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<th>Instructions for use</th>
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The relationship among ultrasound measurements, body fat ratio, and feline body mass index in aging cats

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Abstract
This study compared the subcutaneous adipose tissue thickness between aging cats and young adult cats to confirm the suitability of ultrasonography for measuring feline adiposity. Body weight, body condition score (BCS), head and body length, length from the top of patella to the end of the calcaneus, and chest girth were measured in 14 healthy, intact cats (5 young adult and 9 senior cats). Additionally, feline body mass index and body fat ratio were calculated. Ventral subcutaneous adipose tissue thickness (VSAT, caudal to the 13th rib) and breast subcutaneous adipose tissue thickness (BSAT, between the 6th and 7th ribs) were measured by ultrasonography as the distance from the inferior line of the dermis to the superior line of the latissimus dorsi muscle. The aging group showed significant increases in age, body fat ratio, chest girth, and VSAT but not body weight, feline body mass index, BCS, or BSAT. Additionally, VSAT demonstrated significant positive correlations with body weight, feline body mass index, BCS, body fat ratio, chest girth, and BSAT. In conclusion, the site caudal to the 13th rib can be considered a suitable location for ultrasonographic evaluation of fat accumulation in cats. This measurement enables the evaluation of masked fat deposition in aging cats.

Key Words: Feline body mass index, Sarcopenic obesity, Subcutaneous adipose tissue thickness, Ultrasonography

Introduction
Feline obesity is a widespread concern around the world. In Japan, approximately 56% of cats were diagnosed as obese or overweight. It is well accepted that weight gain is derived from excessive calorie intake, which induces oxidative stress and low-grade inflammation. These metabolic changes contribute to the development of secondary diseases such as diabetes mellitus, lower urinary tract disease, dermatosis, digestive disease, and hepatic lipidosis.

As in humans, aging is one of the risk factors for obesity and adiposity in cats. In sarcopenic obesity, the increase in fat mass is often accompanied by the reduction of lean body mass (LBM). Therefore, body weight (BW) and...
body mass index (BMI) may remain constant, thus masking sarcopenia.

Feline adiposity has been evaluated by body condition score (BCS)\(^{17}\), computed tomography (CT)\(^{19}\), magnetic resonance imaging (MRI)\(^{13}\), and dual-energy X-ray absorptiometry (DEXA)\(^{3,31}\). BCS, which has been demonstrated as a standard technique to evaluate obesity in clinical practice, is diagnosed by inspection and palpation. Therefore, the results are often affected by variations in technicians. CT and DEXA are considered the reference standards for evaluation of adiposity in humans\(^{10,33}\). However, both are invasive in their use of radiation. MRI does not involve any ionizing radiation; however, it has lower availability and is more expensive than CT or DEXA and tends to overestimate fat deposits in humans\(^{33}\). Thus, there is a need for cost-effective and readily available obesity evaluation techniques in veterinary practice.

In a previous study, our group developed an objective criterion, feline body mass index (fBMI)\(^{15}\), that reflects the changes in plasma triglyceride (TG) and non-esterified fatty acid (NEFA) concentrations in early-stage obesity. However, BMI does not distinguish between LBM and fat mass. The increase in fat mass may be masked by LBM reduction in aging cats. Ultrasound shows high reliability in evaluating subcutaneous adiposity in several animals such as beef cattle, pigs, sheep, horses, dogs, and humans\(^{29,35}\). The ultrasound measurement of fat thickness in beef cattle and humans dates back to 1958 and 1965, respectively\(^{29,34}\). The monitoring of adiposity in livestock contributes to improvement in feeding management, and further productivity, and profitability. Perkins et al. (1992)\(^{25}\), using 36 steers, showed a significant relationship between the back fat thickness of live animals measured by ultrasound and carcass fat thickness measured by 2 trained technicians (\(r = 0.87\) and 0.86, respectively). Greiner et al. (2003)\(^{11}\) also showed a significant correlation between ultrasound and carcass fat thickness using 534 steers (\(r = 0.89; P < 0.001\)). In humans, ultrasonography was significantly correlated with needle puncture\(^{4}\), electrical conductivity\(^{2}\), waist calipers\(^{8}\), skinfold calipers\(^{8}\), CT\(^{1}\), MRI\(^{7}\), and DEXA as measures of fat thickness\(^{18}\). Gradmark et al. (2010)\(^{10}\) was placed a probe 5 cm cranial to the umbilicus and measured total subcutaneous adipose tissue thickness from the posterior line of dermis to the outer bowel wall using 29 middle-aged men and women. The ultrasound subcutaneous adipose tissue (SAT) showed significant correlation with SAT by CT (\(r = 0.93; P < 0.0001\)). De Lucia Rolfe et al. (2010)\(^{7}\) defined the SAT as the depth from the cutaneous boundary to the linea alba, and showed strong correlation between ultrasound and CT SAT using 41 men (\(r = 0.63\)) and 33 women (\(r = 0.68\)). However, these ultrasonic methodologies for adiposity measurement haven’t integrated in humans.

