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Evaluation of ultrasound assessments of subcutaneous adiposity and epaxial muscularity in cats: a preliminary study

Eiji Iwazaki[1,*] and Toshihiro Nade[2]

[1] Section of Academic Research, Department of Research and Development, Nippon Pet Food Co. Ltd., Tokyo 140-0002, Japan
[2] Faculty of Regional Innovation, University of Miyazaki, Miyazaki, 889-2192, Japan

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Abstract

A study was conducted in 20 cats to translate an ultrasound procedure for muscle evaluation from bovines to felines. The cats were divided into three groups: Lean, Ideal Body Weight (BW), and Overweight. The groups were compared in terms of zoometry measurements, as well as back fat (BF) and epaxial muscle thickness (EM) measured by ultrasound at the 6-7th ribs and immediately caudal to the 13th rib. A significant reduction in EM at both the 6-7th ribs and immediately caudal to the 13th rib was found for the Lean group compared to the Overweight group. The 13th EM showed a significant correlation with the plasma creatinine concentration along with BW and feline body mass index (fBMI). BF immediately caudal to the 13th rib in the Overweight group was significantly increased compared to that in the Ideal BW and Lean groups. In comparison to the other groups, the Lean group also exhibited a significant reduction in BF immediately caudal to the 13th rib. Additionally, BF at the 13th rib showed significant correlations with BW, fBMI, body condition score, and abdominal girth. These data suggest that ultrasonography is appropriate for the evaluation of adiposity and muscularity in cats. In conclusion, the site immediately caudal to the 13th rib might be suitable for ultrasound assessment of body composition in cats, allowing veterinarians to evaluate both adiposity and muscularity in a single procedure. This technique has the potential for use in evaluations of adiposity and muscularity in veterinary clinical practice.

Key Words: Adiposity, Feline body mass index, Muscularity, Obesity, Ultrasonography

Introduction

The importance of body composition assessments, especially muscle mass/lean body mass (LBM), has been increasing, and the loss of muscle mass/LBM caused by disease and aging is associated with morbidity and mortality, which has been observed worldwide.

An evaluation of body composition is important in terms of diagnosing and managing obesity, cachexia, and sarcopenia. Weight reduction treatment often involves a loss of LBM along with fat reduction in cats.[3,13] To maintain animal performance during weight reduction,

* Corresponding author: Eiji Iwazaki
Section of Academic Research, Department of Research and Development, Nippon Pet Food Co. Ltd., Tennoz First Tower 5F, 2-2-4, Higashishinagawa, Shinagawa-ku, Tokyo 140-0002, Japan
Tel: +81-3-5479-6402, Fax: +81-3-5479-6404, E-mail: e-iwazaki@npf.co.jp
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nutritional intervention should minimize lean tissue loss. Cachexia, which is not usually observed in healthy animals, quickly catabolizes muscle mass/LBM.[14] Several acute and chronic diseases eliminate the ability to switch to fat utilization as a primary energy source for protein preservation.[14,25] Series of these alterations are common in patients with chronic kidney disease, congestive heart failure, and cancer in dogs.[2] In humans, decreased muscle mass/LBM in sarcopenic patients may be due to a reduction in insulin-like growth factor-1 concentration.[4] In addition, energy deficiency resulting from decreased digestibility of protein and lipid may also contribute to the muscle mass/LBM reduction in aging cats.[16,21]

Thus, evaluation of muscle mass/LBM has attracted attention for the early diagnosis of such muscularity statuses in veterinary clinical practice. Several techniques have been developed in the laboratory to estimate the balance of adipose tissue and muscle mass accurately: dual-energy X-ray absorptiometry (DEXA), computed tomography (CT), and magnetic resonance imaging (MRI).

DEXA and CT are considered the reference standards for evaluating adiposity and muscularity in humans.[5,8,24] DEXA enables the measurement of total fat mass, LBM, and bone density.[8] CT also enables researchers to distinguish the areas of adipose tissue and LBM on a 2-dimensional slice image.[24] However, both CT and DEXA involve ionizing radiation. Although MRI does not involve ionizing radiation, it is less widely available and more expensive than CT or DEXA and tends to overestimate fat deposits in humans.[24]

Overall, these techniques have not been used in veterinary practice because of their invasiveness and expense. Body condition score (BCS) and muscle mass score (MMS), which are recognized by the World Small Animal Veterinary Association as standards to evaluate adiposity and muscularity,[26] are subjectively determined by inspection and palpation.[12,15] Although MMS shows a significant correlation with lean mass, the correlation coefficient is somewhat low.[15] It is necessary to develop an objective technique for the accurate evaluation of adiposity and muscularity simultaneously.

