수의학석사학위논문

Abdominal Adipose Tissue Measurement Using Magnetic Resonance Imaging in Dogs

개에서 자기 공명 영상을 이용한

복부 지방 조직의 측정

2014년 2월

서울대학교 대학원

수의학과 임상수의학(수의방사선과학)전공

정선영

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지도교수 최민철

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위원장 윤정희 (인)
부위원장 최민철 (인)
위원 성제경 (인)

Abdominal Adipose Tissue Measurement Using Magnetic Resonance Imaging in Dogs

Director: Professor Mincheolchoi

Sunyoung Jung

Major in Clinical Veterinary Medicine (Veterinary Radiology)

Department of Veterinary Medicine

Graduate School

Seoul National University

Abstract

Obesity is a common nutritional disorder in dogs and has been recognized as a risk factor for variable diseases such as diabetes, pancreatitis, arthritis and cardiovascular disorders.

Magnetic resonance imaging (MRI) along with computed tomography (CT) is considered the gold standard for the quantitative measuring of adipose tissue in humans. The aim of this study is to evaluate usefulness of MRI for the measurement of abdominaladipose tissue in dogs compared with CT and to establish an optimal method for evaluation of abdominal adipose tissue using MRI in dogs.

MR scans with T1- weighted breath hold RSSG (radiofrequency spoiled-steady gradient rewound acquisition) and helical CT scans were performed from the diaphragm to the sacroiliac joint in 19 dogs. The MR images were analyzed by manual and semiautomatic methods for the measurement of abdominal adipose tissue. And abdominal adipose tissue was determined by using the attenuation range from -141 to -93 Hounsfield unit in the CT images.

The mean total adipose tissue volumes of the entire abdomen measured by MRI and CT were no significant difference, which were strongly correlated ($r = 0.958$, $p < 0.01$). And there was good agreement between the total abdominal adipose tissue area determined by semiautomatic single-slice method at L2-3 level and the whole abdominal adipose tissue volume measured by manual multi-slice method using MRI ($r = 0.963$, $p < 0.01$).

MRI acquisition technique and imaging analytic method for measuring abdominal adipose tissue used in this study enable to make a fast and reliable abdominal adipose tissue quantitative measurement. In conclusion, MRI could be a good alternative to CT for the measurement of abdominal adipose tissue in dogs.

Keywords: abdominal adipose tissue, measurement, magnetic resonance imaging, dogs

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CONTENTS

I. Introduction

Obesity is a common nutritional disorder in dogs and has been recognized as a risk factor for variable diseases such as diabetes, pancreatitis, arthritis and cardiovascular disorders (1). Generally, 5-19% above ideal body weight is regarded overweight and more than 20% is considered obesity (2). Recently, 23-41% of domestic dogs are overweight or obesity and 40% of dogs, from 6 to 10yrs of age, are obesity (3-5). Especially it has been reported that visceral adipose tissue is significantly associated with obesity-related disease rather than subcutaneous adipose tissue in human studies (6, 7).

Magnetic resonance imaging (MRI) along with computed tomography (CT) has been regarded as the gold standard for the quantitative assessment of abdominal adipose tissue in humans although there are several kinds of methods for the assessment of that (8). The reliability and the accuracy of the MRI for measuring abdominal adipose tissue have been investigated compared to CT in humans (9, 10). Moreover variable scan parameters such as conventional MR image and water-suppressed T1-weighted image techniques were adjusted and imaging analytic technique such as single-single slice images in specific location and multiple-single slices images were used (8). Based on the variable quantitative assessement of abdominal adipose tissue using MRI in human studies (11-13), this study was conducted to analyze and to measure the abdominal adipose using MRI in dogs. The aim of this study is to evaluate usefulness of MRI for the assessment of abdominal adipose tissue in dogs compared with CT and to establish an optimal method for evaluation of abdominal adipose tissue using MRI in dogs.

