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8	Age and sex influence the activation-dependent stiffness of the pectoralis major				
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- 35 ABSTRACT

36 The pectoralis major fiber regions contribute uniquely to the mobility and the stability of the shoulder complex. It is unknown how age and sex influence the stiffness of these regions 37 during volitional contractions, but this knowledge is critical to inform clinical interventions 38 39 targeting the pectoralis major. The aim of the present study was to determine if the activation-40 dependent stiffness of the pectoralis major fiber regions differs between the sexes and if it is altered with age. Ultrasound shear wave elastography was used to acquire shear wave velocity 41 42 from the clavicular and the sternocostal fiber regions of 48 healthy participants, including 24 43 younger (12 males, 12 females, mean  $\pm$  SD age 25  $\pm$  4.1 years) and 24 older adults (12 males, 12 44 females,  $55 \pm 3.6$  years). Participants performed vertical adduction and horizontal flexion torques in neutral and 90° externally rotated shoulder positions, and one of the two shoulder abduction 45 46 positions (60° and 90°) at varying torque magnitudes (passive, 15% and 30% of maximal 47 voluntary contraction). Separate linear mixed-effects models were run for each fiber region and 48 shoulder position to determine if the activation-dependent stiffness differed between the sexes 49 and was altered in older adults. Age-related alterations in stiffness during volitional contractions 50 were observed in both fiber regions and were dependent on the task. Alterations in activation-51 dependent stiffness due to age were more pronounced in females than males. Additionally, females had greater stiffness than males during volitional contractions in both fiber regions. The 52 53 present findings provide the first line of evidence that the activation-dependent stiffness of the 54 pectoralis major fiber regions is influenced by sex and changes with age.

55

56 Keywords: muscle activation, muscle stiffness, shear wave elastography, ultrasound

57 1. INTRODUCTION

The pectoralis major enables mobility and stability of the shoulder complex. It consists of
two fiber regions, the clavicular and the sternocostal, which have divergent functions (Carlson,
2003; Corten et al., 2007; Fung et al., 2009; Haley & Zacchilli, 2014; Lee et al., 2017; Leonardis

61 et al., 2017; Stegink-Jansen et al., 2011; Tobin, 1985; Wolfe et al., 1992). The clavicular region 62 primarily assists in humeral flexion and horizontal adduction (Ackland et al., 2008; Leonardis et 63 al., 2017; Paton & Brown, 1994), while the sternocostal region assists in humeral vertical 64 adduction, internal rotation, and extension against resistance (Ackland & Pandy, 2011; Leonardis et al., 2017; Paton & Brown, 1994; Wolfe et al., 1992). Our research group previously observed 65 66 greater elasticity of the sternocostal region while at rest and reduced muscle tissue homogeneity 67 with aging, particularly in females (Chodock et al., 2020). Since muscle stiffness is influenced by tissue elasticity and muscle architecture (Cui et al., 2008), it is unknown if these findings 68 69 translate to the stiffness of the pectoralis major during voluntary contractions. Addressing this 70 knowledge gap can provide valuable information on the importance of considering sex and age 71 in designing targeted interventions for the pectoralis major in healthy-aging adults and 72 individuals with neuromuscular pathologies.

73 Age and sex are critical factors to consider when assessing muscle properties. Aging is 74 associated with alterations in the architectural and structural properties of the muscle, impacting 75 functional independence and mobility (Buckwalter et al. 1993; Hill et al., 2010; McPhail et al., 2014; Palazzo et al., 2014). However, alterations in structural properties due to age do not appear 76 77 to be uniform across muscles. For example, under resting conditions, muscle elasticity increases 78 with age in the biceps brachii, the supraspinatus, and the sternocostal region of the pectoralis 79 major (Baumer et al., 2018; Chodock et al., 2020; Eby et al., 2015) and decreases in the lateral 80 gastrocnemius, rectus femoris, and middle trapezius (Akagi et al., 2015; Chodock et al., 2020). 81 No changes in muscle elasticity across the lifespan are observed in the soleus, anterior deltoid, 82 and the clavicular region of the pectoralis major (Akagi et al., 2015; Chodock et al., 2020). 83 During voluntary contractions, muscle elasticity increases with age in the supraspinatus (Baumer 84 et al., 2018). Moreover, differences in the structural properties between the sexes are present for some muscles, but not others. In particular, females have greater muscle stiffness while at rest 85 86 than males in the biceps brachii and the soleus (Eby et al., 2015; Saeki et al., 2019), while no sex differences are observed in lateral and medial gastrocnemius and supraspinatus stiffness (Chino 87 88 & Takahashi, 2016; Saeki et al., 2019).

Examining the pectoralis major fiber regions during voluntary contractions can provide invaluable insights into whether sex and age influence activation-dependent changes in muscle stiffness. Therefore, the purpose of the present study was to determine the effect of sex and age

92 on the stiffness of the clavicular and the sternocostal fiber regions during voluntary contractions. 93 We utilized ultrasound shear wave elastography (SWE) to measure the shear wave velocity 94 (SWV) from each fiber region during voluntary contractions. Shear wave velocity serves as an indirect measure of muscle stiffness, as prior work indicates a direct relationship between shear 95 wave velocity and muscle stiffness during muscular contraction (Bernabei et al., 2020). We 96 97 hypothesized that the older adults would exhibit lower activation-dependent stiffness than younger adults, independent of the fiber region. We further hypothesized females would have 98 99 greater activation-dependent stiffness than males, independent of the fiber region. If age- and 100 sex-related differences exist, this will provide new insights into the structural properties of the 101 pectoralis major fiber regions to consider when examining pectoralis major function in healthy 102 individuals or those with neuromuscular pathologies.