Ultrasonography is an alternative, noninvasive technique to evaluate body composition. Since 1956, the accuracy and reliability of ultrasonography for subcutaneous fat and muscle measurement has been evaluated in several mammals, such as bovines, swine, sheep, rabbits, horses, dogs, and humans.[9,20,27] In beef cattle, an ultrasonographic method has been developed to predict the final carcass composition before slaughter; this method is now in general use in the industry.[17]

In our previous study, the ultrasound procedure for bovines was applied to felines,[10] and the findings revealed that ultrasonography is a viable method for evaluating subcutaneous adiposity in cats. However, the applicability of the method to muscle in cats has not been confirmed. The purpose of this study was to translate the ultrasound procedure for muscle evaluation from bovines to felines. Specifically, the thickness of the back fat and epaxial muscle at the 6-7th rib and immediately caudal to the 13th rib were measured in 20 cats. This study compared the thickness of the back fat and epaxial muscle among cats at different levels of body composition to evaluate adiposity and muscularity. The daily repeatability of fBMI and ultrasonography were also evaluated in this study.

Materials and methods

Experiment 1

Animals: According to the temporary objective criteria for feline body mass index (fBMI), 20 intact cats (1-12 years, non-littermates, 12 males and 8 females) were divided into three groups. 10 cats (fBMI<23.0; 2.6±0.1 kg, 19.6±0.7 kg/m) were defined as the Lean group. 5 cats (23.0≤fBMI<28.0) were defined as the Ideal

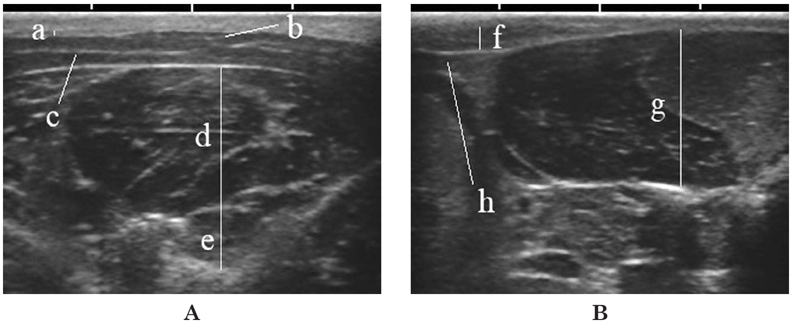

Fig. 1. Measurement of subcutaneous adipose tissue and muscle thickness at the 6-7th ribs (A) and immediately caudal to the 13th rib (B)
a: Back fat thickness at the 6-7th ribs, b: Trapezius muscle, c: Latissimus dorsi muscle, d: Longissimus thoracis muscle, e: Semispinalis capitis muscle, f: Back fat thickness at the 13th rib, g: Longissimus dorsi muscle, and h: Serratus posterior muscle

Body Weight (BW) group (3.5±0.2 kg; 25.9±0.7 kg/m). 5 cats (fBMI≥28.0) were defined as the Overweight group (4.4±0.1 kg; 30.7±0.9 kg/m). These cats were maintained in individual cages under conditions of natural temperature, relative humidity, and light:dark cycle at the Animal Facility of Nippon Pet Food Co. Ltd. The cats were fed a commercially available dry diet once a day to maintain their current BW, and water was available ad libitum. All cats were inspected and palpated by a veterinarian and diagnosed as healthy.

All experiments were approved by the Animal Ethics Committee of Nippon Pet Food Co. Ltd. All procedures and protocols were performed in accordance with the guidelines for Animal Care and Treatment established by the Animal Facility of Nippon Pet Food Co. Ltd.

Zoometric measurements: BW and BCS were measured in all cats. BCS was determined on a 5-point scale: 1, thin; 2, lean; 3, ideal; 4, overweight; and 5, obese.