II. Materials and Methods

1. Experimental animals

Nineteen adult beagle dogs weighing 8-15.6 kg were studied. They were ten males and nine females. All dogs were clinically healthy by the results of physical exam, blood analysis and thoracic radiographs. The BCS (body condition score) of them were variable from 4/9 to 9/9. The experiments employed in this study were performed based on guidelines of the Institute of Laboratory Animal Resources at the Seoul National University.

2. MR scanning

All dogs were fasted from 12h to 16h before MR scans. After sedation by the administration of acepromazine (0.04 mg/kg of body weight, Sedaject, Samu median Co., Seoul, Korea) intravenously, general anesthesia was induced by the intravenous administration of propofol (6mg/kg of body weight, Provive, Myungmoon Pharm Co., Seoul, Korea) and maintained by isofluorane (Ifran, Hana Pharm Co., Seoul, Korea). During MR scanning transient apnea was induced by the intravenous administration of atracurium (0.2mg/kg of body weight, Acrium, Myungmoon Pharm Co., Seoul, Korea) and the apnea was reversed with the intravenous administration of neostigmine (0.04mg/kg of body weight, Neostigmine Methylsulfate, Daihan Pharm Co., Seoul, Korea) after scanning. The respiration was maintained with positive pressure ventilation until the spontaneous breath was coming. MR scans were performed using a 0.3 Tesla permanent MR scanner (Hitachi ARIS Vento, Hitachi Medical Co., Tokyo, Japan) with a human large head coil from the diaphragm to the sacroiliac joint in all dogs. They were positioned in dorsal recumbency. To prevent the signal loss within the coil, five different vertebra levels (T9-10, T12-13, L2-3 and L5-6 levels) were set at isocenter of magnet by altering position of dogs with four scan times on each dogs. The cross sectional images of the entire abdomen was acquired using T1- weighted breath hold 2D-RSSG (radiofrequency spoiled-steady gradient rewound acquisition) sequence in order to reduce acquisition time and motion artifacts. The scan parameters were as follows: repetition time (TR) = 233.3 ms, echo time (TE) = 4.5 ms, flip angle = 60°, number of acquisition (NEX) = 2, slice thickness = 7mm, slice interval = 8mm, field of view(FOV) = 260×260 mm, matrix = 224×146, scan duration = 70 s.

3. CT scanning

The protocol of general anesthesia and transient apnea were the same as MR scan. The CT scans were performed using a single-detector helical CT scanner (GE CT/e, General Electric Medical System, Yokogawa, Japan) as the same range of MR scans in all dogs. They were positioned in dorsal recumbency. The scan parameters were as follows: 120KV, 50mA, 7mm slice thickness, 8mm slice interval and 1.5 pitch.

4. Imaging analysis

The acquired transverse MR images were stored as Tiff format files (image: 256×256 pixel, pixel size = 1.01563×1.01563 mm) and then transmitted on a computer for analysis with NIH image software (ver. 1. 43; National Institutes of Health, USA) for the abdominal adipose tissue measurement. The MR images were analyzed by manual method and semiautomatic methods.

First, manual image analysis was performed. The SAT (subcutaneous adipose tissue) and VAT (visceral adipose tissue) areas in each of the transverse images of the entire abdomen were traced manually (Figure 1). In visceral areas where adipose tissue area was indistinct because of partial volume effect, half of indistinct areas were determined in the tracing.

