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Original Article

Moderate to vigorous physical activity, leucine, and protein intake contributions to muscle health in middle age

Kara A. Stone[1], Allison M. Barry[2], Christopher J. Kotarsky[3], Nathan D. Dicks[4], Sherri N. Stastny[5], Wonwoo Byun[6], Steven Mitchell[5,7], Ryan McGrath[5], Kyle J. Hackney[5]

[1]Department of Kinesiology and Health Studies, University of Central Oklahoma, Edmond, OK, USA;
[2]Health, Human Performance, and Recreation Department, Pittsburg State University, Pittsburg, KS, USA;
[3]Department of Health and Human Physiological Sciences, Skidmore College, Saratoga Springs, NY, USA;
[4]Nutrition, Dietetics, and Exercise Science Department, Concordia College, Moorhead, MN, USA;
[5]Department of Health, Nutrition, and Exercise Sciences, North Dakota State University, Fargo, ND, USA;
[6]Department of Health, Kinesiology, and Recreation, The University of Utah, Salt Lake City, UT, USA;
[7]Department of Radiology, Sanford Health, Fargo, ND, USA

Abstract

Objective: Identify contributors to differences in the muscle size and strength of sedentary and active young and middle-aged adults. **Methods**: This cross-sectional study included 98 participants aged 20-65 years. Participants were categorized based on age and self-reported physical activity (PA) habits. Participants completed a strength assessment of knee extensors (KEPT), knee flexors (KFPT), plantar flexors (PFPT), and dorsiflexors (DFPT), a 3-day dietary intake log, 7-day accelerometry, and a magnetic resonance imaging (MRI) scan for muscle cross-sectional area analysis of the right quadriceps (CSAq). **Results**: There were significant age and activity-related group effects for relative protein intake ($p<0.001$), relative energy intake ($p=0.04$), KEPT ($p=0.01$), CSAq ($p=0.002$), PFPT ($p=0.004$) and DFPT ($p=0.003$). Moderate, moderate-to-vigorous, and vigorous PA were positively associated with CSAq ($R^2=0.69$- 0.71; $p<0.05$), KEPT ($R^2=0.61$-0.63; $p<0.05$), and PFPT ($R^2=0.31$-0.36; $p<0.05$). Relative protein intake and daily leucine intake were significantly and positively associated with CSAq ($R^2=0.70$ and 0.67 respectively; $p<0.05$), KEPT ($R^2=0.62$ and 0.65 respectively; $p<0.05$), and PFPT ($R^2=0.29$ and 0.28 respectively; $p<0.05$). **Conclusion**: Muscle size and strength were lower in middle age relative to younger age, but increased PA, protein intake, and leucine intake was associated with the preservation of muscle size and strength in larger muscle groups of the lower body.

Keywords: Aging muscle, Leucine, Middle-age, Physical activity, Protein

Introduction

Declines in skeletal muscle mass and/or strength and power, defined as sarcopenia, are related to debilitating changes in physical and cognitive impairment, quality of life, and increased risk of early mortality[1,2]. Sarcopenia affects many older adults across sexes, races, and ethnicities supporting the need for recognition as a disease and the recent inclusion in the International Statistical Classification of Diseases and Related Health Problems[1–3]. Though the rate of muscle mass decline has been reported to be 0.65-2.0% per year in those over the age of 50 years, decreases may occur as early as age 30 for men and 40 for women[2,3]. Other data have shown similar decreases in muscle strength accompanied by a three-fold greater decrease in strength than in muscle mass[4]. Reduced abilities to complete activities of daily living and impaired mobility and walking measured

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Corresponding author: *Kara A. Stone, University of Central Oklahoma 100 N. University Dr. Edmond, OK 73034, USA*

E-mail: *kstone7@uco.edu*

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by decrements in the get-up-and-go test, muscular strength, muscular power and muscle quality related to sarcopenia can greatly affect an individual's quality of life[5].