The head and body length (HBL) was determined as the length from the top of the nose to the joint between the sacrum and coccyx. The length from the top of the patella to the end of the calcaneus (PCL) was also determined. The abdominal girth (AG) was determined as the circumference immediately caudal to the 13th rib. All lengths were measured by the same researcher using a commercially available tape measure.

fBMI was calculated by the following formula: BW(kg)/PCL(m).[11]

Measurement of back fat thickness and epaxial muscle thickness: All procedures for ultrasonography followed the protocols of our previous study.[10] Cats were laid in left lateral recumbency. Cross-sectional images were captured using an HS-2100V (Honda Electronics Co. Ltd., Aichi, Japan) and an 11.0/8.5/6.0 MHz HLS-584M transducer (Honda Electronics Co. Ltd., Aichi, Japan). The ultrasonic frequency was set at 8.5 MHz, and 70% ethanol was applied as a couplant. The hair was not clipped.

The ultrasound transducer, which was applied parallel to the rib, was placed on the midpoint between the 6th and 7th rib or immediately caudal 13th rib. The transducer was adjusted to place a full view of the longissimus muscle in the center of the screen (Fig. 1).

The maximum range between the inner limit of the dermis and the superior fascia of the latissimus dorsi muscle on the image from the 6-7th rib was defined as the 6-7th back fat thickness (BF). The maximum range between the inner limit of the dermis and the superior fascia of the serratus posterior muscle immediately caudal to the 13th rib was defined as the 13th BF.

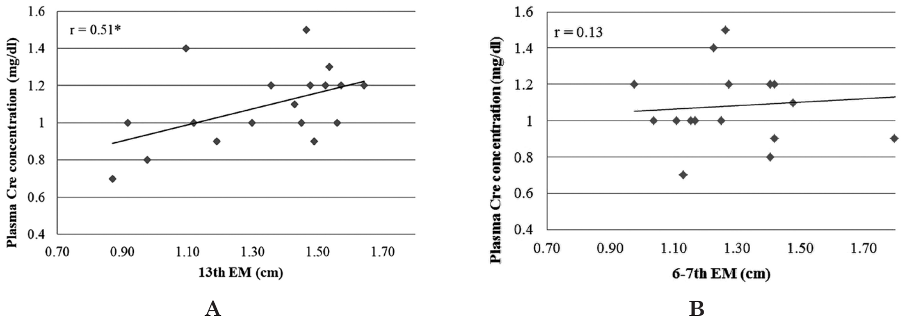

Fig. 2. Relationships between plasma creatinine concentration and 13th (A) or 6-7th (B) epaxial muscle thickness in cats showing IRIS Stage I chronic kidney disease (<1.6 mg/kg of creatinine concentration)
Values are presented as the correlation coefficients.
* Significant correlation based on the Pearson's correlation coefficient (P<0.05).
Cre: creatinine, EM: epaxial muscle thickness

The maximum range between the superior fascia of the longissimus muscle and the bottom sharp angle of the semispinalis capitis muscle at the 6-7th ribs was defined as the 6-7th epaxial muscle thickness (EM). The maximum distance between the dorsal and ventral fasciae of the longissimus muscle immediately caudal to the 13th rib was defined as the 13th EM. An internal electric caliper on these images was applied to free software (Data picker, ver. 1.2, http://www.hp.vector.co.jp/authors/VA019223/) to measure these distances.

Blood sample collection and plasma protein metabolites analysis: Pre-prandial blood was collected from the jugular vein into heparinized plastic tubes. The plasma was recovered by centrifugation (4°C, 3000rpm, 15mins) and stored at -40°C until use.

Plasma creatinine (Cre) concentration was determined using an AU2700 (Olympus Corporation, Tokyo, Japan) with manufacturer's reagents.

Statistical analysis: A one-way ANOVA and Tukey's test were used to reveal the zoometric, plasma Cre concentration, and ultrasonic differences among the three groups (Lean, Ideal BW, and Overweight). The values are expressed as the mean ± standard errors.

To assess the relationships among BF, EM, and zoometric measurements, a Pearson's correlation coefficient was performed, and calculated the correlation coefficients (r).

Then, to assess the relationships between plasma Cre concentration and both EM, a Pearson's correlation coefficient was performed and the correlation coefficient (r) was calculated. To avoid the effect of chronic kidney disease, 18 cats with IRIS grade I were recruited.

The significance was set at P<0.05 for all statistical tests.

