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ORIGINAL RESEARCH

CONTRIBUTION OF PROTEIN INTAKE AND CONCURRENT EXERCISE TO SKELETAL MUSCLE QUALITY WITH AGING

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Abstract: *Background:* The use of magnetic resonance imaging (MRI) derived functional cross-sectional area (FCSA) and intramuscular adipose tissue (IMAT) to define skeletal muscle quality is of fundamental importance in order to understand aging and inactivity-related loss of muscle mass. *Objectives:* This study examined factors associated with lower-extremity skeletal muscle quality in healthy, younger, and middle-aged adults. *Design:* Cross-sectional study. Setting and Participants: Ninety-eight participants (53% female) were classified as younger (20-35 years, n=50) or middle-aged (50-65 years, n=48) as well as sedentary (≤1 day per week) or active (≥3 days per week) on self-reported concurrent exercise (aerobic and resistance). *Measurements:* All participants wore an accelerometer for seven days, recorded a three-day food diary, and participated in magnetic resonance imaging (MRI) of the lower limbs. Muscle cross-sectional area (CSA) was determined by tracing the knee extensors (KE) and plantar flexors, while muscle quality was established through the determination of FCSA and IMAT via color thresholding. *Results:* One-way analysis of variance and stepwise regression models were performed to predict FCSA and IMAT. KE-IMAT (cm^2) was significantly higher among sedentary (3.74 ± 1.93) vs. active (1.85 ± 0.56) and middle-aged (3.14 ± 2.05) vs. younger (2.74 ± 1.25) ($p < 0.05$). Protein intake ($g \cdot kg \cdot day^{-1}$) was significantly higher in active (1.63 ± 0.55) vs. sedentary (1.19 ± 0.40) ($p < 0.05$). Sex, age, concurrent exercise training status, and protein intake were significant predictors of KE FCSA ($R^2 = 0.71$, $p < 0.01$), while concurrent exercise training status and light physical activity predicted 33% of the variance in KE IMAT ($p < 0.01$). *Conclusion:* Concurrent exercise training, dietary protein intake, and light physical activity are significant determinants of skeletal muscle health and require further investigation to mitigate aging and inactivity-related loss of muscle quality.

Key words: Protein, functional CSA, IMAT, physical activity, aging.

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Introduction

Skeletal muscle quality is challenging to define as the cross-sectional area (CSA) of the muscle itself does not reflect health or function (1). For example, inter and intramuscular adipose tissue (IMAT), defined as the visible adipose tissue beneath the muscle fascia and between muscle groups, as well as functional cross-sectional area (FCSA), the area of muscle isolated from fat within the CSA, maybe more useful indicators of muscle quality as they reflect muscle composition (2, 3). IMAT, in particular, has been linked with many metabolic abnormalities (4). Previous research has shown that individuals who are obese or have conditions such as diabetes or peripheral neuropathy have higher amounts of IMAT when compared to healthy adults (5). Additionally, age-associated changes in muscle composition, including increased fatty infiltration and reduced fat oxidation within skeletal muscle tissue, results in elevated levels of IMAT in older adults (6). However, IMAT may be used as an endogenous fuel source as individuals increase their physical activity level (5), and various interventions have been shown to reduce IMAT, including multimodal exercise programs (6-9), dietary interventions (10), and both dietary and exercise cooperatively (11).

Dietary protein intake in combination with exercise, in particular, can influence cross-sectional muscle area and may be related to FSCA in conjunction with interactions with other factors, including physical activity, health status, age, and body mass and composition (12). For instance, a single meal that contains ~30 g of high-quality protein can stimulate muscle protein synthesis in healthy adults, leading to increases in muscle size (13, 14). Protein intake also has a positive association with IMAT in younger individuals (27-31 years) (15), and there is a correlation between muscle size and IMAT regardless of age (16). Thus, the purpose was to identify factors that were related to skeletal muscle quality in healthy, younger, and middle-aged men and women. We hypothesized that concurrent exercise training status, the time within various physical activity intensities, age, sex, and dietary protein intake would be significant contributors to FCSA and IMAT quantity.