### **103 2. METHODS**

## 104 2.1 Study Participants

105 Forty-eight healthy younger and older adults (24 females, 24 males) with no history of 106 orthopedic or neuromuscular shoulder injuries participated in this study (Table 1). This sample 107 size was determined using *a-priori* power analysis, which calculated a minimum sample size of 108 24 individuals based on a conservative effect size (f) of 0.25, four groups (2 age groups by 2 sex groups), an alpha level of 0.05, and 80% power. Additional participants were recruited to 109 110 enhance study power. Forty-four of the 48 participants were right-hand dominant. Participants 111 were recruited through the University of Michigan's online research study portal and flyers 112 posted across campus. The inclusion criteria were: 1) individuals between 18 to 30 years old or 113 between 50 and 60 years old; 2) no history of orthopedic and/or neuromuscular shoulder 114 dysfunction and injuries; and 3) recreationally active. Participants were excluded if they reported 115 a history of neuromuscular shoulder dysfunction and upper extremity injury or surgery. Younger 116 adult group included participants between 18 to 30 years old, while older adults group consisted 117 of individuals between 50 to 60 years old. The University of Michigan Institutional Review 118 Board approved all study procedures (IRB#: HUM00152691), and written informed consent was 119 obtained from each participant before collecting any data.

120 2.2 Experimental Protocol

The participant was seated in a custom designed Biodex chair (Biodex Medical Systems,
Shirley, New York) with their dominant arm placed into a plastic, removable cast, as performed

123 in previous studies (Leonardis et al. 2017; Leonardis et al. 2019; Chodock et al. 2020). The cast 124 extended from the shoulder to the hand, fixing the elbow at 90° and the wrist in a neutral 125 position. Torso movement was restricted using a seatbelt and cushioned plates along the lower, 126 posterior back, and the left and right sides. Glenohumeral joint torques were measured using a 127 six-degrees-of-freedom load cell (45E15A4-M63J, JR3, Inc., Woodland, CA), which was 128 attached to the crank arm of the motor. The motor's axis of rotation was aligned to the 129 glenohumeral joint axis of rotation, approximated as the midpoint of a straight line connecting the acromion process to the anterior-most portion of the axillary fold. The coordinate 130 131 measurement system was defined as the motion of the humerus relative to the thorax (Y-X-Y 132 order) (Wu et al., 2005). The motor was controlled in real-time using Matlab Simulink Real-133 Time (2016a, Mathworks, Inc, Natick, MA).

Maximal voluntary contractions (MVC) were collected at the start of each data collection 134 135 in six different torque directions: vertical adduction and abduction, horizontal adduction and 136 abduction, internal and external rotation with the participant's arm positioned in 90° of elevation, 137  $0^{\circ}$  of the plane of elevation, and  $0^{\circ}$  of axial rotation. Verbal encouragement was provided during 138 MVC performance to ensure maximal exertions were attained. The experimental protocol 139 consisted of four arm positions, including a combination of one of two shoulder rotation positions, neutral and externally rotated 90°, and one of two shoulder abduction positions, 60° 140 141 and 90°. These positions will henceforth be abbreviated as a combination of their rotation angle 142 (N for neutral or ER for externally rotated) and frontal (i.e., coronal) plane of elevation angle (60° or 90°). The order of shoulder positions (N60, N90, ER60, ER90) was randomized first by 143 144 the rotation position (i.e., N or ER) and then by the plane of elevation angle (i.e., 60° or 90°) for 145 each participant. At each shoulder position, the participant was asked to produce and maintain isometric shoulder torques for approximately five seconds in the vertical adduction and 146 147 horizontal flexion. The magnitude of torque produced in each shoulder position was scaled to 0% 148 (e.g., at rest), 15%, and 30% of the participant's MVC. The investigators observed the 149 performance of both maximal and submaximal trials to insure the participant was executing each 150 trial correctly and without the involvement of the trunk. Participants repeated torques in each 151 direction and at each magnitude for a total of 20 trials per shoulder configuration (i.e., N60, N90, 152 ER60, ER90). A computer monitor was placed in front of the participant to provide visual 153 feedback of the target, which assisted with torque accuracy. An Aixplorer ultrasound

elastography machine (Supersonic Imagine, Aix en Provence, France) connected to an SL15-4
linear transducer array was used to collect ultrasound SWE images (Optimization: Standard,
Persistance: Medium, Smoothing: 5, Frame Rate: 12 Hz) from the pectoralis major. Two
ultrasound SWE images were collected per experimental condition from each fiber region,
resulting in 80 images collected per participant.

159 Ultrasound SWE images from the clavicular and the sternocostal fiber region of the 160 pectoralis major were collected during voluntary contractions. For the clavicular fiber region, the 161 ultrasound transducer was initially placed ~1 cm inferior to the clavicle over the muscle's 162 midpoint and shifted inferiorly from the clavicle until it was located mid-belly over the clavicular 163 fiber region. When imaging the sternocostal fiber region in male participants, the transducer was 164 initially placed ~1 cm superior to the nipple. The transducer was then shifted superiorly until it 165 was located mid-belly over the sternocostal fiber region. In females, the sternocostal fiber region 166 was imaged by placing the transducer  $\sim 4$  cm inferior to the sternoclavicular joint and then 167 moving it inferiorly until it was located mid-belly over the sternocostal fiber region. When 168 individual muscle fascicles were identified on the B-mode ultrasound image, the orientation of 169 the ultrasound transducer was considered satisfactory. An elastography color map (2.5 cm x 1 170 cm) positioned within the muscle's belly was superimposed over the B-mode image, which 171 provided pixel by pixel calculations of the participant's SWV. The same investigator collected 172 all ultrasound images.

