-phase gastric emptying in humans,[27-29] and the test has recently been used to assess gastric emptying in cats,[30] mice,[31] and ponies.[a] The test involves monitoring the concentration of ^{13}C-labeled carbon dioxide ($^{13}CO_2$) in expired air collected following ingestion of a test meal mixed with

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^{13}C-octanoic acid. Octanoic acid is a medium-chain fatty acid that is rapidly and completely absorbed in the duodenum and oxidized in the liver to produce CO_2, which enters the bicarbonate pool before being exhaled.[32] Because gastric emptying is the rate-limiting step in the digestion and metabolism of octanoic acid, the amount of exhaled $^{13}CO_2$ reflects the rate and pattern of gastric emptying.[27]

Studies using radioscintigraphy and intestinal fistulation have provided valuable data on solid-phase gastric emptying in dogs under experimental conditions, but the importance of disordered solid-phase gastric emptying in gastric diseases in dogs remains poorly understood because of the lack of a simple and noninvasive test. The aims of the study reported here were to determine whether the ^{13}C-OBT can be used to monitor gastric emptying of a solid test meal in dogs and to develop a suitable breath sampling method, test protocol, test meal, and substrate dose to enable future validation and application of this test in canine medicine and research.

Materials and Methods

Animals—Six (4 castrated males and 2 sexually intact females) healthy adult dogs, weighing between 27 and 33 kg, were used in this study. Before the study, dogs were acclimatized to the breath collection procedure and the test meal. Dogs were housed in individual kennels throughout the study and fed a canine maintenance dry ration[b] when not involved in a test. The study was approved by the Animal Ethics and Welfare Committee of the University of Glasgow.

Study design—Four tests (control, substrate dose, energy density, and reproducibility) were performed. Food was withheld from all dogs for 12 hours (overnight) before each test. Dogs were then allowed 15 to 20 minutes of exercise at a walk and returned to their kennels. Duplicate baseline expiratory breath samples were collected after a 30-minute adjustment period (–30 minutes). Thirty minutes later, dogs ingested the test meal. Duplicate breath samples were collected immediately before (0 minutes) ingestion of the test meal and every 15 minutes for 4 hours and every 30 minutes for an additional 2 hours after ingestion of the test meal. For dogs that ingested labeled test meals (ie, meals containing ^{13}C-octanoic acid as the substrate), at least 1 week was allowed between tests to ensure complete washout of the tracer. Dogs had access to water at all times during each test, but food other than the test meal was withheld until the end of the sampling period. Dogs remained at rest in their kennels throughout the sampling period. Resting CO_2 production was taken as 0.194 L/m^2/min.[33] Body surface area was calculated, using the following formula derived for use in dogs:

$$\text{Body surface area (m}^2\text{)} = 10.1 \times \text{body weight (g)}^{2/3}/10{,}000^{c}$$

Test meal—The standard test meal consisted of 1 slice of whole-meal bread (36 g; 980 kJ/100 g), 200 ml of skim milk (146 kJ/100 ml), and 5 g of sunflower margarine (2,593 kJ/100 g); total energy provided by the meal was approximately 775 kJ. The octanoic acid substrate was added to an egg yolk that was then baked in a microwave oven to increase retention of the substrate in the solid phase,[d] and the baked egg yolk was added to the standard test meal. Either ^{13}C-octanoic acid[e] (labeled test meal) or octanoic acid[f] (unlabeled test meal) was used as the substrate.

Breath collection technique—Breath samples were collected, using an anesthetic mask[g] connected to a 1-way valve[h] attached to a reservoir bag.[i] The mask was fitted snuggly around each dog's muzzle, and dogs were allowed to breath normally until the reservoir bag filled with exhaled air. Expiratory breath samples were collected in duplicate into 20-ml syringes via aspiration through a 3-way tap attached to the reservoir bag. The syringes were sealed with a second 3-way tap, and breath samples were immediately transferred through a 19-gauge 50-mm needle attached to the syringe into collection tubes[j]; collection tube lids were replaced to seal the tubes. Samples were stored at room temperature until analyzed.

