1	Title: Rectus abdominis muscle thickness is a valid measure of cross-sectional area: implications for
2	ultrasound
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26 ABSTRACT

27 Rationale and Objectives: The rectus abdominis muscle exhibits early and significant muscle atrophy, 28 which has largely been characterized using ultrasound measured muscle thickness. However, the validity 29 of rectus abdominis muscle thickness as a metric of muscle size has not been established, limiting 30 precise interpretation of age-related changes. In a heterogeneous cohort of women and men, our 31 objectives were to: 1) evaluate the association between rectus abdominis muscle thickness and cross-32 sectional area (CSA), and 2) examine if the visceral adipose tissue (VAT) compartment confounds the 33 validity of rectus abdominis muscle thickness. Materials and Methods: Abdominal computed tomography scans of the 3rd lumbar from clinical and 34 healthy populations were used to evaluate rectus abdominis thickness and CSA, and VAT CSA. Computed 35 36 tomography scans were utilized due to the limited field of view of ultrasound imaging to capture the 37 rectus abdominis CSA. Results: A total of 348 individuals (31% women) were included in this analysis, with a mean ± standard 38 39 deviation age and body mass index of 51.2 ± 15.4 years and 28.0 ± 5.1 kg/m², respectively. Significant 40 correlations were observed between rectus abdominis thickness and CSA for women (r=0.758; p<0.001) 41 and men (r=0.688; p<0.001). Independent of age, VAT CSA was negatively associated with rectus 42 abdominis thickness in men (p=0.011), but not women (p=0.446). 43 **Conclusion:** These data support the use of rectus abdominis muscle thickness as a measurement of 44 muscle size in both women and men; however, the VAT compartment may confound its validity to a 45 minor extent in men.

46 Key Words: ultrasound, muscle thickness, rectus abdominis, aging, muscle cross-sectional area

47 INTRODUCTION

48 Aging is associated with skeletal muscle atrophy and deleterious deposition of intramuscular 49 adipose tissue (IMAT), which impairs strength and functional capacity of older adults [1,2]. Typically, 50 these age-related changes in skeletal muscle are quantified using whole-body approaches such as dual-51 energy x-ray absorptiometry. However, we, and others [3–9] have demonstrated that the quadriceps 52 and rectus abdominis muscles are particularly prone to age-related atrophy. Moreover, muscle atrophy 53 of the rectus abdominis occurs earlier in life compared with other muscles [10,11]. These age-related 54 declines in the quadriceps and rectus abdominis muscles can lead to increased risk of falls, fractures, 55 physical disability, loss of independence, and mortality [12–14]. 56 Computed tomography (CT) and magnetic resonance imaging (MRI) are considered reference 57 standards for assessing the cross-sectional area or volume of specific muscle groups [12,15,16]; yet they 58 have limited accessibility and portability. Ultrasound is a portable and accessible tool that is increasingly 59 being used to quantify site-specific muscle size in older adults [12,17–21]. However, ultrasound is 60 frequently limited to analyzing muscle thickness, as cross-sectional area (CSA) is challenging for many 61 muscle groups due to a limited lateral field of view (typically 3-5 cm). Although ultrasound is often 62 limited to analysis of muscle thickness, several publications have observed strong associations with 63 muscle CSA or volume in several appendicular muscle groups, such as the rectus femoris and biceps 64 brachii [22–24]. Despite this wide-spread use of rectus abdominis thickness for characterizing age-65 related muscle atrophy [3,5–11], the validity in relation to muscle CSA has not been examined. Studies 66 confirming the validity of rectus abdominis thickness are essential to ensure accurate interpretation of 67 age-related muscle atrophy in this muscle group. 68 Here, our primary objective was to examine the association between rectus abdominis thickness

Here, our primary objective was to examine the association between rectus abdominis thickness
 and CSA in a heterogenous cohort of women and men. Due to ultrasound's limited field of view, we
 utilized abdominal CT scans to compare rectus abdominis muscle thickness and CSA. Furthermore, given

that the rectus abdominis is uniquely adjacent to the visceral adipose tissue (VAT) cavity, as a secondary

objective, we explored if the VAT tissue compartment confounds the interpretation of muscle thickness.

