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## Innovations in the Assessment of Skeletal Muscle Health: A Glimpse into the Future

Jonathan P Beausejour, Kevan S Knowles, Abigail T Wilson, L. Colby Mangum, Ethan C Hill, William J Hanney, Adam J Wells, David H Fukuda, Jeffrey Stout, Matt S Stock.

Affiliations below.

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### Abstract:

Skeletal muscle is the largest organ system in the human body and plays critical roles in athletic performance, mobility, and disease pathogenesis. Despite growing recognition of its importance by major health organizations, significant knowledge gaps remain regarding skeletal muscle health and its crosstalk with nearly every physiological system. Relevant public health challenges like pain, injury, obesity, and sarcopenia underscore the need to accurately assess skeletal muscle health and function. Feasible, non-invasive techniques that reliably evaluate metrics including muscle pain, dynamic structure, contractility, circulatory function, body composition, and emerging biomarkers are imperative to unraveling the complexities of skeletal muscle. Our concise review highlights innovative or overlooked approaches for comprehensively assessing skeletal muscle in vivo. We summarize recent advances in leveraging dynamic ultrasound imaging, muscle echogenicity, tensiomyography, blood flow restriction protocols, molecular techniques, body composition, and pain assessments to gain novel insight into muscle physiology from cellular to whole-body perspectives. Continued development of precise, non-invasive tools to investigate skeletal muscle are critical in informing impactful discoveries in exercise and rehabilitation science.

### Corresponding Author:

Dr. Matt S Stock, University of Central Florida, Institute of Exercise Physiology and Rehabilitation Science, Orlando, United States, matt.stock@ucf.edu

### Affiliations:

Jonathan P Beausejour, University of Central Florida, Institute of Exercise Physiology and Rehabilitation Science, Orlando, United States  
Kevan S Knowles, University of Central Florida, Institute of Exercise Physiology and Rehabilitation Science, Orlando, United States  
Abigail T Wilson, University of Central Florida, Institute of Exercise Physiology and Rehabilitation Science, Orlando, United States  
[...]  
Matt S Stock, University of Central Florida, Institute of Exercise Physiology and Rehabilitation Science, Orlando, United States

# Innovations in the Assessment of Skeletal Muscle Health: A Glimpse into the Future

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## Abstract

Skeletal muscle is the largest organ system in the human body and plays critical roles in athletic performance, mobility, and disease pathogenesis. Despite growing recognition of its importance by major health organizations, significant knowledge gaps remain regarding skeletal muscle health and its crosstalk with nearly every physiological system. Relevant public health challenges like pain, injury, obesity, and sarcopenia underscore the need to accurately assess skeletal muscle health and function. Feasible, non-invasive techniques that reliably evaluate metrics including muscle pain, dynamic structure, contractility, circulatory function, body composition, and emerging biomarkers are imperative to unraveling the complexities of skeletal muscle. Our concise review highlights innovative or overlooked approaches for comprehensively assessing skeletal muscle *in vivo*. We summarize recent advances in leveraging dynamic ultrasound imaging, muscle echogenicity, tensiomyography, blood flow restriction protocols, molecular techniques, body composition, and pain assessments to gain novel insight into muscle physiology from cellular to whole-body perspectives. Continued development of precise, non-invasive tools to investigate skeletal muscle are critical in informing impactful discoveries in exercise and rehabilitation science.

**Keywords:** pain; injury; rehabilitation; athlete performance; blood flow restriction; muscle quality

45 **Introduction**

46  
47 Skeletal muscle is one of the most metabolically active and adaptable tissues in the human body,  
48 comprising up to 40% of total body mass and containing 50-75% of all body proteins [1]. The  
49 dynamic and plastic nature of skeletal muscle enables it to support a wide range of vital functions  
50 for human health and performance, including initiating movement, maintaining posture and body  
51 temperature, stabilizing joints, and storing nutrients [2]. Over recent decades, researchers have  
52 developed and refined a range of techniques to evaluate skeletal muscle structure and function,  
53 non-invasively. These include imaging modalities such as magnetic resonance imaging (MRI)  
54 and CT, dual energy X-ray absorptiometry, anthropometric measurements such as skinfolds and  
55 girths, electromyography (EMG), and isokinetic dynamometry. Application of these techniques  
56 has provided key insights into the adaptability of human skeletal muscle within the context of  
57 aging, disease, injury, exercise, and nutrition [1,3,4]. While current methods have advanced our  
58 understanding of skeletal muscle physiology, continued innovation and optimization are  
59 necessary to develop more feasible assessment tools capable of exploring intricate muscle  
60 morphology responses to different physiological and pathophysiological stimuli [5]. Emerging  
61 areas requiring further research include the influence of individual variation in muscle structure  
62 and function, sensitivity of assessment techniques, the interplay between muscle and other  
63 tissues like fat and bone, and the ideal modes and dosages of exercise, nutrition, and  
64 rehabilitation interventions [6,7].

65  
66 To further promote engagement in these research avenues, scientists must continue honing  
67 current approaches while implementing more viable, novel assessment tools aimed to adequately  
68 assess skeletal muscle properties. The purpose of our review is to briefly highlight emerging,  
69 innovative, and relatively feasible approaches that show promise in assessing skeletal muscle  
70 health. Covered topics in this review include ultrasound-derived dynamic imaging,  
71 tensiomyography, innovative approaches for blood flow restriction administration, utilization of  
72 neoepitope-biomarkers for skeletal muscle structure and function, ultrasound-derived echo  
73 intensity measures for muscle quality, and other novel assessments of body composition and  
74 skeletal muscle pain.

75  
76 **Methods**

77  
78 A comprehensive review of the science literature was conducted to assess the latest approaches  
79 in evaluating skeletal muscle health. The National Institutes for Health National Library of  
80 Medicine (PubMed.gov) and Google Scholar search engines were utilized, specifically, to  
81 identify recent publications related to dynamic imaging of skeletal muscle, peripheral  
82 neuromuscular assessments, muscle echogenicity, blood flow restriction, biomarkers of skeletal  
83 muscle function, skeletal muscle quality, body composition, and pain assessment.

84  
85 **Dynamic Imaging**

86  
87 Muscle function and characteristics can be measured in several ways and many laboratory- and  
88 hospital-based studies utilize EMG, and imaging instrumentation, such as magnetic resonance  
89 imaging. Financial and time constraints can limit access and the ability to employ many of these  
90 techniques when clinicians and practitioners are seeking a deeper understanding of muscle

91 function. A viable alternative to some of the more expensive and less accessible options is  
92 ultrasound imaging (Fig 1) [8]. Specifically for musculoskeletal ultrasound imaging, there are  
93 established methods detailing how to measure muscle thickness, cross-sectional area,  
94 echogenicity, etc. [9]. Most of these methods rely on the patient or participant to be in a static,  
95 rested state, which controls the environment with hopes to avoid any artifact or false  
96 representation of the muscle's morphology. However, many of the patient populations included  
97 in ultrasound imaging studies and clinical scenarios are likely to experience some sort of injury  
98 and/or exhibit symptomology that revolves around pain during movement.