Determination of $^{13}CO_2$ concentration in expiratory breath samples—Breath samples were analyzed by use of continuous-flow isotope-ratio mass spectrometry[k] within 4 weeks of collection. Data obtained from the mass spectrometer were expressed as a **ratio of concentration of $^{13}CO_2$ to concentration of $^{12}CO_2$ ($^{13}CO_2$:$^{12}CO_2$)**. Thereafter, data were expressed as the absolute concentration of $^{13}CO_2$, which was calculated by comparing the measured $^{13}CO_2$:$^{12}CO_2$ with the absolute $^{13}CO_2$:$^{12}CO_2$ of an international standard (Pee Dee belemnite). Concentration of $^{13}CO_2$ in the canine maintenance dry ration and each component of the test meals were also analyzed by use of continuous-flow isotope-ratio mass spectrometry.[34] Concentration of $^{13}CO_2$ was expressed as **parts per million (ppm)** according to the following formula:

$$^{13}C = (^{13}C/[^{13}C + {}^{12}C]) \times 10^{6}$$

Enrichment of $^{13}CO_2$ in expiratory breath samples collected after meal ingestion (ie, concentration of $^{13}CO_2$ in samples after meal ingestion – mean concentration of $^{13}CO_2$ in baseline [–30 minutes] breath samples) was measured with reference to a 3% CO_2 in a nitrogen gas standard that was independently calibrated against an international standard.

Control tests—Three dogs were used to assess basal concentration of $^{13}CO_2$ in expiratory breath samples. Breath samples were collected as described, but dogs did not receive a test meal. To determine the effects of the unlabeled test meal on $^{13}CO_2$ excretion, breath samples were collected from the same 3 dogs before and after ingestion of a standard test meal containing 100 mg of octanoic acid.

Estimation of optimum substrate dose—To estimate the amount of ^{13}C-octanoic acid necessary to result in an adequate increase in concentration of $^{13}CO_2$ in expired air, 3 dogs ingested labeled test meals containing 50 or 100 mg of ^{13}C-octanoic acid during 2 separate tests. Breath samples were collected and analyzed as described.

Effect of energy density of the test meal on gastric emptying—Four dogs were used to assess the effects of energy density of the test meal on results of the ^{13}C-OBT. Energy density of the standard test meal was increased by adding an additional 25 g of sunflower margarine to the standard test meal, resulting in test meals of 2 energy densities: the standard test meal (775 kJ) and a high-energy meal (1,423 kJ). Both test meals were labeled with 50 mg of ^{13}C-octanoic acid. Each dog ingested the high-energy and standard labeled test meals 3 times in random order with at least 1 week between tests. For these tests, single breath samples were collected immediately before (0 minutes) ingestion of the test meal and every 10 minutes for 6 hours and every 20 minutes for an additional 2 hours after ingestion.

Determination of reproducibility of results—Four dogs, the same used in the energy density tests, were used to determine the reproducibility of results of the ^{13}C-OBT. The standard test meal was labeled with 50 mg of ^{13}C-octanoic acid. Three tests were performed with at least 1 week between tests. Data from this series of tests were amalgamated with data obtained from dogs fed the standard labeled test

meal in the energy density study, thus providing data for 4 dogs from 6 tests.

Data analysis—Data analysis was performed, using 2 commercially available software programs.[l,m] For tests in which replicate samples were collected, analysis of test results used the mean of replicate measurements taken at each time point. Results were expressed as either the **percentage of dose recovered (PDR)** per hour or $^{13}CO_2$ enrichment in ppm. Percentage of dose recovered per hour was calculated as:

$$PDR/h = (\text{ppm excess } ^{13}C/10^6 \times V_{CO_2})/\text{mmol } ^{13}C \text{ administered}$$

where V_{CO_2} is mmol of CO_2 exhaled per hour.