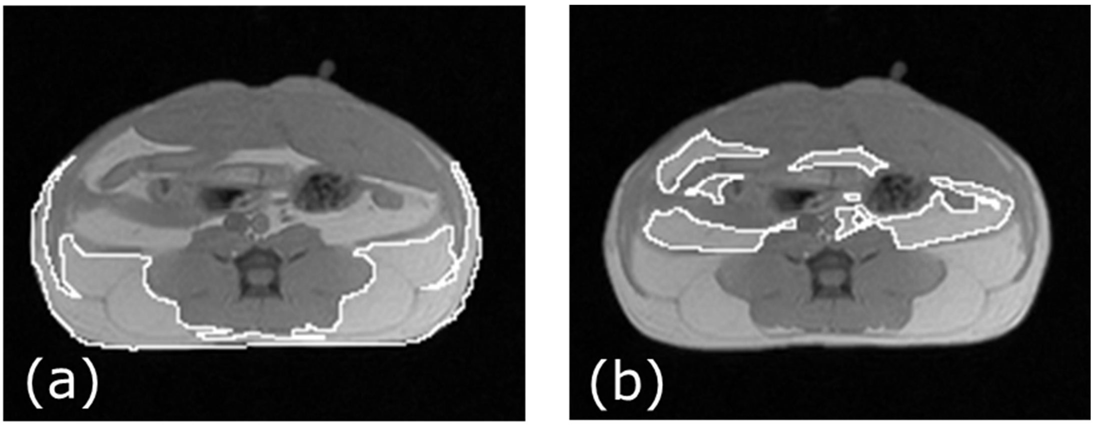

Figure 1.T1 weighted cross sectional images at the L 4-5 level. The areas within white line demonstrate subcutaneous adipose tissue area (a) and visceral adipose tissue area (b) traced manually.

And pixel counts were multiplied by the MR pixel size to obtain fat areas. The SAT and VAT areas in each transverse image were summed to acquire TAT (total adipose tissue) areas. And the volumes of SAT, VAT and TAT in entire abdomen was calculated as the sum of volumes obtained by multiplying the areas of SAT, VAT and TAT in each slice with the slice interval.

And the transverse single image at L2-3 level was analyzed by semiautomatic method obtained by modifying the method described by Lancaster et al. 1991. ROI (region of interest) surrounding total abdomen, subcutaneous tissue and visceral tissue region were drawn and gray value histogram curves were gained in each ROI (Figure 2). Bowel gas and vertebral body region are excluded because these regions affect gray value histogram.

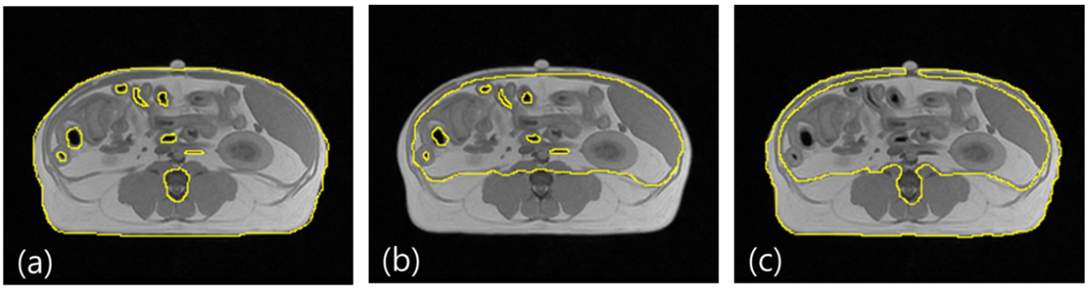

Figure 2.Outer margins of total abdomen (a), visceral area (b), subcutaneous area (c) are demonstrated at the same T1 weighted cross sectional images.

The histograms typically appeared two peaks of gray value and were fitted two Gaussian curves using Matlab software (ver. 2010a, Mathworks Inc., Natick, MA, USA). And the gray value with minimum pixel number between non-fat peak and fat peak was set as a threshold value (Figure 3). The threshold value was calculated semiautomatically using the software. The gray values above this threshold value were determined fat intensity and adipose tissue area was measured (Figure 4).