The European Working Group on Sarcopenia in Older People suggests age as well as specific causes (e.g., inadequate nutrition and low physical activity) contribute to a secondary class of the disease[6,7]. Muscle atrophy originates from catabolism caused by increased protein breakdown, decreased protein synthesis, or a combination of the two[8]. Previous studies have shown that aging affects markers for protein synthesis more than degradation through a blunted activation of the mammalian target of rapamycin (mTOR) in skeletal muscle following exercise and protein intake[8]. Furthermore, sedentary behavior and disuse contribute to anabolic resistance reported with aging[9]. Physical activity of any type (e.g., leisure time, structured exercise, and occupational) has shown to be beneficial to muscle health in older adults[10,11]. Physical activity (PA) has also been reported to reduce the risk of development of functional impairments later in life[12]. These data suggest a need to explore methods of preservation for mass,strength, and muscle quality to maintain the quality of life for individuals as they age.

Recent studies have examined the effect of total protein supplementation and combinations of whey protein, essential amino acids (EAAs), antioxidants, and vitamin D on muscle quality and function[13,14]. Van Dijk et al. showed that replacing casein protein with a whey protein containing additional leucine led to higher force production and improved muscle quality in rats. This suggests that the type of protein and protein turnover might be responsible for greater muscle quality[14]. Similarly, Rondanelli et al. showed significant increases in fat-free mass, handgrip strength, quality of life, and activities of daily living in sarcopenic individuals consuming diets supplemented with a combination of whey protein and EAAs[13]. Through a study specific nutritional assessment, independent of PA levels, it was observed that 68% of the individuals studied improved their classification from sarcopenic to non-sarcopenic. Additionally, it has been stated that EAAs (e.g., leucine) mediate the stimulation of mTOR but only when total dietary protein intake is inadequate[15].

Several studies have examined what amount of total protein is adequate for optimal skeletal muscle health but the results have been inconclusive, ranging from 0.66 g·kg·day^{-1} to 1.8 g·kg·day^{-1} with an additional amount of leucine greater than 2-3 g·kg·2-3 times per day^{-1} [3,15]. It has been reported that protein intake higher than the current recommended dietary allowance (RDA) of 0.8 g·kg·day^{-1} is associated with increased physical performance, lean mass, and muscle strength in elderly populations[16,17]. In addition, the European Society for Clinical Nutrition and Metabolism recommends protein intakes of 1.0-1.5 g·kg·day^{-1} for older adults over 65 years of age[18]. However, approximately one-third of older adults residing in the United States do not meet the current RDA and leucine requirements for the aged are likely two times greater than the recommendations[19,20]. Though there is still concern regarding the protein intake of elderly people, recent literature suggests examining the dietary habit of middle-aged, or "pre-elderly" adults in comparison with young people to identify differences that may be important to understanding interventions to mitigate age-related losses of muscle strength, size, and quality[21]. Thus, the purpose for this study was to identify contributors to differences in the muscle size and strength of sedentary and active young and middle-aged adults.

Materials and Methods

Participants

Our study utilized a cross-sectional design. A sample size of 84 participants was deemed sufficient to detect group differences in muscle size with 80% power using a calculated effect size (f=0.37) from previous data within G.Power (version 3.1.9.7) A volunteer sample of 98 participants were recruited via flyers, email, and word-of-mouth. The North Dakota State University (NDSU) and Sanford Health (Fargo, ND) Institutional Review Boards approved study protocols and the research is in compliance with the World Medical Association Declaration of Helsinki. Participants were excluded if they reported: 1) current pregnancy or believed they could be pregnant; 2) metal fragments, devices, implants, or ink from tattoos that may be affected by magnetic resonance imaging (MRI); 3) claustrophobia given MRI scan; 4) tobacco use-in any form; 5) previous diagnosis of metabolic or cardiovascular co-morbidities, or cancer; 6) significant mobility limitations; 7) taking medications that were known to directly influence muscle protein metabolism; or 8) being third shift workers given alternative daily schedules. Participants were included in this study if they were generally healthy as determined by the Physical Activity Readiness Questionnaire (PAR-Q) and a detailed health history questionnaire[22]. All eligible participants provided written informed consent. Active individuals were predetermined to be 'engaged in self-reported aerobic and resistive exercises three or more times per week at a moderate to vigorous intensity for at least three months prior to participation' Sedentary individuals were defined as those participating in regular, structured, formal aerobic or resistance training one day per week or less . Young and middle-aged adults were defined as 20-35 and 50-65 years of age, respectively. These volunteers were then categorized into the following groups: active young adults (AYA), sedentary young adults (SYA), active middle-aged adults (AMA), sedentary middle-aged adults (SMA)

