Ultrasonographic measurement of kidney-to-aorta parameters in Whippets

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Abstract

In a previous study, an ultrasonographic method to assess kidney size in dogs as a ratio of kidney length to aortic luminal diameter (KL/AoD ratio) was proposed. The main lim- itation of this method was the wide range of normal values (5.5-9.1), which resulted in poor sensitivity and specificity. The aim of this prospective, observational, refer- ence interval study was to determine whether the KL/AoD normal cut-off values in a single breed (Whippets) would have a narrower range than the previously reported normal reference ranges. The influence of sex, age, weight, and side on kidney length (KL) and of sex, age, weight, and scanning plane (longitudinal vs transversal) on aortic luminal diameter (AoD) were also investigated. Thirty-six clinically healthy Whippets (16 males, 20 females) without ultrasonographic renal lesions were included in this study. The 95% confidence interval of mean KL/AoD was found to be narrower than the previously reported range (ie, 6.3-6.9 versus 5.5-9.1). This was considered to be especially notable in that the KL in this breed exhibits marked sexual dimorphism. The KL/AoD ratio did not differ between right versus left sides or male versus female sexes in Whippets ($P > .05$). Findings from the current study provided KL/AoD ratio nor- mal reference range cut-off values for future use in Whippets and supported the use of breed-specific KL/AoD ratio values for characterizing abnormal renal size in other canine breeds.

1. Introduction

Ultrasonography is a standard diagnostic test for evaluating dogs with suspected renal disease, however subjective assessments can be affected by the degree of the operator's expertise.[1] Previous research studies have described quantitative ultrasound methods for more objectively characterizing renal size in dogs. Some studies have correlated renal linear measurements with body weight[2,3] or with the length of the sixth or seventh lumbar vertebra.[4] Other studies have described methods for sonographically estimating the kidney volume.[5–7] In general clinical practice, the time necessary to perform these measurements can be a constraint and therefore renal ultrasonographic dimensions are more commonly evaluated subjectively.[8,9] In 2007, a new method for more quickly quantifying canine renal size was proposed: a ratio of kidney length (KL) to aortic luminal diameter (AoD).[10] That method was found to have good reproducibility when applied by different operators.[10,11] However, the main limitation of this method was the wide range of normal cut-off values, which increased the likelihood of having an overlap in values for dogs with versus without renal pathologies.

One possible reason for the wide range of normal values could have been the use of different breeds, different morphologies (i.e. brachymorphic, mesomorphic and dolichomorphic), and different body weights .[3,12]

We hypothesized that the cut-off values for a single breed of dog would be narrower and thus of greater clinical value. Primary objectives of this study were to determine normal cut-off values of the KL/AoD ratio in a sample of clinically normal Whippets and compare the results with the previously published reference values.[10] Secondary objectives were to test the effects of sex, age, weight, and side on KL and of sex, age, weight, and scanning plane (longitudinal vs transverse) on AoD.

2. Materials and methods

The prospective, observational, reference interval study was approved by the Clinical Ethical Review Board of the University of Naples "Federico II" (n◦ 64674), and performed at the Interdepartmental Center of Veterinary radiology of the University Federico II of Napoli and at three private breeding kennels. The sample size for the study was based on a prospective power analysis (G*Power, v. 3.1.9.2, March 2014, Heinrich- Heine-Universität Düsseldorf, Germany), selecting correlation from the t tests family, applying one tail, an effect size of 0.4 (mean effect size according to Cohen) a significance level (α) = 0.05 and a power of 80%. The number of dogs meeting the inclusion criteria were enrolled in a period of time between March 2017 and December 2017. Only clinically healthy dogs and without ultrasonographic renal lesions were included. Dogs were considered to be clinically healthy if there was no history of signs consistent with renal disease and the clinical examination was unremarkable for diseases related with the urinary system. Final decisions for dog inclusion or exclusion were made by a professor of veterinary medicine (M.P.P.) for the clinical examinations and by a professor of veterinary radiology (L.M.) for the ultrasound examinations.

