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Real-Time Ultrasonographic Evaluation of Canine Gastric Motility in the Postprandial State

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ABSTRACT. Gastric motility is affected by several pathological conditions which may induce upper gastrointestinal clinical symptoms. The pathogenesis of canine gastric motility disorders is poorly understood because of methodological limitations. This study aimed at establishing a simple method for evaluating postprandial gastric motility in dogs. Gastric motility was ultrasonographically assessed in 7 healthy beagles using a technique previously described in humans. The motility index (MI), an indicator of gastric antral motility, was calculated by measuring the area of the gastric antrum in both a contracted and relaxed phase and by counting the number of contractions. The MI was measured every 30 min for 3 hr after feeding and compared with gastric emptying as assessed by a ^{13}C-octanoic acid breath test. The MI at 30 min had the lowest variability in the 7 dogs (mean ± SD, 9.77 ± 0.42; coefficient of variance, 4.25%), and a significant correlation was observed with gastric emptying coefficient (R^2=0.8126, P=0.005) and half-emptying time (R^2=0.654, P=0.027). When atropine was administered, a significant decrease in the MI at 30 min was observed compared with the control (9.77 ± 0.42 vs. 5.19 ± 0.22, P=0.0003). In conclusion, evaluation of the MI at 30 min is suitable for assessing gastric motility and enables us to assess gastric motility simply in a short time. By using this method, further studies for the pathogenesis of canine gastric motility disorders are warranted.

KEY WORDS: ^{13}C-octanoic acid breath test, canine, gastric motility, motility index, ultrasonography.

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Gastric motility disorders are caused secondary by several diseases or drug administration and possibly related with the pathogenesis of upper gastrointestinal clinical symptoms such as anorexia, vomiting, abdominal pain, and emesis. Several methods for evaluating gastric motility in both humans and dogs have been described [25, 29]. Gastric motor activity has 2 characteristic phases: fasting and the postprandial phase. Many of the previous studies have focused on the postprandial phase. Postprandial gastric motility is assessed either by quantifying gastric emptying time and/or gastric antral motility or by detecting the electrical excitement caused by gastric movement. Gastric emptying can be evaluated using scintigraphy [1, 14], a ^{13}C-octanoic acid breath test [8, 31], or radiopaque markers [18, 27], whereas gastric antral motility is examined using force transducers [13] or abdominal ultrasonography [5, 7, 9, 19].

Of these methods, scintigraphy is considered the gold standard for evaluating gastric emptying in both humans and dogs [22, 29]. However, it requires a specialized facility and exposure to radiation. The ^{13}C-octanoic acid breath test is an indirect method of assessing gastric emptying using a non-radioisotope-labeled substrate. The test involves monitoring the rate of $^{13}CO_2$ in expired air following ingestion of a test meal mixed with ^{13}C-octanoic acid. The ^{13}C-octanoic acid breath test has also been established as a method for assessing solid-phase gastric emptying in dogs [31], cats [23], and horses [30]. Although the ^{13}C-octanoic acid breath test has an advantage over scintigraphy in that it can be performed without exposure to radiation, it requires 6 hr of repeated collection of expired air samples. Because of the limitations in these methods, gastric motility disorders in dogs are poorly understood, and a more simple assessment method is required for use in clinical settings.

Ultrasonography is a noninvasive method that allows the veterinarian to assess gastric motility at the bedside. Ultrasonographic evaluation of gastric motility in humans has been applied in clinical settings, and several clinical trials have been performed to estimate the pathogenesis of gastric motility disorders on a large scale [9, 16, 17, 26]. Previous reports have described the ultrasonographic assessment of gastric motility or emptying in dogs [7, 19]. In one of the studies, McLellan *et al.* determined the canine gastric emptying curve by using ultrasonography to measure the area of the stomach [19]. However, this requires making continued measurements over a period of 6 hr. Choi *et al.* used ultrasonography to investigate gastric motility by evaluating gastric contraction and area [7]. However, this method may not be sensitive enough to detect abnormalities in gastric motility because a liquid meal was used as a test meal in the study [22, 29] and the researchers did not compare their results with those found by other methods.