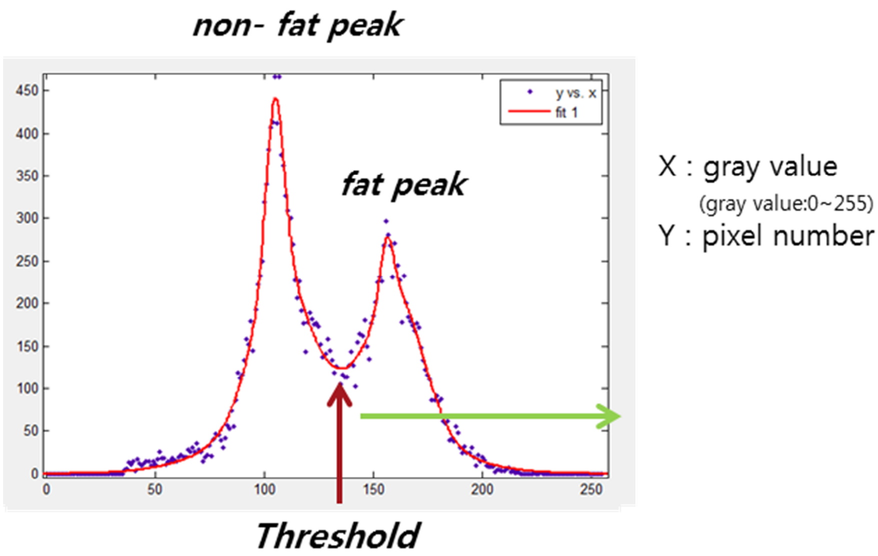

Figure 3.Two Gaussian curve fitted to the histogram. Note threshold is the gray value with minimum pixel numbers between non-fat and fat peaks.

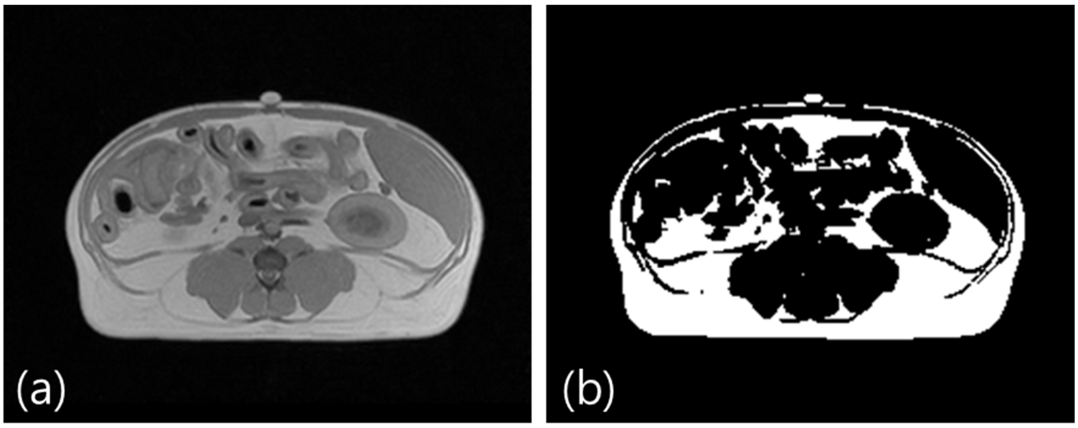

Figure 4.(a) T1 weighted cross sectional MR image. A threshold value is determined signal intensity above which is selected adipose tissue area. (b) Adipose tissue area is illustrated white area and all non adipose tissue is blacked out.

The transverse CT images were analyzed using the adipose tissue HU (Hounsfield unit) value. To define the canine adipose tissue HU range, ROIs surrounding subcutaneous adipose tissue were traced manually on each image at T12-13, L2-3 and L5-6 levels and histograms for adipose tissue were obtained. And by using the method suggested by Tohru et al. 1999, -117 ± 24 HU (mean ± 2SD) was considered as canine adipose tissue HU range. Also within this attenuation range, adipose tissue areas and volumes were measured using built-in software for the helical CT scanner.

A manual image analysis of all MR data was performed by three experienced observers.

5. Statistical analysis

Statistical analysis was conducted by using the SPSS 6.1 statistical package (SPSS Inc., Chicago, ILL). The tests used were the paired t-test, pearson correlation analysis and one-way analysis of variance (ANOVA).