99  
100 Ultrasound imaging when used in a dynamic, innovative manner, capturing images during  
101 contraction and even exercise, provides an understanding of muscle function, not just static  
102 characteristics [8]. When a patient or participant is experiencing pain and dysfunction during  
103 movement, activities of daily living, or exercise, the consequences are well documented,  
104 especially in those with chronic low back pain. Due to the known contribution of abdominal, hip,  
105 and pelvic muscles to low back pain-related dysfunction, ultrasound imaging is a useful tool to  
106 view the complex layering of those muscles [8]. Part of the injury assessment process could  
107 include viewing muscles when individuals move, and they experience pain or avoid movement  
108 due to the fear of pain. Capturing muscle thickness, cross-sectional area, and quality can be done  
109 reliably in many positions [9,10], commonly in the lateral abdominal wall and posterolateral hip.  
110 The application of dynamic ultrasound imaging has been described recently for sport-specific  
111 and body part-focused rehabilitation exercises[11,12] . Visualizing musculature of the lateral  
112 abdominal wall is possible by fixing the ultrasound probe to the anterolateral abdomen with a  
113 belt ensuring the probe stays in the same position even while the individual moves. Documented  
114 methods describing this technique include capturing images and videos while walking,  
115 balancing, squatting, planking, swinging a golf club, etc.[11–14].]. Populations included in these  
116 studies span from healthy, asymptomatic individuals to those experiencing low back pain and  
117 chronic ankle instability.

118  
119 The utility of dynamic ultrasound imaging is shown by recent studies that have reported muscle  
120 thickness changes from a static, rested position to an active, contracted position. Activation ratios  
121 may also be calculated by dividing the contracted thickness by the rested thickness and were first  
122 established in hook-lying tabletop positions. Functional activation ratios divide thickness during  
123 exercise (e.g., peak knee flexion during a single leg squat) by a static, starting position (e.g.,  
124 standing). A preferential activation ratio [15] involves comparing the thickness of one muscle to  
125 others within the same image. For example, when imaging the lateral abdominal wall, the change  
126 in thickness of the transverse abdominis could be divided by the change in thickness of the entire  
127 lateral abdominal wall (Figure 2). This preferential ratio provides insight into how much the  
128 transverse abdominis changes its thickness relative to the other muscles during contraction. A  
129 greater preferential activation ratio indicates the transverse abdominis is the predominant muscle  
130 changing thickness out of the entire lateral abdominal wall. Dynamic ultrasound imaging also  
131 allows for an innovative, clinical approach to visual biofeedback. As the patient visualizes their  
132 muscles during a prescribed exercise, activity of daily living, or pain-provoking position, there is  
133 an opportunity to show the patient how they can contract the muscle either at a different time or  
134 how to increase thickness in general. Methods have been established for sport-specific  
135 ultrasound biofeedback, specifically viewing the obliques during a golf swing [12]. Brightness,  
136 B-mode, and Motion, M-mode can be used for dynamic imaging and biofeedback. Based on the

137 goal of dynamic imaging, B-mode may apply when viewing a reference, static image, or to view  
138 a frame-by-frame breakdown of a muscle moving through a task. M-mode may be appropriate  
139 when synchronizing dynamic ultrasound with other muscle measurement tools capturing in the  
140 time domain, such as timing of activation with electromyography [11].

141

## 142 **Contractile Properties**

143

144 Tensiomyography (TMG) is a non-invasive and objective assessment tool used to evaluate  
145 skeletal muscle contractile properties and peripheral neuromuscular function. It provides  
146 valuable information about muscle contraction characteristics, muscle fiber composition, and  
147 muscle fatigue. TMG has gained popularity in sports science, rehabilitation, and research settings  
148 due to its ability to provide real-time and reliable data on muscle function [16–18]. TMG  
149 involves the application of an external electrical stimulus to the muscle belly, causing a muscle  
150 twitch response that is measured through a displacement sensor tip (Fig 2). It primarily assesses  
151 muscle contractile properties, including muscle displacement, contraction time, and muscle  
152 relaxation time. These parameters reflect muscle stiffness, contractile speed, and muscle fiber  
153 recruitment patterns [19,20]. One of the key advantages of TMG is its ability to provide  
154 objective and quantitative data. Traditional assessment methods, such as manual muscle testing  
155 or electromyography, often rely on more subjective, post-acquisition interpretation or qualitative  
156 measures. TMG, on the other hand, offers standardized numerical values and objective  
157 measurements, reducing the potential for human error and enhancing the reliability of the  
158 assessment [20,21]. By tracking changes in muscle contractile properties, one can evaluate the  
159 effectiveness of training programs and make adjustments accordingly. TMG can also help  
160 identify muscle imbalances and guide targeted interventions to restore balance and optimize  
161 performance.

162

163 TMG involves elicited, involuntary isometric contractions that are generated using a single 1 ms-  
164 wide biphasic wave. By utilizing proprietary computer software, a twitch curve is generated  
165 based on data from the sensor, which allows for the determination of six primary parameters.  
166 The y-axis of this curve represents muscle displacement in millimeters, while the x-axis  
167 represents time in milliseconds. The key TMG parameters comprise displacement (Dm),  
168 contraction time (Tc), delay time (Td), contraction velocity (Vc) ( $Vc = [90\%Dm - 10\%Dm / Tc]$ ),  
169 sustain time (Ts), and half-relaxation time (Tr). (6) Displacement (Dm) pertains to the highest  
170 radial displacement achieved by the muscle and is linked to muscle stiffness. Contraction time  
171 (Tc) represents the duration between 10% and 90% of Dm on the positive slope of the twitch  
172 curve. Delay time (Td) is a temporal parameter that measures the duration from the initiation of  
173 the electrical stimulus to when the muscle belly reaches 10% of Dm or peak displacement. Half-  
174 relaxation time (Tr) refers to the time taken for the muscle displacement to decrease from 90% of  
175 its maximum to 50% of Dm on the negative slope of the curve. Sustain time (Ts) is defined as  
176 the time between 50% Dm on both the negative and positive slopes of the curve. Contraction  
177 velocity (Vc) is a calculated metric that aims to quantify the rate of muscular contraction. Since  
178 Vc is a derived measure, various methods have been employed by authors to compute it. The  
179 most commonly used calculation involves dividing the change in Dm between 10% and 90% by  
180 Tc. This approach enhances the usefulness of the Tc parameter and provides a more reliable  
181 measure of contraction speed by mitigating the influence of Dm. This is important as peak radial



182 displacement values have been shown to impact contraction time values due to the inherent  
183 shape of the twitch curve [20,21].

184  
185 TMG can also help identify specific muscle deficits or imbalances that may contribute to  
186 functional limitations or recurring injuries. With this information, tailored rehabilitation  
187 programs can be developed to address these deficits and promote optimal recovery [18,21].  
188 Moreover, TMG can be a valuable research tool for investigating muscle adaptations to training,  
189 comparing different training protocols, or studying the effects of injury or disease on muscle  
190 function. Researchers can use TMG to examine changes in muscle contractile properties  
191 following specific interventions or to explore how different training modalities affect muscle  
192 performance.

193  
194 While TMG has shown great promise as an assessment tool, it is important to note its limitations.  
195 TMG primarily focuses on muscle contractile properties and does not provide direct information  
196 about neural activation or muscle force production. It is also important to consider that TMG  
197 measurements may be influenced by factors such as skin impedance, adipose tissue thickness,  
198 and anatomical variations. These factors need to be considered during data interpretation to  
199 ensure accurate and meaningful results.

