Comparison of the carbon 13-labeled octanoic acid breath test and ultrasonography for assessment of gastric emptying of a semisolid meal in dogs

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Objective—To compare the rate of gastric emptying of a semisolid meal by use of the carbon 13-labeled octanoic acid breath test (^{13}C-OBT) and gastric emptying ultrasonography (GEU) in dogs.

Animals—10 healthy dogs.

Procedure—Food was withheld from dogs for 12 hours before ingestion of a test meal (bread, egg, and skimmed milk) containing ^{13}C-octanoic acid. The gastric antrum was visualized by use of a 6.5-MHz microconvex transducer, and the area of the ellipse defined by the craniocaudal and ventrodorsal diameters of the stomach was measured. Samples of expired air and antral images were obtained 30 minutes before ingestion of the test meal and then every 15 minutes for 4 hours and every 30 minutes for a further 2 hours. The half-dose recovery time with the ^{13}C-OBT ($t_{½}$[BT]) and the gastric half emtying time withGEU ($t_{50\%}$[GEU]) was calculated.

Results—Mean ± SD values for the $t_{½}$(BT) and $t_{50\%}$(GEU) were 3.44 ± 0.48 hours and 1.89 ± 0.78 hours, respectively. A significant correlation was detected between the $t_{½}$(BT) and $t_{50\%}$(GEU), although there was a large (1.55 hours) mean difference between these indices.

Conclusions and Clinical Relevance—Results indicated that there was a correlation between the rate of solid-phase gastric emptying assessed by use of GEU and the ^{13}C-OBT in dogs. Gastric emptying ultrasonography may be a useful, noninvasive method for assessment of the rate of solid-phase gastric emptying in dogs. (*Am J Vet Res* 2004;65:1557–1562)

Disordered solid-phase gastric emptying has been associated with many pathologic diseases in dogs, including hypertrophic pyloric gastropathy,[1] gastric neoplasia,[2] endotoxemia,[3] and gastric dilatation-volvulus.[4] Delayed gastric emptying may be associated with a number of clinical signs, such as vomiting, abdominal discomfort, and weight loss.

Solid-phase gastric emptying refers to the gastric emptying of solid food and is a function of the trituritive or grinding actions of the stomach. Assessment of the rate of solid-phase gastric emptying in small animal clinical practice is mostly confined to radiographic imaging of the gastrointestinal transit of radiopaque markers.[5] Radiographic methods provide semiquantitative data that are poorly representative of the gastric emptying of solid food.[6,7] Imaging of the passage of a radiolabeled (technetium Tc 99m) test meal by use of a gamma camera (radioscintigraphy) is the standard method for assessing gastric emptying[8,9]; however, this is not generally used in veterinary medicine. A comparative review of methods available for assessment of gastric emptying in dogs has been previously described.[10]

The consequences of delayed gastric emptying in clinical practice are poorly understood, primarily because of the absence of a suitable method for quantification of the rate of solid-phase gastric emptying in dogs. In human medicine, the **carbon 13-labeled octanoic acid breath test** (**^{13}C-OBT**) and **gastric emptying ultrasonography** (**GEU**) are used as simple and noninvasive methods for assessment of solid-phase gastric emptying.[11,12] Both methods have been correlated against the standard method of radioscintigraphy[11,13] and have been used to detect delayed gastric emptying in humans.[14,15] Gastric emptying ultrasonography offers the advantage of permitting real-time (live) visualization of the antropyloric region and provides information on the pathogenesis of disordered gastric emptying (ie, the images of the stomach can be visualized at the time of study, rather than being a recording or there being a delay). The ^{13}C-OBT and GEU have been applied in dogs[16,17] and with further validation may become options for clinical investigation of the rate of solid-phase gastric emptying in dogs. The purpose of the study reported here was to compare the rate of gastric emptying of a semisolid meal by use of the ^{13}C-OBT and GEU in dogs.

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Materials and Methods

Dogs—Ten healthy dogs were used in this study. Body weight of dogs ranged from 18 to 36 kg. The study was approved by the Animal Ethics and Welfare Committee of the University of Glasgow.

