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Muscle function assessment of the hindlimbs in healthy dogs using acoustic myography

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ABSTRACT

Introduction: Impaired muscle function is a frequent consequence of musculoskeletal disorders in dogs. Musculoskeletal disorders, especially stifle joint diseases, are common in dogs and assessment of muscle function in dogs is clinically relevant. Acoustic myography (AMG) is a non-invasive method to assess muscle activity. Quantifying muscle function in normal dogs could help identify clinically relevant changes in dogs with orthopaedic disease and allow targeted interventions to improve recovery in these. The objectives of the study were to characterize hindlimb muscle function in healthy dogs using AMG and to investigate the repeatability and reproducibility of AMG in dogs.

Methods: Healthy dogs (15–40 kg) without musculoskeletal disorders were recruited and screened for eligibility to participate in the study. The muscle activity in four hindlimb muscles related to the stifle was assessed using AMG. The degree of symmetry between the hindlimbs in these dogs was investigated and the reliability of AMG was evaluated.

Results and conclusions: The study population comprised 21 dogs. Reference intervals and symmetry indices for AMG scores of the hindlimb muscles were identified, with highest variability for the *E*-scores. For all AMG-scores, same-day variation was lower than between days variation, and both were lowest for S- and T-scores. Further investigation is needed to establish if AMG can enable discrimination between dogs with altered muscle function and healthy dogs.

1. Introduction

Cranial cruciate ligament disease (CCLD) is a common canine musculoskeletal disorder with a high number of veterinary care claims (Engdahl et al., 2021; Johnson et al., 1994). Muscle function is frequently affected in dogs with CCLD (Adrian et al., 2019), and muscle activation and the balance of muscle forces significantly affect stifle joint stability in ex vivo models (Mazdarani et al., 2022; Ober et al., 2022; Ramirez et al., 2015). Quantifying muscle function in normal dogs could help identify clinically relevant changes in dogs with CCLD and allow targeted interventions to improve recovery in this patient group.

A recent scoping review identified 18 non-invasive methods to assess different aspects of muscle function (Dahl et al., 2023). Among these methods, muscle activity can be quantified using acoustic myography (AMG) (Harrison et al., 2013). Human and veterinary research has shown that AMG can be used to record muscle activity during various physical activities (Bartels et al., 2020; Fuglsang-Damgaard et al., 2021; Harrison et al., 2013; Pingel et al., 2019; Vitger et al., 2021). The output from AMG is somewhat comparable to surface electromyography (Fuglsang-Damgaard et al., 2021; Harrison, 2018). Both techniques assess muscle activity; AMG records pressure waves generated by muscle contractions while surface electromyography measures electrical signals originating from depolarization of muscle fibres (Fuglsang-Damgaard et al., 2021; Harrison, 2018).

From the AMG signal, three scores (E, S, T) can be derived within a user-defined timeframe defining the activity window of each muscle. The *E*-score represents motor efficiency or coordination of muscle work i.e., the duration of motor activity (above a pre-defined threshold). The

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S-score represents the recruitment of muscle fibres i.e., the signal amplitude (spatial summation). The T-score represents the frequency of the pressure waves generated by the muscle contractions i.e., the number of activity peaks per second (temporal summation). Each individual score is given a number from 0 to 10, with low scores (close to 0) reflecting increased muscular effort for a specific movement. Low E-scores have been reported to indicate poor muscle coordination or efficiency, low S-scores indicate high signal amplitudes, and low T-scores indicate high frequency of muscle activity, and vice versa for high scores (Celicanin et al., 2023; Fuglsang-Damgaard et al., 2021; Harrison, 2018).

Other relevant non-invasive methods for assessing muscle function indirectly include pressure sensitive walkway systems (PSW) for kinetic gait analysis, limb circumference for assessment of muscle atrophy, and goniometry to assess the passive range of motion of joints of interest. The AAHA Pain Management Guidelines for Dogs and Cats (Gruen et al., 2022) emphasize kinetic gait analysis and especially PSW as a valid method for assessment of limb pain in dogs. Currently, PSW and force plate systems are considered the reference standard for quantifying lameness in dogs (Walton et al., 2013). Hindlimb muscle asymmetry has been assessed in a clinical study using AMG in dogs with CCLD (Varcoe et al., 2021). However, no healthy controls were included in this study for comparison purposes, and we are not aware of previous research evaluating muscle symmetry of muscles relevant to CCLD using AMG in healthy dogs. The objectives of this study were to characterize symmetry of muscle activity using AMG and establish reference intervals for AMG scores in the hindlimbs of healthy dogs as assessed by objective gait analysis and clinical examination, and to investigate the repeatability and intermediate reproducibility of AMG in this population.