III. Results

1. Comparison of MRI with CT

The representative MR and CT images used for quantification of abdominal adipose tissue are shown in Figure5. The mean TAT volumes of the entire abdomen measured by MRI and CT were 1250.21 ± 685.53 ml and 1272.68 ± 714.99 ml. They were significantly correlated ($r = 0.958$, $p < 0.01$) (Figure 6). The significant correlations between the areas of TAT, VAT and SAT measured by MRI and CT at L2-3 level were shown in table 1. The interobserver reproducibility was excellent for the measurement of VAT and SAT area using MRI at L2-3 level ($r = 0.994$ for VAT, $r = 0.979$ for SAT). And the intraobsever reproducibility was also high significantly (observer Ⅰ: $r = 0.998$ for VAT, $r= 0.999$ for SAT; observer Ⅱ: $r = 0.997$ for VAT, $r = 0.998$ for SAT; observer Ⅲ: $r = 0.999$ for VAT, $r = 0.983$ for SAT).

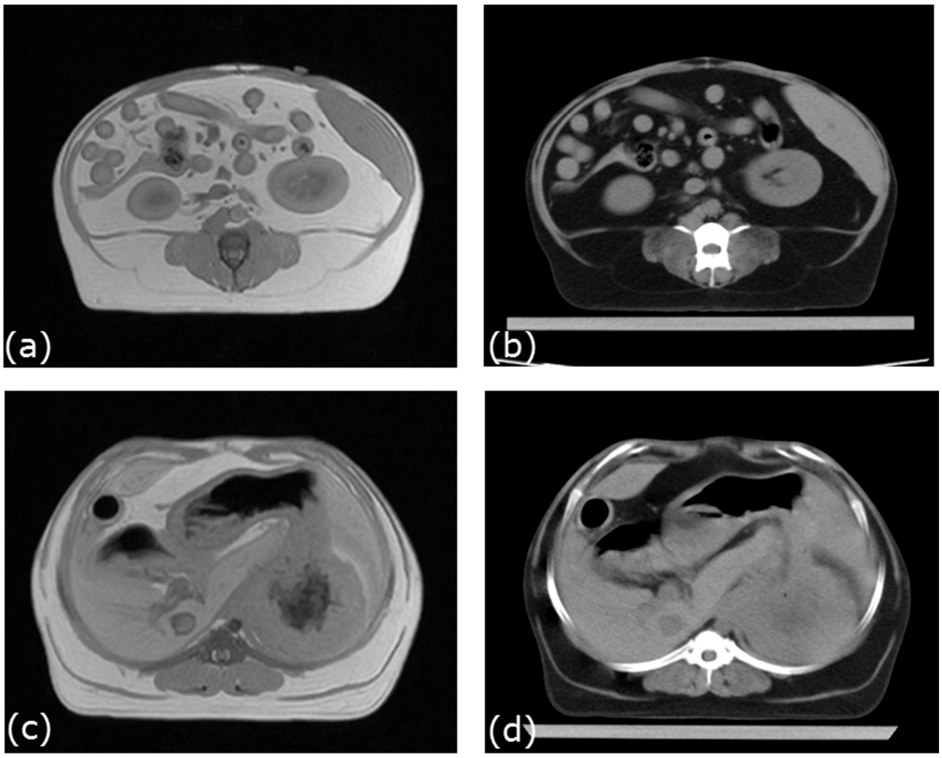

Figure 5.Representative crosss ectional T1 - weighted abdominal MR (a,c) and CT (b,d) images used for quantification of adipose tissue.

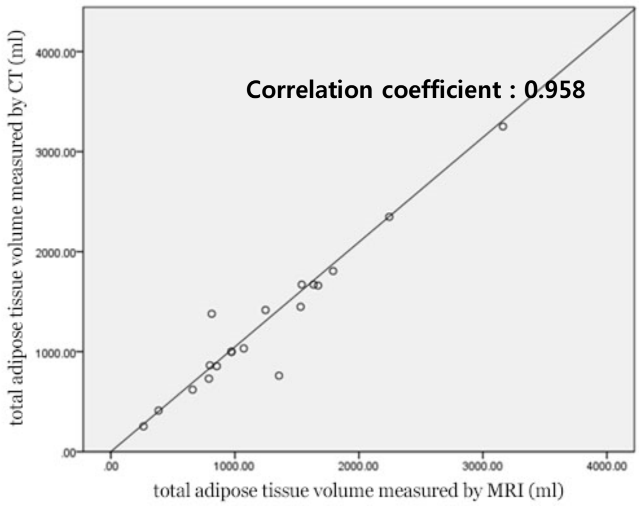

Figure 6.Total abdominal adipose volumes measured by MRI and CT.

