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A DISSERTATION FOR THE DEGREE OF MASTER

Dynamic CT of the Adrenal Glands in Normal Dogs

개에서 역동적 전산화단층촬영을
이용한 부신의 평가

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Abstract

Dynamic CT of the Adrenal Glands in Normal Dogs

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Computed tomography (CT) appearance of the adrenal glands
has been described dogs with pituitary-dependent

hyperadrenocorticism and adrenal neoplasia, and normal dogs. However, to my knowledge, there are no available data on the changes in the contrast-enhancement pattern of the normal canine adrenal glands therefore the contrast enhancement pattern and the attenuation parameters of the normal canine adrenal glands were evaluated, using dynamic CT. Ten healthy beagle dogs were recruited for this study. After survey CT scanning, the dynamic scans (60 scans) were initiated at time of the injection of the contrast medium. The contrast medium with an iodine concentration of 300 mgI/ml was administered with a power injector at a dosage of 640 mgI/kg and a flow rate of 3 ml/s. The time-attenuation curves of the adrenal gland and the aorta were constructed, and based on the changes in the attenuation values, the attenuation parameters were measured. The mean time to adrenal enhancement appearance was 12.3 ± 2.4 seconds after injection, and the mean time to aortic enhancement appearance was 8.55 ± 1.23 seconds after injection. The maximal adrenal enhancement was observed at 21.3 ± 3.15 seconds after injection with a mean attenuation value of 178.9 ± 16.6 HU and the maximal aortic enhancement was observed at 13.95 ± 1.59 seconds after injection with a mean attenuation value of 706.62 ± 140.8 HU. The value for adrenal perfusion by dynamic CT of 2.58 ± 0.67 ml/min/ml is

close to the value for adrenal perfusion by fluorescent microspheres. Dynamic CT is an effective method for assessing the changes in contrast enhancement patterns of normal canine adrenal gland and this attenuation parameters will help future evaluation of canine adrenal gland with CT.

Keywords: Dynamic CT, adrenal gland, contrast enhancement pattern, attenuation parameters, dogs

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Introduction

Advanced imaging modalities such as ultrasonography, CT, and MRI have now greatly enhanced clinician's ability to identify both clinical and subclinical adrenal abnormalities (Schultz *et al.*, 2009; Tidwell *et al.*, 1997). Ultrasonography is the most commonly used modality to assess adrenal morphology in small animals. However, the adrenal glands of large breed, obese dogs and deep chested breed can be difficult to evaluate with ultrasonography and artifacts of intestinal gas can make visualization of the adrenal glands difficult. Moreover, ultrasonography dose not differentiate among types of adrenal gland tumor (Bertolini *et al.*, 2006).

In humans, CT is the primary imaging method for detection and characterization of adrenal tumors. CT provides useful information about anatomic location for masses and gives more precise indication of surround organs, as well as facilitating guidance of needle biopsies. Furthermore, CT allows differentiation between benign and malignant adrenal tumors based on their characteristic attenuation and enhancement (Mayo-Smith *et al.*, 2001).

As in humans, CT has been found to be a useful method for imaging the adrenal glands and detecting adrenal abnormalities in

dogs. The CT appearance of adrenal glands has been described in normal dogs, dogs with pituitary–dependent hyperadrenocorticism, and dogs with primary adrenal neoplasia, using conventional pre– and postcontrast CT (Bertolini *et al.*, 2006; Gregori *et al.*, 2015; Lee *et al.*, 2015; Morandi *et al.*, 2007). In addition, CT is a sensitive and specific preoperative indicator for the assessment of intraluminal vascular invasion of adrenal masses in dogs (Schultz *et al.*, 2009). In this study, sensitivity, specificity, and accuracy of CT for vascular invasion were 92%, 89%, and 94%, respectively.

Until now, there are no available data on the changes in the contrast–enhancement pattern of the normal canine adrenal glands: the mean time to enhancement appearance, the mean peak enhancement, the mean time to peak enhancement. In the present study, the purpose is to measure the attenuation parameters in presumed normal dogs with dynamic CT. And these data will help future CT evaluation of the canine adrenal glands and clinical detection of potential abnormalities.