173

# 174 2.3 Data and Statistical Analyses

The SWE images were exported from the ultrasound machine and analyzed using a 175 176 custom-written MATLAB algorithm (2015a, Mathworks Inc, Natick, MA, USA) to quantify 177 shear wave velocity and corresponding quality maps for each image (Lee et al., 2016; Lee et al., 178 2017; Leonardis et al., 2017). A region of interest encompassing the muscle belly of the fiber 179 region of the pectoralis major was manually selected by the same investigator for each image. 180 Adipose tissue and the aponeurosis were excluded to mitigate bias in the muscle data. The region 181 of interest varied between the participants due to anatomical differences. The quality map for 182 each image was used to determine the accuracy of the SWV measures at each pixel within the 183 region of interest. Only the SWV with a quality map above 0.7 (out of 1.0) were included in 184 analyses. An external trigger was used to obtain an elastography image and collect two-seconds

of torque data. Torque data were low-pass filtered at 500 Hz with a 6<sup>th</sup>-order analog Bessel filter.
Following this, the torque data were averaged across each 2-second trial and normalized to the
maximal voluntary contraction.

188 All statistical analyses were performed in MATLAB (2015a, Mathworks Inc, Natick, 189 MA, USA). The effect of age and sex on shoulder strength in the horizontal and vertical 190 directions was tested using independent t-tests. Linear mixed-effects models were used to test 191 our primary hypotheses that 1) females will have a greater activation-dependent stiffness than 192 males irrespective of the pectoralis major fiber region and that 2) older adults will have a lower 193 activation-dependent stiffness than younger adults. Mean SWV was treated as an outcome 194 measure, while age (older, younger) and sex (male, female) were between-subjects factors. Raw 195 torque was treated as a continuous variable. Separate mixed-effects models were utilized for each region within a posture (i.e., N60, N90, ER60, and ER90). We included age by sex interaction 196 197 terms within our statistical framework to determine if age-related changes in stiffness during 198 volitional contractions differed by sex. Random effects controlled for variability at the subject 199 level. Bonferonni corrected post-hoc comparisons were performed to determine if differences in 200 shear wave velocity existed between the groups and the sexes with increases in torque. For all analyses, statistical significance was set to p < 0.05 and effect sizes for each factor were 201 calculated as partial eta-squared  $(\eta_p^2)$ . 202

203

#### 204 **3.0 RESULTS**

Younger males were stronger than younger females in vertical adduction ( $t_{22} = -5.31$ , p < 0.001; **Table 1**) and horizontal flexion ( $t_{22} = -4.45$ , p < 0.001). Similarly, older males were stronger than older females in vertical adduction ( $t_{22} = -3.50$ , p = 0.002) and horizontal flexion ( $t_{22} = -2.58$ , p = 0.016). No differences in strength existed between the younger and the older males and females in vertical adduction and horizontal flexion strength (all p > 0.05).

The effect of age and sex for both pectoralis major regions during each shoulder position is visualized in shear wave elastography color maps, with representative data from younger and older males and females (**Figure 1**). These images show greater activation-dependent stiffness in both pectoralis major regions in females in comparison to males across tasks. Furthermore, the color maps also show lower activation-dependent stiffness in the clavicular region and greater activation-dependent stiffness of the sternocostal region in older females than younger females in ER60 and N60, respectively. The influence of age and sex on activation-dependent stiffness
within each task was examined using a linear mixed-effects model for each region (Table 2), and
the results of these findings are presented below.

#### 219 The clavicular region

220 Females had greater shear wave velocity with increases in horizontal flexion torque than males in N60 (p < 0.001,  $\eta_p^2 = 0.06$ ; Figure 2A) and N90 (p < 0.001,  $\eta_p^2 = 0.10$ ; Figure 2B). 221 Age did not influence shear wave velocity in N60 (p = 0.21) or N90 (p = 0.88). Age and sex 222 influenced shear wave velocities in ER60 (p = 0.0065,  $\eta_p^2 = 0.03$ ; Figure 2C) and ER90 (p = 223 0.043,  $\eta_p^2 = 0.01$ ; Figure 2D). Younger females had greater shear wave velocities than younger 224 males with increases in horizontal flexion torque in the clavicular region in ER60 (p < 0.001;  $\eta_p^2$ 225 226 = 0.29) and ER90 (p < 0.001;  $\eta_p^2 = 0.32$ ). This was also observed in older adults, where older females had greater shear wave velocities than older males with increases in horizontal flexion 227 torques in ER60 (p = 0.0015;  $\eta_p^2$  = 0.08) and ER90 (p < 0.001;  $\eta_p^2$  = 0.18). Aging only 228 229 influenced activation-dependent stiffness in females in ER60, where older females had lower 230 shear wave velocities than younger females with increased horizontal flexion torques (p < 0.001;  $\eta_p^2 = 0.10$ ). We did not observe such differences in ER90 (p = 0.047). Further, no differences in 231 232 shear wave velocity with increased horizontal flexion torque existed between younger and older 233 males in ER60 (p = 0.62) or ER90 (p = 0.47).