### 200 201 **Innovation Applications to Determine Blood Flow Restriction Occlusion Pressures**

202  
203 Traditional exercise-based approaches are not well tolerated by several clinical populations, an  
204 issue researchers/practitioners continually attempt to circumvent [22–24]. An exciting strategy to  
205 address this issue includes combining exercise with limb occlusion (blood flow restriction  
206 [BFR]) due to its ability to induce similar or even superior benefits compared to traditional non-  
207 occluded exercise [25–27]. During BFR exercise, low exercise loads (e.g., lighter  
208 weights/resistance) are used while completing a standardized 5-min exercise scheme. Blood flow  
209 restriction exercise uses a small inflatable cuff applied to the upper most portion of a limb to  
210 restrict venous blood from exiting the exercising limb which facilitates robust physiological  
211 responses that may underly the subsequent increases in muscle strength, mass, and endurance  
212 (i.e., fatigue resistance) observed following chronic BFR exercise. These adaptations have been  
213 demonstrated in asymptomatic [22–31] and some symptomatic populations [32–34]. Importantly,  
214 BFR resistance exercise has also been shown to be safe and to elicit positive effects in post-  
215 surgical [35,36] older adults [32,37,38] and hospitalized patients [39]. Specifically, the safety  
216 and effectiveness of BFR exercise has been routinely demonstrated with the implementation of  
217 the standard 75 repetition ( $1 \times 30$ ,  $3 \times 15$ ) scheme [28,40]. Additionally, the exercise is  
218 performed at 30% of 1RM (i.e., a low weight/resistance) and with the small, inflated cuff set to a  
219 pressure corresponding to 40-80% of arterial occlusion pressure (i.e., approximately 40-80% of  
220 systolic blood pressure) [41,42].

221  
222 Despite its high versatility and implementation in both research, clinical, and athletic settings, the  
223 application of BFR is limited. Specifically, the gold standard for the determination of total  
224 arterial occlusion pressure (TAOP) and application of BFR requires the use of pulsed wave  
225 Doppler (Fig 3). Typically, this necessitates trained personnel to operate and interpret  
226 ultrasound-based readings (i.e., arterial and venous blood flow) with the concomitant modulation  
227 of pneumatic pressures. Theoretically, minute variations in the determination of TAOP changes

228 the BFR pressure applied which has been demonstrated to alter the physiological responses  
229 [41,43] and discomfort (e.g., higher pressures result in greater discomfort). Therefore, it is  
230 imperative that TAOP is determined accurately and precisely. As a result, the current  
231 applications of BFR exercise are somewhat limited to research and clinical settings whereby  
232 these procedures can be performed accurately and reliably, although data are limited in this  
233 regard. For example, among a small sample of males (n=13), pulsed wave Doppler ultrasound  
234 exhibited moderate/high reliability (intraclass correlation coefficient [ICC] = 0.796), while the  
235 coefficient of variation (COV) was 5.6% (Bezerra et al., 2017). There were, however, no relative  
236 measures of reliability reported (e.g., standard error of the measurement [SEM] or minimal  
237 difference [MD]) which limits the application of these findings [44].

238  
239 An exciting strategy to circumvent the determination of TAOP, is the algorithm-based  
240 determination of TAOP. Specifically, several commercially available devices (e.g. SujiBFR,  
241 Defli, Smartcuffs) independently *estimate* TAOP using proprietary engineering that likely  
242 leverages known variables (e.g., limb width, mean arterial pressure) which affect TAOP. For  
243 example, in a small sample (n=10), one study [45] examined test-retest reliability of an  
244 algorithm-based determination (Delfi PTS, Delfi Medical, Vancouver, BC, Canada) of TAOP  
245 and reported high reliability (ICC > 0.953; COV = 2.97%), but the validity of this device was not  
246 examined. Algorithm-based devices such as the one previously examined and others (e.g., Suji,  
247 SmartCuffs) may exhibit greater utility than current research-based practices for the  
248 determination of TAOP which requires ultrasound and trained personnel, but their algorithms  
249 should be critically examined to prevent potential adverse effects. Specifically, researchers  
250 [42,46,47] have done substantial and meaningful work identifying predictors, equations, and/or  
251 algorithms which can estimate TAOP, but these methods are not without error. Thus, there is an  
252 inherent need to determine if commercially available BFR devices implementing algorithms to  
253 determine TAOP are clinically sound (i.e., reliability and validity). Furthermore, there is  
254 insufficient data examining the reliability of TAOP using pulsed wave Doppler (the criterion  
255 method). Regardless, commercially available devices (e.g., SujiBFR, Delfi, Smartcuffs) may  
256 provide viable alternatives to the criterion determination of TAOP ultimately facilitating the  
257 larger implementation of BFR.

## 258 259 **Novel Serological Neopeptides as Biomarkers of Skeletal Muscle Structure and Function**

260  
261 Biomarkers are defined as measurable indicators of biological processes or responses to an  
262 exposure or intervention [48] . They represent an indispensable tool in human biology that  
263 allows researchers to map the complex physiological pathways that underpin healthy and altered  
264 physiological function [49] . The use of serological biomarkers has the benefit of being relatively  
265 non-invasive and easy to perform [50,51], which has led to the widespread development of  
266 assays for various biological markers. In this regard, neopeptides have emerged as a class of  
267 serological peptide biomarkers with diagnostic and prognostic potential that may also have utility  
268 as minimally invasive indicators of health status, physiological response and/or disease  
269 susceptibility (Fig 4).

270  
271 In skeletal muscle, neopeptides may be exposed following post-translational modifications  
272 (PTMs) to specific muscle proteins [52] . Proteolytic cleavage is a particularly interesting PTM

273 as it creates peptide fragments and the potential for novel neopeptides on the carboxy- or  
274 aminoterminal ends of cleaved peptides [53,54]. Peptide fragments being smaller than their intact  
275 parent proteins can enter the circulation more readily [55] where antibodies can be raised against  
276 specific neopeptides exposed on these fragments. Since serological neopeptide biomarkers  
277 consist of a unique combination of parent proteins and PTMs [56], they are thought to be  
278 reflective of tissue specific physiological or pathological remodeling processes rather than  
279 overall muscle size or quality and may therefore serve as ideal biomarkers for early the detection  
280 of various myopathies [57,58]. It is also thought that serological neopeptide biomarkers have the  
281 potential to provide insight into net changes in protein metabolism, which is currently limited to  
282 operationally complex and invasive stable isotope techniques [58].