Test meal—The test meal consisted of 1 slice of wholemeal bread (36 g; 980 kJ/100 g), 5 g of margarine (2,593 kJ/100 g), and 200 mL of skimmed milk (146 kJ/100 mL); total energy provided by the meal was approximately 775 kJ. Fifty milligrams of the ^{13}C-octanoic acid substrate[a] (octanoic acid-1-^{13}C [a minimum of 99% of the carbon atoms are ^{13}C]) was mixed with 1 egg yolk (octanoic acid is highly soluble in egg yolk), baked, and added to the test meal. This test meal was chosen because it has been previously described for use of the ^{13}C-OBT in dogs.[16]

Gastric emptying ultrasonography—Ultrasonographic examinations were performed by the same sonographer with an ultrasound machine[b] and a 6.5-MHz microconvex transducer. Each dog was gently restrained in the standing position, and the transducer was positioned in a longitudinal orientation on the ventral midline caudal to the xiphoid. The ultrasound beam was maintained in the sagittal plane and directed cranially until the liver was located and the stomach was identified immediately caudal to it. The stomach was observed by use of real-time imaging, which permitted the image to be frozen between peristaltic contractions when the stomach was at a constant, maximal distension. If intraluminal gas caused acoustic shadowing, which obscured the far wall, and the gas did not pass with subsequent gastric contractions, the stomach was assumed to be an oval shape permitting the ventrodorsal measurement to be made. Antral area was calculated by use of software attached to the ultrasound machine to predict the area inside the elliptical shape that was defined by the craniocaudal and ventrodorsal diameters of the stomach. Measurements of antral area (cm^2) were obtained in triplicate at each sample time, and the mean of the 3 measurements was used in further calculations. Baseline antral area was measured before ingestion of the test meal and subtracted from each subsequent measurement. All measurements were expressed as a percentage of the maximal antral area measured during each test and plotted against time. The **gastric half-emptying time with GEU ($t_{50\%}$[GEU])** was defined as the time point at which antral area was < 50% of the maximal value.

^{13}C-OBT—The ^{13}C-OBT was performed according to a previously described method.[16] Briefly, samples of expired air were collected before and after ingestion of the ^{13}C-labeled test meal by permitting the dog to breathe through a plastic mask connected to a nonrebreathing valve[c] and a 50-mL reservoir bag.[c] Expired air samples were transferred from the reservoir bag to sample tubes,[d] and the ratio of $^{13}CO_2$-to-$^{12}CO_2$ was measured by use of isotope-ratio mass spectrometry.

Results of ^{13}C analyses were expressed as **percentage dose recovered per hour (PDR)** and calculated by use of following equation:

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

where V_{CO_2} is mmol of CO_2 exhaled per hour, which was considered to remain stable at 0.194 L/m^2/min.[18] Body surface area was calculated as the following: body surface area (m^2) = 10.1 × body weight (g)$^{2/3}$/10,000.[19] The PDR per hour values were plotted against time, and nonlinear regression analysis was used to model the data according to the formula described by Ghoos et al[13]:

$$y = at^b e^{-ct}$$

where y is the PDR per hour; t is time in hours; and a, b and c are variables predicted by the Solver function of a spreadsheet.[e] This model was used to calculate the **half-dose recovery time with the $^{13}CO_2$-OBT ($t_{1/2}$[BT])** defined as the time when the area under the fitted cumulative excretion curve (expressed as PDR per hour) equals half of the ^{13}C dose recovered in expired air.

Study design—The ^{13}C-OBT and GEU were performed simultaneously in each dog on 1 occasion. Food and water were withheld from dogs for 12 hours before ingestion of the test meal. Expired air samples and ultrasonographic images were acquired 30 minutes before and 5 minutes after ingestion of the test meal and then every 15 minutes for 4 hours and every 30 minutes for a further 2 hours.

Statistical analyses—All data analyses were performed by use of computer software.[e] The association between the rate of gastric emptying assessed with the ^{13}C-OBT and GEU was investigated by comparing $t_{1/2}$(BT) and $t_{50\%}$(GEU) by calculation of a Pearson correlation coefficient (*r*). Concordance of the gastric emptying indices predicted with the ^{13}C-OBT and GEU was assessed by use of the method of Bland and Altman.[20] The difference between $t_{1/2}$(BT) and $t_{50\%}$(GEU) calculated for each dog was plotted against the mean difference between the 2 indices. Limits of agreement were plotted on the graph and defined as d ± 1.96s, where d is the mean difference between $t_{1/2}$(BT) and $t_{50\%}$(GEU) and s is the SD of the differences.