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Methods

Participants completed three lab sessions, including two in the research laboratory at North Dakota State University campus and one at Sanford Broadway Clinic, Fargo, North Dakota, during this cross-sectional study. The initial visit involved completing a training session on the three-day food diary, along with an informed consent and health history questionnaire. The return visit to the laboratory included the provision of a three-day food diary and an accelerometer. Participants' third visit was to the local hospital to complete magnetic resonance imaging (MRI) of the lower extremity. There was an average of four weeks between visits one and two, due to recruitment meetings and the capacity of the research laboratory, and there were less than two weeks between visits two and three.

Participants

Participants were recruited from the local area through information sessions, flyers, and word of mouth over two years. Participants were included in this study if they were generally healthy: 1) without any conditions that limit protein consumption and regular physical activity, 2) were not taking medications that may influence anabolism (e.g., testosterone therapy), and 3) classified into one of the two age groups: younger (20-35 years of age) and middle-aged (50-65 years of age). They were excluded if they did not meet the above criteria. Eligible participants after being categorized into two age groups: younger and middle-aged and were further sub-classified by determining their concurrent exercise training status. Sedentary individuals were not participating in resistance or aerobic exercise more than one time per week. Physically active individuals were participating in three or more days per week of aerobic and resistance exercise at a moderate-to-vigorous intensity. Before starting the study, all participants provided written informed consent approved by the university's Institutional Review Board for the protection of human participants.

Muscle CSA and FCSA Measurements

MRI provides a highly detailed image of skeletal muscle tissue that can be analyzed to quantify IMAT, FSCA, and CSA (2, 16). In this capacity, multi-slice analysis of the mid-thigh has been shown to reduce the standard error of the estimate, increase the improvement of the prediction (4), and to assess CSA, FCSA, and IMAT accurately. For this investigation, serial axial plane MRI scans from a 3.0 T Siemens Skyra Intera whole-body scanner (Siemens Healthcare Headquarters, Erlangen, DE) were obtained at the local hospital (Sanford Broadway Clinic, Fargo, ND). Licensed radiology technicians, in collaboration with researchers, obtained images. Participants were positioned with elevated heels and knees to minimize the distortion of the muscles to be analyzed. The MRI settings were as follows: repetition time = 3730 m/s, 10 mm slice-to-slice interval, 420-500 mm x 328-390 mm field of view. ImageJ version 1.42 (National Institutes of Health, Bethesda, MD) was used to analyze MRI-derived muscle CSA on a personal computer (MacBook Pro, Apple Inc., Cupertino, CA). Images of the left leg from the MRI were analyzed. Knee extensor (KE) images selected for analysis started with the first image in which the rectus femoris was visible proximately to the first slice before the appearance of the gluteal muscles. Depending on the height of the participant, the research started with seven to nine slices. Three slices were used starting with the midpoint slice, with one slice proximal and one slice distal for the upper leg. Plantar flexor (PF) images started with the first analyzable slice for the lateral gastrocnemius and ended when there was no clear distinction between the lateral gastrocnemius and soleus resulting in four to five slices for analysis. Methods for measuring CSA were used by encompassing the KE (vastii group and rectus femoris) and PF (lateral gastrocnemius, medial gastrocnemius, soleus, and flexor longus). Determination of muscle CSA was estimated by carefully tracing the edge of each group of muscles using the freehand tool in ImageJ. The average CSA for each participant was between the selected slices.

FCSA involved an image-analysis thresholding technique, measured using gray-scale thresholding to analyze those regions of the muscle cross-sections corresponding to dark, lean muscle mass. One trained researcher conducted the analysis (NDD). Previous analysis showed that repeated measurements performed by the individual were reliable and reproducible, with an intraclass correlation coefficient of 0.98. The sampling of each slice for pixel intensity of muscle tissue in three locations on both the KE and PF was used to determine the appropriate thresholds for discriminating different tissue types. The threshold value previously collected was applied to the CSA to estimate FCSA, and the difference was the estimated composition of IMAT expressed in cm^2 and a percentage of muscle area.

Dietary Intake and Physical Activity

To examine dietary intake, participants completed a three-day food diary estimating portions sizes from picture series. Participants were asked to log everything they ingested on two typical days (e.g., weekdays) and one atypical day (e.g., weekend day). Once completed, registered dietitians analyzed energy and macronutrients, including protein intake both as grams per subject and $g \bullet kg^{-1}$ per subject, using Food Processor Nutrition Analysis software (ESHA, Salem, OR). Completion of all three days of the food diary was required to be included in the analysis.