2. Material and methods

2.1. Subjects

This study was approved by the local ethical and administrative committee at the Department of Veterinary Clinical Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Denmark, No. 2023–2. Further, the AMG method was approved, No. AEIRB 2023–09-PAS-014 A. A protocol for the study was uploaded and registered prospectively in the Open Science Framework on 14th April 2023 (https://osf.io/rn9gw).

Sample size (n) was calculated for a desired confidence interval width of 10% for the within-subject standard deviation based on 10 repeated measurements for each muscle (McAlinden et al., 2015).

Advertisements on social media and direct contact to owners via the first author's institution were used to recruit healthy medium and large breed dogs (15–40 kg). Inclusion criteria were age between one and four years, body condition score of 4–6/9 and normal muscle condition score (Laflamme, 1997). Exclusion criteria were musculoskeletal diseases or abnormalities, significant prior musculoskeletal injuries, and current use of any pain medications. Significant injury was defined as a condition that required surgery or medical treatment exceeding 10 days. Informed written consent was obtained from owners for their dog's participation in the study prior to any procedures with the right to terminate the participation at any point. All dogs completed the study procedures in the following order: pressure sensitive walkway analysis followed by video recordings of gait, clinical and orthopaedic examination, AMG, and finally goniometry and limb circumference. All clinical and orthopaedic examinations were performed by the same experienced veterinarian (MBMN), and video recordings of each dog's gait were reviewed by the same to confirm absence of musculoskeletal disorders (Witte and Scott, 2011).

2.1.1. Pressure sensitive walkway system

Recordings from a PSW (Tekscan I-Scan model 5101E VH4, Evolution, Tekscan, Inc., *Massachusetts, USA*) were obtained to assess symmetry in the dogs' gait. Four Tekscan Medical #3140 sensors with a total length of 1.95 m protected by a three-meter-long rubber mat, and with a resolution of one sensel/cm^2 were connected to a laptop running Tekscan Walkway 7.66 software. The PSW was equilibrated daily and calibrated weekly. The dogs were given a few minutes to get acquainted with the examination room and equipment before starting. All dogs were walked in one direction across the PSW on a loose leash on the left side of an experienced handler (MBMN) at their preferred velocity. Five successive valid recordings with similar gait velocities were obtained (maximum ±10 cm/s from the average gait velocity). A recording was considered valid when the dogs walked in a straight line across the PSW without overt head movements, with all four paws fully contacting the PSW.

Automatic software identification of each paw was manually checked by one observer (KHD) and corrected if necessary. Outcome variables were peak vertical force, vertical impulse, and peak pressure.

2.1.2. Acoustic myography

Acoustic myography recordings were obtained from four thigh muscles related to the stifle (vastus lateralis, semitendinosus, biceps femoris and cranial sartorius). Piezoelectric sensors with a diameter of 20 mm (CURO-Diagnostic Aps, Bagsværd, Denmark) were placed with the aid of palpation by the same observer (KHD) for each measurement and at the same level on all dogs. Landmarks for placing the sensors were based on previous literature combined with experience from cadaver dissections (Table 1) as shown in Fig. 1 (McLean et al., 2019; Varcoe et al., 2021). Before placing the sensors on the skin above the muscles of interest, the fur was parted, and non-sterile acoustic gel (MyoDynamik Aps, Bagsværd, Denmark) was placed on the skin: sensors were secured in place by an adhesive foam bandage (Snögg AS, Kristiansand, Norway) (Fig. 2).

The AMG recordings were obtained bilaterally for two muscles at a time: first vastus lateralis and semitendinosus, followed by biceps femoris and cranial sartorius.

Each sensor was attached to a separate recording device (CURO unit), containing an amplifier (0 to 32 dB) and a Bluetooth antenna that transmitted the signal to the CURO Canine application on a tablet or smart phone. Recordings were made at a sampling rate of 1000 Hz for each CURO unit, without analogue or digital filtering. In the CURO Canine application, an amplification setting of 21 dB was set based on pilot studies (Appendix A). Each CURO unit's output was linked to a specific muscle within the CURO Canine application.

A test recording was made to confirm satisfactory quality and amplification of the pressure wave signals immediately after attaching the sensors and CURO units to each dog.