The aim of the present study was to establish a novel method for assessing canine gastric motility in the postprandial state using ultrasonography. To determine the reliability of the method, we investigated its correlation with gastric

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emptying as assessed by a ^{13}C-octanoic acid breath test. We also used an atropine-induced gastric hypomotility model to examine the ability of this new method to detect gastric hypomotility.

MATERIALS AND METHODS

Study design: First, the correlation between gastric motility evaluated with ultrasonography and gastric emptying assessed by the ^{13}C-octanoic acid breath test was investigated to determine the reliability of the ultrasonographic method. Ultrasonography and the ^{13}C-octanoic acid breath test were performed simultaneously on 7 healthy beagles. Gastric motility was assessed by ultrasonography every 30 min for 3 hr after the animals were fed the test meal. The motility index (MI), a marker of gastric antral motility, was calculated at each time point. Variability in the MI at each time was estimated to determine the appropriate time of measurement. Correlation with parameters of the ^{13}C-octanoic acid breath test was then investigated. Next, ultrasonography was performed in the same 7 dogs according to an atropine-induced gastric hypomotility model to confirm the ability of the ultrasonographic method to detect gastric hypomotility.

Study animals: Seven healthy beagles were used in this study. The dogs ranged in age from 3 to 5 years (mean: 3.8 years). The body weight of the dogs ranged from 9.4 to 13.0 kg (mean: 11.5 kg). Four dogs were male and 3 were female. No dog showed clinical signs of diarrhea, constipation, vomiting, anorexia, or weight loss. Blood tests (complete blood count, blood urea nitrogen, creatinine, alkaline phosphatase, and alanine aminotransferase) showed no abnormalities. All dogs were fasted for at least 12 hr prior to the study. Experiments and animal care complied with guidelines outlined in the Guide to Animal Use and Care of the University of Tokyo.

Test meal: The test meal consisted of 10 g/kg body weight of commercial wet food (SPECIFICR® CIW, Intervet Schering-Plough Animal Health, Tokyo, Japan), 1 baked egg yolk, and 50 mg ^{13}C-sodium octanoate (Cambridge Isotope Laboratories, Inc., Andover, MA, U.S.A.). After the ^{13}C-octanoate was dissolved in the egg yolk, the wet food was mixed in. The test meal was baked in a microwave to increase retention in the solid phase.

Ultrasonography: We modified a technique described previously in humans [12, 17] to establish a new method of assessing canine gastric motility. Gastric antral motility was assessed using ultrasonography (ProSound SSD-5000 SV, Aloka Co., Ltd., Tokyo, Japan) with a 7.5-MHz phased array sector transducer. The ultrasonography was performed by a single operator. Dogs were restrained in the right recumbent position, and the probe position was adjusted to obtain maximum visualization of transverse image of the gastric antrum close to the left lobe of the liver. The cross-section of antral area was measured by tracing the serosal side of the antrum with the built-in caliper (Fig. 1). The antral area was measured 3 times in both the contracted and relaxed phases. The number of contractions in 3 min was counted. Amplitude (%) was calculated with following formula: (mean area relaxed - mean area contracted)/mean area relaxed. Frequency was defined as the number of antral contractions in 3 min. Motility index (MI), an indicator of gastric motility, was expressed as amplitude multiplied by frequency. Continuous calculation of the MI was performed before feeding and every 30 min after feeding for 3 hr.

^{13}C-Octanoic acid breath test: The ^{13}C-octanoic acid breath test was performed according to previous reports in dogs [19, 31]. A breath bag (Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan) was connected to a plastic mask, and expired air was collected from dogs through the mask. Breath samples were collected before giving the test meal as a baseline, every 15 min after feeding for 4 hr, and every 30