Experiment 2

Animals: 10 intact cats (1-17 years, non-littermates, 5 males and 5 females) were recruited in this study. These cats were maintained in individual cages under conditions of natural temperature, relative humidity, and light:dark cycle at the Animal Facility of Nippon Pet Food Co., Ltd. The cats were fed a commercially available dry diet once a day to maintain their current BW, and water was available ad libitum. All cats were inspected and palpated by a veterinarian and diagnosed as healthy.

Zoometric measurements: The zoometry, BF, and EM measurements were performed twice a day. The first measurement was performed at 9:00 am, and the second measurement was performed at 1:00 pm.

All procedures such as the sites and formula

Table 1. Comparison of the zoometric measurements, subcutaneous adipose tissue and muscle thickness among cats with different feline body mass index levels

	Lean			Ideal BW			Overweight		
	fBMI < 23.0 (10)			23.0 ≤ fBMI < 28.0 (5)			fBMI ≥ 28.0 (5)		
Zoometry measurements									
Age (y)	4.6	±	1.1	5.0	±	1.9	3.4	±	2.2
BW (kg)	2.6	±	0.1*	3.5	±	0.2	4.4	±	0.1*,**
fBMI (kg/m)	19.6	±	0.7*	25.9	±	0.7	30.7	±	0.9*,**
BCS (/5)	2.8	±	0.1*	3.6	±	0.2	4.0	±	0.3**
HBL (cm)	48.0	±	1.3	50.0	±	1.6	55.1	±	0.4**
PCL (cm)	13.2	±	0.3	13.5	±	0.5	14.4	±	0.3
AG (cm)	30.7	±	0.6*	37.5	±	1.9	37.2	±	0.9**
Plasma protein metabolite									
Cre (mg/dl)	1.1	±	0.1	1.2	±	0.2	1.4	±	0.2
Back fat thickness									
13th rib (cm)	0.17	±	0.0*	0.27	±	0.02	0.39	±	0.04*,**
6-7th ribs (cm)	0.02	±	0.01	0.02	±	0.01	0.03	±	0.01
Epaxial muscle thickness									
13th rib (cm)	1.23	±	0.08	1.41	±	0.06	1.53	±	0.04**
6-7th ribs (cm)	1.19	±	0.05*	1.49	±	0.09	1.60	±	0.12**

Values are presented as the means ± standard error.
The numbers in parentheses indicate the number of animals examined.
Cross-sectional images were captured on 6-7th rib and immediately caudal to the 13th rib by ultrasound.
* Significantly different from the control group (One-way ANOVA, $P < 0.05$).
** Significantly different from the fBMI < 23.0 group.
BW: body weight, fBMI: feline body mass index, BCS: body condition score, HBL: head and body length, PCL: length from patella to end of calcaneus, AG: abdominal girth, Cre: creatinine concentration

for zoometric measurements were the same as described for Experiment 1.

Measurement of BF and EM: Cross-sectional images were captured using an LOGIQ P7 (GE Healthcare Japan., Tokyo, Japan) and an L6-12-RS probe (GE Healthcare Japan., Tokyo, Japan). The ultrasonic frequency was set at 12.0 MHz, and 70% ethanol was applied as a couplant. The hair was not clipped.

All procedures such as the patient's posture, measurement sites, range, operation of the probe, and software were same as described for Experiment 1.

Statistical analysis: A paired *t*-test was used to reveal the zoometric and ultrasonic differences between morning and afternoon. The values were expressed as the mean ± standard errors.

To assess the daily repeatability between morning and afternoon, the Pearson's correlation coefficient was performed and the correlation coefficient (r) was calculated. And intraclass correlation coefficient (ICC) (1, 2) was calculated. ICC (1, 2) of >0.75 was associated with excellent reliability. ICC (1, 2) of 0.4-0.75 was associated with good reliability and ICC (1, 2) of <0.4 was associated with poor reliability.

The significance was set at $P<0.05$ for all statistical tests.