## 234 The sternocostal region

235 Age and sex influenced shear wave velocities of the sternocostal region in N60 (Age by Sex interaction: p = 0.0011;  $\eta_p^2 = 0.04$ ; Figure 3A). In terms of age effects, older females had 236 237 greater shear wave velocity with increases in vertical adduction torques than younger females (p = 0.0058,  $\eta_p^2 = 0.06$ ). Shear wave velocity was the same in younger and older males (p = 0.102). 238 239 Sex-related differences were also observed in both older and younger adults in N60. Specifically, 240 older females had greater shear wave velocities with increases in vertical adduction torques than older males (p < 0.001,  $\eta_p^2 = 0.25$ ). Similarly, younger females had greater shear wave velocities 241 than younger males with increases in vertical adduction torques (p = 0.0015,  $\eta_p^2 = 0.08$ ). In 242 ER60, older adults had lower shear wave velocities than younger adults (p = 0.0356,  $\eta_p^2 = 0.01$ ), 243 while females had greater shear wave velocities than males (p < 0.001,  $\eta_p^2 = 0.05$ ; Figure 3C) 244 245 with increases in vertical adduction torques. We also observed sex differences in N90 and ER90, 246 where females had greater shear wave velocities than males with increases in vertical adduction torques (both p < 0.001; N90:  $\eta_p^2 = 0.07$ ; ER90:  $\eta_p^2 = 0.09$ ; Figure 3B and Figure 3D). Shear wave velocities were not influenced by age in N90 (p = 0.52) or ER90 (p = 0.10).

249

## 250 4.0 DISCUSSION

251 This study aimed to investigate if age and sex affect the activation-dependent changes in 252 the stiffness of the pectoralis major fiber regions. First, we hypothesized that the older adults 253 would have lower activation-dependent stiffness than younger adults across tasks, independent of 254 the pectoralis major fiber region. In contrast, we observed reduced activation-dependent stiffness 255 of the clavicular region and greater activation-dependent stiffness of the sternocostal region only 256 in the older females in comparison to younger females in ER60 and N60, respectively. We also 257 observed reductions in the activation-dependent sternocostal region stiffness in older adults in ER60 compared to younger adults. Second, we hypothesized that females would have a greater 258 259 stiffness than males during voluntary contractions, which was supported by our results.

260 The present study provides evidence that sex and age differentially affect the activation-261 dependent changes to the stiffness of each fiber region of the pectoralis major. Older females 262 exhibited greater activation-dependent stiffness than younger females in the sternocostal region 263 when generating vertical adduction torques in N60. In contrast, activation-dependent stiffness was reduced in older females compared to younger females in the clavicular region when 264 265 generating horizontal flexion torques in ER60. These differences cannot be attributed to potential 266 age-related declines in strength, as both groups exhibited similar strength in vertical and 267 horizontal directions. The present findings may reflect task-specific age-related alterations in the 268 activation of the clavicular region given shear wave velocity increases with increases in muscle 269 activation (Chernak et al., 2013; Nordez & Hug, 2010; Yoshitake et al., 2014). While the 270 sternocostal region contribution to vertical adduction torques is greater at higher contractions in 271 N60, both regions equally assist in generating horizontal flexion torques in ER60 (Leonardis et 272 al., 2017). The altered neuromuscular control of the clavicular region in older females may 273 prompt greater utilization of the sternocostal region to generate vertical adduction torques and 274 potentially rely on other shoulder muscles to generate horizontal flexion torques. As such, 275 greater activation of the anterior deltoid or coracobrachialis may be observed in older females 276 during the generation of horizontal flexion torques. We also observed reductions in the 277 activation-dependent stiffness of the sternocostal region in older groups as participants generated vertical adduction torques in ER60. These findings indicate position-specific alterations in the activation-dependent stiffness. The generation of vertical adduction torques in this position relies on the contribution of both pectoralis major fiber regions (Leonardis et al., 2017). Therefore, it may be that older adults utilize differential neuromuscular strategy to generate vertical adduction torques in this position, such as greater reliance on the clavicular fiber region or greater recruitment of other shoulder muscles.

284 Females had greater activation-dependent stiffness than males irrespective of the 285 pectoralis major fiber region in all tasks. Since muscle activity modulates shear wave velocity, 286 differences in the stiffness of these groups may be due to a neurally mediated muscle stiffening 287 effect (Chernak et al., 2013). Sex and age-related differences in muscle activation, architectural 288 or neural properties are presently unknown for the pectoralis major fiber regions. Studies in other 289 muscles reported greater surface electromyography (EMG) amplitudes in females than males in 290 vastus medialis and rectus femoris (Krishnan & Williams, 2009), tibialis anterior (Cioni et al., 291 1994), and adductor pollicis (Visser & De Ruke, 1974) in submaximal tasks. Moreover, recent 292 studies using intramuscular electromyography reported higher motor unit discharge rates in 293 females than males in tibialis anterior and vastus medialis in submaximal tasks (Inglis & Gabriel, 294 2020; Peng et al., 2018). Lastly, differences in architectural properties, such as muscle fiber pennation angles and muscle fiber lengths, may contribute to the observed sex-related 295 296 differences in stiffness during volitional contraction. Lower pennation angles and longer muscle 297 fiber lengths are typically observed in females than males (Chow et al., 2000; Manal et al., 298 2006). Interestingly, females in the present study generated lower torques than males but 299 required greater activation-dependent stiffness, indicating that males require less contribution or 300 activation of the pectoralis major fiber regions to generate greater shoulder torques. These 301 findings further indicate that differences in neural and architectural properties may be playing a 302 key role in the sex-related functional utility of the pectoralis major fiber regions. The neural and 303 architectural properties, however, were not directly measured in the present study, limiting our interpretation of the present findings. 304

