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ORIGINAL RESEARCH

Evaluating the Relationship Between Surface and Intramuscular-Based Electromyography Signals: Implications of Subcutaneous Fat Thickness

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Intramuscular (iEMG) and surface electromyographic (sEMG) signals have been compared previously using predictive regression equations, finite element modeling, and correlation and cross-correlation analyses. Although subcutaneous fat thickness (SCFT) has been identified as a primary source of sEMG signal amplitude attenuation and low-pass filter equivalence, few studies have explored the potential effect of SCFT on sEMG and iEMG signal characteristics. The purpose of this study was to investigate the relationship between normalized submaximal iEMG and sEMG signal amplitudes collected from 4 muscles (rectus femoris, vastus lateralis, infraspinatus, and erector spinae) and determine whether SCFT explains more variance in this relationship. The effect of sex was also explored. Linear regression models demonstrated that the relationship between sEMG and iEMG was highly variable across the muscles examined (adjusted coefficient of determination [Adj R^2] = .02–.74). SCFT improved the model fit for vastus lateralis, although this relationship only emerged with the inclusion of sex as a covariate. Thus, this research suggests that SCFT is not a prominent factor affecting the linearity between sEMG and iEMG. Researchers should investigate other parameters that may affect the linearity between sEMG and iEMG signals.

Keywords: adipose tissue, upper limb, lower limb, muscle activity, ultrasound

Surface electromyography (sEMG) is a relatively quick and noninvasive method of measuring muscle activity compared with more invasive intramuscular-based methods (intramuscular electromyography [iEMG]). iEMG requires the insertion of a small-gauge hypodermic needle into the muscle belly, which can cause pain, muscle cramping, and potentially neural or muscular injury.[1,2] Furthermore, iEMG requires extensive training for high-precision placement, materials can be expensive,[3] and some regions or institutional ethical review boards require medical supervision. As well, iEMG is also limited to recording motor unit activity with high spatial selectivity and only characterizes the activity of a subset of local muscle fibers (approximately 0–20 fibers), which may not correlate with whole muscle activity.[4,5] Thus, when access to deeper musculature is not required, many studies often use sEMG to record muscular activity.[1] However, sEMG signals are affected by several intrinsic and extrinsic factors, including electrode configuration and placement, physiology and anatomy (fiber type, diameter, and location) of the underlying muscles, and subcutaneous fat thickness (SCFT).[6]

Multiple theoretical[7–10] and experimental[11–14] studies have described the equivocal time-spectrum and frequency-spectrum differences between iEMG and sEMG signal properties. Normalization of submaximal EMG amplitudes to maximum voluntary isometric contraction (MVIC) amplitude[15,16] provides a relative comparison of signal amplitude to the maximal voluntary excitation of the particular muscle within each recording method, thereby enabling more relevant time-domain comparisons between the methods. However, the selectivity and sensitivity characteristics of various EMG methods can affect signal frequency, spectral density, iEMG and sEMG signal waveform cross correlations, and low-level exertion amplitude, which may not be relativized appropriately by normalizing to MVIC.[17] iEMG has a more selective recording profile, only recording motor unit activity adjacent to the electrodes, and thus, iEMG has a low risk of falsely indicating that a muscle is active when it is truly inactive. Alternatively, sEMG amplitudes represent an arithmetic sum of all motor units within a 10- to 20-mm range[9,18,19] whereby the signal is dominated by motor unit action potentials within a radial projection of the interelectrode distance.[9] Thus, sEMG methods are more sensitive and have a reduced chance of failing to capture muscle activity when a muscle is active.

Researchers have demonstrated that sEMG amplitude may also increase nonlinearly with respect to iEMG amplitude, often underestimating iEMG amplitude at low contraction intensity and overestimating iEMG magnitude at high contraction intensity.[3,20–23] Specifically, Allen et al[23] used regression analysis to evaluate the relationship between submaximal (20%, 40%, and 60% MVIC) and maximal normalized EMG amplitudes from surface and fine-wire intramuscular recordings of the supraspinatus and infraspinatus (IS) muscles. These regression equations included all submaximal and maximal exertions into a single model, with models separated by muscle and exertion direction. The regression equations indicated that normalized sEMG amplitude appeared to overestimate iEMG amplitude and explain less variance at maximal exertion intensities compared with submaximal exertion intensity.[23] Although previous studies reported sEMG amplitude overestimations of iEMG at maximal exertion intensity for supraspinatus (88.7%) and IS (185.7%),[3] the submaximal exertion intensity regressions by Allen

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et al[23] showed reduced over approximations of 31.4% and 20.5%, respectively. However, at low exertion intensities (≤15% maximal voluntary contraction [MVC]), the opposite interaction seems to emerge, with normalized iEMG amplitude generally overestimating normalized sEMG amplitude.[20,23] These findings suggest that comparisons between normalized sEMG and iEMG amplitude may find optimal agreement near mid-range submaximal contractions (40%–60% MVC), yet these results are currently only extensible to the IS and supraspinatus muscles.[20,24] Other research comparing the relationship between iEMG and sEMG amplitudes at erector spinae (ES) and multifidus,[25] and rectus femoris (RF) and vastus lateralis (VL),[26] have reported more moderate relationships with coefficients of variation between 0.35 and 0.60. However, these studies report using absolute loads; therefore, the sEMG–iEMG linear relationship that is suspected to be most stable at 40% to 60% submaximal exertions[3,20–23] is difficult to confirm in these studies. Due to the incongruity between EMG methods, which can be attributed to several factors, researchers have cautioned against the use of sEMG to estimate deep muscle activity.[1,3,24–27] The Consensus for Experimental Design in Electromyography project states that practitioners should be aware of the inherent differences between surface and intramuscular signals that can influence differences in EMG signal amplitude as well as other signal characteristics like waveform shape and frequency content.[1,16]