283  
284 Much of the research examining neopeptides in skeletal muscle has focused on temporal changes  
285 to extracellular matrix (ECM) collagens following various interventions. Nedergaard et al., 2013  
286 examined changes in collagen type VI fragment degraded by matrix metalloproteinases 2 and 9  
287 (C6M) and type VI collagen N-terminal globular domain epitope (IC6) among young and old  
288 men at baseline after 2-weeks of unilateral immobilization and 4-weeks of remobilization with 3  
289 x weekly resistance training, respectively [59]. They found significant correlations between IC6  
290 and muscle mass at baseline, and between C6M and the change in muscle mass from 2-weeks to  
291 4-weeks of remobilization in young but not old men [59]. The same group also reported  
292 significant associations between lean body mass, IC6, collagen type III synthesis (Pro-C3) and  
293 the IC6/C6M ratio among matched controls in the 25B cohort of the Danish Head and Neck  
294 cancer group (DAHANCA) trial [60]. In another study, Sun et al., 2015 examined the temporal  
295 profile of neopeptide peptides Pro-C3, C-terminus  $\alpha 3(\text{VI})$  chain (Pro-C6) and C6M following 8-  
296 weeks immobilization and remobilization. They reported significant associations between Pro-  
297 C3, C6M and lean body mass at baseline, a significant upregulation of both Pro-C3 and Pro-C6  
298 following immobilization and remobilization indicative of muscle remodeling, and an inverse  
299 relationship between Pro-C6 and changes in muscle mass [61]. Consistent with this, work by  
300 Nielsen et al. 2013 also indicates that higher levels of Pro-C3 predict greater muscle mass in  
301 healthy individuals [62]. More recently, Reule et al., 2016 found that the ratio of type II collagen  
302 collagenase cleavage neopeptide (C2C) to C propeptide of type II procollagen (CP2) was  
303 responsive to leucine-rich amino acid supplementation administered in conjunction with 12-  
304 weeks of combined aerobic strength and balance training [63]. They report a significantly greater  
305 decrease in the acute phase (0-3 hours) C2C/CP2 post-training response to a downhill walking  
306 stress test when compared to the placebo group, indicating a lower disturbance in joint  
307 homeostasis that coincided with a significant attenuation of acute phase quadriceps MVC strength  
308 loss [63]. Several other serological biomarkers, including C-terminal agrin fragment (CAF) and  
309 matrix metalloproteinase-2 degraded titin fragment (titin-MMP2) also appear to be strong  
310 discriminators of normal versus aberrant skeletal muscle outcomes including muscle  
311 wasting/atrophy and protein turnover [61,64].

312  
313 The large dynamic range and complexity of both the proteome and various PTMs [65] represents  
314 a significant challenge for the identification of new muscle specific neopeptide biomarkers and  
315 targeting reagents. Nevertheless, while this area of research is currently in its infancy, the limited



316 current literature indicates that serological neopeptide biomarkers of cleaved circulating peptide  
317 fragments are promising candidates for assessing skeletal muscle structure and function.

### 318 319 **Skeletal Muscle Quality**

320  
321 Studies have increasingly shown that there is a disassociation between skeletal muscle strength  
322 and mass [66,67]. For example, the National Institutes on Aging's longitudinal Healthy, Aging,  
323 and Body Composition study showed that adults  $\geq 70$  years of age lost  $\sim 3\times$  more muscle strength  
324 than mass on an annual basis [66]. Even more compelling, older adults that gained muscle mass  
325 still lost muscle strength [68]. In addition, immobilization and bed rest studies have shown that  
326 muscle strength and mass show divergent timelines, with strength rapidly diminishing before  
327 detectable declines in muscle mass [69–71]. These concepts have given rise to the concept of  
328 muscle quality and its methods of assessment [72].

329  
330 The measurement of echo intensity has emerged as a potential tool for studying skeletal muscle  
331 quality and estimating intramuscular fat content, providing insights that may be unique from  
332 measures of muscle size [73]. Echo intensity is a quantitative measure of brightness in ultrasound  
333 images, reflecting the echogenicity of tissues (Fig 5). In skeletal muscle, echo intensity is  
334 influenced by physiological factors such as muscle fiber arrangement [74], connective tissue  
335 content [75], and the presence of intramuscular fat [76]. Higher echo intensity values are  
336 associated with muscle pathologies like atrophy, fibrosis, and fatty infiltration [77], while lower  
337 values are observed in young, healthy muscles. MRI studies suggest that echo intensity's ability  
338 to estimate intramuscular fat content appears promising [77,78]. Echo intensity is associated with  
339 several functional outcomes [79,80] and it appears to be sensitive in detecting differences  
340 between age groups [81]. Additional research is needed to understand why large differences in  
341 echo intensity are often seen when comparing groups with distinct characteristics like age or  
342 training status, whereas smaller changes are detected in response to exercise or rehabilitation  
343 interventions [82]. One potential explanation is that intrinsic physiological factors like muscle  
344 fiber type distribution, connective tissue content, and intramuscular fat infiltration change slowly  
345 over time. Group differences may represent the cumulative result of prolonged exposure to  
346 factors like aging. In contrast, short-term interventions elicit more modest echo intensity  
347 changes, as muscle structural characteristics do not radically transform within days or weeks.  
348 Longer training studies are needed to determine if more marked echo intensity changes can be  
349 induced over time with sustained exposure to stimuli like exercise. It is also important to  
350 recognize that echo intensity is affected by methodological factors, such as probe tilt [83] and  
351 participant positioning [84]. Recent evidence suggests that researchers new to echo intensity  
352 analyses provide reliable measurements [85] and small adjustments in image depth to  
353 accommodate muscles of different sizes are acceptable [86].

354  
355 Most published echo intensity studies have relied on ImageJ software (National Institutes of  
356 Health, Bethesda, MA, USA), in which investigators manually analyze a muscle's region of  
357 interest. While these approaches are well established, they can be time consuming and  
358 subjective, making it difficult to conduct large-scale analyses quickly. However, emerging  
359 technologies are likely to streamline the measurement process, reduce subjectivity, and enhance  
360 the accuracy of echo intensity analysis. Automated or semi-automated region of interest selection  
361 algorithms have recently been introduced to target specific muscle regions [87,88]. In the future,

362 computerized analysis of ultrasound images will enable the precise quantification of echo  
363 intensity values, ensuring consistent and reproducible evaluations. In addition, small probes and  
364 wireless technology that integrate with laptops, tablets, and smartphones phones will likely make  
365 the analysis of echo intensity much more accessible and rapid.

366  
367 Overall, the measurement of echo intensity has emerged as an innovative approach for studying  
368 skeletal muscle quality and estimating intramuscular fat content. Importantly, these  
369 measurements can be done with ultrasound devices that are portable and less expensive than  
370 MRI, and minimal training is required. These measurements are particularly useful when  
371 complementing measures of muscle size (e.g., cross-sectional area or volume) and physical  
372 function. For more detailed information, the reader is encouraged to review two recent echo  
373 intensity reviews by Stock and Thompson [73] and Wong et al [82].

374

### 375 **Practical Body Composition & Novel Use of Bioelectrical Impedance Analysis (BIA)**

376

377 Laboratory-based methods of body composition estimation potentially offer an error reduction of  
378 approximately 50% in body fat estimation compared to field-based methods. However, these  
379 improvements are partially attributable to the ability to control numerous physiological  
380 assumptions [89]. The inherent error of body composition measurement is further complicated  
381 using either population-specific or generalized prediction equations that likely compound the  
382 error in some individuals or groups. For athletes, the need for dietary restriction, adequate  
383 hydration, and standardized physical activity further exacerbates the issue.

384

385 Whole body estimation of body fat percentage is limited with its greatest utility in large  
386 population/epidemiological evaluations or public health settings. Site- or region-specific values  
387 to evaluate tissue distribution may be more useful for practitioners and athletes. Dual-energy X-  
388 ray absorptiometry enables potential evaluation of total body and site-specific fat mass, lean soft  
389 tissue mass, and bone mineral density [89]. However, these devices are expensive, vary across  
390 manufacturers, require low-dose radiation exposure, all of which may be prohibitive in many  
391 practical settings.