The AMG recordings were obtained while each dog walked at its preferred velocity along a 38 m long corridor. To visualize baseline muscle activity, recordings included a few seconds of standing before and after the walking phase. Ten recordings were obtained for each muscle pairing to evaluate repeatability. A further ten recordings using the same protocol were obtained on a separate day within 30 days to evaluate reproducibility.

Table 1
Landmarks for placement of acoustic myography sensors.

Muscle	Sensor placement
Cranial sartorius	On the cranial border of the medial thigh, half the femur length
Vastus lateralis	On the craniolateral thigh, at the cranial border of femur and 2/3 proximodistal length of the femur from the greater trochanter to lateral condyle
Biceps femoris	On the belly of the cranial head, 1/3 proximodistal length of the femur from the greater trochanter to lateral condyle
Semitendinosus	On the caudolateral border of the thigh, 1/3 proximodistal length from ischiatic tuberosity to calcaneus, lateral to the groove between semitendinosus and semimembranosus

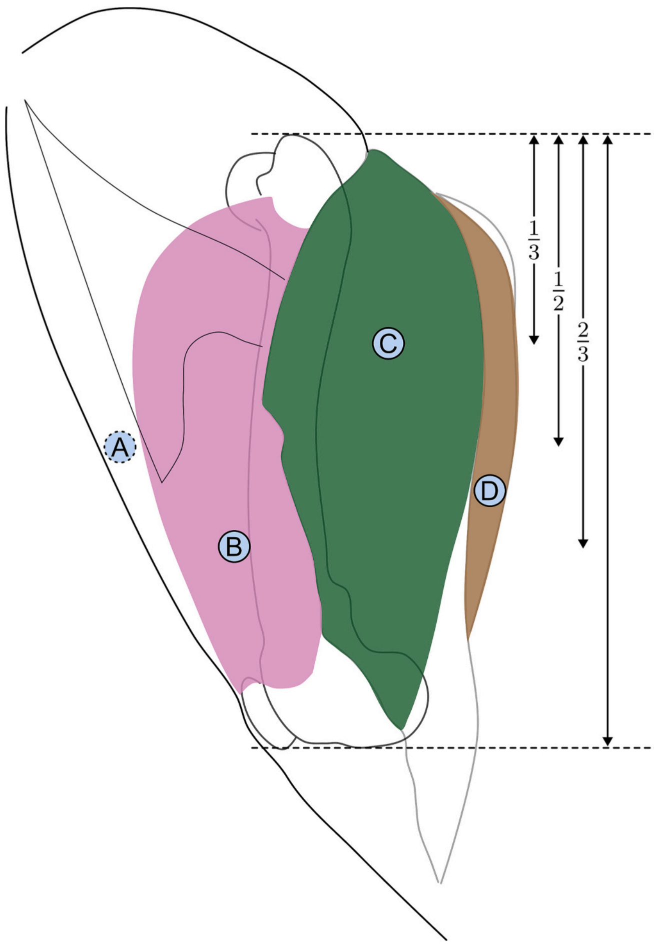

Fig. 1. Locations for placement of acoustic myography sensors in this study. Sensor locations A-C are shown relative to the vertical distance between the greater trochanter and lateral femoral condyle, identified by careful palpation. A - cranial sartorius, placed medially at 1/2 this distance. B - vastus lateralis, placed laterally at 2/3 this distance. C - biceps femoris, placed laterally at 1/3 this distance. D - semitendinosus, placed at 1/3 the distance from the greater trochanter to calcaneus (not shown).

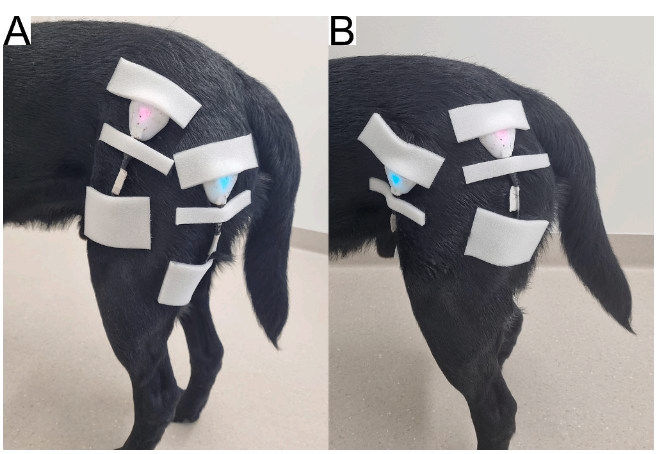

Fig. 2. A Labrador retriever with acoustic myography sensors attached above vastus lateralis and semitendinosus (left picture), and above cranial sartorius and biceps femoris (right picture). The sensor is positioned distally, covered by adhesive bandage, and connected by a short cable to the CURO unit proximally, which contains an amplifier circuit, rechargeable battery, and Bluetooth 4 antenna.