There are limitations that accompany the current study. Our findings may not translate to elderly adults, as we did not evaluate any participants above 60 years old. There are known alterations to neuromuscular properties, fiber quantity, and strength in individuals older than 60 (Baumgartner et al., 1995; Campbell et al., 1973; Fukumoto et al., 2015; Frontera et al., 2000;

309 Hepple & Rice, 2016; Lexell et al., 1988), and it is reasonable to suspect these changes could 310 influence muscle stiffness. Our participants were predominantly recreationally active, and our 311 findings may not translate to sedentary or highly trained individuals. The tasks were performed 312 only in the frontal plane, although the pectoralis major contributes to shoulder joint torque 313 throughout the range of motion. Further, participants generated torques only in two planes of 314 rotation and MVCs were only obtained in a single posture (i.e. N90). Lastly, the tasks were 315 isometric and only examined up to 30% MVC as shear wave elastography has not been validated 316 above 60% MVC (Yoshitake et al., 2014).

These findings collectively provide the first in vivo evidence of age- and sex-related 317 318 differences in stiffness of the pectoralis major fiber regions during voluntary contractions. 319 Alterations in the activation-dependent stiffness of the clavicular fiber region are evident in older 320 females, indicating age-related changes in the role and utilization of the clavicular region. This 321 may influence an older female's ability to produce horizontal flexion torques effectively. Further, 322 females have greater activation-dependent stiffness than males in both regions of the pectoralis 323 major, irrespective of age, but generate substantially less torque. These differences may indicate 324 divergent neural, architectural, and global activation properties of the pectoralis major fiber regions between the sexes. Together, these findings suggest that sex and age are important 325 factors to consider when assessing and designing interventions targeting the function of 326 327 pectoralis major fiber regions in healthy adults and individuals with neuromuscular pathologies. 328 Moreover, differences between males and females characterized in this study may have critical 329 implications in understanding the increased incidence of musculoskeletal injuries in females.

330

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335

# 336 CONTRIBUTIONS

- 337 Research design: CAS, TL, JML, MK, DBL; Data acquisition: CAS, JML, MK; Data analyses:
- 338 CAS, TL, DBL; Statistical analyses: TL, DBL; Drafting manuscript: CAS, TL, DBL; Revising

manuscript: CAS, TL, JML, MK, DBL. All authors have read and approved the final submittedmanuscript.

341

# 342 DATA AVAILABILITY STATEMENT

343 The data that support the findings of this study are available from the corresponding author upon 344 reasonable request.

345

# References

- Ackland DC, Pak P, Richardson M, Pandy MG (2008) Moment arms of the muscles crossing the
  anatomical shoulder. *Journal of Anatomy* 213(4), 383–390. https://doi.org/10.1111/j.14697580.2008.00965.x
- Ackland DC, Pandy MG (2011) Moment arms of the shoulder muscles during axial rotation.
   *Journal of Orthopaedic Research* 29(5), 658–667. https://doi.org/10.1002/jor.21269
- Akagi R, Yamashita Y, Ueyasu Y (2015) Age-related differences in muscle shear moduli in the
   lower extremity. *Ultrasound in Medicine and Biology* 41(11), 2906–2912.
- 353 https://doi.org/10.1016/j.ultrasmedbio.2015.07.011
- Baumer TG, Dischler J, Davis L, Labyed Y, Siegal DS, van Holsbeeck M, Moutzouros V, Bey
- 355 MJ (2018) Effects of age and pathology on shear wave speed of the human rotator cuff.
- *Journal of Orthopaedic Research* **36**(1), 282–288. https://doi.org/10.1002/jor.23641
- 357 Baumgartner RN, Stauber PM, McHugh D, Koehler KM, Garry PJ (1995) Cross-sectional age
- differences in body composition in persons 60 + years of age. *Journals of Gerontology* -
- 359 *Series A Biological Sciences and Medical Sciences* **50**A(6), M307–M316.
- 360 https://doi.org/10.1093/gerona/50A.6.M307
- 361 Bernabei M, Lee SSM, Perreault EJ, Sandercock TG (2020) Shear wave velocity is sensitive to
- 362 changes in muscle stiffness that occur independently from changes in force. *Journal of*
- 363 *Applied Physiology (Bethesda, Md. : 1985)* **128**(1), 8–16.
- 364 https://doi.org/10.1152/japplphysiol.00112.2019
- Buckwalter JA, Woo L-YS, Goldberg VM, Hadley EC, Booth F, Oegema TR, Eyre DR (1993) *The Journal of Bone and Joint Surgery* 75(10), 1533-1548.
- 367 Campbell MJ, McComas AJ, Petito F (1973) Physiological changes in ageing muscles. *Journal* 368 of Neurology Neurosurgery and Psychiatry 36(2), 174–182.

