Limitations and Variability in In Situ Muscle Volume Measurement Using Ultrasound

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INTRODUCTION: Ultrasound imaging is increasingly utilized to quantify skeletal muscle volume in both clinical and research settings due to its portability, safety, and cost-effectiveness. However, in situ muscle volume measurement via ultrasound is subject to several limitations, including variability induced by probe pressure, tissue deformation, acoustic propagation differences, and inconsistencies in probe positioning. These factors can introduce significant errors, particularly when assessing small changes in muscle volume or comparing measurements across time points or between operators. Despite recent advances in 3D ultrasound imaging and automated volume reconstruction, the reliability and accuracy of ultrasound-based volume assessments remain uncertain. This study aimed to quantify and validate small muscle volume measurement using a 3D ultrasound probe, identify potential factors influencing measurement accuracy, and inform best practices to enhance reproducibility and measurement fidelity.

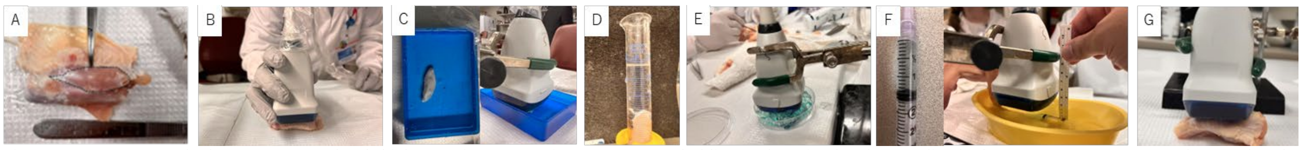

Fig 1. Measurement methods. A) FCU muscle of chicken's wing. B) In-situ measurement holding probe using hand. C) Saline bath measurement: FCU muscle was placed into saline bath, with the probe fixed by a clamp. D) Actual volume measurement using Archimedes' principle. E) Gel bath measurement to identify the differences in propagation between gel and saline. F) Measurement of influence of probe-to-object distance; 1.5 cm^3 of gel preloaded in a syringe was measured at distance increments of 1cm, 2cm and 4cm from the probe. G) In-situ measurement holding probe using clamp.

METHODS: A total of six commercial fresh chicken wings purchased from a local Sam's club vendor were used to conduct this study. Left and right wings were randomly selected, and the flexor carpi ulnaris (FCU) muscle (Fig. 1A) was identified using ultrasound (SuperSonic® MACH™ 30, Hologic, MA). The FCU muscle was chosen, as it best aligned with the 4cm x 4cm field-of-view size constraints posed by the ultrasound probe. The volume of the FCU was first measured in situ using the 3D volume measurement function of the ultrasound system, with the probe being held directly on the skin by the operator's hand. This value was recorded as the "in situ" volume (Fig. 1B). Subsequently, the skin was removed, and the FCU muscle was exposed and carefully dissected. The isolated muscle was placed in a saline-filled bath, and its volume was measured again using the same ultrasound system, this time with the probe held by a clamp to avoid compression. This was recorded as the "saline bath" measurement (Fig. 1C). To determine the actual muscle volume, a method based on Archimedes' principle was used. Each dissected FCU muscle was fully submerged in a container filled with saline, and the displaced fluid volume was measured and recorded as the "actual volume" (Fig. 1D). Intra-class correlation coefficient tests for intra-observer and inter-observer were performed to each measurement method. Further experiments were carried out to specifically determine the effects of propagation, probe positioning, and compression. To evaluate the effect of coupling media on ultrasound-based volume measurement, the same FCU specimen was scanned while immersed in either ultrasound gel or saline, with consistent probe-to-muscle distance and no compression ensured using a clamp (Fig. 1E). To assess the influence of probe-to-object distance, the ultrasound probe was suspended above the target at fixed distances of 1 cm, 2 cm, and 4 cm, using a clamp. In each condition, a standardized volume of ultrasound gel (1.5 cm³), preloaded in a syringe, was used to maintain acoustic continuity (Fig. 1F). The volume of the gel was measured at each distance using the 3D ultrasound function (Fig. 1F). To evaluate the effect of tissue compression, the in-situ measurement (with handheld probe contact) was compared with a clamped measurement which provided for complete elimination of probe pressure against the muscle (Fig. 1G).