73 METHODS

74 Description of cohort

75 Clinically acquired abdominal CT scans were utilized in this study for analysis of rectus abdominis 76 thickness and CSA, as well as VAT CSA. To obtain a diverse range of body composition phenotypes, scans 77 were comprised of liver and renal donors [25] and pancreatic cancer [26], renal cancer [27], liver 78 cirrhosis [25], and critically ill [28] patients. A single investigator visually determined if the rectus 79 abdominis fascial borders were distinct from the lateral abdominal musculature (i.e., internal and 80 external obligues) to ensure precise analysis of rectus abdominis CSA. Of the initial 893 CT scans, 545 81 scans were excluded due to an inability to distinguish the rectus abdominis CSA from the lateral 82 abdominal wall musculature. All research included in this analysis was approved by local and institutional research ethics boards and conducted in accordance with established protocols for human 83 84 research.

Participant's age (range 18 to 88 years), sex, weight, height, and body mass index (BMI) were extracted from medical charts. Of the 348 participants, data was missing for age (n=2), height (n=6), weight (n=4), and BMI (n=6).

88 Muscle and visceral adipose cross-sectional area analysis

Scans of the 3rd lumbar vertebrae were manually landmarked from a series of CT scans. The 3rd
lumbar vertebrae corresponds to a commonly used landmarking site for ultrasound imaging of the
rectus abdominis (umbilicus) [5,29]. CT scans were manually segmented for skeletal muscle and VAT CSA
by trained analysts at the University of Waterloo using SliceOmatic image analysis software
(TomoVision, Montreal, Canada, version 5.0). Using a brush tool, the various tissues were segmented
based on the Hounsfield unit (HU) thresholds of skeletal muscle (-29 to 150 HU) and VAT (-150 to -50

HU). Once tissue compartments were defined, all pixels within the compartment were summed, thenmultiplied by the pixel surface area to determine the tissue CSA.

97 Rectus abdominis cross-sectional area and thickness analysis

98 Rectus abdominis CSA and thickness were measured on participants right side using ImageJ 99 software (Version 1.52e, National Institutes of Health, MD) by two investigators (CRK, MTP). CSA was 100 analyzed using the polygon tool to trace the facial boarders of the entire right rectus abdominis muscle 101 (Figure 1A). Muscle thickness was analyzed at the thickest location where the superficial and deep 102 rectus abdominis fascia borders were parallel. Using the straight tool, the distance between the 103 superficial and deep borders of the rectus abdominis were measured at an angle perpendicular to the 104 parallel fascia (Figure 1B). A sample of 35 (~10%) randomly selected scans for inter-rater reliability 105 yielded a coefficient of variation of 4.79% for CSA and 4.83% for thickness. To examine if IMAT 106 infiltration influences the correlation between rectus abdominis thickness and CSA, IMAT-corrected CSA 107 was analyzed by removing the pixels in the IMAT HU range (-190 to -30 HU) from the original CSA region 108 of interest.

109 Statistical analysis

Normality of data was confirmed using QQ-plots. Student's t-tests were used to compare 110 111 differences in physical and body composition characteristics between women and men. Pearson 112 correlation coefficients were used to evaluate the associations between rectus abdominis thickness and 113 CSA or IMAT-corrected CSA in women and men. Pearson correlation coefficients between rectus 114 abdominis muscle thickness and CSA were further evaluated based on clinical cohort subgroups (donors, 115 cancer, and critically ill; liver cirrhotic patients were not evaluated separately due to small sample sizes). 116 Multiple linear regression analysis was used to examine if VAT CSA was associated with rectus abdominis 117 thickness or CSA, independent of age, in women and men. Age, VAT CSA, and an age by VAT CSA interaction were included in the linear regression models for rectus abdominis thickness or CSA. 118

Statistical analyses were performed using SPSS (version 26, IBM, USA) with p<0.05 defining statistical
 significance.