369 https://doi.org/10.1136/jnnp.36.2.174

- 370 Carlson ER (2003) Pectoralis major myocutaneous flap. Oral and Maxillofacial Surgery Clinics
   371 of North America 15(4), 565–575. https://doi.org/10.1016/S1042-3699(03)00060-8
- 372 Chernak LA, Dewall RJ, Lee KS, Thelen DG (2013) Length and activation dependent variations
- in muscle shear wave speed. *Physiological Measurement* **34**(6), 713–721.
- 374 https://doi.org/10.1088/0967-3334/34/6/713
- 375 Chino K, Takahashi H (2016) Measurement of gastrocnemius muscle elasticity by shear wave
  376 elastography: association with passive ankle joint stiffness and sex differences. *European*377 *Journal of Applied Physiology* 116(4), 823–830. https://doi.org/10.1007/s00421-016-3339-5
- 378 Chodock E, Hahn J, Setlock CA, Lipps DB (2020) Identifying predictors of upper extremity
- muscle elasticity with healthy aging. *Journal of Biomechanics* **103**, 109687.
- 380 https://doi.org/10.1016/j.jbiomech.2020.109687
- 381 Chow RS, Medri MK, Martin DC, Leekam RN, Agur AM, McKee NH (2000) Sonographic
- 382 studies of human soleus and gastrocnemius muscle architecture: Gender variability.
- 383 *European Journal of Applied Physiology* **82**(3), 236–244.
- 384 https://doi.org/10.1007/s004210050677
- Cioni R, Giannini F, Paradiso C, Battistini N, Navona C, Starita A (1994) Sex differences in
   surface EMG interference pattern power spectrum. *Journal of Applied Physiology* 77(5),
- 387 2163–2168. https://doi.org/10.1152/jappl.1994.77.5.2163
- 388 Corten EML, Schellekens PPA, Oey PL, Hage JJ, Kerst A, Kon M (2007) Function of the
- clavicular part of the pectoralis major muscle after transplantation of its sternocostal part.
   *Annals of Plastic Surgery* 58(4), 392–396.
- 391 https://doi.org/10.1097/01.sap.0000238427.18396.ea
- Cui L, Perreault EJ, Maas H, Sandercock TG (2008) Modeling short-range stiffness of feline
  lower hindlimb muscles. *Journal of Biomechanics* 41(9), 1945–1952.
- 394 https://doi.org/10.1016/j.jbiomech.2008.03.024
- 395 Eby SF, Cloud BA, Brandenburg JE, Giambini H, Song P, Chen S, Lebrasseur NK, An KN
- 396 (2015) Shear wave elastography of passive skeletal muscle stiffness: Influences of sex and
- age throughout adulthood. *Clinical Biomechanics* **30**(1), 22–27.
- 398 https://doi.org/10.1016/j.clinbiomech.2014.11.011
- 399 Frontera WR, Hughes VA, Fielding RA, Fiatarone MA, Evans WJ, Roubenoff R (2000) Aging

- 400 of skeletal muscle: A 12-yr longitudinal study. *Journal of Applied Physiology* 88(4), 1321–
  401 1326. https://doi.org/10.1152/jappl.2000.88.4.1321
- 402 Fukumoto Y, Ikezoe T, Yamada Y, Tsukagoshi R, Nakamura M, Takagi Y, Kimura M,
- 403 Ichihashi N (2015) Age-Related ultrasound changes in muscle quantity and quality in
- 404 women. Ultrasound in Medicine and Biology **41**(11), 3013–3017.
- 405 https://doi.org/10.1016/j.ultrasmedbio.2015.06.017
- 406 Fung L, Wong B, Ravichandiran K, Agur A, Rindlisbacher T, Elmaraghy A (2009) Three-
- 407 dimensional study of pectoralis major muscle and tendon architecture. *Clinical Anatomy*
- 408 **22**(4), 500–508. https://doi.org/10.1002/ca.20784
- Haley CA, Zacchilli MA (2014) Pectoralis Major Injuries: Evaluation and Treatment. *Clinics in Sports Medicine* 33(4), 739–756. https://doi.org/10.1016/j.csm.2014.06.005
- 411 Hepple RT, Rice CL (2016) Innervation and neuromuscular control in ageing skeletal muscle.
- 412 *Journal of Physiology* **594**(8), 1965–1978. https://doi.org/10.1113/JP270561
- 413 Hill CL, Gill TK, Shanahan EM, Taylor AW (2010) Prevalence and correlates of shoulder pain
- and stiffness in a population-based study: The North West Adelaide Health Study.
- 415 *International Journal of Rheumatic Diseases* **13**(3), 215–222.
- 416 https://doi.org/10.1111/j.1756-185X.2010.01475.x
- 417 Inglis JG, Gabriel DA (2020) Sex differences in motor unit discharge rates at maximal and
- 418 submaximal levels of force output. *Applied Physiology, Nutrition, and Metabolism* **45**(11):
- 419 1197-1207. https://doi.org/10.1139/apnm-2019-0958
- 420 Krishnan C, Williams GN (2009) Sex Differences in Quadriceps & Hamstrings EMG-Moment
- 421 Relationships. *Medicine and Science in Sports and Exercise* **41**(8), 1652-1660.
- 422 https://doi.org/10.1249/MSS.0b013e31819e8e5d
- 423 Lee SSM, Gaebler-Spira D, Zhang LQ, Rymer WZ, Steele KM (2016) Use of shear wave
- 424 ultrasound elastography to quantify muscle properties in cerebral palsy. *Clinical*425 *Biomechanics* 31, 20–28. https://doi.org/10.1016/j.clinbiomech.2015.10.006
- 426 Lee YK, Skalski MR, White EA, Tomasian A, Phan DD, Patel DB, Matcuk GR, Schein AJ
- 427 (2017) US and MR imaging of pectoralis major injuries. *Radiographics* 37(1), 176–189.
  428 https://doi.org/10.1148/rg.2017160070
- 429 Leonardis JM, Desmet DM, Lipps DB (2017) Quantifying differences in the material properties
- 430 of the fiber regions of the pectoralis major using ultrasound shear wave elastography.