In addition to the aforementioned factors that may interfere with recorded EMG amplitude characteristics, subcutaneous tissue inhomogeneities[8,10] and the electrical resistance of subcutaneous fatty tissue or adipose tissue[7,8,10,27,28] have long been identified as primary factors affecting sEMG signal selectivity. There are 2 main mechanisms by which subcutaneous fat and other subcutaneous tissue affect an EMG signal. First, subcutaneous fat and other inhomogeneous tissues, such as vascular and connective tissue in the subcutaneous space, display poor electrical conductance (high resistive impedance), thereby unevenly attenuating the motor unit electrical signal.[7–9] Researchers have equated subcutaneous fat spectral signal loss to a second-order low-pass filter wherein the filter cutoff decreases proportionally to an increase in SCFT.[7–10,13,27] Increases in SCFT also increase the distance between the electrical source (motor unit) and the surface recording electrode (sEMG), amplifying the spatial filtering (distance-damping function) imparted on the EMG signal.[8] Second, increased SCFT also reduces the comparative distance between (1) the surface electrode and the muscle of interest and (2) the surface electrode and a nearby muscle, which permits increased crosstalk[12] and, thus, reduced sEMG sensitivity to the muscle of interest.

Sex differences in the EMG–force relationship have been noted in the literature,[29,30] yet some evidence suggests that these sex differences are also task dependent and may be influenced by many other factors, such as training history.[31,32] Specifically, some studies using sEMG methods have reported that females display greater motor activity to produce comparable relative contraction intensities compared with males[31] and may also display different muscle activation dynamics between synergists.[29] To our knowledge, one study has investigated sex differences in sEMG and iEMG recordings and reported significant differences in submaximal sEMG amplitude and frequency that were not apparent using simultaneous iEMG.[30] Several factors may explain these sex differences in EMG activity, such as differences in muscle moment arms, morphology, or body fat distributions. Body fat, in particular, has been demonstrated to vary differently across body regions between males and females, and females have also demonstrated more SCFT accrual relative to males, who demonstrate more visceral fat accrual.[33–35] To date, a dearth in sex-based differences in EMG and motor unit behavior has been noted in the literature[36] despite clear evidence that significant differences exist; thus, further investigation is required.

Many studies have simultaneously captured iEMG and sEMG to study differences in signal characteristics[14,23–26,37,38]; however, few have attempted to isolate the effects of SCFT on the relationship between iEMG and sEMG normalized signal amplitudes in vivo. One study examined sEMG amplitude across a range of skinfold thicknesses and found that increasing skinfold thickness from 6 to 21 mm showed a significant decrease in median signal frequency ($P < .001$) and nonnormalized signal amplitude ($P < .025$), whereas waveform cross-correlations were not significantly impacted.[12] In addition, researchers compared iEMG and sEMG signal amplitudes recorded during electrical stimulation and found that increasing SCFT had a strong, positive relationship on the time delay between onset of the applied stimulation and the signal recorded by the needle electrode ($r = .96$, $P < .001$).[10] Furthermore, the effect of SCFT on iEMG and sEMG signal relationships has been studied from a theoretical perspective, using mathematical models to describe how SCFT attenuates and filters transiting motor unit action potentials.[7–10] Kuiken et al[7] previously reported a decrease in nonnormalized sEMG root mean squared amplitude associated with increasing SCFT in a finite element model. Furthermore, increases in SCFT have been shown to be correlated with reduced root mean square normalized signal amplitudes following an exponential relationship in the range of the 1.2 to 1.4 power such that an increased distance between the muscle and the electrode was exponentially related to sEMG amplitude decay.[12] Therefore, although the effects of SCFT on amplitude and spectral characteristics of sEMG have been studied, no studies have investigated whether the relationship between iEMG and sEMG is improved by considering SCFT in the recording area.

The primary purpose of this research was 2-fold: (1) to determine whether normalized iEMG amplitude would display linear relationships to sEMG across 4 muscles in 3 regions of the body—lower limb (RF and VL), trunk (ES at the T4 vertebral level), and shoulder (IS)—during submaximal intensity contractions and (2) to determine whether SCFT explains significantly more variance in the relationship between iEMG and sEMG signal amplitudes. IS was included among the 4 measured muscles to confirm whether a linear relationship would emerge at this muscle when performing submaximal contractions, as suggested previously.[20–23] The secondary purposes of this research were to determine (1) whether sex affected the relationship between normalized iEMG and sEMG signal amplitude when considering SCFT, (2) whether regional differences in SCFT exist in men and women, and (3) whether body mass index (BMI) and SCFT of each muscle were related. We hypothesized that sEMG amplitude would be a significant predictor of iEMG amplitude and that SCFT would be a significant covariate, with higher magnitudes of SCFT related to decreases in relative sEMG signal amplitude. As sex differences associated with body fat distribution exist,[33–35,39] and one study has also reported sex differences in simultaneous sEMG and iEMG amplitude,[30] we expect that sex could be a significant covariate for improving EMG amplitude comparisons in the current study. We expect the quadriceps muscles (lower limb region) to have higher relative SCFT compared with the ES (trunk) and IS (shoulder). Finally, we expect BMI to correlate with SCFT in each body region.