Materials and Methods

Ten adult beagle dogs, including two females and eight males (weight range, 10.0–13.6 kg; mean, 11.8 kg; SD, ± 1.52) were used for this study. A physical examination, complete blood count, serum biochemistry panel, and urinalysis obtained via cystocentesis were performed on each dog. Prior to the CT scan, dogs were fasted for 12 hours. Anesthesia was induced with alfaxalone (3 mg/kg of body weight, Alfaxan[®], Jurox Pty Ltd., Rutherford, NSW, Australia) intravenously to effect and the trachea of the dogs was intubated. General anesthesia was maintained with isoflurane (Forane[®], Choongwae Pharm Co., Seoul, Korea) and oxygen.

The anesthetized dogs were placed in sternal recumbency on the CT table and survey CT images from the diaphragm to the caudal aspect of the left kidney, including the adrenal glands, were obtained with 3 mm slice thickness and a pitch of 1.5 using a single–detector helical CT unit (GE CT/e[®], General Electric Medical System, Yokogawa, Japan). The scanning parameters were as follow: 120 kV, 50 mA and 3 mm intervals. The dynamic study was acquired after the survey scan at the level of the largest adrenal

gland cross-section. A contrast medium (Omnipaque®, GE healthcare Ireland, Cork, Ireland) with an iodine concentration of 300 mg I/ml was administered with a power injector at a dosage of 640 mg I/kg and a flow rate of 3 ml/s through a 22-gauge plastic catheter preplaced in the cephalic vein. The dynamic scans (60 scans) were initiated at time of the injection of the contrast medium. This study was approved by the Institutional Animal Care and Use Committee, Seoul National University, Seoul (SNU-150903-4).

The attenuation values (in Hounsfield unit; HU) of the adrenal gland were measured using a circular region of interest (ROI). The ROI was drawn manually on each dynamic CT image and included at least one-half of the area of the adrenal gland, excluding the gland edges, to minimize partial volume effects and to exclude adjacent periadrenal fat (Figure 1). In the same image, another ROI was drawn in the aorta. Time-attenuation curves of the adrenal gland and the aorta were constructed from these data. Based on the change in attenuation values, the mean peak enhancement, the mean time to peak enhancement, and the mean time to enhancement appearance, the upslope (wash-in) of the adrenal gland and the aorta were determined.

The upslope was defined as the maximal increase of attenuation between two measurements of the time–attenuation curve. The perfusion was given by dividing the upslope by the peak attenuation in the aorta multiplied by 60 seconds (ml/min/ml) (Miles, 1991; Schultz *et al.* 2009).

The attenuation values of adrenal glands and aorta were measured three times by two observers (S.K. and S.H.). Each of three measurements was compared to evaluate the intraobserver repeatability in each observer, and the mean values of three measurements in each observer were compared to evaluate interobserver reproducibility. All data were recorded in Microsoft Excel 2013. SPSS software (IBM SPSS Statistics for Windows, Version 21.0, IBM Corp., Armonk, NY) was used for statistical analysis.