Habitual physical activity was assessed using an Actigraph GT3X+ accelerometer (Actigraph, Pensacola, FL) for seven consecutive days. Participants were instructed to wear the accelerometer on their right hip during all waking hours except for water activities (e.g., bathing or swimming), and to keep a sleep log to record the time that the accelerometer was removed

Table 1

Physical Activity (PA) and Sedentary Behavior (SED) Levels of Participants

	Sedentary (n=49)				Active (n=49)			
	Younger (n=25)		Middle-aged (n=24)		Younger (n=25)		Middle-aged (n=24)	
	Male (n=12)	Female (n=13)	Male (n=11)	Female (n=13)	Male (n=12)	Female (n=13)	Male (n=11)	Female (n=13)
SED /day (min)	638.4 ± 82.52	599.12 ± 69.19	601 ± 83.46	588.99 ±73.29	673.6 ± 84.39	623.8 ± 139.75	637.79 ± 114.6	617.2 ± 83.6
LPA/day (min)	196.14 ± 83.17	168.89 ± 80.25	263.8 ± 86.11	206.3 ± 70.41	150.37 ± 56.24‡	216.73 ± 91.46	258.52 ± 98.14	232.81 ± 70.38
MVPA/day (min)	31.39 ± 11.13	28.97 ± 11.28*	27.33 ± 13.12*	30.05 ± 16.48	42.45 ± 21.02	48.26 ± 16.88	37.49 ± 18.48	46.16 ± 16.03
Steps/day	6137 ± 2360	5907 ± 1496	6611 ± 2715	6771 ± 2512	8010 ± 2421	8394 ± 2747	8070 ± 2239	9113 ± 2505*

Values are presented as mean ± SD. * Significant difference from SYM ($p < 0.05$). ‡ Significant difference from SMM ($p < 0.05$); Note: Sedentary individuals were not participating in resistance or aerobic exercise more than one time per week, Active individuals were participating in three or more days per week of aerobic and resistance exercise at a moderate-to-vigorous intensity, Younger (20-35 years of age), Middle-aged (50-65 years of age), SMM = Sedentary Middle-aged Male, SYM = Sedentary Young Male

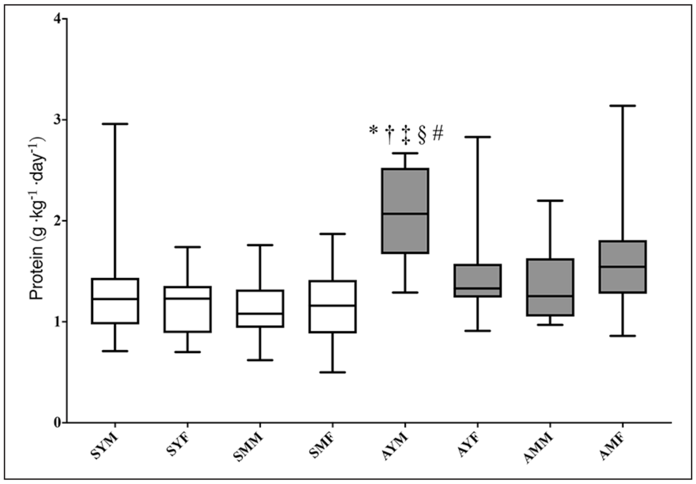
at night and put back on in the morning. The accelerometers were initialized to collect raw acceleration at 80 Hz, and raw acceleration data was processed using the R-package called GGIR, specially designed for reducing multiday raw acceleration data (17). The acceleration summary data were used to calculated the amount of time (min/day) spent in sedentary behavior (<1.5 metabolic equivalents [METs]), light physical activity (>1.5 to < 3 METs), and moderate-to-vigorous physical activity (MVPA, ≥ 3 METs), based on the intensity-specific milli-g cut-points derived from previously validated regression equations (18). Non-wear time was defined as intervals of at least 90 minutes of zero counts, allowing a two-minute interval of non-zero counts with a 30-minute window (19). A minimum wear time of four days with ten hours/day was required to be included in the statistical analysis.