121 **RESULTS**

A total of 348 individuals were included in this analysis, with 31.3% (n=109) being female. Of these, 35.6% were donors, and 38.8%, 22.1%, 3.5% were cancer, critically ill, and liver cirrhosis patients, respectively. The men were significantly older (p=0.009), taller (p<0.001), heavier (p<0.001), and had a higher BMI (p<0.001) than the women (Table 1).

126 Total muscle and VAT CSA were significantly larger in the men compared to the women

127 (p<0.001) (Table 2). Similarly, rectus abdominis CSA, IMAT-corrected CSA, and thickness were

significantly larger in men compared with women (p<0.001) (Table 2).

129 Rectus abdominis thickness was positively associated with CSA in women (r=0.758; p<0.001;

130 Figure 2A) and men (r=0.688; p<0.001; Figure 2B). A similar association was found for IMAT-corrected

rectus abdominis CSA and thickness in both women (r=0.771; p<0.001; Figure 2C) and men (r=0.715;

132 p<0.001; Figure 2D). Subgroup specific associations between rectus abdominis thickness and CSA

displayed similar strength associations across all clinical cohorts, except for women cancer patients

134 (Table S1).

Negative associations were observed between age and either rectus abdominis CSA or thickness
for both men and women (Table S2). However, only men demonstrated negative associations between
VAT CSA and either rectus abdominis CSA or thickness (Table S2). Whereas women had displayed a
negative association between VAT CSA and rectus abdominis thickness, but not rectus abdominis CSA
(Table S2).

140 In women, age, but not VAT CSA, was independently associated with rectus abdominis thickness 141 (p<0.001) and CSA (p=0.011) (Table 3). In men, both age (p<0.001) and VAT CSA (p=0.011) were 142 independently associated with rectus abdominis thickness. However, age (p=0.008), but not VAT CSA

(p=0.299) was associated with rectus abdominis CSA in men (Table 3). Age by VAT CSA interactions were
not significant in women or men for rectus abdominis thickness (women: p=0.924, men: p=0.065) and
CSA (women: p=0.228, men: p=0.850) (Table 3).

146 **DISCUSSION**

The primary objective of this study was to examine the association between rectus abdominis thickness and CSA in a heterogenous cohort of women and men. We observed that rectus abdominis thickness is strongly and positively associated with CSA in both women and men. Furthermore, we observed that age was independently associated with both rectus abdominis thickness and CSA in both sexes, whereas VAT CSA was only independently associated with rectus abdominis thickness in men. To the best our knowledge, this is the first study to confirm that the rectus abdominis thickness is a valid measure of muscle size.

154 The rectus abdominis muscle is prone to age-related skeletal muscle atrophy, which has largely 155 been demonstrated using ultrasound measurements of muscle thickness [3,5–11]. This age-related 156 degradation of the rectus abdominis may predispose older adults to perturbations in gait and posture 157 and are associated with increased risk of metabolic syndrome [30,31]. Despite the increasing prevalence 158 of using rectus abdominis muscle thickness as a metric of muscle size, its association with muscle CSA 159 had not been examined, limiting accurate interpretation of age-related atrophy of the rectus abdominis 160 using ultrasound. In the present study, we observed strong associations between rectus abdominis 161 muscle thickness and CSA in women (r=0.758 to 0.771) and men (r=0.688 to 0.715), indicating that the 162 thickness of this muscle is indeed a valid metric for muscle size. These associations are particularly robust, given that they were observed using a diverse cohort of healthy and clinical populations of a 163 164 wide age-range.