392

393 A viable alternative is the direct evaluation of skinfold thickness either at a specific site or as a  
394 sum of several sites throughout the body, instead of using prediction equations to determine body  
395 density. While standardized protocols and trained evaluators are required, this method seems  
396 least affected by the inherent variation in the typical restrictions recommended for body  
397 composition assessments [90]. Although less commonly reported in the literature, recent  
398 attempts have been made to provide normative data for the summation of skinfold measurements  
399 [90,91].

400

401 As skinfold thickness does not directly assess skeletal muscle, it is recommended to evaluate this  
402 information alongside anthropometric data such as regional circumferences (limb, hip/waist, etc.)  
403 and/or body mass [92,93]. Fig 6 illustrates how changes in skinfold thickness can be translated to  
404 body fat, while circumferences or body mass can serve as a proxy for muscle mass and the  
405 interaction between these values.

406

407 This combined information can also be used to calculate corrected girth values (which account  
408 for an adjustment of skinfold thickness to determine musculoskeletal cross-sectional area) or lean  
409 mass index (a log-based adjustment for body mass and summed skinfolds) [94] . Notably,  
410 DeFreitas et al. [95] demonstrated that, despite underestimating muscle cross-sectional area  
411 compared to peripheral quantitative computed tomography (pQCT), both a corrected girth  
412 equation [96] and a regression equation [97] adequately tracked changes in this value during an  
413 eight-week resistance training program. A recent review by Duarte et al. [98] discusses  
414 numerous validated anthropometric equations for limb-specific muscle mass estimation.

415  
416 BIA offers a unique approach for body composition assessment by estimating the fat-free body,  
417 generally assumed to be ~73% hydrated, rather than fat mass [89]. However, the basic  
418 assumptions of body shape (i.e., the segments of the body are perfect cylinders), as well as the  
419 use of prediction equations (either through published work or developed by the device  
420 manufacturer), introduce similar problems to other measurement methods. Therefore, it is  
421 becoming increasingly common to directly evaluate the raw bioelectric parameters [resistance  
422 (R), reactance (Xc), and phase angle (PhA)] recorded by these devices (typically at 50 kHz).

423  
424 From a practical perspective, R may represent cellular hydration, Xc may represent cell  
425 membrane integrity, and PhA is calculated as the arctangent ratio of Xc to R. The latter variable  
426 may be considered representative of intra- and extra-cellular fluid (or ICW/ECW ratio) and/or  
427 cell body mass, which has been suggested as an indicator of cellular health. These values can be  
428 considered separately or plotted together through bioelectrical impedance vector analysis  
429 (BIVA). The resultant vectors have been shown to differentiate between competitive levels and  
430 types of athletes [99]. Interestingly, Kim et al. [100] demonstrated the discriminative potential of  
431 BIVA by distinguishing between female fashion models, dancers, and gymnasts in a manner  
432 similar to somatotyping with increasing mesomorphy (i.e., muscularity) and decreasing  
433 ectomorphy (i.e., linearity) across these groups. Furthermore, a recent systematic review reported  
434 that PhA increases with age and is higher in athletes than controls as well as in males than  
435 females [101].

436  
437 The evaluation of raw bioelectrical data can also be applied regionally, a process known as  
438 electrical impedance myography or localized BIA. Initially developed to examine diseased tissue  
439 and subsequently, sarcopenic individuals, its application in athletic populations has been  
440 established, focusing on adaptations to exercise and return-to-play situations following injury  
441 [102].

442  
443 In adopting an “innovation through simplification” stance towards body composition assessment,  
444 this section of the paper underscores the importance of understanding and contextually applying  
445 the available methodologies. For a comprehensive overview of available methods, and a practical  
446 decision-making tree readers are encouraged to consult Kasper et al. [90]. Further, a detailed  
447 discussion on related topics is provided by Lukaski & Raymond-Pope [99].

#### 448 **Skeletal Muscle Pain**

449  
450 Pain is a sensory and emotional experience impacted by the interaction of biological and  
451 psychosocial factors [103]. One biologic factor that impacts the perception of pain is skeletal  
452

453 muscle health. In a state of inflammation or musculoskeletal pathology, myofascial trigger points  
454 may develop within the tissue that contribute to a myofascial pain syndrome [104]. Myofascial  
455 pain is prevalent with 30% of individuals seeking care for pain at a primary care office meeting  
456 the criteria for myofascial pain. While the definition of a trigger point can vary, there is general  
457 agreement among clinicians and scientists that myofascial pain is a separate diagnosis from  
458 fibromyalgia [105] and that trigger points contribute to myofascial pain syndrome [106].

459  
460 Myofascial trigger points are localized, taut bands of skeletal muscle tissue (Fig 7). A recent  
461 Delphi survey established that two of the following three criteria should be present to diagnose a  
462 myofascial trigger point: taut band, hypersensitive spot, and referred pain [107]. During direct  
463 compression, a ‘jump response’ may be elicited with or without referred pain [106]. Myofascial  
464 trigger points are often both painful to palpation and can be a generator of pain. While the  
465 mechanisms underlying trigger points are multifactorial, repetitive eccentric contractions or  
466 overuse may result in an abnormal increase in acetylcholine at the neuromuscular junction of the  
467 muscle. Abnormal acetylcholine release may generate a sustained muscle contraction, causing  
468 localized ischemia and a palpable taut band [108]. Although myofascial pain was traditionally  
469 thought to only involve peripheral changes, muscle sensitization is also impacted by central  
470 nervous system sensitization [109].

471  
472 A diagnosis of myofascial pain syndrome relies on the palpation of myofascial trigger points.  
473 However, a limitation in the clinical assessment is varied inter-rater reliability in myofascial  
474 trigger point identification [110]. New approaches have been proposed to improve the  
475 identification of trigger points, including: pressure algometry and imaging. Pressure Pain  
476 Threshold (PPT) is a cost-effective and clinically feasible technique that may be employed to  
477 assess trigger points. During PPT, a device with a small rubber tip (algometer) is applied over the  
478 muscle with an ascending intensity until the individual reports that the sensation changed from  
479 “comfortable pressure to slightly unpleasant pain” (pain threshold). The benefit of this  
480 assessment is the stimulus is quantifiable and, therefore, the threshold for pain perception in  
481 response to pressure is measured. PPT is significantly lower over trigger points and increases in  
482 areas without trigger points [111]. Excellent intra and inter-rater reliability is demonstrated for  
483 PPT application over trigger points [112].

484  
485 Recent advances have also allowed for imaging of trigger points [113]. Imaging methods,  
486 including ultrasound, magnetic resonance imaging, and infrared thermography have been  
487 developed as objective measures to potentially address the limitations with reliability. Imaging  
488 allows for objective characterization of the tissue consistent with the definition of a trigger point.  
489 B-mode ultrasound imaging indicates trigger points present as spherical, hypoechoic regions  
490 [56]. Ultrasound elastography indicates decreased vibration amplitudes within the region,  
491 indicative of localized stiffness of the muscle [114] at the site of the trigger point. Blood flow to  
492 myofascial trigger points is also distinct from healthy tissue [114]. Magnetic resonance imaging  
493 has been used to examine trigger points; however, the evidence remains unclear on the benefit of  
494 this imaging modality [115] for this purpose. Trigger points are important contributors to  
495 myofascial pain and relevant to clinical treatment of patients with myofascial pain. Innovations  
496 in standardizing the definition of trigger points, along with advances in the imaging of muscle,  
497 may help to make this phenomenon more objective.