Beef cattle and cats have the same number of ribs, at 13 per side; therefore, it may be possible to apply the bovine ultrasonography technique to cats. The objective of this study was to extend the ultrasound technique from beef cattle and evaluate its applicability in cats. This study compared the thickness of SAT in aging cats with that in young adults to evaluate adiposity levels.

Materials and Methods

Animals: In total, 14 healthy, intact cats (2–15 years old, non-littermates, 4 females and 10 males) were included in this study. The cats included 9 mixed-breed cats, 2 Munchkins, 1 Scottish Fold, 1 Somali, and 1 Persian.

According to the Feline Life Stage Guidelines of the American Association of Feline Practitioners (AAFP) and the American Animal Hospital Association (AAHA)\(^{14}\), the cats were divided into 2 groups by age. 5 cats aged 10 years or younger were defined as the young adult group, and 9 cats aged 11 years or older were defined as the aging group.
All cats were housed in individual cages at a natural temperature, relative humidity and light:dark cycle at the Animal Facility of Nippon Pet Food Co. Ltd. They were maintained on a commercially available dry diet with appropriate amounts to maintain their current BW, and they had unlimited access to water.

Approval for this work was given by the Nippon Veterinary and Life Science University Animal Research Committee.

**Zoometry measurements:** Before zoometry and ultrasound measurement, the BW and BCS of each cat were measured. BCS was determined using the following five-point scale: 1, thin; 2, lean; 3, ideal; 4, overweight; and 5, obese.

To minimize measurement error caused by patient posture, we based the zoometry measurements on bone length. Zoometry measurements were performed using a commercial tape measure. The length from the top of the nose to the joint between the sacrum and the coccyx was defined as the head and body length (HBL). In addition, the length from the top of the patella to the end of the calcaneus (PCL) was determined. Chest girth (CG) was measured at the 9th rib. All zoometry measurements were performed by the same researcher.

fBMI was calculated by the following formula:

\[ fBMI = \frac{BW (kg)}{PCL (m)^{15}} \]

Body fat ratio (BFR) was calculated by the following formula: BFR = \((CG (cm)/0.7062) - PCL (cm)\) / \((0.9156 - PCL (cm))^{5.12}\)

**Measurement of SAT:** Before the measurements, the back of each animal was shaved with a commercial hair clipper between the 6th and 7th ribs and caudal to the 13th rib, and the cats were laid in left lateral recumbency. To measure the SAT, an HS-2100V (Honda Electronics Co. Ltd., Aichi, Japan) with an 11.0/8.5/6.0 MHz HLS-584M transducer (Honda Electronics Co. Ltd., Aichi, Japan) was used. All of ultrasound images were captured at 8.5 MHz. According to the established procedure for ultrasound measurements in beef cattle\(^{26}\), the ultrasound transducer was placed near the midline, between the 6th and 7th ribs and just caudal to the 13th rib, and was oriented parallel to the ribs. The transducer was moved laterally until the longissimus muscle came into full view on the screen (Fig. 1). Back fat thickness between the 6th and 7th ribs and just caudal to the 13th rib were defined as breast subcutaneous adipose tissue thickness (BSAT) and ventral subcutaneous adipose tissue thickness (VSAT), respectively. 70% ethanol was used as a couplant to obtain adequate acoustic contact.

BSAT was measured as the maximum distance from the inferior line of the dermis to the superior line of the latissimus dorsi muscle. VSAT was measured as the maximum distance from the inferior line of the dermis to the superior line of the serratus posterior muscle. These lengths were determined by free software (DataPicker, ver. 1.2, http://www.hp.vector.co.jp/authors/VA019223/) using the internal electronic calipers of the ultrasonic unit.

**Statistical analysis:** The values are expressed as the means ± standard errors. Significant differences between the young and aging groups were determined using Student’s t-test.