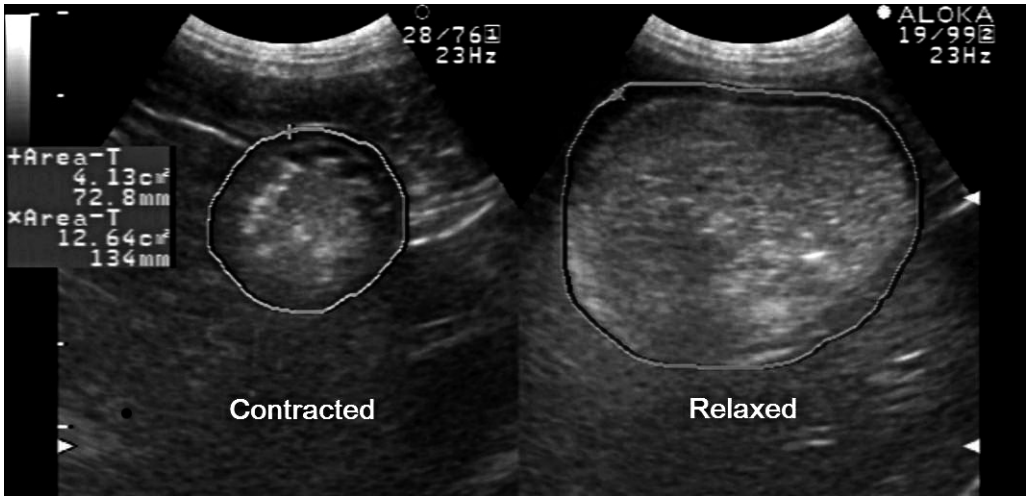

Fig. 1. Ultrasonographic scanning of the cross-section of the gastric antrum in a contracted (left) and relaxed (right) phase 30 min after having given the test meal.

min for additional 2 hr. The breath samples were analyzed using a $^{13}CO_2$-infrared spectrophotometry analyzer (POC one, Otsuka Pharmaceutical), which was previously used to assess gastric emptying in horses [20]. The amount of ^{13}C in the samples was expressed as the change ($\Delta^{13}CO_2$, ‰) in the $^{13}CO_2/^{12}CO_2$ ratio before and after feeding of the test meal. Resting $^{13}CO_2$ production was assumed stable at 0.194 *l*/(m^2•min) [31]. Body surface area was calculated using the following formula: body surface area (m^2)=10.1 × body weight $(g)^{2/3}$/10000 [21]. ^{13}C excretion rate (^{13}C %dose/hr) was calculated according to Ghoos [10] by fitting the following formula: $y=at^be^{-ct}$ (y, %dose/hr; t, time; a, b, and c, constants; and e, exponential). Cumulative ^{13}C recovery in the breath (C%D, %dose) was determined using the following formula: $C\%D=m(1-e^{-kt})^{\beta}$ (t, time; k, m, and β, constants). Lag phase (t_{lag}), the gastric emptying coefficient (GEC), and half-emptying time ($t_{1/2}$) were regarded as gastric emptying parameters. t_{lag}, GEC, and $t_{1/2}$ were calculated using the following formulae: $t_{lag}=b/c$, GEC=ln (a), and $t_{1/2}=(-1/k) \times \ln(1-2^{-1/\beta})$. t_{lag} is the time of the maximum emptying speed of the substrate. GEC is an index of gastric emptying rate. $t_{1/2}$ is the estimated time during which half of the total $^{13}CO_2$ is excreted. These parameters were calculated using excel program software.

Evaluation of atropine-induced gastric hypomotility by ultrasonography: Ultrasonography was performed on the same 7 dogs with atropine-induced gastric hypomotility. Atropine (atropine sulfate, Mitsubishi Tanabe Pharma, Co., Ltd., Osaka, Japan) was administered intramuscularly at a dose of 0.04 mg/kg just before giving the meal. The dose and route of administration were set according to a previous report that confirmed atropine-induced gastric antral hypomotility in healthy beagles [3]. Ultrasonography was performed 30 min after feeding, and the MI was calculated. The results were compared with those obtained without atropine administration (control).

Statistical analysis: The coefficient of variance (CV) was calculated for the MI at each time point to determine the time point at which the variance in the MI was lowest. The correlation between the MI and gastric emptying parameters (t_{lag}, $t_{1/2}$, and GEC) as determined by the ^{13}C-octanoic acid breath test was compared using Pearson's correlation coefficient. The MI of atropine-induced gastric hypomotility was compared with that of the control using Paired *t*-test. All data were expressed as mean ± SD. Significance was set at $P<0.05$.