The $^{13}CO_2$ excretion curve (PDR/h vs time) was fitted, using the formula:

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

where y is PDR per 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 used for modeling the ^{13}C-octanoic acid gastric emptying curve in humans.[d] Values for a, b, and c were calculated by use of nonlinear regression analysis.[35]

Three variables were calculated from the ^{13}C-enrichment curve: **gastric emptying coefficient (GEC)**, **time to peak $^{13}CO_2$ concentration (t_{max})**, and **half-dose recovery time ($t_{½}$)**. The GEC is a global index of the rate of gastric emptying calculated as the natural log of a (ln a).[d] The $t_{½}$ is the time at which the area under the fitted cumulative $^{13}CO_2$ excretion curve equals half the ^{13}C dose recovered and was calculated according to the formula:

$$t_{1/2} = \int_0^{0.5} f(t)dt$$

or by use of a commercial software package[n] (0.5; b + 1; 1/c) where b and c were predicted by the formula, $y = at^be^{-ct}$. The t_{max} was calculated according to the formaula[27,d]:

$$t_{max} = \int_0^{max} f(t)dt$$

or by use of a commercial software package[n] (b/c).

Statistical analyses—The effects of test meal on gastric emptying indices were assessed by use of ANOVA. Intrasubject coefficients of variation (SD/mean × 100%) were calculated to enable comparison of results of the present study with results of previous studies.

To simplify the test protocol, the optimum set of breath samples that could be used to predict the recovery of $^{13}CO_2$ was estimated by use of multiple linear regression analysis as described by Choi et al.[36] Results of the ^{13}C-OBT performed following ingestion of both the high-energy and standard test meals were used in this multiple linear analysis to provide a range of values for $^{13}CO_2$ recovery. This resulted in a reduced number of time points, which were then used to recalculate GEC, $t_{½}$, and t_{max} for each test. The derived coefficients were compared with values determined for all time points by use of a Student paired *t*-test. Results were expressed as mean ± SD, and $P \leq 0.05$ was considered significant.

Results

Control tests—Dogs fed the unlabeled test meal ingested the entire meal within 2 minutes, and the breath collection procedure was well tolerated by all dogs. There was good correlation ($\rho = 0.98$) between concentrations of $^{13}CO_2$ in replicate expiratory breath samples. The pattern of $^{13}CO_2$ excretion remained relatively constant and low during the 6-hour sampling period of both tests. Mean concentrations of $^{13}CO_2$ in expiratory breath samples over the 6-hour sampling period were 10,845.8 ± 2.0 ppm for dogs that were not fed and 10,839.7 ± 2.0 ppm for dogs that ingested the unlabeled standard test meal (**Fig 1**). At most time points during the sampling period, values determined were less in dogs fed the unlabeled test meal, compared with dogs that were not fed; however, these differences were probably attributable to the lower ^{13}C abundance of the test meal, compared with the normal diet. Concentration of $^{13}CO_2$ in the canine maintenance dry ration was 10,833.9 ppm, whereas concentration in the unlabeled test meal was 10,793.7 ppm (weighted mean).[37] The difference in concentration of $^{13}CO_2$ between the regular diet and the test meal (40.2 ppm) may reflect the fish component of the regular diet[37] and helps explain the slight decrease in concentration of ^{13}C in expiratory breath samples collected from dogs after ingestion of the unlabeled test meal.

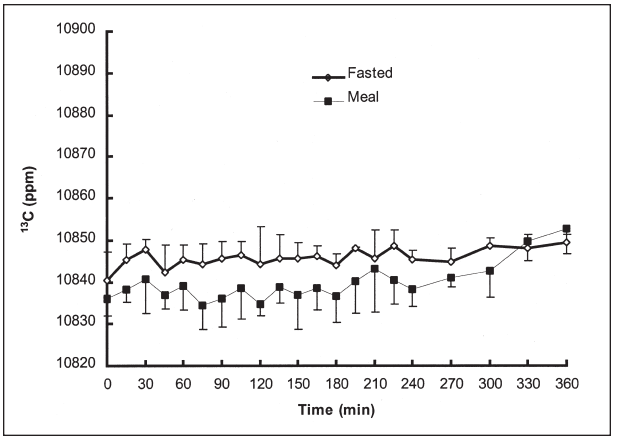

Figure 1—Mean (± SD) basal concentration of $^{13}CO_2$ in expired air versus time for 3 dogs that received or did not receive (fasted) a standard test meal containing unlabeled octanoic acid. Food was withheld from all dogs for 12 hours prior to each test, and the test meal was ingested at 0 minutes. ppm=Parts per million