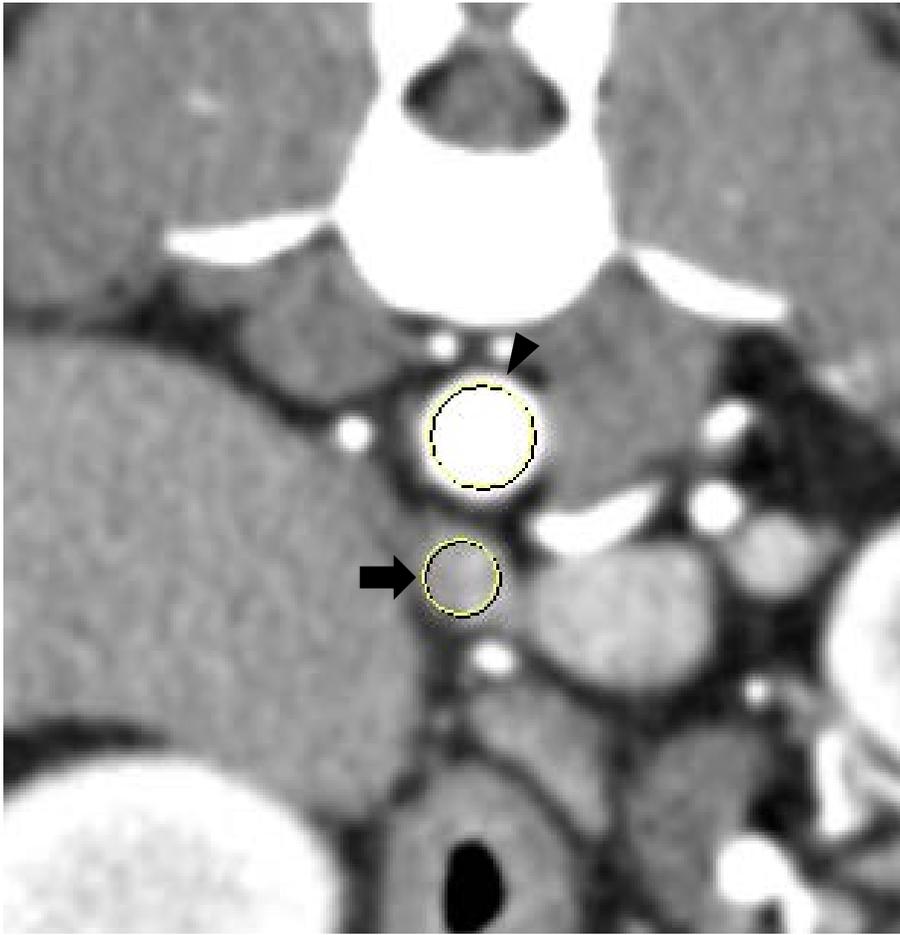


Figure 1. ROI was drawn on each dynamic CT image included at least one-half of the area of the adrenal gland (arrow) excluding gland edges. In the same image, another ROI was drawn in the aorta (arrow head).

Results

Dynamic CT was applied successfully in canine adrenal glands in addition to the conventional CT. Adrenal glands were well visible in dynamic CT images, and relationship of the adrenal gland with vessels (caudal vena cava, aorta and phrenico–abdominal arteries and veins) or other abdominal organs adjacent to adrenal glands (e.g., kidney, liver, pancreas and spleen) in all transverse planes proved to be accurate, allowing vessels and surrounding organs to be excluded from the ROI when determining the attenuation values. Figure 2 shows the dynamic CT images of the adrenal gland and the aorta with time. Figure 3 illustrates the time–attenuation curves from ROI of the adrenal gland and the aorta of a normal beagle dog.

Attenuation parameters of the adrenal gland and the aorta are shown in Table 1. The mean time to adrenal enhancement appearance was 12.3 ± 2.4 seconds after injection, and the mean time to aortic enhancement appearance was 8.55 ± 1.23 seconds after injection. The maximal adrenal enhancement was observed at 21.3 ± 3.15 seconds after injection with a mean attenuation value of 178.9 ± 16.6 HU and the maximal aortic enhancement was observed

at 13.9 ± 1.59 seconds after injection with a mean attenuation value of 706.6 ± 140.8 HU. The mean adrenal attenuation values at aortic enhancement appearance and aortic peaks were 29.2 ± 6.86 HU and 77.3 ± 21.1 HU, respectively. The mean adrenal upslope was 28.8 ± 6.78 , and the mean adrenal perfusion was 2.58 ± 0.67 ml/min/ml. The time to mean peak enhancement was 22.5 seconds and the mean clearance half-life was 57.6 ± 12.8 seconds.

Intra- and interobserver repeatability and reproducibility showed excellent correlation (ICCs of all measurements > 0.9).