RESULTS

All dogs were able to take part in all studies. The test meal was eaten immediately by all dogs. Cross-sectioning of the gastric antrum revealed a round shape close to the left lobe of the liver. Although there was a tendency for gastric gas to increase after 90 min of feeding, all gastric antrum were well visualized. The mean ± SD of amplitude (%) for the 7 dogs was 33 ± 11 before feeding and 61 ± 4 at 30 min, 59 ± 4 at 60 min, 61 ± 6 at 90 min, 51 ± 7 at 120 min, 47 ± 10 at 150 min, and 49 ± 11 at 180 min after feeding. The mean ± SD of frequency (number/3 min) was 9.14 ± 4.81 before feeding and 16.57 ± 0.53 at 30 min, 16.86 ± 0.9 at 60 min, 16.29 ± 0.76 at 90 min, 15.57 ± 1.51 at 120 min, 15.14 ± 1.77 at 150 min, and 15.57 ± 1.4 at 180 min after feeding. The time course of the MI is shown in Fig. 2. The mean ± SD of MI for the 7 dogs was 3.05 ± 2.77 before feeding and 9.77 ± 0.42 at 30 min, 9.90 ± 0.86 at 60 min, 9.85 ± 1.1 at 90 min, 7.92 ± 1.51 at 120 min, 7.32 ± 1.76 at 150 min, and 7.32 ± 1.76 at 180 min after feeding. The mean CV of the MI for each time point was 90.92% before feeding and 4.25% at 30 min, 8.64% at 60 min, 11.14% at 90 min, 19.03% at 120 min, 25.41% at 150 min, and 24.6% at 180 min after feeding. The mean MI was at its peak at 30 min and gradually decreased until 180 min. The CV of the MI was the lowest at 30 min.

The mean ^{13}C-octanoic excretion curve for the 7 healthy dogs is shown in Fig. 3. The mean ± SD of t_{lag}, GEC, and $t_{1/2}$ were 81 ± 23.3 min, 4.28 ± 0.43, and 110 ± 22.4 min, respectively. The MI tended to be negatively correlated with t_{lag} (R^2=0.472, P=0.087; Fig. 4A). The MI at 30 min after feeding was positively correlated with GEC (R^2=0.812, P=0.005; Fig. 4B). Furthermore, there was a negative correlation between the MI at 30 min and $t_{1/2}$ (R^2=0.654, P=0.027; Fig. 4C).

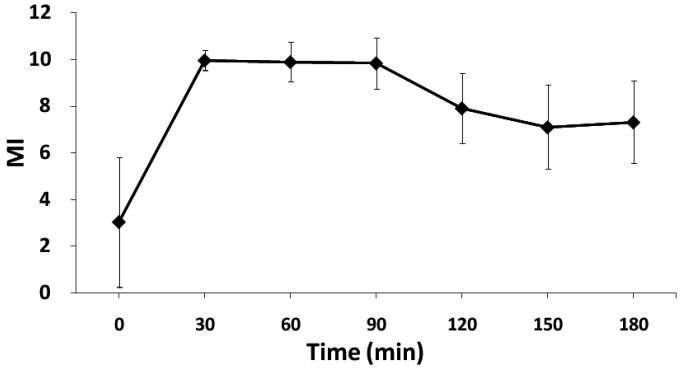

Fig. 2. Time course of the motility index after ingestion of the test meal. Data are given as mean ± SD.

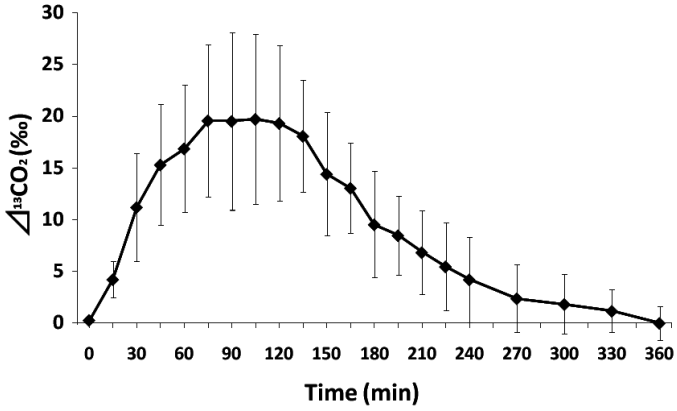

Fig. 3. $^{13}CO_2$ excretion curve in 7 healthy dogs as assessed with the ^{13}C-octanoic acid breath test. Data are given as mean ± SD.