2.1. Data recording

For each included dog, the following clinical characteristics were recorded by a first-year PhD student (D.C.): sex, weight (in kg), age (in months), and findings from the physical examination eventually compatible with systemic disease and/or renal function impairment (ie, hyperthermia/hypothermia, muscle wasting, lethargy, weakness, etc.). Ultrasonography examinations were performed by a professor of veterinary radiology with 24 years of experience in ultrasonography (L.M.). Dogs were physically restrained in the right and left lateral recumbent position or in a standing position at the discretion of the ultrasonographer. No sedatives were administered. Transducer-skin contact was achieved after first moistening the skin with alcohol and then applying acoustic coupling gel. The US exams were performed using one of two devices (MyLab Class C Vet or MyLab 30 Vet, Esaote, Genova, Italy), each equipped with a 3.5−10 MHz microconvex electronic transducer. Each kidney was preliminarily evaluated in order to rule out parenchymal alterations. Using previously described protocols,[10] the KL was measured on still images acquired in the dorsal plane (Figure 1A), when the distance between the two poles was maximum and, the renal pelvis clearly visible, to avoid oblique scans and consequently a possible underestimation of the kidney length. The AoD was assessed from the left side, in transversal (AoDT) and longitudinal (AoDL) scans, just caudal to the origin of the left renal artery. During the examination the operator was careful not to apply excessive pressure on the abdominal wall and compress the aorta. Measurements were made from still images acquired at the maximum luminal diameter, after reviewing cine-loop frames to account for aortic pulsation. Measurement cursors were placed at the borders of the lumen, after excluding the vessel walls (Figure 1B). Both the kidneys and the aorta diameters were measured in triplicate and the average values were used for statistical analyses.

2.2. Data analyses

Statistical analyses were performed by an observer with a Ph.D. degree and eight years of expertise in statistics (L.A.). Data were entered into an electronic spreadsheet (Microsoft Excel ver.16.10 2016, Microsoft Corp., Redmond, WA, USA) and statistical analyses were performed using dedicated software (IBM SPSS Statistics, v. 26.0 IBM Corporation, Armonk, NY, USA; Prism ver.7.0, GraphPad Software, Inc. La Jolla CA USA). The normality of data distribution was evaluated using the Shapiro–Wilk test. Descriptive statistics, including the mean, range (minimum to maximum), standard deviation and 95% confidence interval (CI) of the mean were calculated for KL, AoDT, AoDL, and the ratios KL/AoDL and KL/AoDT (Table 1). A mixed linear model was applied to evaluate the effects of sex, age (considered as a continuous variable), and bodyweight (as fixed principal effects) and of subject and side (as random factorial effects) on KL, KL/AoDL, and KL/AoDT; AoDT and AoDL were also assessed after adjusting for the effects of sex, age, and bodyweight (fixed effects) and of the subject (random effects). Since sex, age, and bodyweight significantly affected KL, AoDT, and AoDL, the effects of age and sex were also tested within sex in a general linear model, prior to further analysis. Post hoc tests were selected according to variable type (continuous vs. categorical) and distribution. Differences in KL, AoDL, and AoDT between males and females were tested using the pooled Student's *t*-test with Levene's test. Correlations of age with KL, AoDL, and AoDT were studied using Spearman's rank correlation coefficient (r_s) within males and with Pearson's product moment test (r) within females. The correlation between bodyweight and KL was studied with Spearman's rank correlation coefficient (r_s). Correlations of bodyweight with AoDL and AoDT were studied using Pearson's product-moment test (r) within both sexes and in the whole sample. AoDL and AoDT were compared using a Bland–Altman plot and the intraclass correlation coefficient (ICC) for absolute agreement was calculated with 95% CI, using a two-way mixed model for single measurements. KL was normalized to the AoD derived from both lon- gitudinal (KL/AoDL) and transversal (KL/AoDT) scans, and the ratios were studied using the same statistical approaches used for AoDL and AoDT. In all analyses, $P < .05$ was considered statistically significant.

3. Results

The power analysis yielded a sample size goal of 34. A total of 39 Whippets (17 males and 22 intact females) met initial criteria for inclusion. Three dogs (2 females and 1 male) were excluded due to the presence of mild pyelectasia (2) and irregular renal contour (1) on ultrasound. The remaining 36 dogs ranged from 10 months to 14 years of age and weighed between 10 and 20 kg (mean 14.12 ± 2.38 kg).