Methods

Participants

Twelve men (23.4 [3.5] y, 24.3 [2.0] $kg \cdot m^{-2}$) and 12 women (22.9 [3.4] y, 22.2 [3.5] $kg \cdot m^{-2}$) were recruited for a single 2-hour

collection. Participants were selected from the local university population to be comparable with previously reported sample demographics.[23,40] A suggested sample size of 10 was calculated in G*Power (version 3.1.9.7)[41] based upon an $\alpha = .05$, $\beta = 0.90$, and a previously suggested coefficient of determination of $R^2 = .70$ from the regression equations published by Allen et al[23] and Waite et al.[3] Participants were excluded from participating in the study if they reported any of the following: discomfort with needles, blood clotting disorders or currently taking blood thinning medications, HIV, Hepatitis B or C, diabetes, or respiratory conditions (asthma, chronic obstructive pulmonary disease, etc), allergies to isopropyl alcohol, betadine, latex, or nickel, any current physical injuries that were currently painful during movement, any diagnosed neurological disorders that would affect muscular control (myasthenia gravis, Parkinson disease, etc), or if they were pregnant.

This research was approved by the York University Office of Research Ethics. All participants provided informed written consent.

Data Acquisition

SCFT local to each EMG recording site (RF, VL, ES, and IS) was captured using a GE Logiq E r6 ultrasound (12-MHz linear array transducer, GE Logiq E, GE Medical Systems) in B-mode. The transducer was oriented in the direction of the muscle fibers, with the midpoint located over the intended intramuscular insertion area.[42] Images were collected for each muscle at 12 MHz frequency, with a gain of 50 and a depth setting of 7.5 cm. The focus adjustment setting was modified to the mid-point of the subcutaneous fat layer to maximize resolution of the borders of this layer for SCFT measurement purposes. Care was taken to not apply any more force than necessary to minimize compression of the SCFT.

EMG signals were recorded from each of the 4 muscles (RF, VL, ES, and IS) on the participants' dominant side using both sEMG and iEMG methods. For intramuscular recording, a 30-mm length, sterile, stainless surgical steel hypodermic needle housing a barbed, staggered pair of 304 series stainless steel wires (0.051 mm diameter, 2 mm bent-tip exposure, 200 mm length wire, nylon coating; Motion Lab Systems Inc) was inserted into the muscle bellies of all 4 muscles of interest, adhering to the intramuscular insertion protocol,[42–44] while also using ultrasound guidance and visual EMG feedback. Intramuscular signals were sampled at 4370 Hz (10–2000 Hz band pass), and electrode pairs were differentially amplified (bipolar) and grounded with a dual on-board stabilizing reference using Trigno spring-lead wireless sensors (Delsys Corporation).

The sEMG signal was recorded from all muscles of interest at the site of intramuscular insertion.[3,45] This was done by affixing a pair of Ambu Bluesensor N single-use surface electrodes (Ag/AgCl with electrolytic conductive gel, Kego Corporation) on either side of the intramuscular insertion point, at an interelectrode distance of 20 mm, oriented in the direction of the muscle fibers.[23] Surface signals were sampled at 2148 Hz (20–450 Hz bandpass), and electrode pairs were differentially amplified (bipolar) and grounded with a dual on-board stabilizing reference using Trigno snap-lead wireless sensors (Delsys Corporation).

Participants completed 4 MVIC for the knee extensors,[46] 2 MVIC for the ES,[47] and 2 MVIC for IS[47] (Table 1). All maximal and submaximal exertions were recorded using an ergoFET (Hoggan Scientific) linear force transducer. A rigid, height-adjustable steel frame was positioned to resist all isometric contractions (Figure 1). Each MVIC trial was 5 seconds, separated by a 30-second break. After completing the MVIC tests, participants completed a series of submaximal exertions for each muscle scaled to 30% and 50% of their MVIC force output; the 50% MVIC submaximal contractions were analyzed in this research as 50% MVIC exertions optimize the observed linear relationship between iEMG and sEMG that demonstrably develops higher nonlinearity and variability at low (<15% MVIC) and high (100% MVIC) exertions.[3,20–23] Exertions were block randomized for each participant. For each submaximal exertion, participants ramped up to the force level associated with 50% of their previously recorded MVIC using visual feedback from the force transducer. Once the participant reached the 50% threshold, they were instructed to maintain this force output as accurately as possible for 5 seconds. EMG recording commenced when the participant reached the 50% force threshold and continued for 5 seconds while they maintained this force level, following which the participant ramped their force back down.

