**Ultrasonographic monitoring of feline epaxial muscle height as part of an annual wellness examination to assess for the development of sarcopenia**

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**Keywords:** cat, feline, mature, sarcopenia, muscle condition, muscle measurement

**Abstract**

**Objectives:** To determine if epaxial muscle height (EMH) could be reliably incorporated into annual routine wellness screenings, and also determine its relationship to age, body condition score (BCS), subjective muscle assessment (SMA), breed and sex in mature cats.

**Methods:** EMH was determined independently by three observers from ultrasonographic examinations collected by an additional trained individual of cats enrolled at the Feline Healthy Ageing Clinic, University of Liverpool, UK. Age, body weight, BCS and SMA data were also collected.

**Results:** A total of 92 cats were included, 35 of which had repeat ultrasonographic examinations taken 12 months apart. Cats enrolled had a median age of 8 years 9 months at the time of the first measurement. Variation in the quality of ultrasonographic images collected did not impact the muscle depth measurements (*P* = 0.974). Further, there was good intra- and inter-observer repeatability for all observations, (ICC range 0.97 - 0.99). There was a moderate positive association between EMH and body weight (R = 0.49, *P* <0.001), but no association with age (R = -0.05, *P* = 0.680). There were also positive associations in EMH amongst cats with different BCS (*P* = 0.001) and SMA (thoracic spine, *P*= 0.021, lumbar spine, *P =* 0.014), but breed (*P* = 0.429) and sex (*P* = 0.187) had no effect. Finally, there was no change in EMH measurements in the paired samples (*P* = 0.145) or correlation between percentage weight and EMH change over 12 months.

**Conclusions and relevance:** Accuracy of EMH measurement using ultrasonographic imaging is good, irrespective of observer experience and provided the ultrasonographer has some training. This suggests that ultrasonographic measurement of EMH could have a major practical impact as a non-invasive determination of muscle mass in pet cat populations. Further research is required to assess longitudinal changes in muscle mass over time in senior pet cats.

**Introduction:**

Sarcopenia is a term first proposed by Rosenberg in 1989 from the Greek *sarx*, meaning flesh, and *penia,* meaning loss.1 It is defined as the progressive, age-related loss of muscle condition and strength which, in turn, is associated with adverse health-related outcomes in older people.2 In the 2018 operational definition of sarcopenia in humans, likely sarcopenia is identified by low muscle strength, sarcopenia is confirmed by the presence of low muscle quantity or quality, and severe sarcopenia is used for cases meeting the above two criteria in addition to low physical performance.3 This is a change from an earlier consensus that put loss of muscle mass alone as the first sign of sarcopenia.4 The predominant feature of primary sarcopenia is that it occurs in the absence of any other definable cause.3 Secondary sarcopenia can occur due to systemic disease, physical inactivity (due to a sedentary lifestyle or disease related immobility) and inadequate energy or protein intake.3 Reversal of sarcopenia in ageing humans can be difficult and, therefore, early intervention with appropriate nutritional support and exercise is important to slow its progression.5

There is currently a paucity of data regarding the identification, quantification, and treatment of sarcopenia in cats. Muscle wasting has been reported in 22% of cats aged 7-10 years, 54% of cats aged 11-14 and 77% of cats greater than 15 years of age.6 In ageing cats, weight loss is associated with shorter survival times when chronic disease (e.g., cancer, kidney disease, hyperthyroidism) is present; however, clinical assessment of sarcopenia in cats is not currently standardised.7,8 Weight measurement alone is a poor measure of sarcopenia9, but can be used clinically in conjunction with body condition scoring (BCS) and muscle condition scoring (MCS). Both of these measurements have been validated against dual-energy X-ray absorption (DEXA) in cats10,11, and can be used to identify possible changes in body composition and muscle wastage, respectively.12 However, an additional non-invasive method allowing for measurements of muscle mass would be helpful for clinicians, because both BCS and MCS are subjective estimations.12