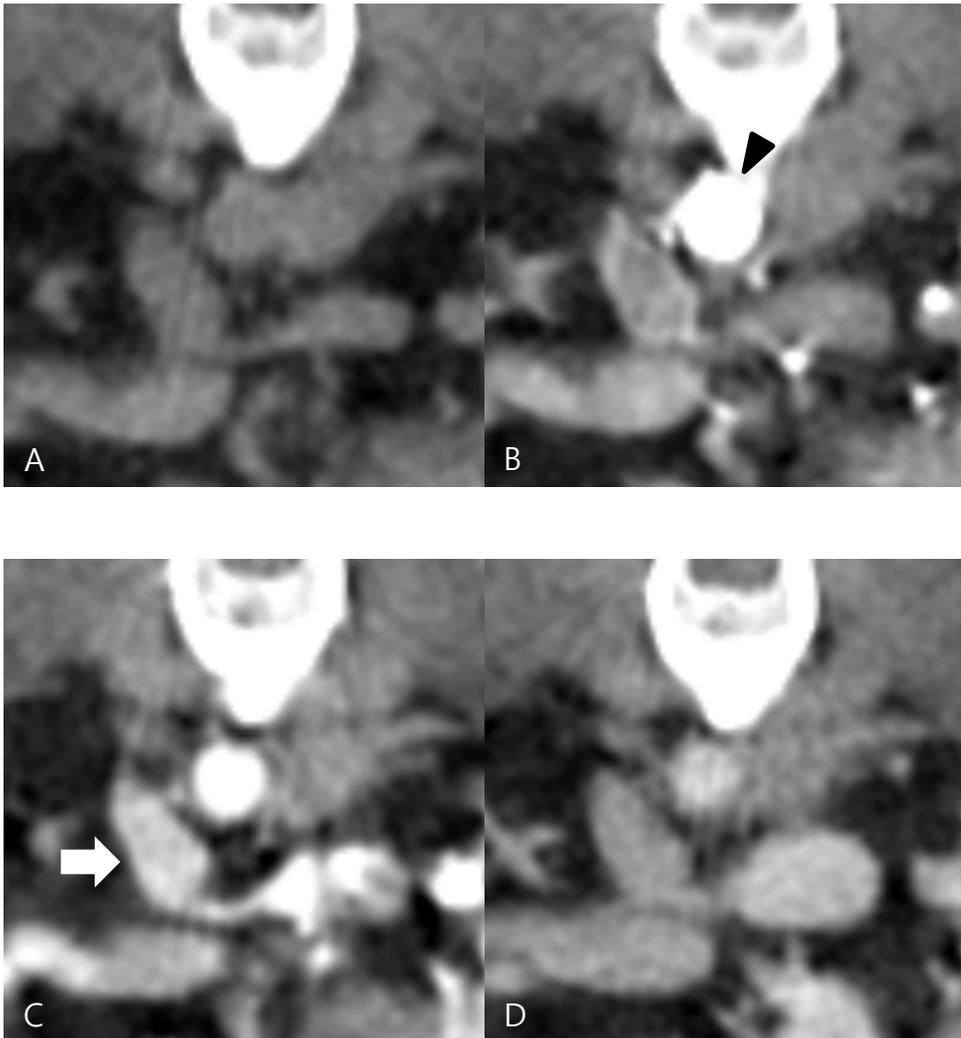


Figure 2. Dynamic CT images of the adrenal gland in a normal beagle dog. (A) Immediately after contrast medium injection. (B) The maximal aortic enhancement (arrow head) appeared approximately 14 seconds after injection. (C) The maximal adrenal enhancement (arrow) appeared approximately 21 seconds after injection. (D) The adrenal and aortic enhancement fade rapidly 33

seconds after injection.