Data Analysis

SCFT was measured from each ultrasound image using an open-source image analysis software, ImageJ.[48] SCFT measurements

Table 1 Maximum Voluntary Isometric Muscle Tests

Muscle	MVIC test procedure	References
Infraspinatus	Participants sat upright with their lower back supported. From anatomical position, they were asked to flex their elbow to 90° and internally rotate their elbow 90° (measured via a goniometer) such that their palm was facing medially. Participants were instructed to pinch a towel between their elbow and the side of their torso while also externally rotating at the shoulder isometrically such that their wrist would apply force to the ergoFET linear force transducer reinforced by an immovable vertical frame.	McDonald et al[47]
T4 erector spinae	Participants lay prone on a clinical plinth with their torso overhanging one end of the plinth from just below T4 height. The participants' waist and ankles were secured to the plinth using straps. The participant was instructed to "raise their head, shoulders, and chest to table level," which was isometrically resisted by a strap around their back at T4 level. The strap was attached to an ergoFET linear force transducer to determine force output.	Hislop et al[46]
Rectus femoris	Participants sat upright with their lower back supported. Participants were instructed to isometrically extend their knee at a 90° knee angle (measured via a goniometer) such that the motion would be resisted by an ergoFET linear force transducer reinforced by an immovable horizontal frame.	Hislop et al[46]
Vastus lateralis	Participants sat upright with their lower back supported. Participants were instructed to isometrically extend their knee at a 90° knee angle (measured via a goniometer) such that the motion would be resisted by an ergoFET linear force transducer reinforced by an immovable horizontal frame.	Hislop et al[46]

Abbreviation: MVIC, maximum voluntary isometric contraction.

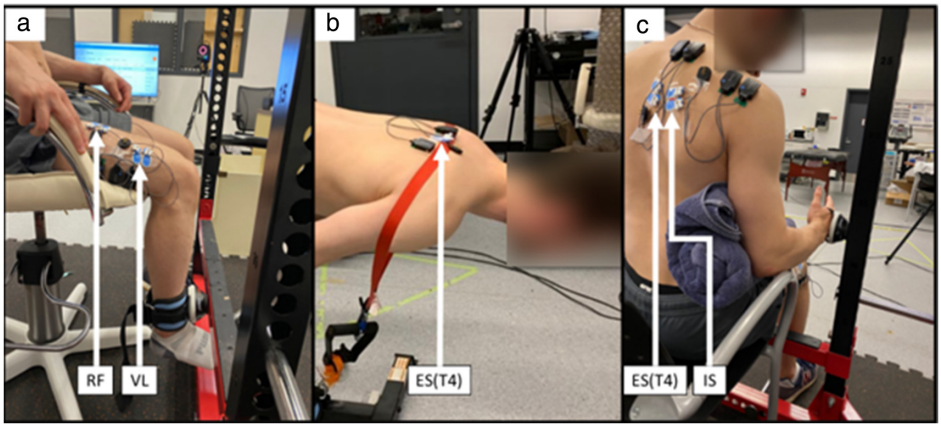

Figure 1 — Maximum voluntary isometric contraction testing postures. (a) Knee extension, (b) T4 thoracic extension, and (c) external rotation. Arrows indicate the location of electrode pairs (sEMG electrodes in blue oriented on either side of iEMG electrodes). ES indicates erector spinae; iEMG, intramuscular electromyography; IS, infraspinatus; RF, rectus femoris; sEMG, surface electromyography; VL, vastus lateralis. (Color figure online).

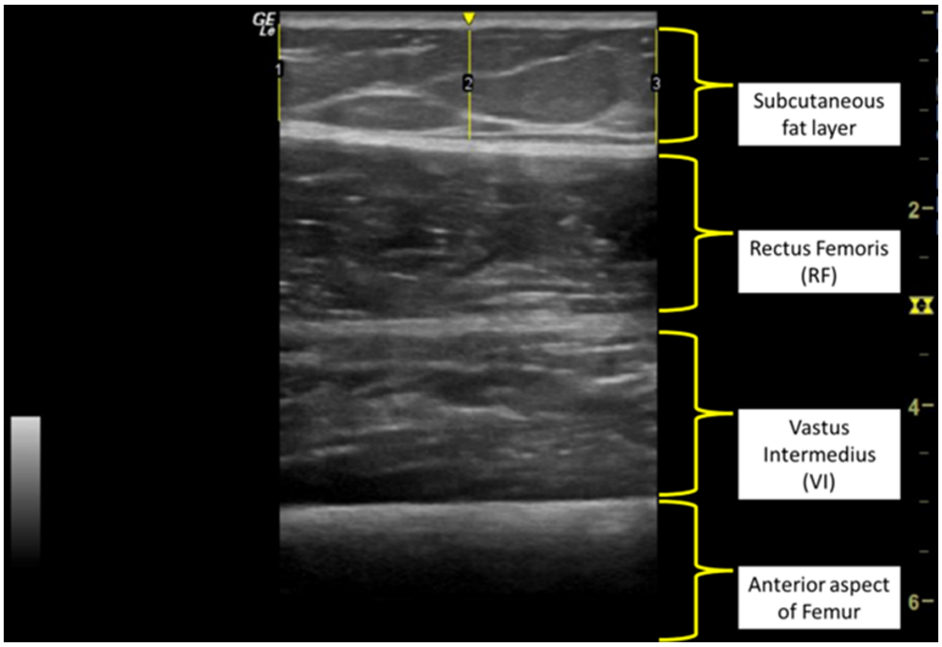

Figure 2 — Subcutaneous fat thickness measurement at rectus femoris iEMG/sEMG recording site. Vertical yellow lines indicate the subcutaneous fat thickness as measured at the left (1), middle (2), and right (3) sections of the image. iEMG indicates intramuscular electromyography; sEMG, surface electromyography. (Color figure online).