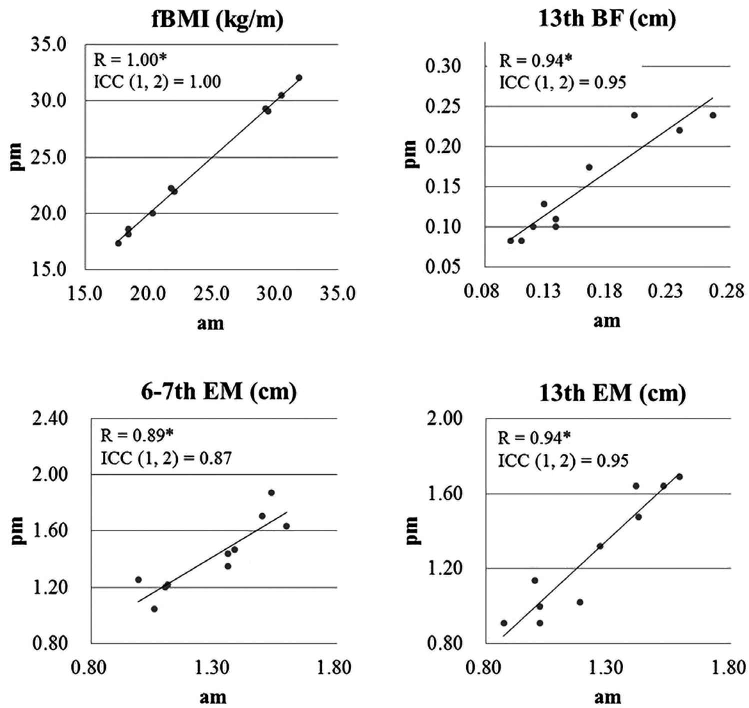

Fig. 3. Relationships of each parameter between morning and afternoon in cats
Values are presented as the correlation coefficients.
* Significant correlation based on the Pearson's correlation coefficient (P<0.05).
ICC (1, 2) of > 0.75 was associated with excellent reliability.
fBMI: feline body mass index, BF: back fat thickness, EM: epaxial muscle thickness, ICC: intraclass correlation coefficient

Results

Experiment 1

Zoometric measurements: The BW, fBMI, BCS, HBL, and AG were significantly higher in the Overweight group than in the Lean group (P<0.05) (Table 1). The BW and fBMI were also significantly higher in the Overweight group than in the Ideal BW group (P<0.05). The BW, fBMI, BCS, and AG were significantly lower in the Lean group than in the Ideal BW group (P<0.05).

Plasma Cre concentration: Significant differences were not observed among each obese group (Table 1).

Subcutaneous adipose tissue thickness: The 13th BF of the Overweight group was significantly higher than that of the Ideal BW group or the Lean group (P<0.05) (Table 1). The 13th BF of the Lean group was also significantly lower than that of the Ideal BW group (P<0.05).

Muscle thickness: Significant muscle wasting was evident from the 13th EM and 6-7th EM of the Lean group compared to those of the Overweight group (P<0.05) (Table 1). Although the mean values of the 13th and 6-7th EM were similar in the Lean group, only the 6-7th EM was significantly lower than that of the Ideal BW group (P<0.05).

Relationships between zoometric measurements, BF, and EM: The value of the 13th BF showed significant correlations with the BW, fBMI, BCS, HBL, PCL, and AG (r = 0.73, 0.74, 0.69, 0.55, 0.45, and 0.59, respectively) (P<0.05) (Table 2). The value of the 6-7th BF showed significant correlations with the BW, fBMI, and AG (r = 0.53, 0.53, and 0.72, respectively) (P<0.05).

The value of the 13th EM showed significant

Table 2. Correlations between subcutaneous adipose tissue thickness, muscle thickness and zoometric measurements in cats

	Age	BW	fBMI	BCS	HBL	PCL	AG
Back fat thickness							
13th rib	-0.09	0.73*	0.74*	0.69*	0.55*	0.45*	0.59*
6-7th ribs	0.32	0.53*	0.53*	0.36	0.30	0.33	0.72*
Epaxial muscle thickness							
13th rib	-0.05	0.68*	0.68*	0.53*	0.51*	0.45*	0.45*
6-7th ribs	-0.13	0.69*	0.68*	0.28	0.52*	0.45*	0.71*

Values are presented as correlation coefficients.
*Pearson's correlation coefficient is significant (P<0.05)
BW: body weight, fBMI: feline body mass index, BCS: body condition score, HBL: head and body length, PCL: length from patella to end of calcaneus, AG: abdominal girth

correlations with the BW, fBMI, BCS, HBL, PCL, and AG (r = 0.68, 0.68, 0.53, 0.51, 0.45, and 0.45, respectively) (P<0.05) (Table 2). The value of the 6-7th EM also showed significant correlations with the BW, fBMI, HBL, PCL, and AG (r = 0.69, 0.68, 0.52, 0.45, and 0.71, respectively) (P<0.05).