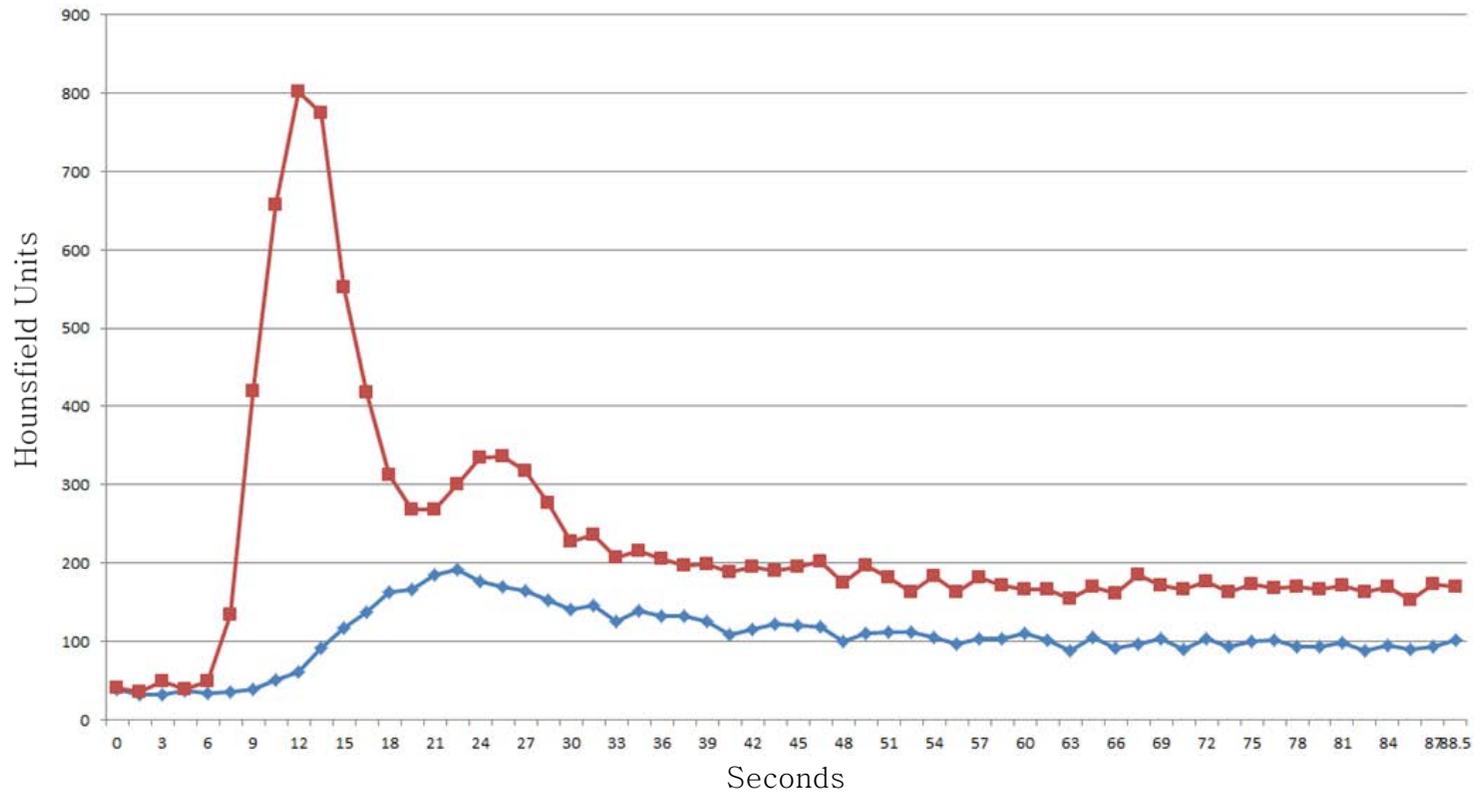


Figure 3. Time attenuation curve from ROI of the adrenal gland (◆) and the aorta (■) that have been applied to the dynamic imaging data set from a normal beagle dog.