Ultrasonography is an imaging modality that is gaining increasing recognition as a useful method for assessment of epaxial musculature in companion animals with attempts to standardise measurements.13–15 Ultrasonographic measurements of the epaxial muscle height (EMH) at the level of 13th thoracic vertebrae were positively correlated with body weight in clinically normal cats (aged 1-6 years, BCS 4-6/9)14, whilst MCS was positively correlated with epaxial muscle height in cats of various ages (mean 10.3 years ± 3.9 years)15. However, to date, there have been no studies investigating either the use of this methodology as part of a routine wellness appointment, or associations between EMH and different variables in mature (7-10 years)16 pet cats.

Therefore, the first aim of this study was to determine if ultrasonographic EMH measurements are repeatable amongst observers and could readily be incorporated into a routine wellness screening consultation for mature cats. The second aim was to determine if EMH measurements were associated with variables such as age, body weight, BCS, subjective muscle assessment (SMA), breed and sex in mature cats, and also whether EMH measurements changed over time.

**Methods**

*Selection criteria*

Cats attending the Royal Canin Feline Healthy Ageing Clinic (FHAC) at the University of Liverpool, between 2017 and 2020, were included at the time they attended either their enrolment or subsequent annual examinations. Eligibility criteria included being of a temperament that was amenable to having a conscious ultrasonographic examination, additional criteria for enrolment to the FHAC included age (7-9 years).17 Cats were excluded where recorded cine-loop ultrasonographic images were not of a sufficient quality to allow measurement of the epaxial muscles using predefined landmarks.

*Clinical assessment*

Baseline data including age, sex, and breed were recorded at enrolment. Bodyweight was measured using the same set of electronic scales (V20 scales, Burtons, UK), which were calibrated on a weekly basis using 2.5 kg fitness weight plates. To assess BCS, a validated 9-unit system18 was used, whilst SMA was based on a previously-validated system whereby a score of 3 indicated no muscle wastage, a score of 2 indicated mild muscle wastage, a score of 1 indicated moderate muscle wastage and a score of 0 indicated marked muscle wastage.10 However, to distinguish scores from those of BCS, the terminology used for the categories was that recommended by WSAVA, 3 to 0 was replaced by letters A to D.19 Although SMA was assessed at 10 different points of the body, for the purposes of this study, only the scores for the epaxial muscles (thoracic spine and lumbar spine) were used rather than a global score, as this is where muscle loss is first typically noticed in cats 20. Bodyweight, BCS and SMA were collected at the time of each ultrasonographic assessment, by the investigator (ND).

*Ultrasonographic assessment*

All ultrasonographic examinations were performed by ND, a veterinarian and PhD student on the FHAC, who had been trained by an EBVS® European Specialist in Veterinary Diagnostic Imaging (TM). Training consisted of a 40-minute session optimising the settings on the ultrasound machine and standardising the protocol for the ultrasonographic probe placement. Cats were either held in a standing position or positioned in sternal recumbency. The hair was parted over either the left or right thoracolumbar junction, with the side of the cat imaged determined solely by the cats’ comfort and posture. Before measurements were taken, ultrasound gel (Blue ultrasound gel, Henleys Medical Supplies Ltd., UK) was used to saturate the fur, and a 75L38 5-10 MHz 38mm footprint linear probe (Mindray DP-50, IMV Ltd., UK) was then used to collect measurements. Landmarks for ultrasonographic probe placement were determined by palpating the junction of the 13th rib and vertebrae. Multiple ultrasonographic cine-loops were recorded and saved as digital imaging and communications in medicine (DICOM) files for analysis.