498



499 **Summary**

500  
501 Accurate and precise assessment of skeletal muscle health is imperative for diagnosis of disease  
502 and optimization of exercise and rehabilitation interventions. Our review has highlighted several  
503 viable, novel techniques with potential to advance these aims. Although not comprehensive, we  
504 have focused on select emerging approaches based on their promise for impactful discoveries  
505 and feasible implementation. Rapid technological innovations and subsequent adoption seem  
506 poised to accelerate and expand prior methods. Despite progress, outstanding questions remain  
507 regarding individual variation in exercise responsiveness, organ crosstalk, biomarker  
508 development, and monitoring and enhancing athletic performance. It is our hope that continued  
509 technical advances in assessing skeletal muscle health will provide insights into these critical  
510 topics in exercise and rehabilitation science.

511  
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## 850 Figure Legends

- 851  
852 Figure 1. Dynamic imaging (B-mode) of lateral abdominal wall musculature using an elastic belt to keep  
853 linear transducer fixed to the abdomen throughout movement. Created with BioRender.com.
- 854 Figure 2. Muscle belly displacement versus time the phases of contraction elicited by TMG with key  
855 parameters comprise displacement (Dm), contraction time (Tc), delay time (Td), contraction velocity (Vc)  
856 ( $Vc = [90\%Dm - 10\%Dm / Tc]$ ), sustain time (Ts), and half-relaxation time (Tr). (Top). An example of  
857 (TMG) placement for the lumbar erector spinae (bottom).
- 858 Figure 3. Illustrates the gold standard for the determination of TAOP which requires at least one  
859 technician. More recently, innovative approaches have been developed which leverage proprietary  
860 algorithms to determine TAOP and do not require a trained technician(s). These devices can often be

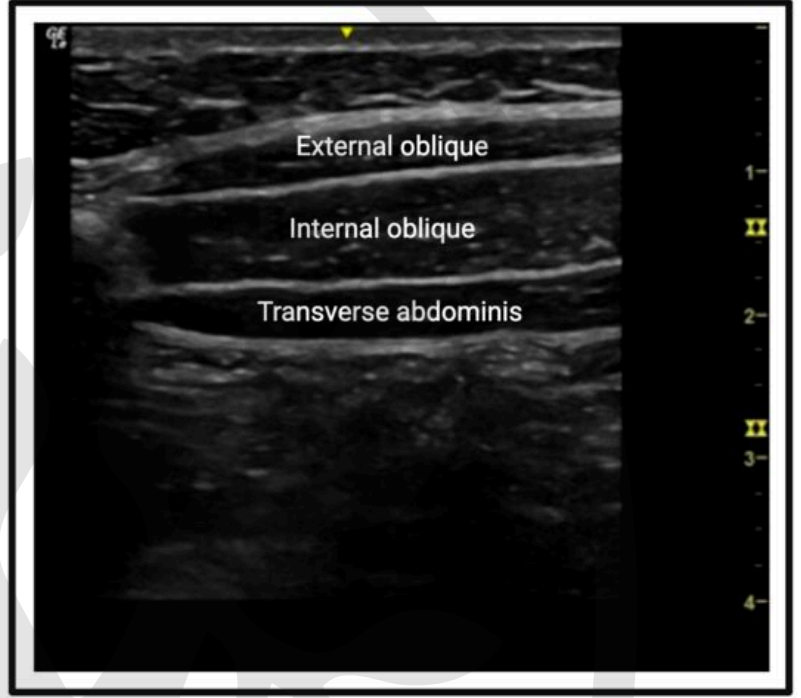
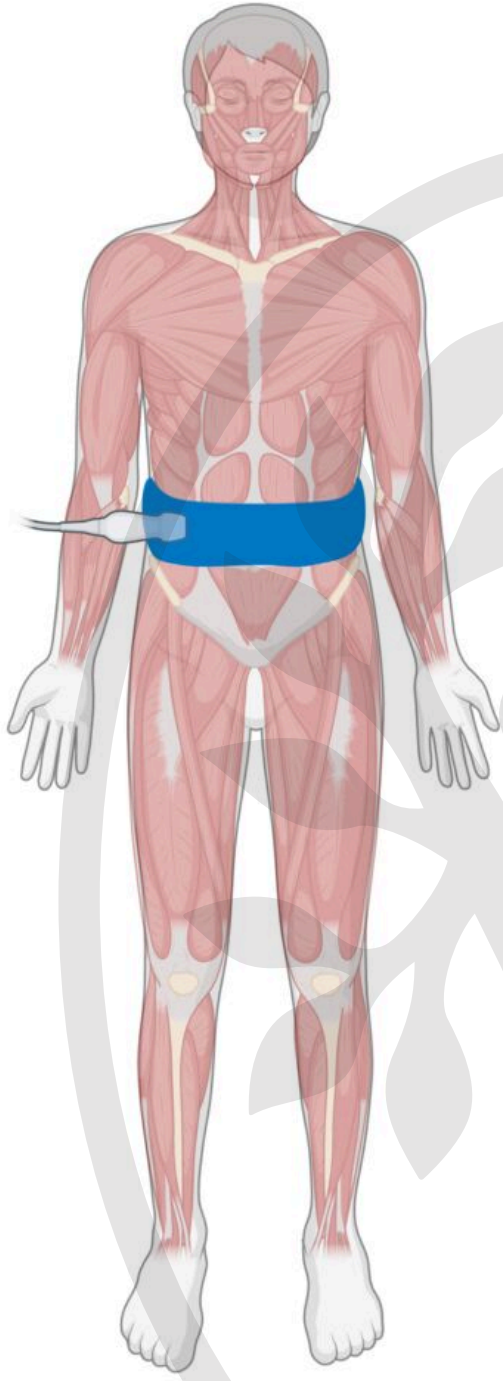
861 controlled wirelessly from a mobile app and detached from the air source facilitating, greater utility in  
862 exercise and rehabilitation settings. Created with BioRender.com.

863 Figure 4. Detection of serological peptide fragments. 1. Proteolytic cleavage of collagen protein 2.  
864 Peptide fragments enter blood stream, 3. Blood sample obtained, 4. Antibodies raised against neoepitope  
865 markers, 5. Quantification vis assay e.g., flow cytometry. Created with BioRender.com.