- 431 *Journal of Biomechanics* 63, 41–46. https://doi.org/10.1016/j.jbiomech.2017.07.031
- 432 Leonardis JM, Lyons DA, Giladi AM, Momoh AO, Lipps DB (2019) Functional integrity of the
  433 shoulder joint and pectoralis major following subpectoral implant breast reconstruction. J
  434 Orthop Res 37(7), 1610-1619. https://doi.org/10.1002/jor.24257
- 435 Lexell J, Taylor CC, Sjöström M (1988) What is the cause of the ageing atrophy?. Total number,
- size and proportion of different fiber types studied in whole vastus lateralis muscle from 15-
- to 83-year-old men. *Journal of the Neurological Sciences* **84**(2–3), 275–294.
- 438 https://doi.org/10.1016/0022-510X(88)90132-3
- 439 Manal K, Roberts DP, Buchanan TS (2006) Optimal pennation angle of the primary ankle
- 440 plantar and dorsiflexors: Variations with sex, contraction intensity, and limb. *Journal of*
- 441 *Applied Biomechanics* **22**(4), 255–263. https://doi.org/10.1123/jab.22.4.255
- 442 McPhail SM, Schippers M, Marshall AL (2014) Age, physical inactivity, obesity, health
- 443 conditions, and health-related quality of life among patients receiving conservative
- 444 management for musculoskeletal disorders. *Clinical Interventions in Aging* **9**, 1069–1080.
- 445 https://doi.org/10.2147/CIA.S61732
- 446 Nordez A, Hug F (2010) Muscle shear elastic modulus measured using supersonic shear imaging
- 447 is highly related to muscle activity level. *Journal of Applied Physiology* 108(5), 1389–1394.
  448 https://doi.org/10.1152/japplphysiol.01323.2009
- 449 Palazzo C, Ravaud JF, Papelard A, Ravaud P, Poiraudeau S (2014) The burden of
- 450 musculoskeletal conditions. *PLoS ONE* **9**(3). https://doi.org/10.1371/journal.pone.0090633
- 451 Paton ME, Brown JMM (1994) An electromyographic analysis of functional differentiation in
- 452 human pectoralis major muscle. *Journal of Electromyography and Kinesiology* **4**(3), 161–
- 453 169. https://doi.org/10.1016/1050-6411(94)90017-5
- 454 Peng YL, Tenan MS, Griffin L (2018) Hip position and sex differences in motor unit firing
- 455 patterns of the vastus medialis and vastus medialis oblique in healthy individuals. *Journal of*456 *Applied Physiology* 124(6), 1438–1446. https://doi.org/10.1152/japplphysiol.00702.2017
- 457 Saeki J, Ikezoe T, Yoshimi S, Nakamura M, Ichihashi N (2019) Menstrual cycle variation and
- 458 gender difference in muscle stiffness of triceps surae. *Clinical Biomechanics* 61, 222–226.
  459 https://doi.org/10.1016/j.clinbiomech.2018.12.013
- 460 Stegink-Jansen CW, Buford WL, Patterson RM, Gould LJ (2011) Computer simulation of
- 461 pectoralis major muscle strain to guide exercise protocols for patients after breast cancer

- 462 surgery. *Journal of Orthopaedic and Sports Physical Therapy* **41**(6), 417–426.
- 463 https://doi.org/10.2519/jospt.2011.3358
- Tobin GR (1985) Pectoralis major segmental anatomy and segmentally split pectoralis major
   flaps. *Plastic and Reconstructive Surgery* 75(6), 814–824.
- 466 https://doi.org/10.1097/00006534-198506000-00009
- Visser SL, De Ruke W (1974) Influence of sex and age on EMG contraction pattern. *European Neurology* 12(4), 229–235. https://doi.org/10.1159/000114623
- Wolfe SW, Wickiewicz TL, Cavanaugh JT (1992) Ruptures of the pectoralis major muscle. *Surgery* 10(2), 309–312. https://doi.org/10.1177/036354659202000517
- 471 Wu G, Van Der Helm FCT, Veeger HEJ, Makhsous M, Van Roy P, Anglin C, Nagels J, Karduna
- 472 AR, McQuade K, Wang X, Werner FW, Buchholz B (2005) ISB recommendation on
- 473 definitions of joint coordinate systems of various joints for the reporting of human joint
- 474 motion Part II: Shoulder, elbow, wrist and hand. *Journal of Biomechanics* **38**(5), 981–992.
- 475 https://doi.org/10.1016/j.jbiomech.2004.05.042
- 476 Yoshitake Y, Takai Y, Kanehisa H, Shinohara M (2014) Muscle shear modulus measured with
- 477 ultrasound shear-wave elastography across a wide range of contraction intensity. *Muscle*
- 478 *and Nerve* **50**(1), 103–113. https://doi.org/10.1002/mus.24104



## 479 Tables and table captions

- 480 **Table 1**. General participant information (mean  $\pm$  SD). The asterisk indicates the significance
- 481 level between younger females and males, and older females and males (\* p < 0.05, \*\*\* p < 0.05)
- **482** 0.001).