The ultrasonographic cine-loops were first assessed for visibility of landmarks for EMH measurement by Observer 1 (AL), a veterinarian, and Observer 2 (NS), a final-year veterinary student. Landmarks assessed for visibility were the junction of the 13th rib and vertebrae and the junction of the muscle and subcutaneous fat. If the required landmarks were not visible, the cine-loops were discarded. For cats with multiple cine-loops, the best quality cine-loop (i.e., the one that showed the required landmarks most clearly) was selected, and three measurements were made using the measurement callipers of a freely available DICOM viewer (Horos Project version 4.0.0, Purview, USA). For each measurement, one cursor was positioned at the junction of the 13th rib-vertebral junction and the ventromedial aspect of the epaxial muscle; the second cursor was positioned at the junction between muscle and subcutaneous fat at the dorsolateral aspect of the muscle (Figure 1) as has been previously described.14,15 Mean muscle height for each observer was calculated from the three measurements taken from each cat, as previously described.14,15

*Method validation*

Observer 2 independently graded all cine-loops for quality using a 3-point grade scale following consultation with ND and TM. High-quality images, where anatomical landmarks were fully visible, were graded 1; reasonable quality images, where some areas of the cine-loop were unclear but landmarks were still visible, were graded 2; and poor-quality images, where the landmarks were not fully visible but measurements could still be made, were graded 3. To determine intra- and inter-observer association and agreement in scoring, Observers 1 (veterinarian) and 2 (veterinary student) measured EMH from the ultrasonographic cine-loops, following a short period of training by TM. Additionally, a third observer, Observer 3, veterinary diagnostic imaging specialist (TM) also assessed 30 cine-loops (validation image set), selected from all the cases using a random number generator (Excel version 16.57 [22011101], Microsoft® Corp).

*Data handling and statistical analysis*

Statistical analysis was performed using an online open-access statistical language and environment (R, version 4.11; The R Foundation for Statistical Computing, <http://www.R-project.org>) and the following packages ‘ggplot 2’21, ‘ggpur’22, ‘tidyverse’23, ‘psych’24. Datasets were assessed for normality using the Shapiro-Wilk test and visual assessment of histograms. Coefficient of variation (CoV) was used as a measure of relative variability in the individual observer measurements. Parametric tests were used for normally-distributed data, with results presented as mean ± standard deviation (SD). Non-parametric tests were used for data that were not normally distributed, with results presented as median and minimum and maximum range. The level of statistical significance was set at *P ≤*0.05, and all reported *P*-values are two-sided.

Bland-Altman plots were created to assess the agreement of measurements between observers. Intra and inter-class correlation coefficients were calculated, a two-way mixed-effects model based on single rating for the intra-observer calculation and a two-way random-effect model based on single ratings and absolute agreement for inter-observer calculations were used.

Using the primary image set (first images collected from each cat, regardless of quality and not including any repeat measures), associations between mean EMH measurements (mean EMH was calculated from all 6 measurements from both Observer 1 and 2) and continuous baseline variables (age, bodyweight) were examined using scatter plots and Pearson’s correlation coefficient. The effect of categorical variables (BCS, SMA, sex) on mean EMH and of CoV in different image quality categories were assessed with box plots in conjunction with either Kruskal-Wallis tests (BCS, CoV in different quality grades), Mann-Whitney tests (SMA [scores A vs. B]) or unpaired *t*-tests (sex [male vs. female] and breed [pedigree vs domestic]). Finally, where repeat measurements 12 months apart were available, change in mean EMH, body weight and BCS were assessed, using either paired *t-*tests or Wilcoxon signed rank tests when the data were parametrically or non-parametrically distributed, respectively. The relationship between changes in body weight and EMH was examined using scatter plots and Kendall’s Tau (Kendall rank correlation coefficients).