were captured at 3 points on the ultrasound image: leftmost region of the image, rightmost region of the image, and center of the image. At each point, SCFT was measured from the inferior border of the dermis layer to the superior border of the superficial aponeurosis (Figure 2).[49] The average SCFT was then calculated as the average depth across these 3 measurement sites (Figure 2). All images were independently measured by 2 separate researchers to evaluate interrater reliability and measured twice by a single researcher to evaluate the intrarater reliability. Interrater and intrarater intraclass correlation (ICC) models both comprised 24 comparisons each, between 2 raters, using an absolute agreement definition.[50] SCFT remeasurement for intrarater reliability was assessed 4 weeks after the first assessment.

EMG signals were high-pass filtered using a fourth-order dual-pass Butterworth filter with 30 Hz cutoff.[51] The average amplitude across all resting trials was subtracted from all signals, after which signals were linear enveloped using a dual-pass fourth-order 4-Hz low-pass Butterworth filter. Submaximal signal amplitudes were then normalized to muscle-specific peak amplitudes across MVIC trials.

Statistical Analysis

Linear regression models for each muscle (RF, VL, ES, and IS) were used to determine the normalized sEMG signal amplitude relationship to iEMG. Normalized iEMG amplitude was the

outcome variable in each of the models, and sEMG was the predictor variable.[3,23] Additional models were then developed that included (1) SCFT and (2) SCFT and sex as model covariates to address our primary and secondary research objectives, respectively. Likelihood ratio tests were computed between all 3 regression models for each muscle to determine whether SCFT or sex significantly impacted model fit. To identify whether regional differences in SCFT existed, 1-way analyses of variance were used to compare SCFT across the 4 muscle locations in both men and women. Pairwise correlation coefficients with Sidak adjustment for multiple comparisons were calculated between SCFT and BMI for each muscle to test for linear relationships between BMI and SCFT. Finally, a 2-way ICC model with random effects was used to calculate the absolute agreement between SCFT captured by 2 raters (interrater reliability; ICC [2,1]) and within repeated measures of the first rater (intrarater reliability; ICC [2,1]). Strength of ICCs was interpreted based on the guidelines by Koo and Li.[50] Statistical analyses were conducted using Stata/IC (version 16.0, StataCorp LP).

Results

sEMG amplitude accounted for a significant amount of variance in iEMG amplitude at ES (Adj $R^2 = .277$, $P = .010$) and IS (Adj $R^2 = .741$, $P < .001$), whereas regression models for RF (Adj $R^2 = -.007$, $P = .362$) and VL (Adj $R^2 = .010$, $P = .078$) were not statistically significant (Table 2; Equations 1–4). Including SCFT as a model covariate did not significantly affect the relationship between normalized sEMG and iEMG for any muscle, with likelihood ratio tests computed between models not shown to be significant. The VL model with both SCFT and sex included as model covariates explained significantly more variance than sEMG alone (Table 2, Figure 3). In addition, including SCFT and sex as model covariates reduced ES model fit compared with the model with no covariates (Table 2).

The following are linear regression equations for normalized intramuscular EMG amplitude ($\%MVIC_{sEMG}$) for each measured muscle (RF, VL, IS, and ES) given normalized sEMG amplitude ($\%MVC_{iEMG}$) as a fixed factor. Model covariates were included if they contributed to a significant increase in explained variance.

$$\text{RF}: \%\text{MVIC}_{\text{iEMG}} = [0.34 \times (\%\text{MVC}_{\text{sEMG}})] + 16.00, \quad (1)$$

$$\text{VL}: \%\text{MVC}_{\text{iEMG}} = [0.35 \times (\%\text{MVC}_{\text{sEMG}})] - [16.00 \times (\text{SCFT in mm})] + [7.31(\text{if male})] + 19.55, \quad (2)$$

$$\text{ES}: \%\text{MVC}_{\text{iEMG}} = [0.93 \times (\%\text{MVC}_{\text{sEMG}})] - 3.68, \quad (3)$$

$$\text{IS}: \%\text{MVC}_{\text{iEMG}} = [1.01 \times (\%\text{MVC}_{\text{sEMG}})] - 2.89. \quad (4)$$

Mean SCFT was as follows for each muscle: RF (M: 40.5 [20.9] mm; F: 103.7 [46.8] mm), VL (M: 31.8 [18.6] mm; F: 62.3 [28.5] mm), ES (M: 31.0 [32.5] mm; F: 29.9 [36.4] mm), and IS (M: 37.7 [18.4] mm; F: 68.3 [61.1] mm). When dichotomized by sex, women demonstrated significant regional differences in SCFT ($F_{3,44} = 5.69$, $P = .002$, $\eta^2 = .28$), whereas men did not. Specifically, for women, RF SCFT was significantly larger than ES ($P < .001$; Figure 4).

Pearson correlations between BMI and SCFT identified no significant relationships for the 4 muscles assessed (RF: $R = -.12$, $P = .581$; VL: $R = -.10$, $P = .632$; ES: $R = .47$, $P = .020$; IS: $R = .26$, $P = .225$).