The mixed linear model identified significant effects of sex ($P < .0001$) and bodyweight ($P = .029$) on KL, but no effects of age ($P = .66$), subject ($P = .30$), or side ($P = .78$). The effect of bodyweight was independent of sex ($P = .07$). KL differed significantly between males and females, and was longer in males ($P < .0001$). There was no correlation between KL and age. However, there was a positive linear correlation between KL and bodyweight (rs = 0.64, $P < .0001$, Figure 2).

For both AoDT and AoDL, the mixed linear models found significant effects of sex ($P < .0001$), age ($P < .0001$), and bodyweight ($P < .0001$). In male Whippets, both AoDL (Figure 3A) and AoDT were positively correlated with age ($r_s = 0.56$, $P = .03$, for both). A positive correlation was also identified in females for AoDT ($r = 0.46$, $P = .04$, Fig- ure 3B), but not for AoDL ($r = 0.16$, $P = .49$). In all dogs combined, a significant positive correlation with age was found for both AoDL ($r = 0.44$, $P = .007$,) and AoDT ($r = 0.30$, $P = .07$,). A Bland–Altman plot showed minimal bias (−0.03 ± 0.11; 95% CI −0.25 to 0.17, Figure 4A) and an ICC of 0.63 ($P < .0001$; 95% CI 0.40−0.77) between AoDL and AoDT.

None of the effect variables included in the mixed linear model had any effect on either KL/AoDL or KL/AoDT. The Bland–Altman plot showed a bias of 0.27 (± 0.73; 95% CI −1.15 to 1.70, Figure 4B) and an ICC of 0.69 ($P < 0.0001$; 95% CI 0.49−0.81) between KL/AoDL and KL/AoDT.

4. Discussion

Breed-specific findings from the current study of Whippets, as hypothesized, contributed narrower KL/AoD cut-off values than those previously proposed by Mareschal et al.[10] This is consistent with findings from studies of other morphometric indexes such as the Vertebral Heart Score.[13,14] Use of ratios with adjacent structures, categorization by body conformation (i.e. brachymorphic, mesomorphic, and dolichomorphic), or determining breed-specific values can help to maximize clinical utility.[3,12]

The ultrasonographer for the current study considered the KL and AoD to be relatively easy to obtain, as previously reported.[10,11] The cranial margin of the right kidney was sometimes more difficult to clearly outline, due to its more cranial position within the rib cage, particularly when imaging was performed with the dog in a standing position. This position was used in some dogs to reduce the stress associated from placing them in the lateral recumbent position.

Similar to previous reports,[4,10] the absolute length of the kidney was not significantly different between the right and left kidneys, although the right kidney was slightly larger than the left one in other studies.[2,11,12]

The positive correlation between KL and bodyweight, as previously reported,[2] was also identified in our study. This was particularly notable given the fact that the bodyweights showed a fairly narrow range in our sampled dogs (10−20 kg). It has been previously reported that end-stage kidney disease and aging are associated with reductions in renal dimensions.[15] This would suggest that a negative correlation would occur between age and kidney dimensions. However, we found no such correlation in our study.

The KL/AoD did not differ between the right and left kidneys in our sampled dogs. Furthermore, even though Whippets show marked sexual dimorphism, the KL/AoD ratio was not significantly different between the two sexes. These findings supported the normalization of KL using AoD to help minimize outside sources of variability based on differences between sexes and facilitating the comparability of results. Findings also supported using only one range of the KL/AoD ratio for both kidneys and both sexes.

In a previous radiographic study that compared KL relative to the body length of the second lumbar vertebra (L2), there were significant differences between dogs grouped by skull type, particularly between dolichocephalic and brachycephalic dogs.[3] Therefore, it is possible that the KL/AoD cut-off values obtained in the present study could be used as a reference for other dolichocephalic breeds, particularly sighthound breeds. However, given the differences in body conformation among breeds within the same skull types, and considering the high correlation between bodyweight and KL; this theory would need to be confirmed in studies of other dolichocephalic and/or dolichomorphic breeds.