**Results**

*Study animals and ultrasonographic cine-loop image sets*

Ultrasonographic cine-loops were initially available from 94 cats, but cine-loops from two cats were excluded because the required anatomical landmarks were not visible; therefore, sets of cine-loops available from 92 cats were in this primary image set. Repeat measurements, 12 months apart, were also available for 35 cats (secondary image set). The breeds represented were 78 domestic cats (long and short haired) and 14 pedigree cats (Persian, 3; Ragdoll, 2; Siamese, 2) and individual cats from seven other breeds (American exotic, Bengal, Egyptian Mau, Maine Coon, Oriental, Russian Blue and Sphynx). There were 49 male cats (48 neutered) and 43 female cats (all neutered), whilst median age at first measurement was 105 months (8 years 9 months) (range 80 to 143 months), median body weight was 4.8 kg (range 3.1 to 9.2 kg) and median BCS was 6/9 (range 3/9 to 9/9). Epaxial SMA was graded B in 6/92 cats over the thoracic spine and 7/92 cats over the lumbar spine at first assessment, and 1/35 cat at the second assessment; all other cats received a SMA of A at both locations and time points. Mean EMH was 13.6 ± 2.60 mm at first assessment and 13.9 ± 2.49 mm at the second (Table 1).

*Agreement amongst observers*

When assessing intra-observer variability (Table 2), relative variability was low in Observers 1 (veterinarian: median CoV 1.9, range 0.2 to 8.3) and 2 (student: median CoV 1.7, range 0.4 to 6.3). Additionally, intra-observer agreement in EMH measurements was excellent for both Observers 1 and 2 (Table 2). Visual inspection of Bland-Altman plots revealed excellent inter-observer agreement with no obvious systematic or proportional bias (Figure 2). Between Observers 1 and 2 (veterinarian vs. student), the mean bias was -0.08 mm (95% limits of agreement: 0.51 to -0.67; Figure 2A); between Observers 2 and 3 (student and imaging specialist), the mean bias was -0.09 mm (95% limits of agreement: 1.34 to -1.52; Figure 2B); whilst between Observers 1 and 3 (veterinarian vs imaging specialist), the mean bias was -0.004 mm (95% limits of agreement: 1.21 to -1.22; Figure 2C). The median absolute magnitude of difference amongst Observers 1 and 2 was 0.140 mm (range 0.003-1.740), between Observers 2 and 3 was 0.402 mm (range 0.023-1.393) and between Observers 1 and 3 was 0.402 mm (range 0.020-1.820). Furthermore, there was good inter-observer agreement between Observers 1 (veterinarian) and 2 (student), and good inter-observer repeatability amongst all three Observers (ICC 0.97, 0.95 to 0.98, *P* <0.001, Table 2).

*Ultrasonography cine-loop quality*

Mean EMH measurements did not differ amongst different quality grades of ultrasonographic images in the primary image set (*P* = 0.974). Variability in muscle height measurements did not differ between cine-loops of different quality when assessed either by both Observer 1 (*P* = 0.961, Figure 3A) or 2 (*P* = 0.546; Figure 3B).

*Associations between EMH and other variables*

In the primary image set, there was no significant difference in mean EMH between cats of different sex (*P* = 0.187) or domestic versus pedigree breed (*P* = 0.429). No correlation was observed between EMH and age (R = -0.05, *P* = 0.68; Figure 4a), but there was a moderate positive correlation between EMH and body weight (R = 0.49*,* P = <0.001; Figure 4b). Mean EMH was significantly greater in cats with a BCS of either BCS 7 or 8 than in cats with a BCS of 4 or 6; furthermore, cats with a BCS of 8 had a significantly greater mean EMH than cats with a BCS of 5 (*P* =0.001, Figure 5). Mean EMH was significantly greater in cats with a SMA of A compared with SMA B for both the thoracic spine (*P* = 0.021) and lumbar spine (*P =* 0.014) regions (Figure 6a-b).

*Variation in cats over 12 months*

In the cats (n=35)with repeat assessments 12 months apart, there were no significant differences in either bodyweight (*P* = 0.474) or BCS (*P* = 0.516) over time. No significant difference was observed in median EMH between the first (14.7 ± 2.82 mm) and second (13.9 ± 2.48 mm) measurements (*P* = 0.145, Figure 7), mean change in EMH was -0.8 ± 3.02 mm. Median change in individual cat body weight for the 35 cats was 0.07 kg (-3.63 to 0.91 kg), no correlation was observed between percentage change in body weight and epaxial muscle height (R = 0.19, *P* = 0.110; Figure 8). SMA was unchanged in all but two of the 35 cats (6%), where SMA improved from B to A at both sites for both cats. In one of these cats, mean EMH increased from 12.51 mm to 14.49 mm, whilst weight (4.29 to 5.2 kg) and BCS (6 to 7) also increased; in the second cat, mean EMH decreased from 14.55 mm to 11.83 mm, whilst weight (3.70 to 3.19 kg) and BCS (5 to 4) also decreased.