Interrater reliability between researchers for SCFT ultrasound measurement was ICC (2,1) = .97 (.92–.99) for RF, ICC (2,1) = .97 (.92–.99) for VL, ICC (2,1) = .84 (.10–.95) for ES, and ICC (2,1) = .91 (.61–.97) for IS. Intrarater reliability computed between repeated SCFT measurements made by the primary researcher were as follows: ICC (2,1) = .97 (confidence interval [CI]: .91 to .99) for RF, ICC (2,1) = .97 (CI: .94 to .99) for VL, ICC (2,1) = .84 (CI: .09 to .95) for ES, and ICC (2,1) = .97 (CI: .61 to .99) for IS. Based on ICC thresholds published by Koo and Li,[50] RF and VL displayed "excellent" interrater and intrarater reliability (excellent ICC = .90–1.00), whereas ES displayed "poor" to "excellent" interrater and intrarater reliability (poor ICC = .00–.50), and IS displayed "moderate" to "excellent" reliability (moderate ICC = .50–.75).

Discussion

This research sought to determine whether normalized iEMG signal amplitude is linearly related to normalized sEMG at an isokinetic submaximal exertion intensity of 50% MVIC and whether the relationship between recording methods could be improved by including regional SCFT as a model covariate. Our results were highly variable, with relationships differing by recording site (RF, VL, ES, and IS). The strongest relationship between sEMG and iEMG was identified at IS (Adj $R^2 = .74$; $F_{1,22} = 66.9$, $P < .001$). SCFT was shown to explain significantly more model variance for VL. However, when sex was not included as a covariate, SCFT alone did not significantly improve model fit. Secondary aims of this research were (1) to determine whether regional differences exist in SCFT and (2) to determine the interrater and intrarater reliability of SCFT measurements from RF, VL, ES, and IS. Regional differences in SCFT did exist in

Table 2 *F* Statistic, *P* Statistic, Adjusted Coefficient of Determination (Adj R^2), and Associated Cohen f^2 Effect Size Statistic for Each Computed Linear Regression Model: No Covariates, SCFT as a Covariate, and SCFT and Sex as Covariates

	No covariates			Covariate: SCFT			Covariates: SCFT and sex		
Muscle	$F_{1,21}$	*P*	Adj R^2; f^2	$F_{2,20}$	*P*	Adj R^2; f^2	$F_{3,19}$	*P*	Adj R^2; f^2
Rectus femoris	0.87	.362	−.007; 0.007	0.62	.548	−.041; 0.043	1.56	.238	.081; 0.088
Vastus lateralis	3.44	.078	.010; 0.010	2.86	.081	.145; 0.170	3.28	**.043**	.235; 0.307
T4 erector spinae	8.29	**.010**	.277; 0.389	3.93	**.039**	.236; 0.309	2.74	.077	.216; 0.276
Infraspinatus	66.90	**<.001**	.741; 2.861	32.06	**<.001**	.730; 0.270	21.98	**<.001**	.732; 2.731

Abbreviation: SCFT, subcutaneous fat thickness. Note: Bold indicates significance at $P < .05$.