Participants completed two testing sessions. Anthropometric and muscle strength tests were completed during the initial subject visit to our labs. Body mass was measured using a digital scale to the nearest 0.1 kg (Denver Instruments DA-150, Denver, Colorado), height to the nearest 0.5 cm using a stadiometer (Seca 703 scale, Chino, CA), and waist circumferences were completed using a Gulick

measuring tape to the nearest 0.1 cm (Fitness Mart, Gay Mills, WI). At this session, participants were sent home with an accelerometer and 3-day dietary log to be completed and returned at their follow-up session one week later. The final session took place at Sanford Health and included a magnetic resonance imaging (MRI) scan and return of materials.

Measures

Serial axial plane MRI scans from a 3.0 T Siemens Skyra Intera whole-body scanner (Siemens Healthcare Headquarters, Erlangen, DE) were obtained at Sanford Broadway Clinic, Fargo. Images were obtained by licensed radiology technicians in collaboration with researchers. Participants were positioned with elevated heels and knees to minimize the distortion of the analyzed muscle. The MRI settings were: repetition time=3730 m/s, 10 mm slice-to-slice interval, 420-500 mm x 328-390 mm field of view[21]. Image J version 1.42 (National Institutes of Health, Bethesda, MD, US) was used to analyze MRI-derived muscle cross sectional area (CSA). Quadriceps CSA (CSAq) was determined for the rectus femoris and vastii by using the free-hand tool. Subcutaneous fat of the right upper and lower leg was also determined using the free-hand tool. Muscle CSA analyses were performed by three different researchers andreliability (ICC >0.98) had been previously reported[23].

Muscle function of the upper and lower right leg was assessed using a Biodex Pro4 System dynamometer (Biodex Medical Systems, Shirley, NY, US). To examine the isokinetic strength and endurance of the knee flexors and extensors the participants were seated in an upright position and would move the leg through flexion and extension at angular velocities of 60 and 180°·sec^{-1}, respectively. For both assessments, the upper leg moved through a range of motion of 95° flexion and 20° extension. In two instances, participants were limited to 25° extension due to self-reported tension in the hamstrings. A back pad was used to achieve a trunk angle of 90° when necessary. The center of the dynamometer was aligned with the subjects' lateral epicondyle and the shin pad was placed approximately 3-5 cm above the tongue of the shoe, just above the lateral malleolus. After being provided with consistent, verbal instruction, participants completed a linked protocol.

The protocol consisted of one warm-up set of four repetitions at 60°·sec^{-1} with the participants contributing no more than 85% effort on the final warm-up repetition followed by 30 seconds of rest and three maximal effort repetitions to determine peak torque (N-M) during knee extension and flexion. After another rest period of 30 seconds, participants completed one warm-up set of five repetitions at 180°·sec^{-1} followed by 30 seconds of rest and 21 maximal effort repetitions to determine total work (J). Upon completion of this protocol, participants were released from the dynamometer while the researchers set up for assessment of the lower leg. To examine the strength and endurance of the ankle dorsiflexors and plantar flexors the participants were seated in an upright position at 70° tilt with the hamstrings supported. Participants would move the ankle at angular velocities of 30° and 60° sec^{-1} to assess strength and endurance, respectively. The center of the dynamometer was aligned with the individuals' lateral malleolus. The heel was supported by a heel cup and the foot was strapped tightly to the foot plate. After being provided with consistent, verbal instruction, participants completed a second linked protocol. The protocol consisted of one warm-up set of four repetitions at 30°·sec^{-1} with the participant contributing no more than 50-75% effort on the final repetition followed by 60 seconds of rest and three maximal effort repetitions to determine peak torque (N-M) during plantar flexion and dorsiflexion. After another rest period of 60 seconds, participants completed a warm-up set of 5 repetitions at 60°·sec^{-1} followed by 60 seconds of rest and then 21 maximal effort repetitions to determine total work.