MI was also evaluated in the 7 dogs with atropine-induced gastric hypomotility and compared with that of dogs that were not administered atropine (control). The mean ± SD of MI at 30 min after atropine administration was 5.19 ± 0.22. In all dogs, the MI decreased compared with control (9.77 ± 0.42) and this decrease was statistically significant (P<0.05; Fig. 5).

DISCUSSION

In the present study, we established a ultrasonographic method for evaluating postprandial gastric motility in dogs. There was a significant correlation between the MI of antral contractions and gastric emptying as assessed by the ^{13}C-octanoic acid breath test. Furthermore, gastric hypomotility induced by atropine was detected by this method. These results indicate that the simple assessment of MI at 30 min after feeding is useful in screening postprandial gastric motility in dogs.

In the present method, gastric antral tone and frequency were evaluated ultrasonographically by observing real-time gastric movement. Amplitude, or the intensity of antral contraction, was estimated from changes in the antral area during contractions, whereas frequency was determined by the number of contractions. The MI, an indicator of gastric antral motility, reflects both the frequency and intensity of contractions. To reduce the degree of technical variability in the experiment, we measured antral area 3 times and defined the average as the amplitude. Although the use of force transducers or myograms provides more accurate information about antral motor activity, the ultrasonographic method can screen for antral motility with minimum invasion in dogs.

In this study, the MI was ultrasonographically measured every 30 min for 3 hr to compare variability at each time point. The mean MI in the healthy beagles reached its peak at 30 min after feeding, and its variability was lowest among the time points. The MI then gradually decreased until 180 min, and the variability tended to increase with time. In a human report, difference in MI between cases with functional dyspepsia, which is known to cause gastric hypomotility, and healthy volunteers is prominent in the early postprandial state [17]. Therefore, 30 min after feeding is an appropriate time point at which to detect gastric motility abnormalities, because this time point is in the early postprandial phase and shows the lowest variability in healthy dogs. Thus, the method described here will enable clinicians to assess postprandial gastric motility more simply in a short time compared to previous methods described in dogs.

The present study investigated the correlation between postprandial antral motility as evaluated by ultrasound and gastric emptying as assessed by the ^{13}C-octanoic acid breath test to confirm the validity of the ultrasonographic method. Gastric emptying is regulated by the tonic contraction of the proximal stomach, antral contraction, and the inhibitory forces of pyloric and duodenal contraction. Among them, gastric antral motility is regarded as one of the main regulators of gastric emptying of solid meals [15]. A previous report described that emptying rates for the entire gastric contents are correlated with antral motor activity during the active emptying phase for solids in humans [4]. We adopted the ^{13}C-octanoic acid breath test as a comparative test because this method correlates with a gold standard of gastric emptying, scintigraphy, in humans [6]. It also enabled us to perform the study with minimum stress to the dogs. The results showed that the MI at 30 min was negatively correlated with $t_{1/2}$, or the time of gastric emptying, and positively correlated with GEC, an indicator of the rate of gastric emptying [10]. These results indicate that the MI of antral contraction is correlated with gastric emptying, demonstrating the validity of the ultrasonographic method.

In general, gastric hypomotility is clinically important and may be a therapeutic target of gastroprokinetic agents. Therefore, we confirmed the ability of the ultrasonographic method to detect atropine-induced gastric hypomotility. Atropine is a competitive antagonist for the muscarinic acethylcholine receptor, which acts on postganglionic para-