If the data are normally distributed, 95% of the differences between the 2 methods will fall between the limits d + 1.96s and d – 1.96s, thus defining the range containing most of the differences. Similarly, the repeatability of ultrasonographic measurement of antral area was assessed by plotting the difference between each of 3 consecutive measurements in each dog (n = 651) against their mean. Data are given as mean ± SD, and values of $P \leq 0.05$ were considered significant.

Results

Both techniques (^{13}C-OBT and GEU) were simple to perform and tolerated well by all dogs. In all dogs, the stomach was consistently imaged as an oval-shaped structure surrounded by echogenic mesenteric fat. The region being examined corresponded with the junction between the body of the stomach and the pyloric antrum. In preprandial images in which the stomach was empty, it was difficult to determine the location of the lumen and the rugal folds caused a cartwheel-like appearance (**Figure 1**). Distension caused by gastric secretions permitted visualization of the lumen as a hyperechoic region, whereas, less commonly, gas in the lumen caused a distal acoustic shadow, which obscured the far wall of the stomach. Immediately after ingestion of the test meal, the gastric contents were extremely hyperechoic and caused marked shadowing that reflected the presence of gas bubbles within the ingesta. By 5 minutes after ingestion of the test meal, the contents resumed an echogenicity similar to that of gastric secretions and the lumen could be visualized because of distension of the stomach.

Repeatability of the ultrasonographic measurement of antral area at each time point was poor, as indicated by a wide spread of data outside of the range defined by the mean difference ± 1.96 SD, when mean replicate measurements (n = 651) were plotted against their difference (**Figure 2**). In our study, > 5% (29%) of the differences were outside of this range, although the mean difference was not different from zero.

The change in antral area and the ^{13}C-excretion curve for each test were determined (**Figure 3**). Mean ± SD for $t_{1/2}$(BT) and $t_{50\%}$(GEU) was 3.44 ± 0.48 hours and 1.89 ± 0.78 hours, respectively. These data were plotted on a histogram and were normally distributed. Although there was a significant (r = 0.68; $P < 0.02$) correlation between $t_{1/2}$(BT) and $t_{50\%}$(GEU), there was a difference of 1.55 hours between the overall mean of $t_{1/2}$(BT) and $t_{50\%}$(GEU) (**Figure 4**). Results

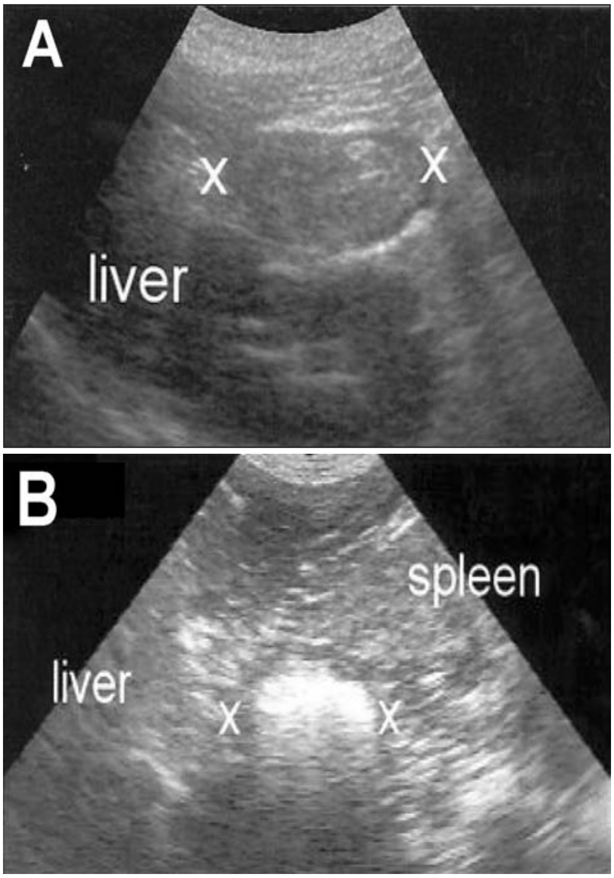

Figure 1—Ultrasonographic images of the abdomen of a dog. A—Before ingestion of a semisolid test meal, the lumen of the stomach is difficult to locate and the rugal folds cause a cartwheel-like appearance. B—Five minutes after ingestion of a semisolid test meal, the stomach contents resumed an echogenicity similar to that of gastric secretions and the lumen can be visualized due to distension of the stomach.