Characteristic	Young Female	Young Male	Older Female	Older Male
	(n=12)	(n=12)	(n=12)	(n=12)
Age (years)	$25.1 \pm 4.3$	$25.0\pm4.0$	$55.0\pm4.4$	$55.1 \pm 2.7$
Height (cm)	$165.4\pm7.1$	$177.6\pm7.2$	$156.3\pm11.5$	$179.2\pm5.5$
Weight (kg)	$59.1\pm5.0$	$74.5\pm6.0$	$70.4\pm16.6$	$79.9 \pm 12.4$
BMI (kg/m²)	$21.5\pm1.9$	$23.8\pm2.0$	$27.4\pm5.5$	$24.6\pm3.1$
Upper Arm Length (cm)	$29.3\pm2.5$	$32.1\pm3.0$	$28.7\pm3.9$	$33.0\pm2.3$
Forearm Length (cm)	$26.0\pm1.6$	$28.1\pm1.6$	$25.9\pm2.7$	$29.3\pm2.3$
Vertical Adduction Strength (Nm)	53.9 ± 15.6***	103.4 ± 28.2***	53.0 ± 10.5***	88.3 ± 33.2***
Horizontal Flexion Strength (Nm) 484	37.4 ± 14.7***	66.9 ± 17.6***	44.3 ± 8.9*	65.3 ± 26.6*

Table 2. Parameter estimates of mean shear wave velocity with the standard error shown for 486 each variable within the linear mixed-effects model for each of the pectoralis major fiber regions 487 in each task. Significant parameters are bolded. The adjusted R<sup>2</sup> for each model is also shown. 488 The asterisk indicates the significance level (\* p < 0.05, \*\* p < 0.01, \*\*\* p < 0.001). 489

		Mean Shear Wave Velocity (m/s)		
Task	Measures	Clavicular	Sternocostal	
N60	Intercept	3.94 (0.36)***	2.74 (0.30)***	
	Torque	0.21 (0.02)***	0.12 (0.01)***	
	Age*Torque	-0.034 (0.024)	-0.024 (0.014)	
	Sex*Torque (Reference: Female)	0.13 (0.03)***	0.056 (0.02)***	
	Age*Sex*Torque (Reference: Female)	0.019 (0.046)	0.099 (0.03)***	
	Adjusted R <sup>2</sup> for model	0.77	0.78	
N90	Intercept	4.12 (0.31)***	3.33 (0.33)***	
	Torque	0.19 (0.02)***	0.093 (0.010)***	

	Age*Torque	0.003 (0.021)	0.009 (0.014)
	Sex*Torque (Reference: Female)	0.16 (0.03)***	0.094 (0.021)***
	Age*Sex*Torque (Reference: Female)	-0.016 (0.041)	0.015 (0.030)
	Adjusted R <sup>2</sup> for model	0.79	0.76
ER60	Intercept	4.71 (0.30)***	3.36 (0.28)***
	Torque	0.17 (0.02)***	0.11 (0.01)***
	Age*Torque	-0.009 (0.023)	-0.030 (0.014)*
	Sex*Torque (Reference: Female)	0.22 (0.03)***	0.071 (0.019)***
	Age*Sex*Torque (Reference: Female)	-0.12 (0.05)**	0.054 (0.028)
	Adjusted R <sup>2</sup> for model	0.75	0.78
ER90	Intercept	4.81 (0.29)***	4.37 (0.27)***
	Torque	0.14*** (0.02)	0.07 (0.01)***
	Age*Torque	0.016 (0.021)	-0.021 (0.013)
	Sex*Torque (Reference: Female)	0.22 (0.03)***	0.097 (0.019)***
	Age*Sex*Torque (Reference: Female)	-0.081 (0.040)*	0.012 (0.028)
	Adjusted R <sup>2</sup> for model	0.78	0.69

490

# 491 Figure captions

492 Figure 1. Representative shear wave elastography maps for each pectoralis major fiber region. 493 Each row shows the images from a representative young female, young male, older female, and 494 older male participant. Each column represents the resultant color map for a given task (N60, 495 N90, ER60, and ER90). Warmer colors indicate higher shear wave velocities, and cooler colors 496 indicate lower shear wave velocities. Note the high shear wave velocities in females in 497 comparison to males, irrespective of the task. These color maps indicated lower shear wave 498 velocities were observed for the clavicular region in older females in ER60 when compared to 499 younger females. Higher shear wave velocities were observed in the sternocostal region in older females in N60 when compared to younger females. 500

501

Figure 2. Influence of age and sex on mean shear wave velocity in the clavicular fiber region for
 each task while producing horizontal flexion torques at rest, 15%, and 30% MVC. White filled
 circles represent young females; yellow filled circles represent young males; red filled squares

represent older females, and orange filled squares represent older males. The resultant fit of the linear mixed effects model for each group is presented as lines of best fit with shaded regions (white solid line: young females; yellow solid line: young males; red solid line: older females; orange solid line: older males). A: N60. Females had higher shear wave velocities than males. B: N90. Females had higher shear wave velocities in comparison to males. C: ER60. Older females had lower shear wave velocities than younger females, and females had higher shear wave velocities than males. D: ER90. Females had higher shear wave velocities than males.

512

Figure 3. Influence of age and sex on mean shear wave velocity of the sternocostal fiber region 513 514 for each task while producing vertical adduction torques at rest, 15% and 30% MVC. White 515 filled circles represent young females; yellow filled circles represent young males; red filled 516 squares represent older females, and orange filled squares represent older males. The resultant fit 517 of the linear mixed effects model for each group is presented as lines of best fit with shaded 518 regions (white solid line: young females; yellow solid line: young males; red solid line: older 519 females; orange solid line: older males). A: N60. Note the higher shear wave velocities in older 520 and younger females in comparison to older and younger males. Older females also had higher 521 shear wave velocities in comparison to younger females. B: N90. Higher shear wave velocities 522 were observed in females than males. C: ER60. Older adults had lower shear wave velocities 523 than younger adults, and females had higher shear wave velocities in than males. D: ER90. 524 Higher shear wave velocities were observed in females in comparison to the males.

# Autho



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