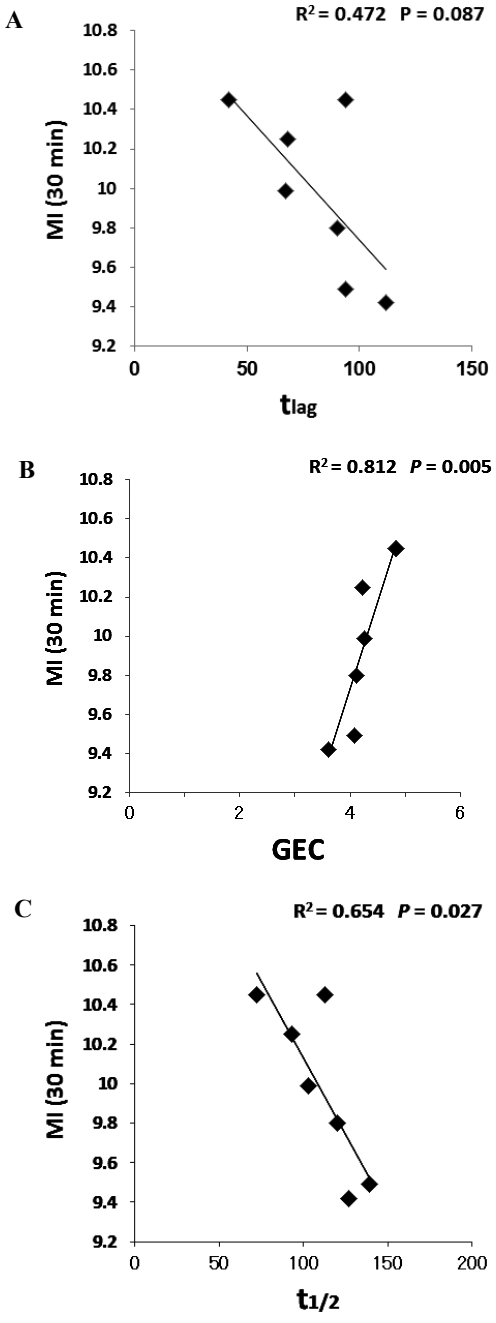

Fig. 4. Correlations between the motility index (30 min) and t_{lag} (Fig. 4A), GEC (Fig. 4B), and $t_{1/2}$ (Fig. 4C).

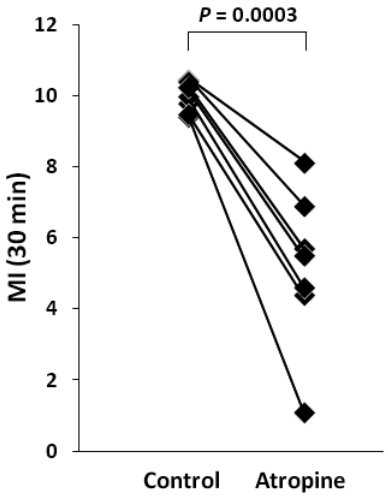

Fig. 5. Comparison of the motility index (30 min) with and without atropine administration in 7 dogs.

sympathetic neuroeffector sites and inhibits peristalsis in the gastrointestinal tract. An obvious decrease in the MI was observed in all dogs after atropine administration, indicating the validity of the ultrasonographic method for detecting gastric hypomotility in dogs.

Gastric motility is affected by many physiological, pharmacological, and pathological conditions, and abnormalities in gastric motility may be associated with upper gastrointestinal clinical symptoms [2, 11]. In humans, many reports have investigated the effect of gastroprokinetic agents in the treatment of gastric motility disorders [24, 28]. A better understanding of the pathogenesis of gastric motility will contribute to the management of upper gastrointestinal clinical symptoms. Among the various methods of assessing gastric motility or emptying, the advantage of ultrasonography is that it is non-invasive and is readily available in daily practice. Ultrasonography has already been used clinically on humans, and gastric motility has been investigated in patients with functional dyspepsia [17], gastric ulcer [9], and diabetes with metabolic syndrome [26]. In addition, the prokinetic effect of $5HT_4$ receptor agonist mosapride citrate was investigated using this ultrasonographic method [16]. In contrast, the significance of canine gastric motility is poorly understood because there have been limits to the use of previous assessment methods in clinical settings. Like in humans, this ultrasonographic method will be able to evaluate the canine gastric motility disorders and efficacy of gastroprokinetic agent easily. A limitation of the present study is that gastric motility was assessed only in beagle. Further study is required to determine the normal range of motility index by performing the ultrasonographic method in various breeds.

In conclusion, we have established a simple ultrasonographic method to evaluate postprandial gastric antral motility in dogs. This method enables the screening of canine gastric motility simply in a short time. By using this method, further studies are required for the better understanding of the pathogenesis of gastric motility disorders in dogs.

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