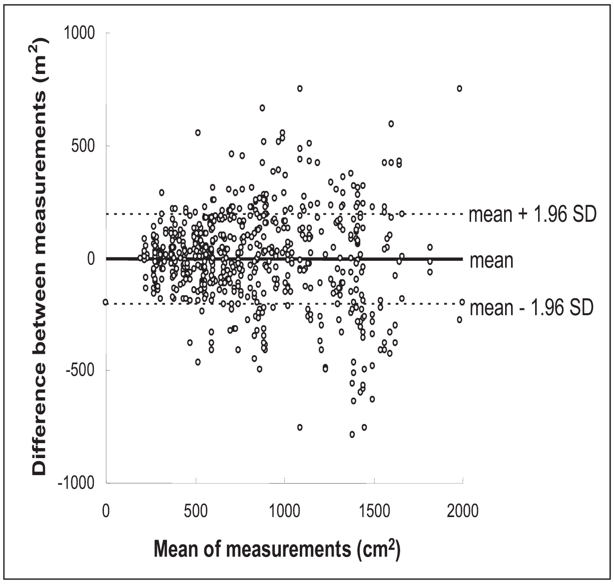

Figure 2—Mean of 3 replicate measurements (n = 651) plotted against the difference between each of the 3 measurements obtained in 10 dogs. Antral area was calculated by use of the software attached to the ultrasound machine to predict the area inside the elliptical shape defined by the craniocaudal and ventrodorsal diameters of the stomach. The spread of > 5% of the data points outside of the range defined by the mean difference ± 1.96 SD indicates the poor repeatability of the measurement.