To examine dietary intake, participants completed a 3-day dietary intake log. Participants were asked to log everything they ingested on two typical days (i.e. weekdays) and one atypical day (i.e. weekend day) in the week following muscle function testing. Once completed, registered dietitians analyzed the protein (both grams per subject and g·kg^{-1} per subject) and other nutrients using Food Processor Nutrition Analysis software (ESHA, Salem, OR).

Habitual physical activity (PA) 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, swimming), and to keep a sleep log to record the time that the accelerometer was removed at night and put back on in the morning. The accelerometers were initialized to collect activity counts in 60-second epochs, and activity counts data were converted into the amount of time (min/day) spent in sedentary (<100 counts/min), light physical activity (LPA) (100-1951 counts/min), moderate physical activity (MPA) (1952-5724 counts/min), moderate-to-vigorous (MVPA) (>1952 counts/min), and vigorous physical activity (VPA) (5725-9498 counts/min) intensities using previously validated cut points[24]. Non-wear time was defined as intervals of at least 90 minutes of zero counts with allowance of two-minute interval of non-zero counts with 30-minute window[25]. A minimum wear time of four days with 10 hrs/day was required to be included in the statistical analysis[26].

Statistical Analysis

All statistical analyses were performed using SPSS version 24 (IBM, Armonk, NY) and SAS version 9.4 (SAS Institute; Cary, NC). Descriptive statistics are reported as mean (95% confidence intervals). Mahalanobis distance was used to remove outliers for total leucine intake, energy intake (kcals·kg·day^{-1}), and protein intake (g·kg·day^{-1}). Separate one-way analysis of variance (ANOVA) with Bonferroni adjustments were used to examine group

	AYA	SYA	AMA	SMA
N	25	25	24	24
Female (%)	52	54	52	54
Age (yrs.)	23.0 (21.4, 24.7)	**26.3 (24.6, 27.9)**	**57.3 (55.6,58.9)**	**57.9 (56.2, 59.5)**
BMI ($kg \cdot m^{-2}$)	23.7 (22.2, 25.3)	24.1 (22.6, 25.7)	24.7 (23.1, 26.3)	**26.8 (25.3, 28.4)**
Sedentary Behavior ($m \cdot day^{-1}$)	647.7 (610.5, 684.9)	618.0 (580.8, 655.1)	626.6 (588.7, 664.6)	594.9 (557.0, 632.9)
Light PA ($m \cdot day^{-1}$)	184.9 (152.3, 217.5)	182.0 (149.4, 214.6)	244.6 (211.3, 277.9)	232.6 (199.4, 265.9)
MPA ($m \cdot day^{-1}$)	42.4 (36.5, 48.3)	29.4 (23.5, 35.3)	38.4 (32.4, 44.4)	28.6 (22.6, 34.6)
VPA ($m \cdot day^{-1}$)	3.1 (1.6, 4.5)	0.7 (-0.7, 2.2)	3.7 (2.3, 5.2)	0.2 (-1.3, 1.6)
MVPA ($m \cdot day^{-1}$)	45.5 (39.2,51.7)	30.1 (23.9, 36.4)	42.2 (35.8, 48.6)	**28.8 (22.4, 35.2)**
Height (cm)	176.1 (171.9, 180.3)	173.1 (169.1, 177.1)	172.7 (168.7, 176.8)	174.5 (171.7, 177.3)
Body mass(kg)	74.0 (68.4, 79.6)	72.7 (66.5, 78.9)	74.4 (66.9, 81.9)	81.9 (75.4, 88.4)
Protein ($g \cdot kg \cdot day^{-1}$)	1.7 (1.5,1.9)	**1.2 (1.0, 1.4)**	1.5 (1.3, 1.7)	**1.1 (1.0, 1.4)**
Leucine ($g \cdot day^{-1}$)	4.7 (3.6, 5.8)	**2.8 (2.0, 3.6)**	3.9 (3.3, 4.6)	3.8 (3.1, 4.6)