To identify the relationships between adipose tissue thickness (breast and ventral), fBMI, age, BW, various obesity criteria, BFR, and zoometry measurements, we performed regression analysis and obtained Pearson’s correlation coefficient. Statistical significance was set at \(P < 0.05\).

**Results**

**Age**

The mean age (12.9 ± 0.5 years vs. 5.2 ± 1.1 years), BFR (22.3 ± 2.1% vs. 14.3 ± 1.0%), CG (35.0 ± 1.6 cm vs. 29.2 ± 0.7 cm), and VSAT (0.42 ± 0.08 cm vs. 0.22 ± 0.01 cm) of the aging group were significantly higher than those of the young adult group (\(P < 0.05\)) (Table 1).
Correlation

VSAT demonstrated significant positive correlations with BW (r = 0.55), fBMI (r = 0.69), BCS (r = 0.69), BFR (r = 0.81), CG (r = 0.73), and BSAT (r = 0.69) (P < 0.05) (Table 2).

BSAT demonstrated significant positive correlations with BCS (r = 0.59) and BFR (r = 0.58) (P < 0.05).

fBMI demonstrated significant positive correlations with BW (r = 0.95), BCS (r = 0.92), BFR (r = 0.77), and CG (r = 0.81) (P < 0.05).

Discussion

Ultrasonography is a quick, easy-to-learn, safe, cost-effective, non-invasive imaging technique that does not use ionizing radiation. It is one of basic equipment used for visceral screening in veterinary clinical practices. While ultrasonographic evaluation of adiposity has not been developed in small animals, ultrasonography has been used to evaluate SAT before slaughter in beef cattle. In general, the Japan Meat Grading Association grades Japanese beef cattle on the basis of back fat thickness between the 6th and 7th ribs; this measure was applied in the present study as BSAT. Although back fat thickness between the 12th and 13th ribs has been evaluated in the US, cats tend to accumulate excessive fat in their abdominal adipose tissue. Thus, back fat thickness immediately caudal to the 13th rib was measured as BSAT. The present study showed that VSAT and BSAT were positively correlated with BW, fBMI, BCS, BFR, CG, and BSAT.

Table 1. Comparison of zoometric measurements and thickness of subcutaneous adipose tissues between young adult (1-10 years) and aging (11-15 years) cats

<table>
<thead>
<tr>
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<th>Young (n = 5)</th>
<th>Aging (n = 9)</th>
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<tr>
<td><strong>Age</strong> (years)</td>
<td>5.2 ± 1.1</td>
<td>12.9 ± 0.5*</td>
</tr>
<tr>
<td><strong>BW (kg)</strong></td>
<td>2.8 ± 0.2</td>
<td>3.5 ± 0.3</td>
</tr>
<tr>
<td><strong>fBMI (kg/m)</strong></td>
<td>20.4 ± 1.1</td>
<td>24.1 ± 1.6</td>
</tr>
<tr>
<td><strong>BCS (/5)</strong></td>
<td>3.0 ± 0.3</td>
<td>3.8 ± 0.5</td>
</tr>
<tr>
<td><strong>BFR (%)</strong></td>
<td>14.3 ± 1.0</td>
<td>22.3 ± 2.1*</td>
</tr>
<tr>
<td><strong>CG (cm)</strong></td>
<td>29.2 ± 0.7</td>
<td>35.0 ± 1.6*</td>
</tr>
<tr>
<td><strong>HBL (cm)</strong></td>
<td>51.2 ± 1.4</td>
<td>52.4 ± 1.7</td>
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<tr>
<td><strong>PCL (cm)</strong></td>
<td>13.9 ± 0.3</td>
<td>14.5 ± 0.4</td>
</tr>
<tr>
<td><strong>VSAT (cm)</strong></td>
<td>0.22 ± 0.01</td>
<td>0.42 ± 0.08*</td>
</tr>
<tr>
<td><strong>BSAT (cm)</strong></td>
<td>0.01 ± 0.01</td>
<td>0.03 ± 0.01</td>
</tr>
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</table>

Values are presented as the means ± standard error. The numbers in parentheses indicate the number of animals examined.

*Significantly different from the young adult group (Student’s t-test, P < 0.05).