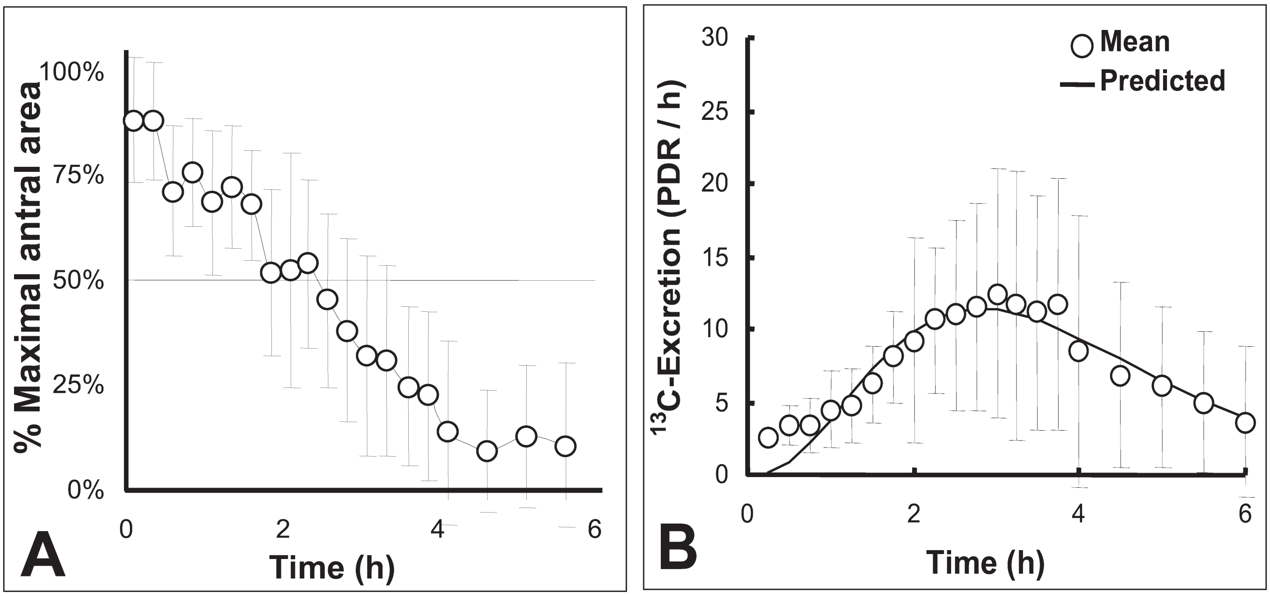

Figure 3—Percentage decrease in antral area (A) and the carbon 13 (^{13}C)-excretion curve (B) observed in 10 dogs after assessment of gastric emptying of a semisolid test meal by use of gastric emptying ultrasonography (GEU) and the ^{13}C-octanoic acid breath test (^{13}C-OBT). Data are given as mean ± SD. The horizontal line in A line represents a 50% decrease in antral area. The time point at which the maximal antral area was observed varied between dogs; therefore, the mean data for all dogs did not reach 100%. PDR = Percentage of dose recovered per hour. The predicted curve in B is data modeled by use of nonlinear regression analysis.

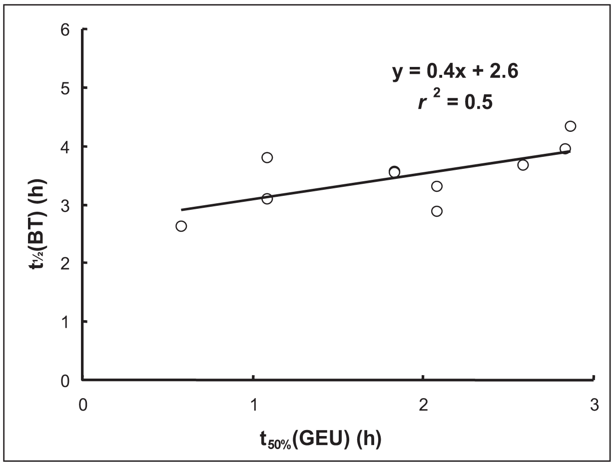

Figure 4—Association between gastric half-emptying time as assessed by gastric emptying ultrasonography ($t_{50\%}$[GEU]) and the [13]C-OBT ($t_{½}$[BT]) in 10 dogs. Two points on the graph overlap.

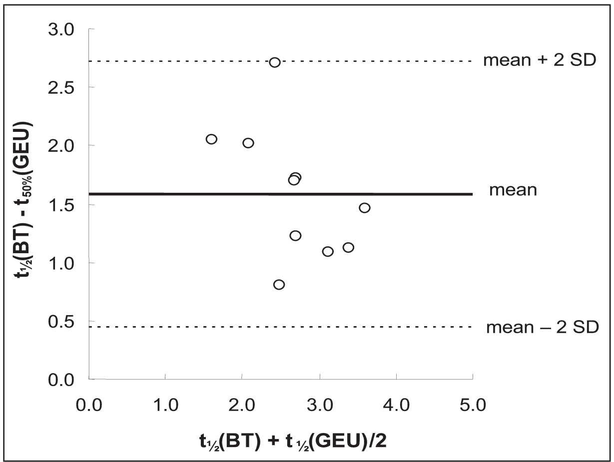

Figure 5—Bland-Altman plot illustrating the concordance between $t_{½}$(BT) and $t_{50\%}$(GEU) in 10 dogs after assessment of gastric emptying of a semisolid test meal using GEU and the [13]C-OBT. The difference between $t_{½}$(BT) and $t_{50\%}$(GEU) is plotted against the mean value of $t_{½}$(BT) and $t_{50\%}$(GEU) for each dog. The dotted lines represent the mean difference and 95% limits of agreement for $t_{½}$(BT) and $t_{50\%}$(GEU) for all dogs. *See* Figure 3 for key.

indicated that the rate of gastric emptying was slower when measured by use of the [13]C-OBT, compared with GEU. The time point at which the maximal antral area was observed varied between dogs (range, 5 to 65 minutes; median, 18.5 minutes), and for this reason, the mean data for all dogs did not reach 100% (Figure 3). The concordance of the results obtained by use of both methods was examined by plotting the data as described by Bland and Altman[20] (**Figure 5**). The difference between $t_{½}$(BT) and $t_{50\%}$(GEU) was within the limits of agreement for all dogs, although a large mean difference between the methods (1.55 hours) was evident.