A data recording document was created for each dog to add notes to each AMG recordings (such as reluctance to walk, pulled at the leash, jumped, trotted, etc.) to assist in setting the reading frame during analysis.

The AMG data analysis was performed online.[1] Outcome measures from the AMG recording were *E*-, S- and T-scores. Maximum frequency (max T) was set to 160 Hz and maximum amplitude (max S) to 0.99 (Varcoe et al., 2021). To quantify the E-, S- and T-scores, a reading frame and a threshold were specified for each recording. The reading frame covered the pressure waves related to the muscle activity during walking omitting the first and last two to three steps of the recording and varied in duration between different sizes of dogs and associated gait velocities. The threshold was set to 0.06–0.07 to filter out baseline muscle activity, equivalent to filtering 6% - 7% of the signal. The threshold was adjusted upwards from 0 until the AMG scores approached a steady state, ideally without any of the scores reaching 0 or 10. For each dog and each muscle, the threshold was set bilaterally to the same value for all AMG recordings to be able to evaluate the symmetry and reliability of AMG.

2.1.3. *Limb circumference*

Thigh girth was measured in centimetres using a spring weighted tape with dogs in lateral recumbency and the leg in a fully extended position. A coloured pen was used to mark the fur at 70% of the femoral length, measured from the greater trochanter to the lateral epicondyle (McCarthy et al., 2018). Two consecutive measurements of limb circumference were made bilaterally at this mark by the same observer (KHD), to obtain a mean value for each limb.

2.1.4. *Goniometry*

Goniometric measurements of passive range of motion of the stifle and tarsal joints in both hindlimbs were obtained with dogs in lateral recumbency. A conventional plastic goniometer (model G300, Whitehall Manufacturing, City of Industry, CA, USA) was used. Maximum flexion and extension of the stifle joint were assessed as the angles formed when the static arm of a goniometer was placed from the greater trochanter along the femoral longitudinal axis with the vertex above the lateral epicondyle. The mobile arm was placed along the tibial long axis on a line to the lateral malleolus. Maximum flexion and extension of the tarsal joint were assessed as the angles formed when the static arm of a goniometer was placed along the tibial long axis and the mobile arm followed the lateral axis of metatarsal bones III and IV (Jaegger et al., 2002). Two consecutive goniometric measurements were made for each joint by the same observer (KHD), to obtain a mean value for each limb.

2.2. *Statistics*

Symmetry indices for outcome variables were calculated as left/right limb ratios. Data were presented as mean values ± standard deviation (SD) and as 95% reference intervals (mean ± 1.96*SD). For AMG scores, repeatability was assessed using the within-subject standard deviations (wsSD) to quantify the variation of the repeated measures (Bland and Altman, 1999). Reproducibility was assessed similarly using the within-subject standard deviations corrected for repeated measurements on both days (corrwsSD). Analyses were performed in Excel (Microsoft 365, Redmond, WA) and R Statistical Software v4.3.2 with package *referenceIntervals*. Data were assessed for normality using a Shapiro-Wilk test. Reference intervals for the AMG scores were calculated using a robust method with and without removal of outliers identified following Box-Cox transformation using Tukey's interquartile fences (Horn et al., 2001; Horn et al., 1998).

[1] http://www.app.myodynamik.com

3. Results

3.1. Demographics

Twenty-five dog owners responded positively to the advertisements and inquiries regarding recruitment. Of these, three dogs were excluded due to logistical challenges, and one dog was excluded because of signs of pain when manipulating the hip joint (Fig. 3). The study population comprised 21 dogs. Included breeds were Labrador retriever (*n* = 4), Golden retriever (n = 4), Border collie (*n* = 2), Small Münsterländer (n = 2), mixed breed (n = 2), and one each of Nova Scotia Duck Tolling retriever, Weimeraner, Cockerpoo, Vizla, Australian shepherd, Dobermann Pinscher, and standard poodle. Of these, 8/21 were intact females, 3/21 were neutered females, 8/21 were intact males, and 2/21 were neutered males. Mean age was 2.2 years (SD 1.2 years) and mean body mass was 25.7 kg (SD 5.6 kg).