Relationships between plasma protein metabolites and EM: 2 cats that showed IRIS grade II were excluded. The 13th EM exhibited a significant correlation with the plasma Cre concentration (r = 0.51) (P<0.05) (Fig. 2A). However, significant differences were not observed between the 6-7th EM and plasma Cre concentration (r = 0.13) (Fig. 2B).

Experiment 2

Daily repeatability: The 6-7th EM was significantly lower in the morning than in the afternoon (P<0.05) (Table 3). The BW, fBMI, BCS, HBL, PCL, AG, 13th BF, and 13th EM were not significantly different between the morning and afternoon.

Relationships of the fBMI, BF, and EM: The fBMI, 13th BF, 6-7th EM, and 13th EM were significantly correlated between the morning and afternoon (P<0.05) (Fig. 3); the correlation coefficients were as follows: r = 1.00, 0.94, 0.89, and 0.94, respectively. And the ICC (1, 2) were as follows: ICC (1, 2) = 1.00, 0.95, 0.87, and 0.95, respectively. All ICC (1, 2) were considered as excellent reliability.

Discussion

To our knowledge, this is the first trial to reveal the availability of ultrasound evaluations of muscularity in cats. As the fattening in bovine, obese level reflected muscularity in cats. The increase of 13th EM may due to maintain animal’s posture and mobility against overweight. Then, our results showed that ultrasonography immediately caudal to the 13th rib enables the evaluation of adiposity and muscularity in a single procedure. In this study, the 13th EM was correlated with the plasma Cre concentration along with the BW and fBMI. The plasma Cre concentration, which is often used as a biomarker of chronic kidney disease,[19] reflects LBM.[7] Similarly, our previous report also validated the availability of ultrasonography for the evaluation of subcutaneous adiposity immediately caudal to the 13th rib in cats.[10] That study showed a significant correlation with BW, fBMI, BCS, body fat ratio, and chest girth, which was consistent with our current study. And the 13th BF and EM, in contrast to the 6-7th EM, didn’t show the daily fluctuation. According to the result of ICC (1, 2), the 13th EM also showed higher reliability than the 6-7th EM. Thus, the 13th EM rather than

Table 3. Comparison of the zoometric measurements, subcutaneous adipose tissue and muscle thickness between morning and afternoon in cats

	Morning (10)			Afternoon (10)		
Zoometry measurements						
Age (y)	7.6	±	1.7	7.6	±	1.7
BW (kg)	3.3	±	0.3	3.2	±	0.3
fBMI (kg/m)	24.0	±	1.8	23.9	±	1.8
BCS (/5)	2.9	±	0.3	3.0	±	0.3
HBL (cm)	48.5	±	1.5	48.2	±	1.5
PCL (cm)	13.4	±	0.4	13.4	±	0.4
AG (cm)	31.2	±	1.1	31.7	±	1.1
Back fat thickness						
13th rib (cm)	0.16	±	0.02	0.15	±	0.02
Epaxial muscle thickness						
6-7th ribs (cm)	1.30	±	0.07*	1.42	±	0.08
13th rib (cm)	1.23	±	0.08	1.27	±	0.10

Values are presented as the means ± standard error.
The numbers in parentheses indicate the number of animals examined.
Cross-sectional images were captured on 6-7th rib and immediately caudal to the 13th rib by ultrasound.
* Significantly different from Afternoon (A paired t-test, $P<0.05$).
BW: body weight, fBMI: feline body mass index, BCS: body condition score, HBL: head and body length, PCL: length from patella to end of calcaneus, AG: abdominal girth

the 6-7th EM may be available for evaluation of muscularity and adiposity in cats. Although fBMI includes the effects of both adiposity and muscularity, ultrasonography enables the distinction of adiposity effects with muscularity effects on BW changes. In addition, a one-site procedure may contribute to lessening the patient's and veterinarian's stress or activity in clinical practice.