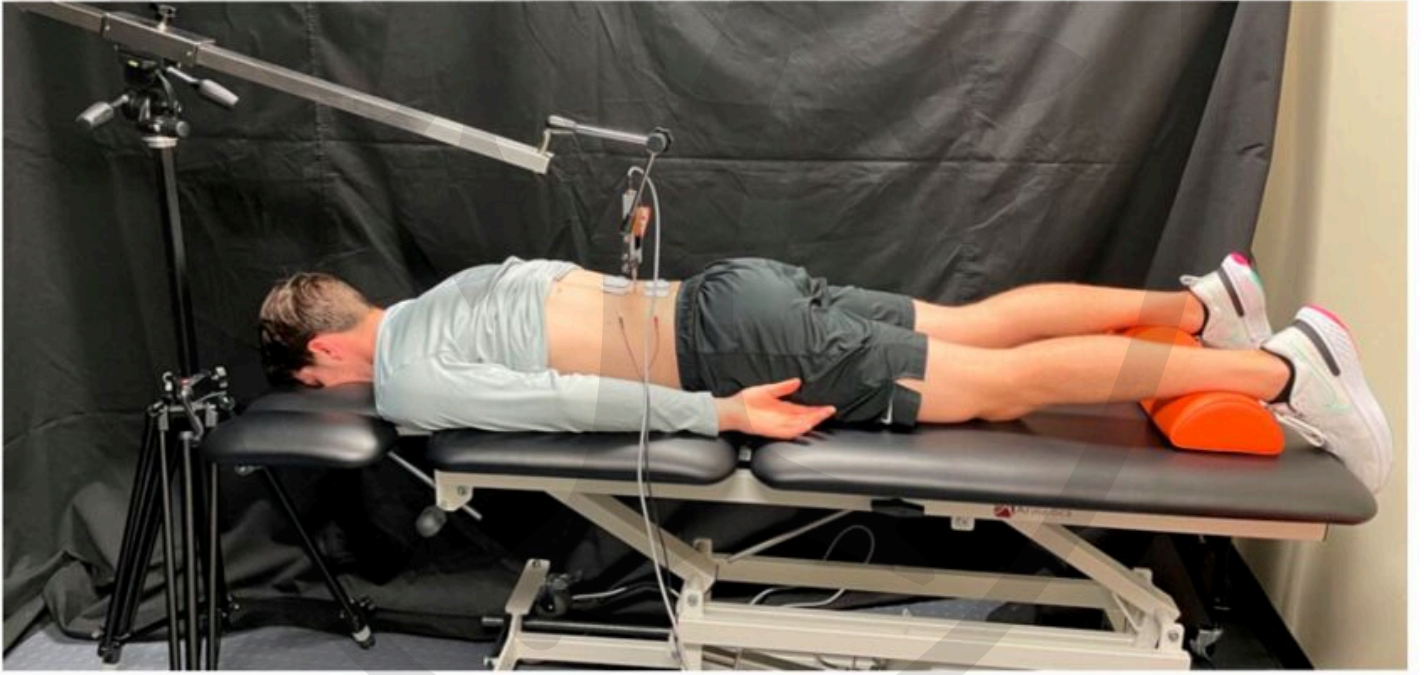
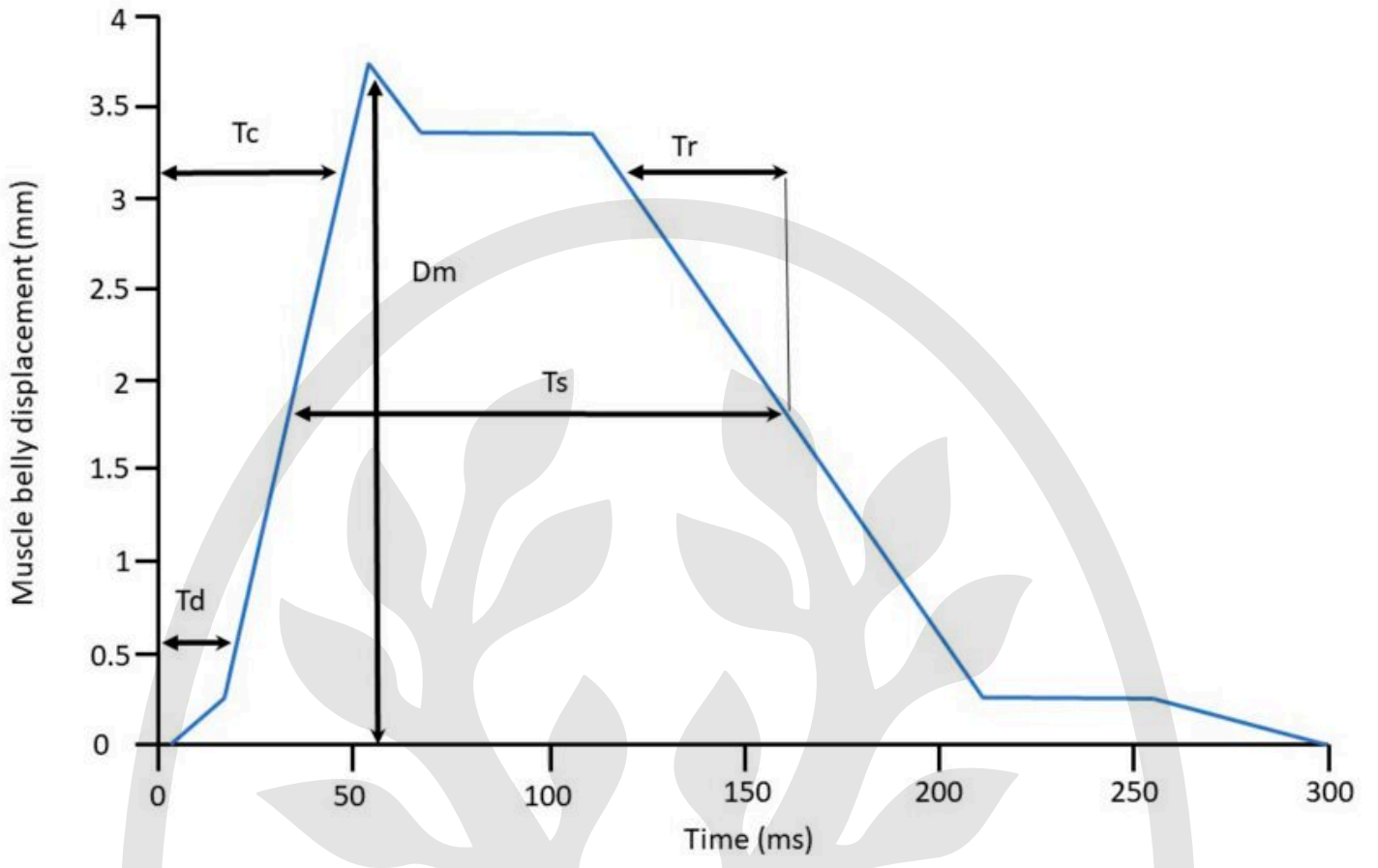
866 Figure 5. Example B-mode ultrasound images and echo intensity (EI) analyses of the vastus lateralis for  
867 an older (top) and younger (bottom) male. Note the vastly different pixel distributions for the two images.  
868 These images are a fairly accurate depiction of published findings, as many studies have reported higher  
869 echo intensity among older adults.