**Discussion**

Findings from this study support those of other studies where there was correlation between EMH and body weight, and also between EMH and SMA in cats.14,15 Together, these studies support the potential for ultrasonographic assessment of muscle loss, providing an additional valuable tool for assessing the health of ageing cats.

This is the first study to look at repeat measurements of EMH in cats. There was no significant decline in EMH over a period of 12 months and further longitudinal assessment of EMH in cats is required.

There were also differences in EMH in cats with different BCS, whereby cats with greater body fat mass (BCS 7 or 8) had a significantly greater EMH than cats with less body fat mass (e.g., BCS 4-6). This is, perhaps, not unexpected because lean tissue mass is greater in cats with obesity.11 However, this increase in EMH might also be due to compensatory fat deposition within and around myocytes, as seen in elderly (>70 years) people, especially, women, with an increased body fat mass.25,26 This condition is called ‘sarcopenic obesity’, and is associated with an increased risk of compromised mobility.4,26 Similar findings have been shown to occur in cats with obesity27, and may compromise identification of sarcopenia using either SMA or ultrasonography. Advanced diagnostic imaging modalities, such as computed tomography or magnetic resonance imaging, would be required to assess such intra-muscular fat deposition.26

Both intra- and inter-observer ICC were good, suggesting good agreement amongst observers with differing levels of training, from a veterinary student to a diagnostic imaging specialist. This finding is also supported by Bland-Altman plots, whereby the 95% limits of agreement were 0.51 to -0.67, 1.34 to -1.52 and 1.21 to -1.22 respectively. Given these findings, and the fact that image quality had limited impact on EMH results, EMH measurements could reliably be used to monitor cats in primary care practice provided that veterinary professionals receive a modest amount of appropriate training. However, a limitation of this study is that all EMH measurements were performed on one set of images collected by a single examiner, variability may be greater within and between Observers if they had to collect images themselves or they were measuring images collected by several different examiners.

There was no significant correlation in mean EMH with age, which may be due to the small age range of the cats enrolled. No cats with severe muscle loss were included in this study. Muscle strength or performance was not assessed, so the physiological significance of sarcopenia in the cats with mild muscle wastage could not be fully evaluated. As sarcopenia is an age-related condition, further study with a larger cohort of older animals and greater range of ages would therefore be useful.

In addition to the narrow age range, other limitations associated with the demographics of the study population include the small number of pedigree cats and also those with moderate or severe muscle wastage. Documenting co-morbidities would be critical to interpret sarcopenic effects clinically.28

Additional limitations include the operator skill during ultrasonographic image collection, as manual manipulation risks excessive muscle compression (artefactually reducing muscle height) during the examination and may also compromise the identification of anatomical landmarks for accurate measurements.15 However, this study demonstrated that repeatable results could still be obtained from poorer quality images. However, several images were removed from the data set as landmarks could not be identified, creating bias in the dataset. The training required for EMH measurements is straightforward, including the appropriate application and direction of the ultrasound probe as well as identification of the pre-defined landmarks. Posture and inconsistency when measuring the left side or right side of the spine would preferably be standardised although another study demonstrated no difference in measurements between standing or crouching, and left or right epaxial muscle measurements.14 Finally, normalisation of EMH to body size was not performed. Normalisation has been previously described in cats using the forelimbs circumference measurement 14,15 and will be useful to include in future work where comparison between cats is required.