Mean (95% confidence intervals). AYA=active young adults, SYA=sedentary young adults, AMA=active middle-aged adults, SMA=sedentary middle-aged adults. BMI=body mass index. MPA=moderate physical activity, VPA=vigorous physical activity, MVPA moderate-to-vigrous physical activity. Note: Boldface indicates statistical significance compared to AYA (p<0.05).

Table 1. Participant Demographics.

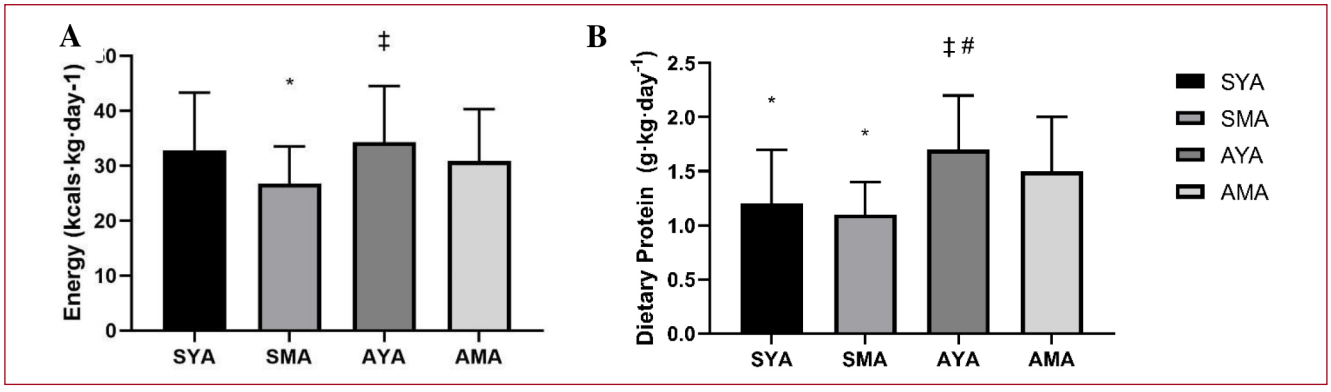

Figure 1. Nutrient Intake by Group. A) Energy Intake by Age and Activity B) Protein Intake by Age and Activity. Values are represented as mean SD. SYA= sedentary young adults, SMA=sedentary middle-aged adults, AYA=active young adults, AMA=active middle-aged adults. *denotes significance from AYA, #denotes significance from SYA, ‡ denotes significance from SMA. All significance levels set at p<0.05.

differences in protein intake ($g \cdot kg \cdot day^{-1}$), energy intake ($kcals \cdot kg \cdot day^{-1}$), leucine intake ($g \cdot day^{-1}$), CSAq, KEPT, KFPT, PFPT, and DFPT. Individual simple linear regression models were used to evaluate relationships between PA and nutrient intake and muscle size and strength. Sedentary behavior, LPA, MPA, MVPA and VPA, were separately used as independent variables for PA. Protein intake ($g \cdot kg \cdot day^{-1}$), energy intake ($kcals \cdot kg \cdot day^{-1}$), and total leucine intake (g) were likewise used as separate independent variables for nutrient intake. The outcome variable for muscle size was CSAq. For muscle strength, the independent variables KEPT, KFPT, PFPT, and DFPT were used. These models were adjusted for age, body mass index, and biological sex. Stepwise regression models were used to examine the relationship of age, protein intake, energy intake, total leucine intake, and PA with CSAq, KEPT, KFPT, DFPT, and PFPT. An alpha level of 0.05 was used for all analyses.