The validity and reliability of ultrasonography for meat quality have been reported since 1956.[20] The importance of grading systems in the beef industry has precipitated the development of ultrasonography in beef cattle. This technique was available for determining the ideal nutritional interventions to improve trait performance in live animals before slaughter. According to the beef quality grading system provided by the United States Department of Agriculture,[22] cross-sectional images of the longissimus muscle area at the 12-13th ribs are often used for evaluation of subcutaneous adiposity and muscularity in beef cattle in the US.[1,18] Several reports have validated the availability and repeatability of ultrasonography at or near the 13th rib for the accurate evaluation of subcutaneous fat thickness and longissimus muscle area in live animals.

Perkins *et al.* (1992)[18] examined the technician effects and repeatability using 36 steers. The BF and muscle area at the 12-13th ribs were compared between carcass measurements and ultrasonography by two trained technicians. The two technicians showed significant correlations in both BF (r=0.87 and 0.86) and longissimus muscle area (r=0.76 and 0.82). Greiner et al. (2003)[6] also confirmed the repeatability of the technique in successive years using 534 steers. The study showed strong relationships between the carcass-measured and ultrasonographic values of both BF (r=0.89) and longissimus muscle area (r=0.86) at the 12th rib. The differences between years were small for both fat (carcass and ultrasonography: r=0.86 and 0.90, respectively) and longissimus muscle area (r=0.91 and 0.79, respectively). In addition, the daily repeatability of the 13th BF and EM were also confirmed in this study.

The availability of ultrasonography for muscle measurement at the 13th rib has also been reported in dogs. Hutchinson *et al.* (2012)[9] measured the maximal transverse epaxial muscle area at the 13th rib in 20 Labrador Retrievers. The report showed that the muscle area measured by ultrasound was decreased in old dogs, as was the muscle area measured by CT. Thus, the site around the 13th rib is considered a suitable site for the ultrasonic evaluation of subcutaneous adiposity and muscularity in mammals.

Freeman *et al.* (2017)[2] developed a similar technique called the Vertebral Epaxial Muscle Score (VEMS) in dogs. The study revealed higher reproducibility for epaxial muscle height divided by the length of the 4th thoracic vertebra (T4) compared to the area divided by T4 when the muscle was compared between breeds of different sizes. The EM height showed greater reproducibility than muscle area and can be performed in real time, and this variable is easy to evaluate in veterinary practice. However, the epaxial muscle in that study was measured with a curvilinear transducer, which inherently deforms the subcutaneous adipose tissue and longissimus muscle.[9] Additionally, T4 was measured by a computed radiography system, which is invasive because it uses ionizing radiation. In cats, the morphological differences between breeds are negligible. Normalization of the muscle height or area is not needed. The ultrasonographic method developed in our study never uses any ionized radiation. The linear transducer minimized the deformation of subcutaneous adipose tissue and epaxial muscle. Thus, our ultrasound technique is more useful than Freeman's technique.

The main limitations of this study include the lack of reference standards (DEXA, CT, or MRI) for adiposity and muscularity. For the determination of adiposity, fBMI was applied for grouping as an objective criterion for obesity to avoid inter-technician errors from the grouping by BCS or MMS. Although the validity of daily repeatability was confirmed in this study, fBMI is not the gold standard criterion for obesity in cats. The muscularity biomarker of plasma Cre is often affected by several factors, such as chronic renal disease and physical/nutritional status.[23] Then, our data also included no severely obese or cachexic cats in this study. And, the nonsignificance of the 13th EM between the Lean and Ideal BW groups may have been affected by the lack of statistical power. Further studies will be needed to confirm the validity and reliability of various nutritional statuses in cats.

In conclusion, the 13th EM rather than the 6-7th EM may be suitable for evaluating muscularity and adiposity in cats. The 13th EM was significantly correlated with the plasma Cre concentration along with the BW and fBMI. And the 13th BF significantly correlated with BW, fBMI, BCS, and AG, which was consistent with our previous report. And the daily repeatability was also confirmed. Thus, performing ultrasonography immediately caudal to the 13th rib enables the evaluation of adiposity and muscularity in a single procedure. This technique has the potential for use in evaluations of adiposity and muscularity in veterinary clinical practice.

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Supplemental data

Supplemental data associated with this article can be found, in the online version, at http://dx.doi.org/10.14943/jjvr.68.1.31

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