Statistical Analyses

All values are reported as means and standard deviations. One-way analysis of variance (ANOVA) with Bonferroni corrections for multiple comparisons was used to compare variables of physical activity levels and FCSA and IMAT measures between groups defined by concurrent exercise training status, sex, and age. Standard Q-Q plots were consulted to check for the variables' normal distribution. Stepwise regression models were used to examine the relationship of sex, protein intake, concurrent exercise training status, physical activity intensity, and age with FCSA and IMAT. The sample size was selected based on previous studies using regression methods to predict muscle size/quality (20). The two-tailed level of significance was set at $p < 0.05$. All statistical analyses were performed using IBM SPSS Statistics (version 24, SPSS, Inc., Chicago, IL).

Figure 1

Protein intake in Active and Sedentary Younger and Middle-Aged Adults. Values are presented as mean ± SD



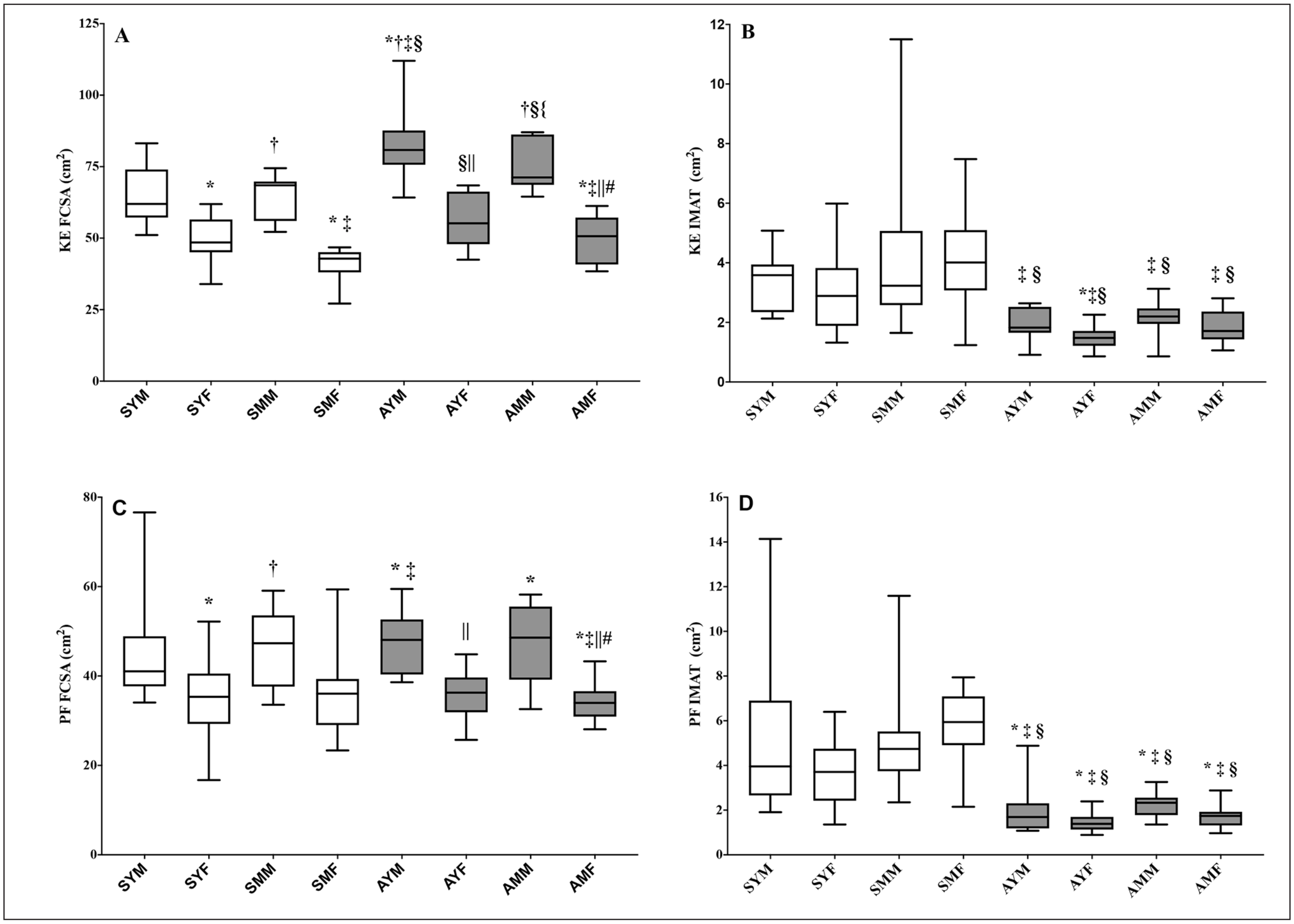
* Significant difference vs. SYM ($p < 0.05$). † Significant difference vs. SYF ($p < 0.05$). ‡ Significant difference vs. SMM ($p < 0.05$). § Significant difference vs. SMF ($p < 0.05$). # Significant difference vs. AMM ($p < 0.05$). White and gray bars represent sedentary and active, respectively; AMF = Active Middle-Aged Female, AMM = Active Middle-Aged Male, AYF = Active Younger Female, AYM = Active Younger Male, SMF = Sedentary Middle-Aged Female, SMM = Sedentary Middle-Aged Male, SYF = Sedentary Young Female, SYM = Sedentary Young Male