BW: body weight
fBMI: feline body mass index
BCS: body condition score
CG: chest girth
HBL: head and body length
PCL: length from patella to end of calcaneus
VSAT: ventral subcutaneous adipose tissue thickness
BSAT: breast subcutaneous adipose tissue thickness

Fig. 1. Ultrasound measurement of subcutaneous adipose tissue thickness at the breast (A) and abdomen (B). a: breast subcutaneous adipose tissue thickness; b: trapezius muscle; c: latissimus dorsi muscle; d: longissimus thoracis muscle; e: ventral subcutaneous adipose tissue thickness; f: longissimus dorsi muscle; g: serratus posterior muscle
measured as VSAT in this study.

According to previous research, humans and steers tend to accumulate excessive energy on their lower trunk as SAT. Leahy et al. (2012) compared ultrasound and DEXA as techniques for measuring whole-body fat and segmental body fat in five regions (SAT of triceps, iliac crest, abdomen, front thigh, and medial calf) using 135 healthy 18- to 29-year-old adults. In that study, ultrasonically measured ventral adiposity showed the highest correlation with BFR by DEXA ($r = 0.905$, $P < 0.01$). Greiner et al. (2003) also found, using 534 steers, that carcass fat thickness at the 12th rib was more strongly correlated with ultrasonic measures of SAT at the 12th rib than with rump or body wall fat thickness. Cats also showed higher fat accumulation caudal to the 13th rib than between the 6th and 7th ribs in this study. Therefore, the caudal edge of the 13th rib appears to be a suitable site to evaluate fat accumulation by ultrasonography in cats.

In general, BW decreases after 11 years in cats. The main cause of the reduction is LBM loss derived from decreased protein and lipid digestibility, resulting in energy deficiency. The loss of LBM may mask increases in adiposity. Therefore, BW and fBMI may not significantly change, thus masking sarcopenia and adiposity in this study. By contrast, ultrasonic fat measurement enabled us to estimate masked fat deposition in aging cats. This technique thus facilitates the accurate diagnosis of fat deposition in aging cats.

However, our research did not include any muscle mass measurements. BFR was also determined by indirect calculation. Additionally, our study is the first research to apply the ultrasound technique from beef cattle to cats. Thus, minimum number of cats was included according to animal ethics in this study. Further direct measurement of muscle and fat mass will be needed to reveal the sarcopenic reduction of LBM and increase in fat deposition with accuracy.

In conclusion, the caudal edge of the 13th rib appears to be a suitable site for ultrasonic evaluation of fat accumulation in cats. Compared with young adult cats, aging cats showed a significant increase in VSAT but no significant increase in fBMI. Additionally, VSAT showed significant correlations with BW, BCS, fBMI, BFR, and CG. Ultrasound measurement of VSAT

### Table 2. Correlations between adipose tissue thicknesses, feline body mass index, and zoometric measurements in cats

<table>
<thead>
<tr>
<th></th>
<th>Age</th>
<th>BW (kg)</th>
<th>fBMI (kg/m)</th>
<th>BCS (/5)</th>
<th>BFR (%)</th>
<th>CG (cm)</th>
<th>HBL (cm)</th>
<th>PCL (cm)</th>
<th>BSAT (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VSAT (cm)</td>
<td>0.26</td>
<td>0.55*</td>
<td>0.69*</td>
<td>0.69*</td>
<td>0.81*</td>
<td>0.73*</td>
<td>−0.02</td>
<td>0.01</td>
<td>0.69*</td>
</tr>
<tr>
<td>BSAT (cm)</td>
<td>0.10</td>
<td>0.43</td>
<td>0.53</td>
<td>0.59*</td>
<td>0.58*</td>
<td>0.51</td>
<td>0.08</td>
<td>−0.04</td>
<td>−</td>
</tr>
<tr>
<td>fBMI (kg/m)</td>
<td>0.24</td>
<td>0.95*</td>
<td></td>
<td>0.92*</td>
<td>0.77*</td>
<td>0.81*</td>
<td>0.53</td>
<td>0.46</td>
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Values are presented as regression coefficients.

*Significant correlation by Pearson’s regression analysis ($P < 0.05$)

BW: body weight
fBMI: feline body mass index
BCS: body condition score
CG: chest girth
HBL: head and body length
PCL: length from patella to end of calcaneus
VSAT: ventral subcutaneous adipose tissue thickness
BSAT: breast subcutaneous adipose tissue thickness
enables the evaluation of masked fat deposition in aging cats. Overall, ultrasound measurement of VSAT appears to be a valid method to evaluate fat deposition in aging cats.

Acknowledgements

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