**Conclusion**

Measurements of EMH are associated with body weight, BCS and SMA, but not associated with age, breed or sex in this cohort of cats. There was no relationship between changes in weight and EMH in individual cats over 12 months but further longitudinal research on the changes to epaxial muscle depth measurements as individual cats age is warranted. This study suggests that ultrasonographic measurement of EMH could have major practical impact as a non-invasive determination of muscle mass in pet cat populations.

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**Conflicts of interest**

At the time the study was performed, ND was undertaking a post-graduate studentship funded by Royal Canin, a division of Mars Petcare. Since October 2020, ND has been employed by International Cat Care, but also holds a part-time post-doctoral research position at the University of Liverpool, funded by Royal Canin. AJG is an employee of the University of Liverpool whose position is currently funded by Royal Canin. AJG has also received financial remuneration and gifts for providing educational material, speaking at conferences, and consultancy work. NS was undertaking an Inspire studentship funded by the Academy of Medical Sciences at the time of her involvement in the study.

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**Ethical approval**

The work described in this manuscript involved the use of non-experimental (owned or unowned) animals. Established internationally recognised high standards (‘best practice’) of veterinary clinical care for the individual patient were always followed and/or this work involved the use of cadavers. Ethical approval from a committee was therefore not specifically required for publication in JFMS. Although not required, where ethical approval was still obtained, it is stated in the manuscript. Ethical approval for this study was granted by the University of Liverpool Veterinary Research Ethics Committee (VREC491abcd) and the Royal Canin Ethical Committee.

**Informed consent**

Informed consent (verbal or written) was obtained from the owner or legal custodian of all animal(s) described in this work (experimental or non-experimental animals, including cadavers) for all procedure(s) undertaken (prospective or retrospective studies). No animals or people are identifiable within this publication, and therefore additional informed consent for publication was not required. At enrolment to the FHAC all owners were provided with a detailed information sheet explaining the purpose of the study before giving written consent for their cat to be enrolled.

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**Table 1.** Baseline data and mean epaxial muscle height measurements in cats at different time points

|  |  |
| --- | --- |
| **Descriptor** | **Image set** |
| **Primary** | **Secondary** |
| *Number* | 92 | 35 |
| *Age (months)* | 105 (80 – 143) | 113 (85 – 145) |
| *Weight (kg)* | 4.8 (3.1 – 9.2) | 4.8 (3.2 – 7.2) |
| *BCS* | 6 (3 – 9) | 6 (3 – 9) |
| *SMA-LS* | A (A – B) | A (A – B) |
| *SMA-TS* | A (A – B) | A (A – B) |
| *EPH (mm)* | 13.6 (2.60) | 13.9 (2.49) |

Data are median and range for all variables except epaxial height measurements (EMH), which are reported as mean (SD). BCS: body condition score; SMA-LS: subjective muscle assessment measured over the lumbar spine; SMA-TS: subjective muscle assessment measured over thoracic spine.

**Table 2:** Agreement between observers for epaxial muscle ultrasound height measurements**.**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | **ICC** | **95% CI** | **p-value** | **Median CoV** | **Range** |
| Intra-observer |  |  |  |  |  |
|  *Observer 1* | 0.98 | 0.98-0.99 | <0.001 | 1.9 | 0.2-8.3 |
|  *Observer 2* | 0.98 | 0.98-0.99 | <0.001 | 1.7 | 0.4-6.3 |
|  |  |  |  |  |  |
| Inter-observer |  |  |  |  |  |
|  *Observer 1 vs 2* | 0.99 | 0.99-1.00 | <0.001 | 2.5 | 0.7-9.3 |
|  |  |  |  |  |  |
|  *Observer 1 vs 2 vs 3* | 0.97 | 0.95-0.98 | <0.001 | 1.9 | 0.5-7.9 |

ICC: Intra class correlation; CI: confidence interval; CoV: coefficient of variation. Observer 1: veterinarian; observer 2: veterinary student; observer 3: veterinary diagnostic imaging specialist