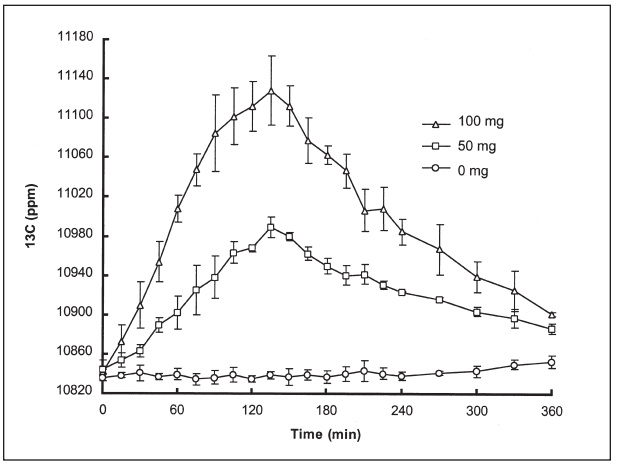

Figure 2—Mean (± SD) concentration of $^{13}CO_2$ in expired air determined immediately before (0 minutes) and at various times after ingestion of the standard test meal containing unlabeled octanoic acid (0 mg; n = 3) or 50 or 100 mg of ^{13}C-octanoic acid (3).

Table 1—Effects of energy density of the test meal on indices of gastric emptying determined by use of the ^{13}C-octanacic acid breath test in 4 healthy adult dogs

Test meal	GEC	$t_{½}$ (h)	t_{max} (h)
Standard	2.70 ± 0.41[a]	3.58 ± 0.45[a]	2.83 ± 0.37[a]
High energy	2.21 ± 0.46[b]	4.02 ± 0.37[b]	3.26 ± 0.36[b]

Data are reported as mean ± SD.
GEC = Gastric emptying coefficient. $t_{½}$ = Half-dose recovery time. t_{max} = Time to peak $^{13}CO_2$ concentration.
[a,b]Within a column, values with different superscripts are significantly ($P < 0.05$) different.

Table 2—Reproducibility of the ^{13}C-octanacic acid breath test determined by performing 6 tests in 4 healthy adult dogs

Index	Mean ± SD	Range	CV (%)
GEC	2.91 ± 0.47	2.12–3.66	16
$t_{½}$ (h)	3.43 ± 0.50	2.40–4.36	14
t_{max} (h)	2.68 ± 0.44	1.74–3.42	16

CV = Intrasubject coefficient of variation.

Estimation of optimum substrate concentration—Ingestion of labeled test meals was associated with significant increases in concentration of $^{13}CO_2$ in expiratory breath samples, compared with basal values. Increases were proportional to the amount of ^{13}C-octanoic acid in the test meal (**Fig 2**).

Energy density tests—Results of ^{13}C-OBT performed after ingestion of the high-energy labeled test meal were significantly different, compared with results obtained after ingestion of the standard labeled meal. The GEC was significantly ($P = 0.028$) decreased following ingestion of the high-energy labeled test meal, whereas $t_{1/2}$ and t_{max} were significantly ($P = 0.032$ and $P = 0.022$, respectively) increased (**Table 1**).

Reproducibility tests—Reproducibility of the ^{13}C-OBT was assessed by comparing values determined during 6 separate tests in 4 dogs (**Table 2**). Coefficients of variation for GEC, $t_{1/2}$, and t_{max} were 16, 14, and 16%, respectively.

Protocol simplification—Multiple linear analysis of the cumulative $^{13}CO_2$ curve predicted that analysis of breath samples collected at 20, 80, 140, 220, 280, 400, and 420 minutes after ingestion of the test meal could accurately predict cumulative $^{13}CO_2$ recovery at 480 minutes ($r^2 > 0.99$). These 8 breath samples were used to recalculate gastric emptying indices for the 24 energy density tests. Significant differences in GEC, $t_{1/2}$, and t_{max} were not detected between recalculated and original values (ie, values calculated from all time points [n = 41]).