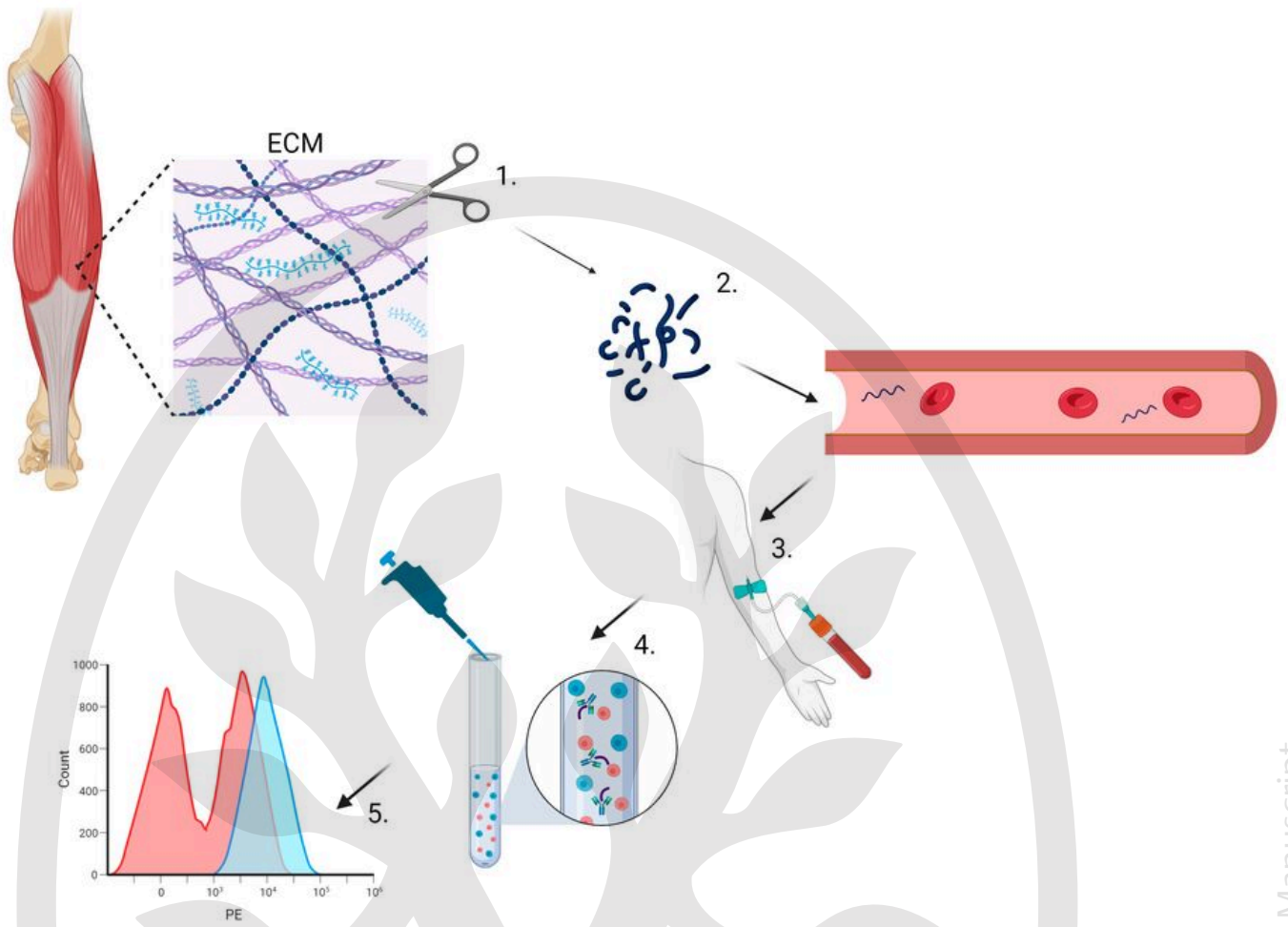
870 Figure 6. Changes in site-specific skinfold thickness and circumference (or body mass) as potential  
871 proxies of body fat and muscle mass, respectively. The examples provided indicate trends toward and  
872 away from A) muscle growth/hypertrophy, B) adiposity, C) muscle growth/hypertrophy with leanness,  
873 and D) muscle growth/hypertrophy with adiposity. Created with BioRender.com.

874 Figure 7. Overview of Methods to Identify a Myofascial Trigger Point. Myofascial trigger points may be  
875 identified with palpation or novel imaging techniques. Although not comprehensive, novel imaging  
876 techniques may include ultrasound or magnetic resonance imaging (MRI). Created with BioRender.com.



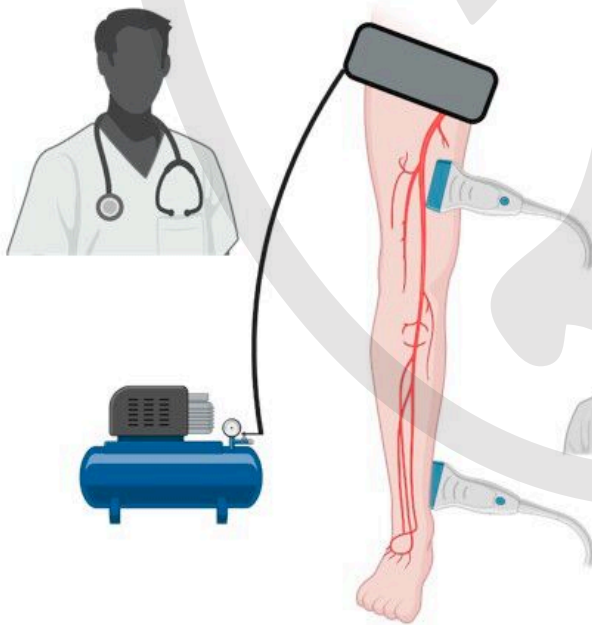




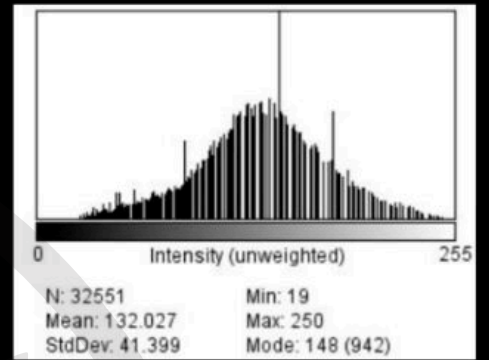
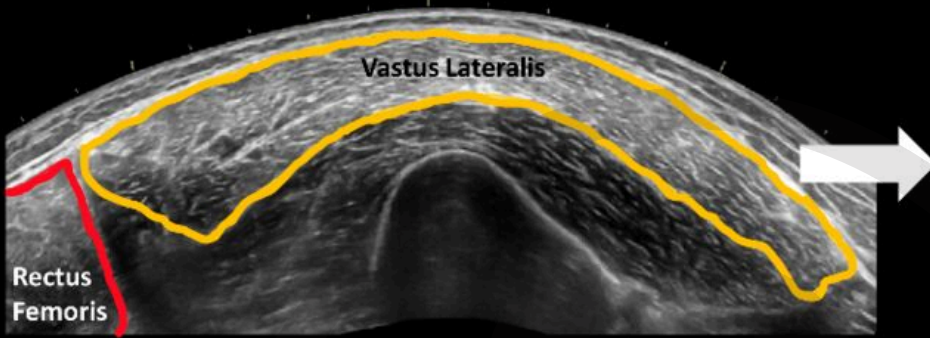


### Traditional TAOP Determination

### Proprietary Algorithms



### High EI (74-year-old male)



### Low EI (19-year-old male)