Results

All of the recruited 98 participants (46 male, 52 female) completed the study, including 49 sedentary individuals (n = 25, age = 26.3 ± 4.7 years; n = 24, age = 57.9 ± 4.5 years) and 49 active individuals (n = 25, age = 23.0 ± 3.1 years; n = 24, age = 57.3 ± 4.0 years). Protein intake (g•kg•day^{-1}) was significantly higher in active (1.63 ± 0.55) vs. sedentary (1.19 ± 0.40) ($p < 0.05$) and among the active-younger group compared to all of the sedentary groups (Figure 1). Figure 2 depicts the FCSA and IMAT in the KE and PF. IMAT (cm^2) was significantly higher in sedentary (3.74 ± 1.93) vs. active (1.85 ± 0.56) as well as in older (3.14 ± 2.05) vs. younger (2.74 ± 1.25) ($p < 0.05$). Table 1 shows the differences in physical activity intensity of participants based on accelerometry. Sex,

Figure 2

A) Knee Extensor Muscle Functional Cross-sectional Area, B) Knee Extensor Muscle Intermuscular Adipose Tissue, C) Plantar Flexor Muscle Functional Cross-sectional Area, and D) Plantar Flexor Muscle Intermuscular Adipose Tissue in Active and Sedentary Younger and Middle-Aged Adults. Values are presented as mean ± SD



* Significant difference vs. SYM ($p < 0.05$). † Significant difference vs. SYF ($p < 0.05$). ‡ Significant difference vs. SMM ($p < 0.05$). § Significant difference vs. SMF ($p < 0.05$). || Significant difference vs. AYM ($p < 0.05$). { Significant difference then AYF ($p < 0.05$). # Significant difference vs. AMM ($p < 0.05$). White and grey bars represent sedentary and active, respectively; AMF = Active Middle-Aged Female, AMM = Active Middle-Aged Male, AYF = Active Younger Female, AYM = Active Younger Male, SMF = Sedentary Middle-Aged Female, SMM = Sedentary Middle-Aged Male, SYF = Sedentary Young Female, SYM = Sedentary Young Male

age, concurrent exercise training status, and protein intake significantly predicted 71% of the variance in FCSA (F (1, 94) = 58.12, $R^2 = 0.714$, adjusted $R^2 = 0.702$, $p < 0.001$, Table 2). Physical activity intensity indicators were removed from the stepwise model, given they were not significant predictors: sedentary (t=-0.483, p =0.629); light (t=-0.093, p =0.926); MVPA (t = -0.355, p = 0.724). Concurrent exercise training status and light physical activity predicted 34% of the variance in KE IMAT (F (1, 95) =24.84, $R^2 = 0.343$, R^2 adjusted = 0.330, $p < 0.001$, Table 2). Sex was the only significant predictor of PF FCSA (F (1, 96) =47.23, $R^2 = 0.330$, R^2 adjusted = 0.323, $p < 0.001$, Table 3). Concurrent exercise training and age significantly predicted PF IMAT (F (1, 96) = 39.04, $R^2 = 0.451$, R^2 adjusted = 0.440, $p < 0.001$, Table 3).