Kinetic gait analysis and AMG data were obtained for all 21 dogs (Table 2). One dog was non-compliant with goniometry and limb circumference measurements and data are reported for 20/21 dogs.

All 20/21 dogs appeared symmetrical between left and right limbs for stifle and tarsus goniometry and for thigh circumference, indicating no loss of range of motion or muscle atrophy that could be associated with orthopaedic disease.

Symmetry indices for kinetic gait parameters were within previously reported intervals (Krotscheck et al., 2016; Nielsen et al., 2020) indicating no preference for limb loading which could be associated with musculoskeletal disorders.

3.2. Reliability of Acoustic myography

Qualitative assessment of the AMG recordings in the software system combined with notes made for each recording gave a clear view of the correlation between activity level and the intensity or size of the pressure waves. Inactivity at the beginning of a recording was easily visualized, as were brief periods of trotting which produced pressure waves with increased frequency and amplitude (Fig. 4).

The mean interval between day 1 and day 2 AMG recordings was 13.0 days (range 1–28 days). Due to temperament, one dog was excluded from the second AMG recording day, and data from 20/21 dogs are reported in Table 3. For each AMG score (E, S and T), values were similar for all four muscles. Mean *E*-scores were lower and exhibited greater variability based on SD than mean S- and T-scores, which had comparable and higher values. Repeatability was likewise poorer for E-scores than S- or T-scores, based on higher wsSD estimates for the E-scores (Table 3). A similar pattern was seen for measurement reproducibility between day one and two (Table 3).

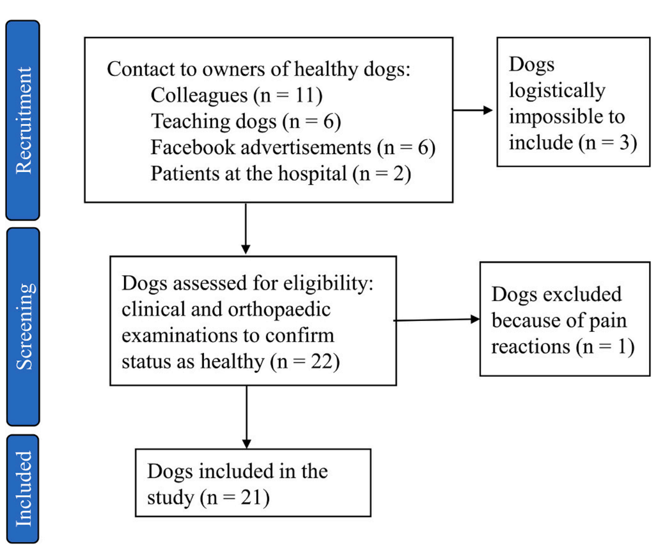

Fig. 3. Flow diagram of the recruitment process.

Table 2
Hindlimb symmetry indices and reference intervals for gait analysis, goniometry, limb circumference, and acoustic myography in healthy dogs. Symmetry indices were calculated as left limb values divided by right limb values. Reference intervals (RI) were calculated using a robust method with and without removal of outliers.

		Symmetry index	95% RI	95% RI without outliers
Pressure sensitive walkway (*n* = 21)	Peak vertical force (N)	1.03 ± 0.08		
	Vertical impulse (N**sec*)	1.04 ± 0.09		
	Peak pressure (kPa)	1.02 ± 0.08		
Goniometry and limb circumference (*n* = 20)	PROM of the stifle (degrees)	0.98 ± 0.05		
	PROM of the tarsus (degrees)	1.01 ± 0.04		
	Limb circumference (cm)	1.00 ± 0.04		
Acoustic myography (n = 21)				
E-score	Vastus lateralis	1.07 ± 0.71	0* – 2.42	0* - 1.95
	Biceps femoris	1.53 ± 1.45	0* - 4.17	0* - 2.18
	Semitendinosus	1.73 ± 3.04	0* - 7.43	0* - 7.43
	Sartorius	2.52 ± 3.62	0* - 9.08	0* - 3.68
S-score	Vastus lateralis	1.03 ± 0.07	0.86–1.17	0.86–1.17
	Biceps femoris	1.06 ± 0.14	0.72–1.34	0.72–1.34
	Semitendinosus	0.98 ± 0.07	0.86–1.14	0.92–1.08
	Sartorius	0.99 ± 0.08	0.82–1.18	0.82–1.18
T-score	Vastus lateralis	1.04 ± 0.13	0.76–1.29	0.91–1.15
	Biceps femoris	1.03 ± 0.12	0.79–1.27	0.94–1.12
	Semitendinosus	0.98 ± 0.14	0.70–1.28	0.70–1.28
	Sartorius	0.99 ± 0.20	0.58–1.44	0.75–1.30

RI – reference interval; PROM – passive range of motion; * - lower limit censored to 0.