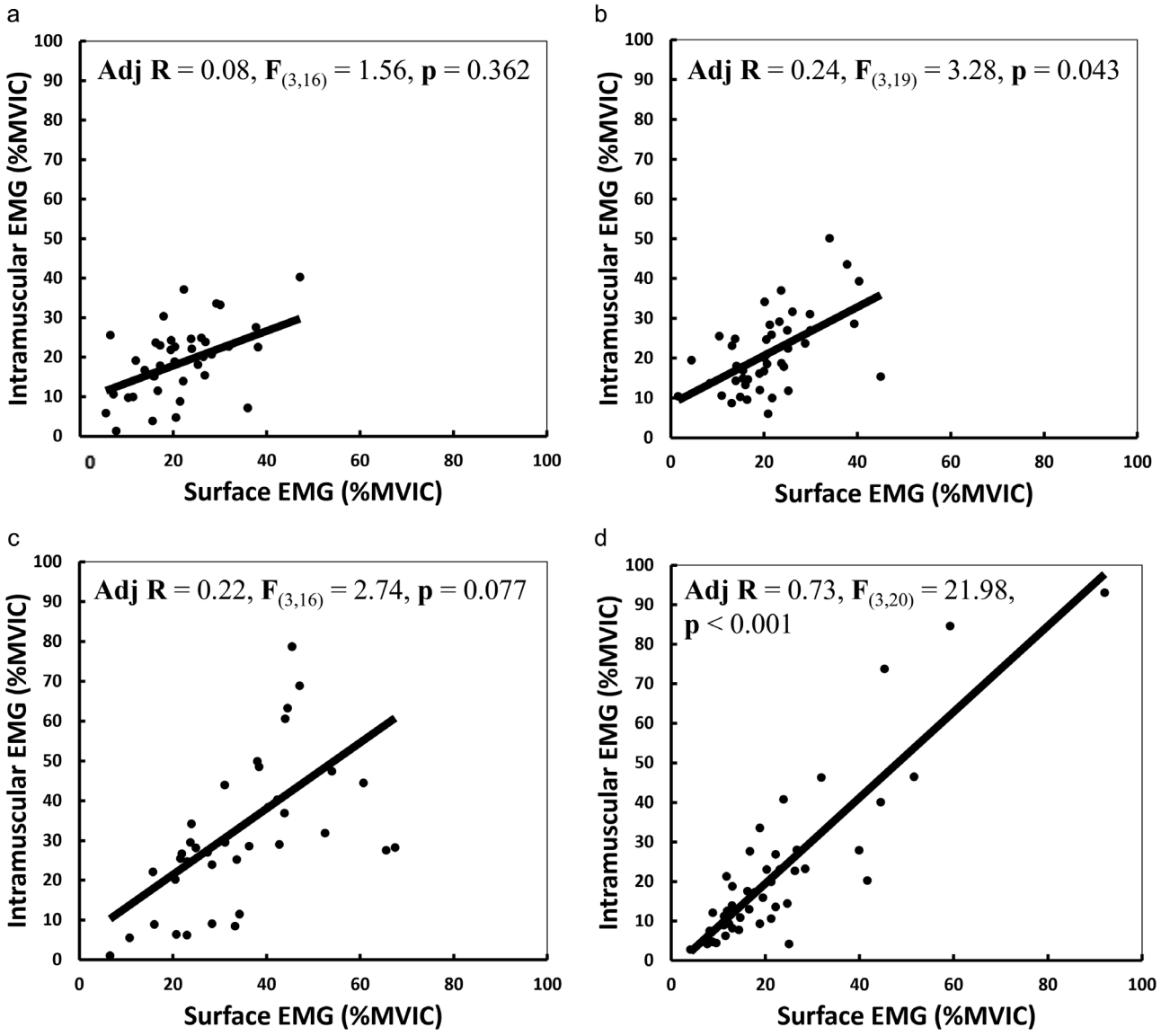

Figure 3 — Linear regression models between intramuscular (*y*-axis) and surface (*x*-axis) normalized EMG amplitude peak with SCFT and sex as covariates during submaximal (50% MVIC) contraction. (a) Rectus femoris, (b) vastus lateralis, (c) erector spinae, and (d) infraspinatus. EMG indicates electromyography; MVIC, maximum voluntary isometric contraction; SCFT, subcutaneous fat thickness.

women but not men, with RF demonstrating significantly larger SCFT magnitude compared with ES. There were no significant correlations demonstrated between participant BMI and regional SCFT. Average SCFT thickness may be related to SCFT measurement reliability as RF and VL displayed "excellent" interrater and intrarater reliability, while IS displayed "moderate" to "excellent" interrater and intrarater reliability and ES displayed "poor" to "excellent" interrater and intrarater reliability.[50]

A strong, positive correlation coefficient and positive regression coefficient between sEMG and iEMG was shown for IS (Adj $R^2 = .74$, $P < .001$; Figure 3). The stronger relationship identified for IS relative to other muscles may be attributed to the regional anatomy of the muscle whereby the superficial position of the muscle articulating with the scapula may potentially insulate the IS electrodes from the crosstalk of neighboring muscles. The reduction in model fit with the inclusion of covariates for ES (Table 2) may be related to the high variance of the sex covariate, which presented very large CIs at each muscle (RF [−1.7 to 21.6], VL [−0.9 to 15.6], ES [−27.2 to 13.0], and IS [−4.9 to 15.8]). This suggests that sex was likely underpowered as a variable in our current sample (N = 12 per sex), although sex-based variance also appeared higher at ES than other muscles. Conversely, the regression model of VL showed a significant improvement in model fit with the inclusion of SCFT as a covariate. The isolated effects of SCFT at VL may be attributed to its sensitivity to crosstalk, particularly from vastus intermedius. Researchers have previously reported that vastus intermedius can be collected using sEMG just superior to the lateral edge of the patella, very close to the recommended sEMG placement for VL.[38] Thus, VL sEMG may be particularly sensitive to vastus intermedius crosstalk, although researchers reported that vastus intermedius root mean square amplitude was not significantly influenced by VL root mean square amplitude.[52] In the current study, SCFT only significantly influenced model fit with the inclusion of sex. This finding may point

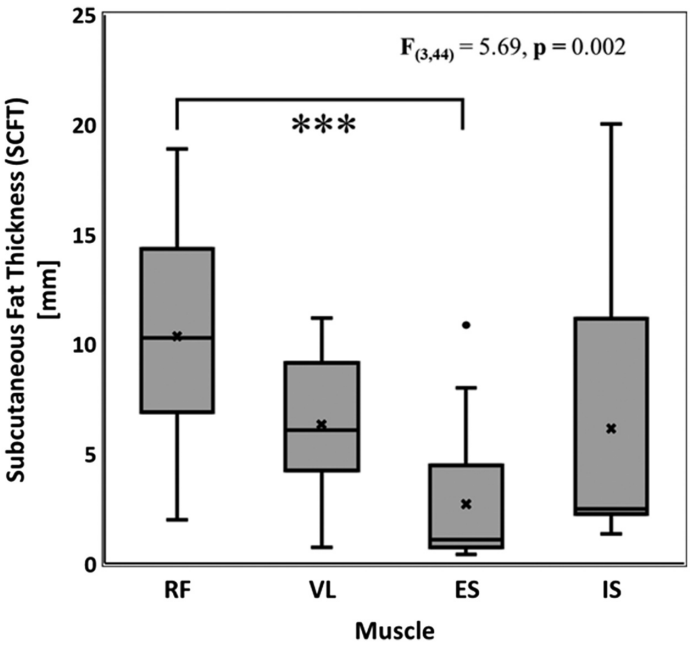

Figure 4 — Regional differences in subcutaneous fat thickness in females. ***Significance <.001. ES indicates erector spinae; IS, infraspinatus; RF, rectus femoris; VL, vastus lateralis.