Table 1. Attenuation parameters for the adrenal gland and aorta in normal beagle dogs (mean \pm SD)

Measurement	Adrenal gland	Aorta
Time to enhancement appearance (seconds)	12.3 \pm 2.40	8.55 \pm 1.23
Time to peak enhancement (seconds)	21.3 \pm 3.15	13.9 \pm 1.59
Pre contrast medium attenuation (HU)	33.4 \pm 3.29	38.1 \pm 7.51
Peak attenuation (HU)	178.9 \pm 16.6	706.6 \pm 140.8
Upslope of TAC	28.7 \pm 6.78	–
Perfusion (ml/min/ml)	2.47 \pm 0.67	–
Clearance half-life (seconds)	57.6 \pm 12.8	–

Discussion

The contrast enhancement patterns and attenuation values with contrast enhancement of the adrenal glands have been studied in humans. Variable CT protocols allow distinguishing benign from malignant adrenal lesions. Since majority of human adenomas contain a significant amount of intracellular lipid, they have low density value at nonenhanced CT (Boland *et al.*, 1998). In addition, both adenomas and malignancies enhanced rapidly and intensely, however, malignant lesions usually have slower wash-out due to capillary leakage. Thus, when combination of low density values (<10 HU) in nonenhanced CT images and early contrast wash-in/wash-out of the adrenal mass in delayed enhanced CT, a sensitivity of 98% and specificity of 92% was found for characterizing 159 (96%) of 166 adrenal masses (Caoli *et al.*, 2002). A less common, experimental CT protocol of adrenal imaging used to differentiate adenomas and non-adenomas is quadriphasic imaging. In the study, the accuracy of differentiating adenomas from non-adenomas was 86.5% for unenhanced scans (≤ 10 HU threshold), 90.1% for relative peak washout calculations

($\geq 30\%$ threshold), 85.7% for absolute washout calculations (≥ 12 HU threshold), and 83.5% for percentage enhancement wash-out ($\geq 30\%$ threshold) (Foti *et al.*, 2012). Dual-phase CT is used to distinguish pheochromocytomas from lipid-poor adenomas. Pheochromocytomas tend to have higher degrees of enhancement in the arterial phase (>110 HU), however, adenomas tend to have equivalent degree of enhancement across the arterial and venous phases (15%) or increased enhancement on venous imaging compared with the arterial imaging (85%) (Northcutt *et al.*, 2013). Adrenal gland perfusion analysis is another less common investigational technique to evaluate the blood flow perfusion parameters of adenomas and non-adenomas. Adenomas have a greater permeability surface-area production value than non-adenoma. In addition, the blood volume value of ≥ 9.325 ml/min per 100 g predicted adrenal adenoma with sensitivity of 76.9% and specificity of 73.2% (Qin *et al.*, 2012).

As in humans, the CT appearances of canine adrenal tumors have been described in unenhanced and enhanced scans (Gregori *et al.*, 2015; Lee *et al.*, 2015; Morandi *et al.*, 2007). Attenuation values of pheochromocytoma (52.3 ± 5 HU and 30.1 ± 4.6 HU), adenoma (39

± 8 , 40.8 ± 14.3 and 31.8 ± 11.2 HU), adenocarcinoma (36.7 ± 11.8 and 31.8 ± 11.2 HU) and myelolipoma (-71.4 ± 2.6 and between -56 ± 9 and -34 ± 22 HU) were measured in unenhanced phase. Enhanced attenuation values were described in pheochromocytoma (103 ± 21.7 and 104.4 ± 28.6 HU), adenoma (234 ± 20 , 66.7 ± 8.3 and 228 ± 25.5 HU), adenocarcinoma (83.9 ± 52.7 and 137.0 ± 8.6 HU) and myelolipoma (-34.1 ± 1.8 and between 11 ± 27 and 26 ± 48 HU). Delay enhanced attenuation values of pheochromocytoma (93.4 ± 14.3 and 115.3 ± 7.9 HU), adenoma (55.5 ± 22.4 and 134.1 ± 10.6 HU), adenocarcinoma (76.9 ± 28.4 and 90.8 ± 3.3 HU) and myelolipoma (-34.1 ± 1.8 HU).