4. Discussion

In healthy dogs, the best repeatability and reproducibility estimates for AMG measurements were observed for the S- and T-scores, and reference intervals for these scores were correspondingly narrower.

Symmetry indices for S- and T-scores indicated hindlimb symmetry in agreement with the other methods used in this study, in contrast to the E-scores. However, due to the increased variability of the E-scores as well as poor repeatability and reproducibility, the S- and T-scores may be more useful for assessing muscle symmetry in this population. The large variation in the E-scores may be because the software calculations of this score are more influenced by small changes in the AMG-recordings than the other scores. The AMG-score equations are described in the literature (Fuglsang-Damgaard et al., 2021) but the scores output from the software system cannot be independently checked by the user. Physiological explanations might include motor cortex control of S- and T-scores resulting in similar variability, whereas E-scores are more dependent on muscle performance and condition. Since the reference intervals for all four muscles overlapped for each

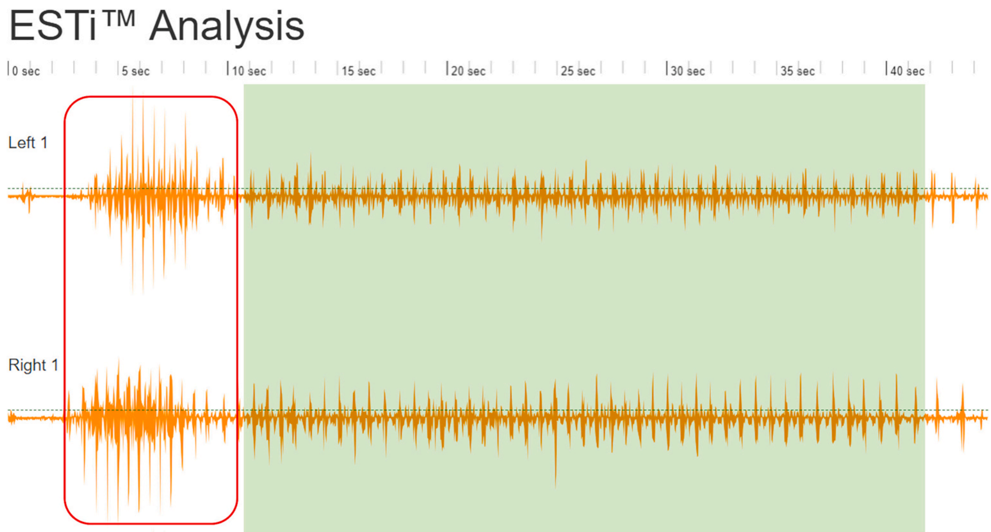

Fig. 4. A screenshot from http://www.app.myodynamik.com visualizing AMG recordings from vastus lateralis as an example of how the reading frame was set. Here the dog started trotting for a few steps (the box) and therefore the reading frame (green area) was set to start when the dog walked with a steady gait velocity.

Table 3
Acoustic myography measurements for four hindlimb muscles from 20 healthy dogs. Scores are presented as mean with standard deviation for day 1 and day 2 measurements, 95% reference intervals (RI) calculated using a robust method with and without removal of outliers. Repeatability for day 1 and reproducibility between days 1 and 2 are reported as within-subject standard deviations with 95% confidence intervals.