Discussion

In this cross-sectional investigation, younger and middle-aged adults were classified as active or sedentary based on the self-reported concurrent exercise training status, physical activity intensity of participants was quantified with accelerometry, and dietary protein intake was estimated from three-day food diaries. Muscle quality was examined using MRI derived FSCA and IMAT measurement. The main finding of this study was that 71% of the variance in KE FCSA was predicted by age, sex, concurrent exercise training, and protein intake; leaving only 29% of factors unaccounted. Dietary protein intake increases skeletal muscle protein synthesis, and over time, can increase and maintain muscle mass as we age

(21). Protein intake, in this investigation, was highest in active, younger, males (2.05 ± 0.46) compared to all other groups (1.32 ± 0.43), and all groups were well above the recommended daily allowance (g • kg • d^{-1}) (22). These data, in particular, emphasize the importance of concurrent exercise training in combination with adequate dietary protein intake for muscle quality with early aging as recent groups have advocated for higher protein intake when both healthy (1.0 -1.2 g) and when malnourished or with illness (1.2 -1.5 g) (23).

Table 2

Stepwise regression determinants for predicting KE muscle quality

	Unstandardized β	**SE**	**p-value**
KE FSCA (n=98)			
Constant	24.88	3.89	<0.001
Gender	20.39	2.01	<0.001
Concurrent Training Exercise	8.56	1.94	<0.001
Age	-0.17	0.05	0.002
Protein Intake	0.076	0.03	0.004
R-squared =.714			
Adjusted R-squared =.702			
KE IMAT (n=98)			
Constant	2.99	0.39	<0.001
Concurrent Training Exercise	-1.92	0.28	<0.001
Light physical activity	0.004	.002	0.033
R-squared =.343			
Adjusted R-squared =.330			

Note: FCSA = Functional Cross-Sectional Area, IMAT = Intramuscular Adipose Tissue, KE = Knee Extensors

Table 3

Stepwise regression determinants for predicting PF muscle quality

	Unstandardized β	**SE**	**p-value**
PF FSCA (n=98)			
Constant	23.62	2.56	<0.001
Gender	11.49	1.67	<0.001
R-squared = .033			
Adjusted R-squared =.323			
PF IMAT (n=98)			
Constant	4.02	.519	<0.001
Concurrent Training Exercise	-3.09	.366	<0.001
Age	0.22	.011	0.45
R-squared =.451			
Adjusted R-squared =.440			

Note: FCSA = Functional Cross-Sectional Area, IMAT = Intramuscular Adipose Tissue, PF = Plantar Flexor

Despite established health benefits of increased regular physical activity, the time spent within the various intensities was not associated with reduced IMAT in younger or middle-aged adults. In particular, given the relationship between higher light physical activity was positive (β=0.004) with IMAT; rather than negative, which would disagree with other investigators exploring more extreme types of reduced physical activity models (e.g., limb suspension) (24). Most of the previous research, exploring IMAT content of KE has been among groups of individuals with obesity, diabetes, or peripheral neuropathy. Therefore, given this study explored generally healthy aging adults, any elevated IMAT levels may still have been well below any threshold that could interfere with metabolism and muscle quality.

Although we found that concurrent exercise training and protein intake were essential factors for KE FCSA, only sex was associated with PF FCSA. Further, only concurrent training exercise and age were related to PF IMAT content. Differences in muscle protein synthesis rates based on fiber type may explain the inability to identify as many predictive factors of muscle quality in the PF. In the analysis of the PF, the inclusion of the soleus, that is predominately slow-twitch muscle is contrasting to the faster twitch gastrocnemius and knee extensors muscle group that was analyzed. Mettendorfer et al. (2005) found small (>15%) but significant differences in myofibrillar and sarcoplasmic muscle protein synthesis rates between slow-twitch versus fast-twitch muscles (25). Further, the muscles of the lower limb do not hypertrophy to the same degree as the KE; thus, limiting the predictability of muscle quality in the PF with aging (25).

The strengths of this investigation were the determination of IMAT and FSCA from MRI images and the objective measure of PA through accelerometry. The limitations were the assessment of self-reported concurrent exercise training status that could have been assessed with objective measures, and the estimates of protein intake were based off participant estimates in their food logs associated analysis in dietary software.

Conclusion and Implications

Concurrent exercise training, protein intake, and light physical activity are significant determinants of skeletal muscle health in younger and middle-aged adults. This knowledge may help mitigate age- and inactivity-related loss of muscle quality. Understanding the factors that may impact muscle quality with aging represent important areas of investigation in the future, given the healthcare-related burden that will be present as a higher proportion of the population becomes 65 years and older.

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Conflicts of Interest: There are no conflicts of interest.

Ethics declaration: The host university's Institutional Review Board for the protection of human participants approved all procedures.

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