Discussion

To the authors' knowledge, use of ultrasonography for assessment of solid-phase gastric emptying in dogs has not been previously reported, despite widespread use of this method in human medicine.[11,12,15,21-23] Results of our study indicated that there was a significant correlation in the rate of solid-phase gastric emptying measured with the [13]C-OBT and GEU in dogs.

The advantages of GEU are that it is noninvasive and the equipment required is available at most veterinary practices. The procedure was easy to perform, and the antral region of the stomach was easily identified in all dogs in the study. In dogs, ingestion of a meal results in the body of the stomach moving caudoventrally,[24] unlike humans in which the gastric antrum moves towards the right.[25] Therefore, the position of the transducer should have ensured that the plane of the stomach under investigation remained constant despite alterations in the degree of gastric distension. Reportedly, intraluminal gas is a problem when imaging the stomach in dogs,[26] particularly immediately after ingestion of a meal; however, in our study, dogs were examined in a standing position to permit gas to rise into the fundus and thus minimize interference with antral measurement. To the authors' knowledge, repeatability of ultrasonographic measurements of antral area has not been reported in human or veterinary medicine. In the present study, measurement of antral area in dogs by use of ultrasonography was poorly repeatable and use of the mean of 3 replicate measurements of antral area at each time point was required.

The [13]C-OBT is a simple and noninvasive method for assessment of gastric emptying that can be used in veterinary practice and does not require exposure to radiation. This test has been used in dogs and cats,[16,27] and comprehensive studies[13,28,29] have validated this test for assessment of gastric emptying in humans and horses. The [13]C-OBT provides an index of the rate of gastric emptying as determined by retention of the [13]C-substrate in the solid phase, rapid digestion and absorption in the duodenum, hepatic oxidation to $^{13}CO_2$, absorption in the bicarbonate pool, and finally excretion of $^{13}CO_2$ in expired air.[13] The premise of the test is that intra- and interindividual variation in the rate of these processes remains small and that the rate of delivery of the [13]C-substrate to the duodenum is the factor that regulates the production of $^{13}CO_2$ in expired air. Consequently, the [13]C-OBT is accepted as an indirect measure of gastric emptying, and consistent with this is the documented agreement between the results of the [13]C-OBT and other measures of gastric emptying, including the gold standards of radioscintigraphy[13,28] and ultrasonography.[12]

The rate of gastric emptying measured by use of ultrasonography relies on a linear association between the rate of gastric emptying and changes in antral area. The distal regions of the stomach are believed to be primarily responsible for trituration of solid food and emptying of particles into the duodenum,[30,31] and it is likely that postcibal antral dilatation is related to the rate of solid-phase emptying. However, the rate of passage of ingesta from the proximal regions of the stomach and redistribution of ingesta within the stomach may also be important in regulating the postcibal antral area. This may affect the point of maximal antral dilatation, an important point in ultrasonographic assessment of gastric emptying, because all other data points are expressed as a ratio of the maximal antral dilatation. The point of maximal antral dilatation varied between dogs in this study. The physiologic importance of this point is unknown; however, it may be of some interest that maximal antral dilatation is delayed in human patients with functional dyspepsia.[22] The point of maximal antral dilatation may be of importance in the assessment of healthy and diseased individuals, and further research is required to establish the physiologic process represented by this point. The rate of solid-phase gastric emptying assessed by GEU describes a unique pattern that is not adequately addressed by mathematical models reported for assessment of gastric emptying by use of scintigraphy or the ^{13}C-OBT. Future research should be addressed towards derivation of a mathematical model that can be used to plot data obtained by GEU and a definition of appropriate mathematical indices, such as the point of maximal antral area.