Table 1. Comparison of the cross sectional abdominal adipose tissue areas measured by MRI and CT at L2-3 level

Comparison	Correlation coefficient	Mean ± SD (mm^2) by **MRI**	Mean ± SD (mm^2) by **CT**	Significance of correlation
TAT area	0.996	6936.82±3206.16	6904.05±3239.01	P<0.01
VAT area	0.995	2682.03±1747.77	2683.20±1768.37	P<0.01
SAT area	0.990	4254.79±1744.63	4220.85±1759.64	P<0.01

TAT: total adipose tissue, VAT: visceral adipose tissue, SAT: subcutaneous adipose tissue

2. Distribution of abdominal adipose tissue

There was no significant difference among the total abdominal adipose tissue areas manually measured by MRI at different vertebra levels. The VAT and SAT areas manually measured by MRI at different vertebra levels were shown in Figure 7. The VAT areas were significantly different among the vertebra levels ($P < 0.01$). And they were significantly lower at T11-12 level and L6-7 level compared to the other levels. By contrast, they were significantly higher at L2-3 level compared to the other levels. The SAT areas were also significantly different among the vertebra levels ($P < 0.05$). They were significantly lower at T13-L1 level and higher at L6-7 level than the other levels.

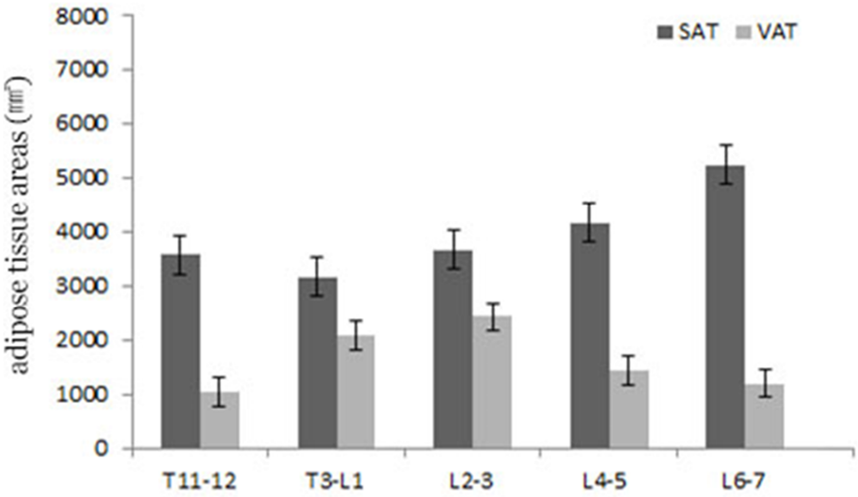

Figure 7.Abdominal adipose tissue areas measured by MRI at different vertebralevels.

Table 2.Correlation coefficents between the cross sectional adipose tissue areas at different vertebra levels and entire abdominal adipose tissue volumes measured by MRI

comparison	T11-12	T13-L1	L2-3	L4-5	L6-7
TAT area/TAT volume	0.925**	0.981**	0.966**	0.985**	0.972**
VAT area/ VAT volume	0.654**	0.835**	0.864**	0.831**	0.781**
SAT area/SAT volume	0.908**	0.906**	0.937**	0.944**	0.959**

** Correlation is significant at the 0.01 level

Correlations between cross-sectional adipose areas and abdominal adipose volumes were shown in table 2. The TAT areas and total adipose volumes in all vertebra levels were significantly correlated ($P < 0.01$). And the best correlation between the VAT areas and VAT volumes was obtained at L2-3 level ($P < 0.01$).