We did identify a positive correlation between age and AoD in our sampled dogs. This could have been due to an increase in systemic arterial pressure that occurs with aging.[16,17] One study found that systemic arterial pressure is significantly greater in males and in sighthound dogs (e.g. Whippets).[17] Another explanation could involve degeneration of vessel walls, as reported in healthy humans.[18] Blood pressure was not measured in dogs for the current study and may therefore war- rant further investigation. The positive correlation between AoD and bodyweight likely reflected an expected relationship between AoD and the size of the dog.

We also found that the AoD measured in longitudinal and transversal scans displayed minimal bias and a high ICC. In the original study that proposed the KL/AoD ratio,[10] the measurements obtained from longitudinal scans showed a higher degree of agreement between different operators. That result indicated greater variability of measurements obtained using transversal scans. A proposed reason was that the ultrasound beam is never perpendicular to the long axis of the vessel in this orientation. For this reason, the authors concluded that it is preferable to measure the AoD on longitudinal scans.[10] In another study of dogs aged ≤18 months, the KL/AoD ratio was easier to obtain using transversal scans than with longitudinal scans.[11] The authors reported that this was due to motion artifacts and the small sizes of the anatomical structures. Previous studies that measured vessel diameters suggested that transversal scans may be used as exploratory scans, and recommended obtaining the measurements using longitudinal scans, since the transversal scans are affected by multiple refraction artifacts that reduce their quality.[19,20] In our study, we found no significant differences in AoD between the longitudinal and transversal scans, with minimal bias on a Bland–Altman plot and a large ICC. Regarding the KL/AoD ratio, although the Bland–Altman plot showed greater bias, the ICC was slightly larger and had a narrower 95% CI. In order to establish whether longitudinal or transversal scans are bet- ter for measuring AoD, we think that other imaging modalities, such as CT or MRI, are needed to provide a more objective measurement of AoD.[21]

The main limitations of our study may include the relatively small number of dogs used and their classification as healthy on the basis of the physical examination and ultrasonographic examination alone. For the a priori power analysis we selected a power of 80%, which should be considered fair in view of the inclusion of a single pure breed, and on statistical considerations per se. Yet, our results should be confirmed on larger samples. The dog's state of hydration and any subclinical cardiac pathologies that can affect blood pressure and, consequently, the AoD was not investigated. Furthermore, although the dogs did not show any clinical or US signs of kidney disease, we cannot exclude the possibility of subclinical kidney disease.

In conclusion, findings from the current study supported the use of breed-specific normal reference values for KL/AoD ratios. In this sample of Whippets, taking into account the 95% CI of the mean KL/AoD, a value of < 6.3 could indicate decreased renal size, whereas a value > 6.9 could indicate an enlarged kidney. This range (6.3-6.9) is narrower than the previously reported range (5.5-9.1),[10] and may help to minimize possible overlap with pathological values for future clinical patients. Although Whippets show marked sexual dimorphism, the KL/AoD ratio was not significantly different between the two sexes. Further studies are needed to obtain reference range measurements for each breed or class of dogs. Studies are also needed in order to determine the effect of hydration status and systemic arterial pressure on aorta diameter and therefore on the KL/AoD ratio.

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List of Author contributions

Concept and Design: Costanza, Meomartino, Greco. Acquisition of Data: Meomartino. Analysis and Interpretation of Data: Auletta, Pasolini, Costanza, Mennonna, Lamagna. Drafting the Article: Costanza, Meomartino, Pasolini, Auletta. Revising Article for Intellectual Content: Greco, Mennonna, Meomartino, Lamagna. Final Approval of the Completed Article: Costanza, Pasolini, Greco, Auletta, Mennonna, Lamagna, Meomartino.

Conflict of interest

The authors have no conflicts of interest to declare.

Abbreviations:

AoD, aortic luminal diameter;

AoDL, aortic luminal diameter on longitudinal scan;

AoDT, aortic luminal diameter on transversal scan;

CI, confidence interval;

ICC, intraclass correlation coefficient;

KL, kidney length.

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Tables

TABLE 1. Descriptive statistics for the kidney lengths, aortic luminal diameter and the kidney-to-aorta ratios in a sample of clinically normal Whippets.