Different physiologic factors regulate the ^{13}C-OBT and GEU, although both methods are considered to be reliable methods for assessment of gastric emptying.[11,13] For example, the ^{13}C-OBT may be influenced by bicarbonate kinetics, CO_2 production, and small intestinal function, whereas GEU may be affected by intraluminal redistribution of food in the stomach. A more important discordance between these methods is the fact that the ^{13}C-OBT measures the rate of gastric emptying of solids (^{13}C-octanoic acid in egg), whereas GEU measures the contribution of gastric secretion, ingested fluid and saliva, and duodenal reflux to the antral area. However, it is considered likely that the rate of gastric emptying of a test meal is the most influential factor regulating both the appearance of $^{13}CO_2$ in expired air and the change in antral area. Correlation between the ^{13}C-OBT and GEU may then be expected, especially if the range of gastric emptying rates measured is large. Accordingly, a moderate significant association has been detected between the rate of gastric emptying measured by use of the ^{13}C-OBT and GEU in the dogs in this study and a study[12] in humans.

In the study reported here, the rate of gastric emptying was slower when assessed by use of the ^{13}C-OBT, compared with GEU in dogs. This finding is in agreement with other studies that compared the ^{13}C-OBT with real-time methods for assessment of gastric emptying, such as scintigraphy[13,28] and ultrasonography.[12] Postgastric processing of ^{13}C-octanoic acid imposes a delay on the excretion of $^{13}CO_2$ in expired air that results in an apparent slower rate of gastric emptying by use of the ^{13}C-OBT.[13] Results of studies[28,f] in humans indicate that this delay is highly reproducible between and within healthy individuals. Results of our study further substantiate these findings because the mean difference between $t_{½}$(BT) and $t_{50\%}$(GEU) was 1.55 hours, similar to the 1.33 hours previously reported for the difference between gastric half-emptying time of the ^{13}C-OBT and a real-time measure of gastric emptying (the deuterium-labeled-octanoic acid saliva test) in dogs.[32] The application of a correction factor to account for the postgastric processing of ^{13}C-octanoic acid in dogs or, indeed, whether such a factor is appropriate requires further analysis.

The ^{13}C-OBT and GEU may be simple and noninvasive methods for assessment of solid-phase gastric emptying in dogs that can be easily used in veterinary practice. Results of the study reported here indicated that there was a moderate but significant association between the rate of gastric emptying of a semisolid test meal assessed by use of the ^{13}C-OBT and GEU. This study was limited by a small sample size, and considerable variability was evident in the measurement of antral area by use of ultrasonography. Further studies are required to validate these methods as procedures facilitating quantitative assessment of the rate of solid-phase gastric emptying in veterinary clinical practice.

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[a]Isotec Inc, CK Gas Products Ltd, Manchester, UK.
[b]Justvision, Toshiba America Medical Systems Inc, Tustin, Calif.
[c]QuinTron Instrument Co, Milwaukee, Wis.
[d]Exetainer, Labco Ltd, Cressex Business Park, High Wycombe, Buckinghamshire, UK.
[e]Microsoft Excel for Windows 1995, version 7a, Microsoft Corp, Redmond, Wash.
[f]Maes BD. *Measurement of gastric emptying using dynamic breath analysis*. PhD thesis, Department of Medicine, University of Leuven, Leuven, Belgium, 1994.

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Correction: Effect of trilostane on serum concentrations of aldosterone, cortisol, and potassium in dogs with pituitary-dependent hyperadrenocorticism

In the article "Effect of trilostane on serum concentrations of aldosterone, cortisol, and potassium in dogs with pituitary-dependent hyperadrenocorticism," published September 2004 (2004;65:1245–1250), the following items were incorrect.

On page 1247, the third line of the legend for Figure 2 incorrectly stated ACTH stimulation in 17 dogs with primary hyperadrenocorticism (PDH). The correct statement is ACTH stimulation in 17 dogs with pituitary-dependent hyperadrenocorticism (PDH).

On page 1247, the third sentence of the second paragraph in the second column incorrectly stated that throughout the study, serum aldosterone concentrations after ACTH stimulation remained significantly lower than the concentrations before ACTH stimulation. The correct statement is that throughout the study, serum aldosterone concentrations after ACTH stimulation remained significantly lower than the concentrations before ACTH stimulation prior to treatment.

On page, 1247, the end of the fourth sentence of the second paragraph in the second column incorrectly stated the time frame was weeks 1 to 4, respectively. The correct statement is for follow-up evaluations 1 to 4, respectively.