3. Comparison of semiautomatic measurement with manual measurement

The abdominal adipose tissue areas at L2-3 levels measured by semiautomatic method and the manual method were shown in table 3 and the relative differences were assessed between manual measurements and semiautomatic measurements. The semiautomatic measurement using the threshold obtained from the histogram of ROIt (ROI of total abdomen, Method 1) has the smallest relative difference compared with maual measurement among the semiautomatic methods. And the best correlation semiautomatic method for characterizing total adipose tissue volume was the method using the threshold obtained from the histogram of ROIt (Table 4).

Table 3.Abdominal adipose tissue areas measured by manual method and semiautomaticmethodsat L2-3 level

	Manual measurement	Semiautomatic measurement		
		Method 1	Method 2	Method 3
TAT area	6936.82±3206.16	6985.71±3199.93	7184.22±3189.74	7093.11±3215.46
VAT area	2682.03±1747.77	2699.06±1723.28	2861.53±1666.31	2787.62±1724.65
SAT area	4254.79±1744.64	4286.65±1774.48	4322.68±1775.98	4305.49±1782.36

TAT: total adipose tissue, VAT: visceral adipose tissue, SAT: subcutaneous adipose tissue area
Method 1: using threshold obtained from histogram of ROIt (ROI of total abdomen)
Method 2: using threshold obtained from histogram of ROIv (ROI of visceral tissue)
Method 3: using threshold obtained from histogram of ROIs (ROI of subcutaneous tissue)
All values are listed as Mean ±SD (mm^2)

Table 4. Correlation coefficients between adipose tissue areas measured by semiautomatic methods at L2-3 level and manual adipose tissue volumes

Comparison	Method 1	Method 2	Method 3
TAT area/TAT volume	0.963**	0.941**	0.948**
VAT area/ VAT volume	0.853**	0.849**	0.791**
SAT area/SAT volume	0.948**	0.930**	0.945**

** Correlation is significant at the 0.01 level

IV. Discussion

There are several methods for the adipose tissue assessment in dogs such as anthropometry, dual energy X-ray absorptiometry (DEXA), ultrasound, CT and MRI (14, 15). Anthropometry is accessible easily, but the specificity and accuracy for the adipose tissue assessment is very low (8). And DEXA is much less accessible. The MRI along with CT provides the quantitative assessment of adipose tissue with the very high specificity and accuracy (8). In addition, the MR scan is performed without the exposure of radiation. However MRI is limited due to long scan time and variable artifacts compared to CT. So a few research has been reported for the adipose tissue assessment of dogs using MRI (16, 17). These limitations can be reduced by T1-weighted breath hold RSSG sequence and change position within the coil. T1 - weighted breath hold RSSG sequence images require a shorter scan time than do spin echo images (about 4minute). In this study, the good contrast images could be acquired with fewer motion and respiratory artifact using this sequence. Although the recent studies about quantification of adipose tissue has been performed using T1-weighted breath hold sequence, the MR images using this sequence for the measurement of adipose tissue were not analyzed and not compared with the CT images in animals. In addition, reduced intensity within the coil due to an influence of the coil sensitivity distribution can be prevented by setting the interesting area in the center of the coil. By obtaining good contrast MR images with these procedures, abdominal adipose tissue with MR images could be measured and the measurement could be compared to CT images.

There was a small gap in the measurement of adipose tissue measured by MRI and CT. This distinction is associated with technical problems such as partial volume effect, intestinal movement and contents and positional change. Nevertheless there was no significant difference indicating that the accuracy of adipose tissue measurement using MRI is sufficient for clinical application. Moreover MRI may be better than CT for characterizing abdominal adipose tissue because fat has a very low HU value range and this HU value range is similar with that of air and bowel gas in CT (18). Excellent soft tissue contrast with the distinction from signal intensity of air and bowel gas could be obtained because signal intensity of fat on T1-weighted MRI is bright (19).