Also the attenuation values of adrenal glands in normal dogs were measured in both unenhanced and enhanced series with a helical CT, and the mean unenhanced and enhanced attenuation values were 36.0 ± 5.3 HU, 101.5 ± 10.6 HU for left adrenal gland and 34.3 ± 7.0 HU, 97.4 ± 12.4 HU for right adrenal gland, respectively (Bertolini *et al.*, 2006). These attenuation parameters have helped evaluation of the adrenal lesions in the previous studies by comparison with the attenuation values of normal adrenal glands and those of the adrenal lesions. In addition, there was no significant difference in

unenanced attenuation value of the gland between this study and present study. However, distribution of the enhanced attenuation values in normal dogs was large (Bertolini *et al.*, 2006). It was difficult to obtain the enhanced series at precisely the same time for each patient, thus CT scannings were not initiated with peak enhancement in the aorta. Although the duration of delayed scanning was sometimes only a few seconds, a considerable difference in attenuation value was noted (Bertolini *et al.*, 2006). In the present study, the adrenal gland became enhanced 1.6 seconds before aortic peak, and then enhanced attenuation values of the adrenal gland were significantly increased from 30.7 HU to 178.9 HU during 9 seconds. Therefore, these results were consistent with the results of the previous study.

Contrast enhancement at CT is affected by interacting factors: patient factors, contrast medium factors, and CT scanning factors. In addition, the contrast medium and patient factors contribute to the distribution of contrast medium after injection and the resulting dynamics of contrast enhancement (Bae, 2010). Therefore, for more objective comparison with contrast enhancement of the previous studies, it was important to use a fixed contrast injection

protocol. However, in the previous studies, the contrast medium dosage and the injection rate were variable: a dosage of 528 mg I/kg (Morandi *et al.*, 2007), a dosage of 640 mg I/kg and an injection rate of 3 ml/s (Bertolini *et al.*, 2006; Bertolini *et al.*, 2008), a dosage of 700 mg I/kg and an injection rate of 2 ml/s (Gregori *et al.*, 2015), and 840 mg I/kg and manual administration (Schultz *et al.*, 2009). In the present study, the contrast injection protocol of the previous study by Bertolini was chosen, because this study also evaluated adrenal glands in normal dogs, and the patient factors (healthy adult beagle dogs) were almost equal to ours. Furthermore, perfusion can only be correctly estimated with a bolus of a volume <50 ml administered over 6–7 seconds, and our injection protocol satisfied this precondition to estimate perfusion of adrenal glands (Nitzl *et al.*, 2009).

Values for perfusion of canine adrenal cortex and medulla obtained from fluorescent microspheres were 1.8 ± 0.9 ml/min/g and 2.9 ± 1.8 ml/min/g, respectively (Jasper *et al.*, 1990). The adrenal cortex receives a blood supply from cortical capillaries, otherwise medulla has two blood supplies, one via cortical capillaries and the other directly from medullary arteries (Breslow, 1992). Thus, the

medulla has intense vascularization with a rich medullary capillary circulation (Hullinger and Evans, 1993). This explains why the value for perfusion of the medulla is higher than that of the cortex. In the present study, the value for adrenal perfusion by dynamic CT of 2.48 ml/min/ml is between the value of the cortex and that of the medulla obtained from fluorescent microspheres. On the dynamic CT images, the adrenal cortex and the medulla were not clearly separated. Therefore, ROI probably included both the cortex and the medulla of the adrenal glands on the dynamic CT images, and this might influence the values for dynamic CT adrenal perfusion.