toward sex-related differences in muscle mass, muscle morphology and moment arm, muscle activity, subcutaneous fat, intramuscular fat, and visceral fat, which could potentially explain unique variance to better predict iEMG amplitude given sEMG amplitude and SCFT.[39] These sex-related differences in body composition suggest that using BMI may pose a limitation for accurately representing body composition compared with other more accurate measurements, such as body fat percentage.[39] This may explain why regression modeling between SCFT and BMI was not significant and advocates for the use of more accurate body composition and body fat measurement tools for future studies investigating sex differences in EMG.

The regression model fit for IS was slightly stronger than previously reported,[23] which may be due to differences in exertion intensities between studies. Allen et al[23] previously described an explained variance (R^2) of .64 ($P < .001$) between sEMG and iEMG magnitudes, whereas we achieved an adjusted R^2 of .74 ($P < .001$) before considering the inclusion of model covariates. Both studies measured data from a sex-balanced, university-aged population, with an average BMI within the "Healthy"/"Normal" range.[53,54] Furthermore, with moderate strength correlation coefficients at IS observed in our study and previous studies,[23] we were surprised by the weak correlation coefficients observed at RF, VL, and ES.[11,40] Hofste et al[40] previously reported ES correlation coefficients (R) in a sample of healthy/unaffected participants, 18–65 years, ranging from .40 to .60 ($R^2 = .16–.36$) during various static and dynamic contractions.[42] This range of coefficient of variation values is also comparable with the adjusted R^2 value in our ES model of 0.277 ($P = .010$) with no model covariates. However, a substantially higher coefficient of determination between sEMG and iEMG amplitudes has been previously reported at VL and RF.[11] Byrne et al[11] previously reported a regression coefficient of 0.731 ($R^2 = .53$) at VL during knee extension contractions designed to isolate VL and a regression coefficient of 0.579 ($R^2 = .32$) during contractions designed to isolate RF. These findings suggest that crosstalk among the quadriceps muscles may have contributed to the reduced correlation strength in the current study as the knee extension protocol was not designed to principally recruit an individual muscle. Although controlling for SCFT did not appear to improve this model relationship as intended, this may point to other factors, such as intramuscular fatty infiltration, contributing to sEMG and iEMG normalized amplitude discrepancies.[55]

Certain limitations should be considered when interpreting these results. First, our study used B-mode ultrasound imaging methods to assess the SCFT. Ultrasound methods to measure SCFT are widely used[49,56–58] and reported to be reliable,[59] reproducible,[60] and accurate[61]; however, SCFT measurement techniques are not yet standardized.[62,63] Second, the iEMG insertion protocol used in our study adhered to published guidelines.[42] However, future studies may wish to take additional steps to reduce variation between sEMG and iEMG amplitudes inherent in their selectivity and sensitivity characteristics. Some possible improvements to this methodological approach include the application of high-density sEMG electrodes combined with crosstalk reduction analyses,[64–66] controlling for intramuscular fatty infiltration,[55] using multiple sites of intramuscular recording,[67] and using additional SCFT measurement tools, such as skinfold measurement, BMI, and bioelectric impedance or hydrostatic weighing.[27] The current study also selected a young cohort with an average BMI within the "Healthy" range of 18 to 24.9.[53,54] This benefited aspects of our study by allowing for comparisons with previous work[23]; however, future studies may want to compare a greater spectrum of BMI or consider comparing across discrete groups (eg, "Healthy" BMI and "Overweight" BMI).[53,54] Furthermore, with a sample size of n = 12 for men and women, the sex-based analysis in the current study may have been underpowered. Thus, future studies exploring sex-based analysis should recruit a larger sample to enable statistical comparisons between groups. Finally, temporal alignment of signal amplitudes and spikes was not explored in the current study; therefore, sampling iEMG at a rate that is indivisible by the sEMG sampling rate was not a limitation of this research. However, these sampling rates reflect the iEMG and sEMG sampling rates available with our EMG acquisition system that exceed the necessary Nyquist frequency; thus, future studies interested in the temporal alignment of iEMG and sEMG features may need to consider an alternative signal acquisition setup.

This study represents an initial exploration of the effects of SCFT and sex on sEMG and iEMG relationship linearity. Findings from this research demonstrated variability in the strength of relationship between sEMG and iEMG across different anatomical regions despite having significantly different regional SCFT. Furthermore, although SCFT was expected to explain significantly more model variance, our results suggest that SCFT is not a significant parameter affecting the relationship between sEMG and iEMG. Future research should explore other potential sources of sEMG signal attenuation, notably intramuscular fatty infiltration and sex, on a larger sample to determine whether other muscle and/or experimental parameters affect the relationship between EMG recording methods.

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