Discussion

Results of this study suggest that the ^{13}C-OBT is a reproducible and simple method for the assessment of solid-phase gastric emptying in dogs. Tests that monitor $^{13}CO_2$ concentration in expired air following ingestion of a ^{13}C-labeled substrate are used for clinical diagnosis and research in human gastroenterology.[38] Such tests have also been used in dogs to measure orocecal transit time (hydrogen breath test)[39] and detect *Helicobacter* spp in the stomach (^{13}C-urea breath test).[40] In the present study, breath samples were collected, using a face mask attached to a reservoir bag by a unidirectional valve. This ensured that only exhaled air could enter the reservoir bag and be collected through the sampling port. The accuracy of this method was demonstrated by the good correlation in $^{13}CO_2$ concentrations between replicate samples. The breath sampling procedure could be completed in each dog in < 2 minutes and was well tolerated by all dogs in the study.

Because carbon naturally comprises 1.1% ^{13}C, all ^{13}C breath tests are performed against a background concentration of the naturally occurring isotope.[41] For this reason, it was necessary to establish basal concentrations of $^{13}CO_2$ in expiratory breath samples before attempting to enrich expired $^{13}CO_2$ concentrations by administration of an exogenous isotope source. Basal $^{13}CO_2$ concentrations were monitored in 3 dogs over 6 hours; concentrations were low and remained stable throughout this period. This finding confirmed that for the purposes of the ^{13}C-OBT and other ^{13}C-labeled breath tests, expiratory $^{13}CO_2$ concentrations can be enriched in dogs.

Metabolism of a test meal can result in a shift in basal concentrations of $^{13}CO_2$ in expired air. This shift is dependent on the amount of ^{13}C in the meal, compared with that naturally occurring in carbon (ie, 1.1%).[38] To ensure that ingestion of the test meal did not affect basal $^{13}CO_2$ excretion in this study, 3 dogs were fed a test meal with 100 mg of unlabeled octanoic acid, and the concentration of $^{13}CO_2$ in expired air was monitored for 6 hours. We found no significant alteration in the concentration or pattern of basal $^{13}CO_2$ excretion following ingestion of the unlabeled meal, indicating that the standard test meal was suitable as a carrier for administration of ^{13}C-octanoic acid. In in vitro[d] and in vivo studies[27,36] in humans, ^{13}C-octanoic acid remained bound to the solid phase of the same standard test meal that we used in the present study.

After administration of the labeled test meal, concentration of $^{13}CO_2$ in expiratory breath samples significantly increased in all dogs. This increase was dose related; higher concentrations were detected in dogs fed meals labeled with 100 mg of ^{13}C-octanoic acid, compared with meals labeled with 50 mg of ^{13}C-octanoic acid. These data were fit by application of the mathematical model derived from data obtained during human studies. Moreover, the pattern of recovery of $^{13}CO_2$ in expiratory breath samples in dogs was comparable to that described in humans. Reproducibility of results between and within dogs was assessed by repeating the test 6 times in 4 dogs under identical conditions of meal timing and composition. Intersubject variation was comparable to results of previous reports of gastric emptying in dogs evaluated by use of radioscintigraphy.[15] Mean intrasubject coefficients of variation for GEC (16%) and $t_{1/2}$ (14%) that we determined in the present study were comparable to those determined in humans by use of the ^{13}C-OBT (GEC, 10.82%; $t_{1/2}$, 26.71%).[27]

Because the rate of gastric emptying is proportional to the energy density of the ingested meal, oil-enriched test meals have been used to induce delayed gastric

emptying, which was then assessed by use of the ^{13}C-OBT.[o] In dogs, the rate of gastric emptying of oil is dependent on the release of lipolytic products in the small intestine. Oil-induced slowing of gastric emptying did not occur in the absence of pancreatic and gastric lipases.[42] The ability of the ^{13}C-OBT to detect delayed gastric emptying was assessed in the present study by performing the test in dogs fed test meals with high- and standard-energy densities. Ingestion of the high-energy meal was associated with significant alterations in rate and pattern of recovery of $^{13}CO_2$ in expired air; $t_{½}$ and t_{max} were increased, whereas GEC was decreased, compared with values obtained after ingestion of the standard meal. These findings are an indication of the sensitivity of the ^{13}C-OBT in detecting delayed gastric emptying in dogs and confirm the potential use of this test for investigation of altered gastric emptying associated with gastrointestinal tract disease.