165 Similar to our findings, others have also observed strong associations between thickness and 166 cross-sectional area in various limb and trunk muscles [24,32,33]. Miyatani et al. (2004) observed

167 significant associations between ultrasound muscle thickness and MRI muscle volume for the elbow 168 flexors (r=0.893), elbow extensors (r=0.734), knee extensors (r=0.469), and ankle plantar flexors 169 (r=0.806). Abe et al (1997) also demonstrated a strong correlation (r=0.91, p<0.001) between ultrasound 170 anterior mid-thigh muscle thickness and MRI quadriceps CSA in men. Additionally, trunk musculature, 171 such as the supraspinatus [34], pectoralis major [33], and psoas major [35], have demonstrated 172 significant associations (r=0.76-0.95) between ultrasound muscle thickness and MRI muscle CSA. Overall, 173 the strength of associations we observed for the rectus abdominis muscle thickness and CSA are similar 174 to those previously observed for a variety of limb and trunk muscles. 175 Unlike limb or upper trunk muscles, the lower abdominal wall musculature (e.g., rectus 176 abdominis) is uniquely located adjacent to the VAT compartment. Consequently, as the VAT 177 compartment expands, the rectus abdominis, and the lower abdominal wall musculature in general, 178 would be required to encapsulate a larger circumference. While this circumferential expansion may not 179 influence the overall muscle CSA, it may artificially reduce the thickness of the rectus abdominis, which would confound its association with CSA. After controlling for age, we observed that VAT CSA was 180 181 negatively associated with rectus abdominis thickness in men, but not women; however, these associations were not observed for rectus abdominis CSA for either men or women. The presence of an 182 183 associations between VAT CSA and thickness in men, but not in women, may be related to the 184 significantly larger VAT CSA in the men compared to the women (193.8 \pm 109.6 cm² vs. 89.8 \pm 69.7 cm², 185 p<0.001). It should be noted that a small change in VAT CSA (1 cm²) will only be associated with a minor 186 decrease in rectus abdominis thickness (~0.17 % reduction); although, given the large variation in VAT 187 CSA in men, $(\pm 109.6 \text{ cm}^2)$, it may confound the validity of rectus abdominis muscle thickness. Despite 188 the influences of VAT CSA on rectus abdominis thickness, univariate correlation analysis still indicates a 189 strong association.

190 There are several limitations associated with this study. While the implications of these findings 191 are important for ultrasound imaging of skeletal muscle, analysis of muscle thickness and CSA were 192 performed using CT scans. However, previous work has demonstrated that thickness and CSA analysed from CT and ultrasound are similar [36]. While the 3rd lumbar vertebrae is a similar site to the commonly 193 194 used umbilicus landmark used for ultrasound imaging of the rectus abdominis, individual variability 195 exists for the exact lumbar location of the umbilicus (e.g., L3-L4). Therefore, the exact landmarks 196 between CT and ultrasound imaging may not completely align. While the inclusion of a diverse cohort 197 ensures that the associations we observed between thickness and CSA are robust, it may add further 198 confounding factors, which would weaken the strength of association that would be expected in more 199 homogeneous cohorts (e.g., older vs younger adults).

200 CONCLUSION

201 We observed strong associations between rectus abdominis thickness and CSA in both women

and men. VAT CSA was independently associated with rectus abdominis thickness in men, but not

203 women, which may marginally confound the validity of thickness as a measure of muscle size. Overall,

this work demonstrates that rectus abdominis thickness is a valid measurement of CSA.

205 Conflict of Interests

206 The authors declare there are no conflicts of interest.

207 Funding

208 Acquisition and analysis of renal cancer CT scans was supported by a grant from the National Cancer

209 Institute (R01 CA233885) and Memorial Sloan Kettering Cancer Center Core Grant (P30-CA008748). MTP

210 was supported by a CIHR doctoral award.