Because absolute standard criteria as HU using in CT is absent in MRI, imaging analysis using MRI can be performed by manual method. But this method is time-consuming and is affected by analyzer. Therefore human study on adipose tissue measurement using MRI with automatic analysis has been reported compared to manual analysis in humans (9). And it is a critical point of automatic analysis in MRI to find an adequate threshold gray value between the gray values of non-fat peak and fat peak pixels. Some kinds of methods for setting a threshold value has been suggested in human reports (10,20). In this study, the semiautomatic methods, modifying the method as reported, using the thresholds determined from three different ROIs were done and was compared with manual method. The adipose tissue measured by the semiautomatic method using ROI of total abdomen was no significant difference with that measured by CT. However there was significant difference between the adipose tissue measured by semiautomatic method using ROI of visceral region and that measured by CT. This result may be associated with indistinct threshold between non-fat and fat peaks because there are many organs with variable gray scale within abdominal cavity or abdominal fat is not enough to shape peak.

The visceral adipose tissue area measured manually at L2-3 level among five different vertebra levels was the best correlation with visceral adipose volume. And the total adipose tissue area measured semiautomatically at L2-3 level was highly comparable to volume manual measurements of abdominal adipose tissue. So a single slice scan at L2-3 level with semiautomatic analysis could be suggested as the best method for evaluating abdominal adipose volume as the umbilicus level is used for single slice scan in humans (9, 21). But the other vertebra levels may be able to be used because the total adipose tissue areas at all vertebra level were strong correlation with total adipose volume. A further study with a larger population about single slice scan for evaluating whole volume is considered with the reliability. Although abdominal adipose tissue measurement was obtained only by MRI and

CT, the quantification of adipose tissue with MRI has been shown to be accurate compared to chemical analysis in pigs and has been found to be in good agreement with under water weighing in humans (22-24).

In conclusion, MRI acquisition technique and imaging analytic method for measuring abdominal adipose tissue used in this study enable to make a fast and reliable abdominal adipose tissue quantitative measurement. Therefore, MRI could be a good alternative to CT for the measurement of abdominal adipose tissue in dogs.

V. References

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VI. 국문초록

개에서 자기 공명 영상을 이용한 복부 지방 조직의 측정

서울대학교 대학원

수의학과 임상수의학 (수의 방사선과학) 전공

정선영

개에서 비만은 흔한 영양장애이며 당뇨, 췌장염, 관절염, 심혈관계 질환과 같은 다양한 질병의 위험인자로 인식되어 왔다. 자기 공명 영상은 컴퓨터 단층촬영과 함께 인의에서 지방을 정량적으로 측정하는 표준으로 여겨지고 있다. 본 연구의 목적은 컴퓨터 단층촬영과 비교하여 복부 지방 측정에 있어서 자기 공명 영상의 유용성을 평가하며 최적의 평가 방법을 확립하는 것이다.

19마리의 비글견에서 T1- weighted breath hold RSSG (radiofrequency spoiled gradient rewound acquisition) 시퀀스를 사용한 자기 공명 영상 장치와 나선형 컴퓨터 단층촬영를 이용하여 횡경막 앞쪽 경계부터 천골 장골 관절

수준까지 전 복부 스캔 하였다. 가로 단면 자기 공명 영상을 가지고 수동적 방법과 반자동적 방법으로 복부 지방을 측정하였다. 컴퓨터 단층촬영 영상은 −141 HU 값부터 −93 HU 값까지의 범위를 지방으로 정하고 복부 지방을 측정하였다. 자기 공명 영상과 컴퓨터 단층촬영으로 측정된 총 복부 지방량의 각각의 평균값끼리 유의적인 차이는 없었으며 강한 상관관계를 나타내었다 ($r = 0.958$, $p < 0.01$). L2−3 수준의 단면에서 반자동 방법으로 측정된 복부 지방 면적과 수동적 방법으로 측정된 총 복부 지방량 역시 높은 상관관계를 보였다 ($r = 0.963$, $p < 0.01$).

본 실험에서 사용한 자기 공명 영상 촬영 기법과 영상 분석 방법은 빠르고 신뢰할 수 있는 복부 지방측정을 가능하게 한다. 결론적으로 자기 공명 영상은 복부 지방 측정에 있어서 컴퓨터 단층촬영을 대체할수 있는 좋은 진단적 도구이다.

주요어: 측정, 복부 지방 조직, 자기 공명 영상, 개

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