To assess whether the sampling protocol could be simplified, we estimated the minimum number of breath samples necessary for accurate prediction of $^{13}CO_2$ recovery. We found that $^{13}CO_2$ concentrations in breath samples collected at 8 optimum times could accurately predict the pattern of $^{13}CO_2$ recovery and be used to calculate gastric emptying indices. Optimizing the frequency and duration of breath sampling would simplify the test protocol and result in a reduction in the cost of sample analysis. In addition, simplification would make the test more suitable for clinical application. However, data obtained for the healthy dogs in the present study are not representative of the range of gastric emptying rates likely to be found in diseased dogs. Further studies are necessary before the simplified sampling protocol should be applied in the clinical setting.

The appearance of $^{13}CO_2$ in expired air is subject to an inevitable time shift arising from the postgastric processing of octanoic acid and absorption of the label into the bicarbonate pool.[27] This delay precludes direct comparison of results of the ^{13}C-OBT with radioscintigraphy or other real-time methods for assessing gastric emptying unless appropriate correction factors are used. Postgastric processing of octanoic acid is thought to be consistent between and within individuals[p] but results in a time-shift between recovery of the isotope in breath and absorption of octanoic acid in the duodenum. In humans and horses, a correction factor has been derived by correlating results of the ^{13}C-OBT with results of radioscintigraphy.[27,q] Further investigation is necessary to derive a correction factor specifically for use in dogs.

The ^{13}C-OBT offers several distinct advantages over other methods designed to assess solid-phase gastric emptying in dogs. Noninvasive methods typically require that dogs are sedated or restrained, either of which could affect the rate of gastric emptying.[19,43] The ^{13}C-OBT is completely noninvasive, and breath samples can be collected with minimal disturbance to the dogs. Radioscintigraphy is considered the gold standard for assessment of gastric emptying. However, this method requires access to a nuclear medicine facility and experienced personnel. Moreover, dogs tested by use of radioscintigraphy are exposed to ionizing radiation. Local radiation regulations may require that these dogs be housed in isolation kennels for approximately 48 hours after radioscintigraphy. In contrast, the ^{13}C-OBT requires no special equipment or expertise and can be performed in the field, because expired $^{13}CO_2$ can be stored in sealed sample tubes for up to 60 days,[42] and samples can be sent to a laboratory for analysis. Data analysis is automated, and the test yields data describing the rate and pattern of gastric emptying that are quantitative and nonsubjective. The standard test meal used in the ^{13}C-OBT is similar to food normally ingested by dogs. However, any food can potentially be used, provided that sufficient tracer is administered so that a significant increase in $^{13}CO_2$ is detected. Neither unlabeled nor ^{13}C-labeled octanoic acid poses any risk to health.

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[f]n-Octanoic acid, Sigma Chemical Co, St Louis, Mo.

[g]Cenvet, Veterinary Instrumentation, Welshpool, Australia.

[h]Disposable nonrebreathing valve, QuinTron Instrument Co, Milwaukee, Wis.

[i]Disposable 250-ml mini-collection bag, QuinTron Instrument Co, Milwaukee, Wis.

[j]Exetainer, Labco Ltd, High Wycombe, UK.

[k]ABCA, Europa Scientific, Crewe, UK.

[l]Microsoft Excel for Windows, version 7a, Microsoft Corp, Redmond, Wash.

[m]Minitab 7, Minitab Headquarters, State College, Pa.

[n]Microsoft Excel function Gammainv, Microsoft Corp, Redmond, Wash.

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