	Muscle	Day1	95% RI	95% RI without outliers	Repeatability	Day2	Reproducibility
E-score	L Vastus lateralis	4.5 ± 2.3	0* - 9.2	0* - 9.2	1.0 (0.9–1.1)	4.7 ± 1.4	2.2 (1.5–2.9)
	R Vastus lateralis	4.7 ± 1.7	0.8–7.8	1.9–6.6	0.7 (0.7–0.8)	4.4 ± 2.8	2.2 (1.5–2.9)
	L Biceps femoris	5.0 ± 2.0	1.1–9.2	2.5–7.9	0.8 (0.7–0.9)	4.3 ± 1.1	2.3 (1.6–3.1)
	R Biceps femoris	4.4 ± 1.7	0.2–8.8	0.2–8.8	0.8 (0.7–0.9)	4.5 ± 0.7	1.6 (1.1–2.1)
	L Semitendinosus	4.0 ± 2.8	0* - 10*	0* - 10*	0.8 (0.8–0.9)	3.9 ± 3.8	3.0 (2.0–3.9)
	R Semitendinosus	4.4 ± 2.8	0* - 10*	0* - 10*	0.9 (0.8–1)	4.8 ± 3.4	2.8 (1.9–3.6)
	L Sartorius	4.4 ± 3.3	0* - 10*	0* - 10*	1.1 (0.9–1.2)	3.0 ± 3.6	3.6 (2.5–4.7)
	R Sartorius	4.3 ± 3.3	0* - 10*	0* - 10*	0.9 (0.8–1.0)	4.1 ± 4.5	2.7 (1.9–3.6)
S-score	L Vastus lateralis	8.6 ± 0.5	7.6–9.8	7.8–9.6	0.2 (0,2–0.3)	8.2 ± 0.7	0.9 (0.6–1.1)
	R Vastus lateralis	8.4 ± 0.7	7.0–9.9	7.0–9.9	0.2 (0.2–0.2)	7.9 ± 1.1	1.0 (0.7, 1.3)
	L Biceps femoris	8.5 ± 0.6	7.5–9.9	7.7–9.7	0.2 (0.2–0.2)	7.9 ± 0.4	1.0 (0.7–1.3)
	R Biceps femoris	8.1 ± 0.9	6.4–10*	6.8–9.9	0.3 (0.3–0.3)	8.0 ± 0.4	0.8 (0.6–1.1)
	L Semitendinosus	8.6 ± 0.7	7.4–10*	8.1–9.7	0.3 (0.3–0.4)	8.5 ± 0.9	0.8 (0.6–1.1)
	R Semitendinosus	8.8 ± 0.3	8.0–9.5	8.0–9.5	0.2 (0.2–0.3)	8.7 ± 0.4	0.8 (0.5–1.0)
	L Sartorius	8.6 ± 0.7	7.4–10*	7.6–10*	0.3 (0.3–0.4)	8.1 ± 0.7	1.0 (0.7–1.3)
	R Sartorius	8.8 ± 0.4	7.8–9.7	7.8–9.7	0.3 (0.2–0.3)	8.4 ± 0.4	1.1 (0.8–1.4)
T-score	L Vastus lateralis	8.6 ± 0.8	7.1–10*	7.7–9.9	0.4 (0.4–0.5)	8.5 ± 0.7	1.0 (0.7–1.3)
	R Vastus lateralis	8.5 ± 0.7	6.8–10*	7.1–10*	0.3 (0.3–0.4)	7.9 ± 1.0	1.1 (0.7–1.4)
	L Biceps femoris	9.0 ± 0.6	7.8–10*	7.8–10*	0.3 (0.3–0.3)	8.8 ± 0.4	0.8 (0.5–1.0)
	R Biceps femoris	8.7 ± 0.7	7.2–10*	7.2–10*	0.3 (0.3–0.3)	8.6 ± 1.0	0.8 (0.5–1.0)
	L Semitendinosus	8.6 ± 1.1	6.4–10*	6.4–10*	0.4 (0.4–0.5)	8.5 ± 1.5	1.1 (0.8–1.5)
	R Semitendinosus	8.8 ± 0.8	7.2–10*	7.2–10*	0.4 (0.3–0.4)	9.0 ± 0.7	0.9 (0.6–1.2)
	L Sartorius	8.3 ± 1.4	5.8–10*	5.8–10*	0.7 (0.6–0.8)	8.2 ± 2.0	1.6 (1.1–2.1)
	R Sartorius	8.6 ± 1.2	6.1–10*	6.1–10*	0.5 (0.4–0.5)	8.7 ± 0.8	1.4 (1.0–1.9)

RI – reference interval; L – left; R – right; * - lower limit censored to 0 or upper limit censored to 10.

AMG score, these muscles cannot be differentiated in spatial summation, temporal summation, or efficiency with the use of AMG.