Contrast-enhancement ultrasonography was also used to reveal the enhancement pattern in the adrenal glands of normal dogs. Furthermore, in these studies, comparisons between normal dogs and dogs with pituitary-dependent hyperadrenocorticism were described (Pey *et al.*, 2014; Bargellini *et al.*, 2013). Contrast-enhanced ultrasonography of the adrenal glands in dogs with pituitary-dependent hyperadrenocorticism revealed a delayed time to peak (time of arrival of ultrasonography contrast agent to its maximal signal intensity) in the adrenal gland, compared with values for control dogs. Glucocorticoids inducing mineralocorticoid activity

and complex vasodilation and vasoconstriction activity might influence those changes. In addition, compression of the medulla by hyperplastic adrenal tissue might also influence the delayed time to peak in hyperplastic adrenal glands (Pey *et al.*, 2014). A similar study will be needed in CT and the attenuation parameters of the present study, such as time to peak enhancement, will help future evaluation of CT imaging.

Endocrine testing (e.g., a low-dose dexamethasone suppression test, or an ACTH stimulation test) to definitively rule out hyperadrenocorticism was not done. The consensus statement from the American College of Veterinary Internal Medicine on diagnosing canine hyperadrenocorticism states that hyperadrenocorticism is a clinical diagnosis and specific testing for hyperadrenocorticism is not warranted in dogs without clinical signs of this disease. It was determined that none of the dogs were likely to have hyperadrenocorticism based on the physical examination, complete blood count, serum biochemistry panel, and urinalysis, and therefore, specific endocrine testing was not performed (Behrend *et al.*, 2013).

Limitations of our study included the low sampling rate. Because of

the technical limitations of the CT machine, the images were obtained every 1.5 seconds but newer machines have possible imaging rates of 1 second or less, which will reduce the problem but at the expense of greater radiation exposure. The main problem of low sampling rate is underestimation of the height of the time-attenuation curves, and this can also influence the attenuation parameters including perfusion.

In conclusion, dynamic CT is an effective method for assessing the changes in contrast enhancement patterns of the adrenal glands. The attenuation parameters of the normal canine adrenal glands will help future evaluation of canine adrenal gland, using CT.

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국문 초록

과거의 연구에서 뇌하수체 의존성 부신피질기능항진증에 이환된 개의 부신과 부신 종양 그리고 정상 개의 부신을 CT를 이용하여 평가하였다. 하지만 정상 개의 부신에서 조영 증강 패턴의 변화에 대한 연구는 지금까지 보고된 바가 없는 바, 본 연구에서 정상 개의 부신의 조영 증강 패턴과 attenuation parameter를 dynamic CT를 이용하여 평가하게 되었다. 10마리의 건강한 비글견을 사용하였으며, Survey scan을 실시한 후 60개의 scan으로 구성된 dynamic CT 촬영을 조영제의 주입과 동시에 시작하였다. 300 mg I/ml의 농도의 조영제를 640 mg I/kg의 용량으로 power injector를 이용하여 3 ml/s의 속도로 정맥 혈관에 주입하였다. 부신과 대동맥의 time-attenuation curve를 만들었으며 attenuation value의 변화와 attenuation parameter를 평가하였다. adrenal enhancement appearance 평균 시간은 조영제 주입 후 12.3 ± 2.4 초였으며 aortic enhancement appearance 평균 시간은 조영제 주입 후 8.55 ± 1.23 초였다. 최대 adrenal enhancement 평균 시간은 조영제 주입 후 21.3 ± 3.15 초였으며 평균 attenuation value는 178.9 ± 16.6 HU였다. 최대 aortic enhancement 평균 시간은 13.95 ± 1.59 초였으며 평균 attenuation value는 706.62 ± 140.8 HU였다. 그리고 부신의 perfusion value는 2.58 ± 0.67 ml/min/ml이었으며, 이는 fluorescent

microsphere를 이용한 이전 연구의 수치에 가까웠다. Dynamic CT는 정상 개의 부신의 조영 증강 패턴의 변화를 평가하는데 유용하였으며 본 연구의 attenuation parameter는 향후 CT를 이용한 개의 부신의 평가에 도움이 될 것으로 생각된다.

주요어: Dynamic CT, adrenal gland, contrast enhancement pattern, attenuation parameters, dogs

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