RESULTS: The in-situ muscle volume measurements (1.86 ± 0.33 cm³) were significantly lower than both the saline bath (2.37 ± 0.39 cm³) and actual volumes (2.65 ± 0.28 cm³); the saline bath measurement was also significantly lower than the actual volume (P = 0.04) (Fig 2A). Intraclass correlation coefficient (ICC) analysis revealed similar low reliability in the in-situ condition. The intra-observer ICC (3,1) for in situ measurements was 0.44 (95% CI: 0.059–0.64), and the inter-observer ICC (2,1) was 0.29 (95% CI: -0.80–0.93). In contrast, both saline bath and actual volume conditions showed higher ICCs, with intra-observer ICCs of 0.82 and 0.95, and inter-observer ICCs of 0.66 and 0.91, respectively (Table 1). No significant difference in measured muscle volume was observed between ultrasound gel (3.02 ± 0.57 cm³) and saline (3.12 ± 0.78 cm³) conditions (P = 0.288, Fig 2B). The mean difference between the two media was 0.098 ± 0.46 cm³, suggesting that the choice of coupling medium did not significantly affect the measurement under controlled conditions. Measured muscle volume increased significantly with greater probe-to-skin distance. Specifically, the measured volume was 1.52 ± 0.22 cm³ at 0 cm, 1.79 ± 0.10 cm³ at 1 cm, and 2.99 ± 0.16 cm³ at 2 cm, indicating a strong positive enlargement between probe elevation and apparent volume ($p < 0.01$, Fig 2C). The muscle volume was significantly greater in the clamp holding group (3.39 ± 0.35 cm³) compared to the hand holding group (3.10 ± 0.50 cm³) (*p* = 0.0042, Fig 2D).

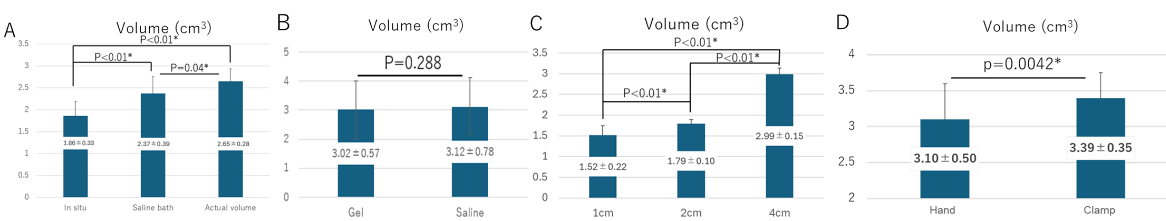

Fig 2. Results. A) Result of 3-way muscle volume measurement. B) Result of comparing gel-bath and saline-bath measurement. C) Result of comparing distance between probe and object. D) Results of comparing hand holding and clamp holding.

	In situ	Saline bath	Actual vol.
Intraclass ICC(3,1)	0.44, 95%CI [0.059, 0.64]	0.82, 95%CI [0.27, 0.91]	0.95, 95%CI [0.60, 0.98]
Interclass ICC(2.1)	0.29, 95%CI [-0.80, 0.93]	0.66, 95%CI [-0.53, 0.98]	0.91, 95%CI [0.11, 0.99]

Table 1. ICC (3.1) and (2.1) values of in situ, saline bath and actual volume measurements.

DISCUSSION: Our findings demonstrated that ultrasound measurements performed in situ significantly underestimated muscle volume compared to saline bath measurements and actual dissected volumes. Several technical aspects of the ultrasound system may explain these discrepancies. The probe used in this study utilizes a 1D array coupled with a motorized wobbler to acquire 3D volume data. This is particularly relevant for this curved probe design, which emits ultrasound beams in a sector (fan) pattern. As a result, scan lines are denser near the transducer surface and sparser at greater depths resulting in enlarging the volume of farther objects. Additionally, holding the probe by hand may have compressed the muscles, making them appear smaller.

SIGNIFICANCE/CLINICAL RELEVANCE: Volume measurements using a 3D probe that emits ultrasonic waves in a sector pattern may be subject to errors depending on the distance to the target muscle, especially when the probe is hand-held.