Given that symmetry indices for PSW, goniometry and limb circumference were consistent with previous reports for healthy dogs (Formenton et al., 2019; Krotscheck et al., 2016; Marrero et al., 2022; Nielsen et al., 2020), and the absence of findings on thorough clinical and orthopaedic examination, we believe that the included dogs could be defined as healthy subjects. The methods used in this study can be considered complementary and each of the included methods contributed to the evaluation of symmetry and orthopaedic health in different ways. Goniometry identified the passive range of motion in the stifle and tarsus, the limb circumference was a simple measure of hindlimb muscle mass, the PSW evaluated limb loading, and the AMG assessed muscle activity.

Reproducibility estimates were markedly higher than those for repeatability. Given the short interval between day 1 and day 2 measurements, it seems unlikely that this increase was caused by development of musculoskeletal disease during this time. Possible explanations could be difficulties in placing the sensors at the exact same location despite clearly described landmarks, and differences in gait velocity between the two days. In addition, canine skin elasticity is high (Ahmed et al., 2019), which could affect the position of the AMG sensors when the dogs move as has been reported for skin markers in kinematic gait analysis (Schwencke et al., 2012) Further, the adhesive bandage holding the AMG sensors in place might result in movement restrictions for the dogs. A few dogs tried instinctively to shake off the equipment immediately after placement, though without showing signs of pain. In these dogs the acclimatization period was extended slightly, and after short practice periods all dogs accepted the equipment without any obvious signs of movement restriction. We decided not to test AMG reproducibility on the same day due to the risk of being biased by the dried-up gel in the fur when replacing the sensors. Since the repeatability of AMG appears good, it may be advantageous to study muscle activity over time or during different activities without changing the position of the sensors, as reported by Vitger et al. (Vitger et al., 2021).

A limitation of this study was the use of a single observer (KHD): use of multiple observers might better reflect real-world measurements. However, a single observer gives consistency, and previous studies have found good agreement for both inter- and intra-observer variability for limb circumference measurements and goniometry (Jaegger et al., 2002; McCarthy et al., 2018). Another limitation of this study and studies applying AMG in general is that the exact AMG scores of a muscle can only be directly compared within subjects and between subjects if they are obtained with the same amplification and comparable settings. Regarding the threshold setting, minimal variation in calculated scores was seen when applying the threshold to filter away signal noise. Guidelines on how to record AMG for specific muscles and specific activities in dogs could be advantageous for future studies. Based on our pilot studies (Appendix A), 21 dB was chosen as the amplification level for this study, but for higher activity levels such as trotting, lower amplification settings are needed for the software system to produce valid scores. The AMG study using dogs with CCLD (Varcoe et al., 2021) and using an older version of the CURO unit utilized a signal amplification of 6 dB. This makes comparison with our reference intervals difficult, and symmetry indices were not established in that study. Few published studies have assessed muscle activity in dogs using AMG. One study assessed forelimb muscles and did not evaluate bilateral symmetry (Weber et al., 2022). Two other studies have used AMG to assess hind limb muscle activity in healthy dogs (Fuglsang-Damgaard et al., 2021; Vitger et al., 2021). Neither evaluated symmetry and both used an older CURO unit as described by Varcoe et al. with a fixed signal amplification of 6 dB. One study assessed different muscles to those reported here (Fuglsang-Damgaard et al., 2021), but the other assessed the biceps femoris and vastus lateralis (Vitger et al., 2021), and identified lower AMG scores with higher gait velocities, in agreement with our pilot data (Appendix A). Additionally, lower variation was seen for S-scores compared with *E*- or T-scores, consistent with the findings presented here.

5. Conclusions

For all AMG-scores, repeatability was better than reproducibility. The E-scores showed higher variability for symmetry indices, and poorer repeatability and reproducibility than the S- and T-scores. Further investigation is needed to establish if the reference intervals can enable discrimination between healthy dogs and dogs with altered muscle function.

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CRediT authorship contribution statement

Kathrine Højte Dahl: Conceptualization, Investigation, Methodology, Project administration, Writing – original draft. **Michelle Brønniche Møller Nielsen:** Investigation, Writing – review & editing. **Tine Alkjær:** Visualization, Writing – review & editing. **Anne Désiré Vitger:** Writing – review & editing. **James Edward Miles:** Conceptualization, Formal analysis, Writing – review & editing.

Declaration of competing interest

One of the first author's supervisors is Adrian Paul Harrison, who has established the company CURO Diagnostics ApS aiming to commercialize the equipment for AMG: however, he was not involved in the data analysis.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.rvsc.2024.105135.

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