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Table 1. Physical characteristics

	All	Women	Men		
	(n=348)	(n=109)	(n=239)	p-value	
Age, (years)	51.2 ± 15.4	47.9 ± 15.0	52.6 ± 15.5	0.009	
Proportion female (%)	31.3	-	-		
Height (m)	1.72 ± 0.10	1.63 ± 0.06	1.76 ± 0.08	<0.001	
Weight (kg)	83.1 ± 18.2	68.5 ± 10.3	89.6 ± 17.2	<0.001	
BMI (kg/m²)	28.0 ± 5.1	25.8 ± 3.85	28.9 ± 5.3	<0.001	
Cohort					
Cancer (n)	135 (38.8 %)	34 (31.2 %)	101 (42.3 %)	-	
Donors (n)	124 (35.6 %)	54 (49.5 %)	70 (29.3 %)	-	
Critically ill (n)	77 (22.1 %)	18 (16.5 %)	59 (24.7 %)	-	
Liver cirrhosis (n)	12 (3.4 %)	3 (2.8 %)	9 (3.8 %)	-	

319 Values expressed as mean ± standard deviation. Abbreviations: BMI, body mass index.

Table 2. Body composition characteristics

	All	Women	Men	
	(n=348)	(n=109)	(n=239)	p-value
Total muscle CSA (cm ²)	160.1 ± 40.8	117.2 ± 18.7	179.7 ± 32.3	<0.001
VAT CSA (cm²)	161.2 ± 109.9	89.8 ± 69.7	193.8 ± 109.6	<0.001
Rectus abdominis thickness (cm)	1.09 ± 0.30	0.97 ± 0.25	1.15 ± 0.31	<0.001
Rectus abdominis CSA (cm ²)	7.3 ± 2.4	5.4 ± 1.3	8.6 ± 2.2	<0.001
IMAT-corrected rectus	6.9 ± 2.4	5.0 ± 1.3	7.8 ± 2.2	<0.001
abdominis CSA (cm²)				

322 Values expressed as mean ± standard deviation. Abbreviations: CSA, cross-sectional area; IMAT,

323 intramuscular adipose tissue; VAT, visceral adipose tissue

	Women			Men				
	B-coefficients	p-value	Model		B-coefficients	<u> </u>	Model	
	(SE)		R ²	Partial R	(SE)	p-value	R ²	Partial R
Rectus abdominis								
thickness		<0.001	0.37			<0.001	0.32	
Age	-0.008 ± 0.002	<0.001		-0.33	-0.012 ± 0.002	<0.001		-0.36
VAT CSA	-0.001 ± 0.001	0.446		-0.08	-0.002 ± 0.001	0.011		-0.17
Age x VAT CSA	0.000 ± 0.000	0.924		0.01	0.000 ± 0.000	0.065		0.12
Rectus abdominis								
CSA		<0.001	0.20			<0.001	0.11	
Age	-0.032 ± 0.012	0.011		-0.25	-0.047 ± 0.017	0.008		-0.17
VAT CSA	0.012 ± 0.007	0.089		0.17	0.005 ± 0.005	0.299		0.07
Age x VAT CSA	0.000 ± 0.000	0.228		-0.12	0.000 ± 0.000	0.850		0.01
Age VAT CSA Age x VAT CSA	0.012 ± 0.007 0.000 ± 0.000	0.089		-0.23 0.17 -0.12	0.005 ± 0.005 0.000 ± 0.000	0.299 0.850		_

Table 3. Multiple linear regression analysis of rectus abdominis thickness and cross-sectional area

327 Abbreviations: CSA, cross-sectional area; VAT, visceral adipose tissue

Figure 1.



334 Figure Captions

- 335 Figure 1. Depiction of A) cross-sectional area and B) thickness CT analysis of the rectus abdominis at the
- level of the 3rd lumbar vertebra. A) CSA tracing of right rectus abdominis muscle using ImageJ polygon
- tool. B) Thickness measurement of right rectus abdominis muscle using ImageJ straight tool. Images are
- 338 magnified on the right rectus abdominis.
- **Figure 2.** Pearson correlation comparing rectus abdominis muscle thickness and cross-sectional area or
- 340 IMAT-corrected cross-sectional area for men and women. A) thickness vs. cross-sectional for women, B)
- 341 thickness vs cross-sectional area for men, C) thickness vs. IMAT-cross-sectional area for women, and D)
- 342 thickness vs. IMAT-cross-sectional area for men. Abbreviations: CSA, cross-sectional area; IMAT,
- intramuscular adipose tissue. All correlations p<0.001.