	MEAN ± SD (n=36)	**95% C.I.**	**RANGE (Min-Max)**
KL (cm)	6.2 ± 0.7	6 - 6.4	4.9 - 8.2
AoL (cm)	0.94 ± 0.11	0.91 - 0.98	0.71 - 1.3
AoT(cm)	0.98 ± 0.1	0.95 - 1	0.78 - 1.3
KL/AoDL	6.7 ± 0.83	6.5 - 6.9	5 - 9.1
KL/AoDT	6.5 ± 0.78	6.3 - 6.7	4.1 - 8.2

Values in the sample were normally distributed.

Abbreviations: 95% CI = confidence interval; AoDL = aortic luminal diameter measured on longitudinal scans; AoDT = aortic luminal diameter measured on transversal scans; KL = kidney length; KL/AoDL = kidney-to-aorta ratio obtained from longitudinal scans of the abdominal aorta; KL/AoDT = kidney-to-aorta ratio obtained from transversal scans of the abdominal aorta; Max = maximum.; Min = minimum; SD = standard deviation.

Figures

Figure 1. A, Measurement of the kidney length (7.15 cm) on a dorsal ultrasound scan (patient in right lateral recumbency; multifrequency microconvex probe, 6.5 MHz). B, The abdominal aorta is scanned from the left side and the aortic luminal diameter is measured just caudal to the emergence of the left renal artery. The aortic luminal diameter is measured on longitudinal (1.13 cm) and transversal (1.11 cm) scans by placing the electronic calipers at the border of the lumen, after excluding the vessel walls.

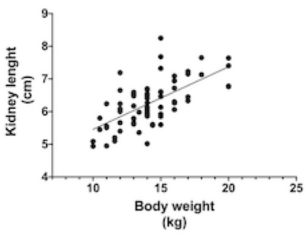

Figure 2. Correlation between kidney length (in cm) and bodyweight (in kg).

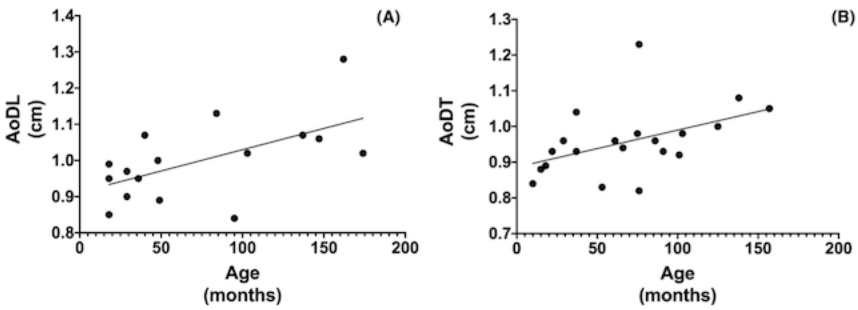

Figure 3. A, Correlation between aortic luminal diameter measured on longitudinal scans (AoDL) and age (in months) of male Whippets. B, Correlation between the aortic luminal diameter measured on transversal scans (AoDT) and age (in months) of female Whippets.

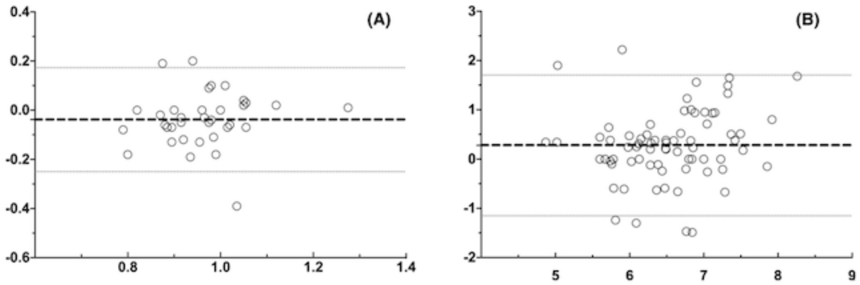

Figure 4. A, Bland–Altman plot comparing the aortic luminal diameters, measured on longitudinal and transversal scans (AoDL and AoDT). B, Bland–Altman plot comparing the kidney-to-aorta ratio obtained from longitudinal and transversal scans of the abdominal aorta (KL/AoDL and KL/AoDT). The y axis shows the difference between the two measurements and the x-axis shows the average of both measurements. The dotted lines represent the 95% confidence intervals and the dashed line represents the bias