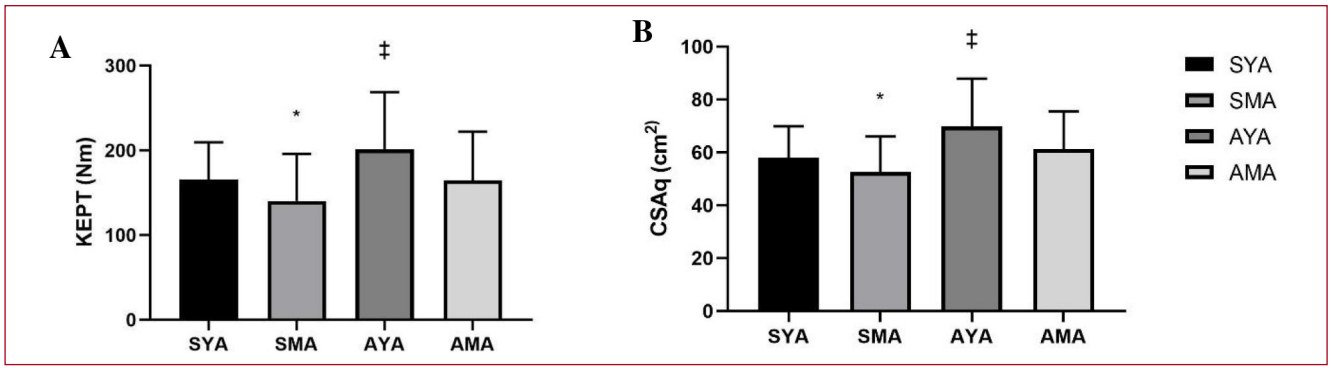

Figure 2. Knee Extensors Strength and Size by Group. A) Knee Extensor Peak Torque by Age and Activity B) Quadriceps Cross-Sectional Area by Age and Activity. Values are represented as mean SD. KEPT= knee extensors peak torque, CSAq=quadriceps cross-sectional area, SYA=sedentary young adults, SMA= sedentary middle-aged adults, AYA=active young adults, AMA=active middle-aged adults. *denotes significance from AYA, ‡ denotes significance from SMA. All significance levels set at p<0.05.

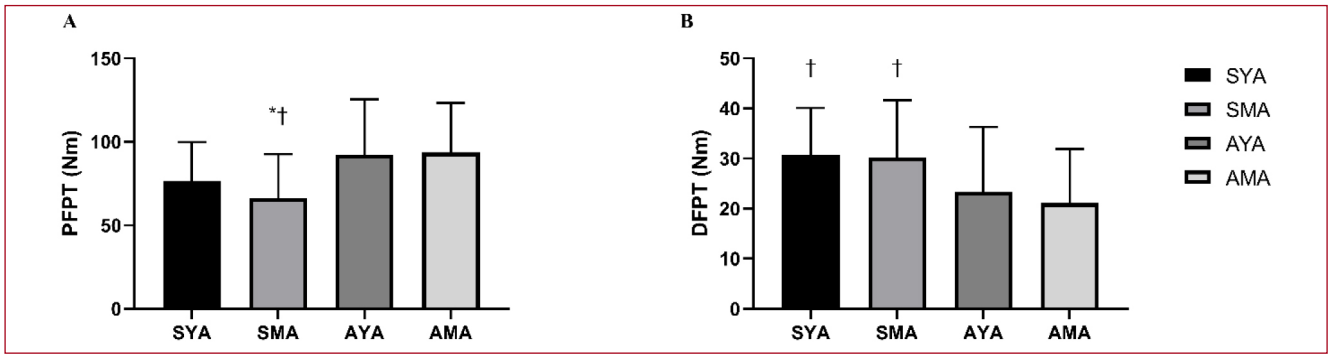

Figure 3. Plantar Flexors and Dorsiflexors Strength by Group. A) Plantar Flexors and B) Dorsiflexors Peak Torque by Age and Activity. Values are represented as mean SD. PFPT=plantar flexors peak torque, SYA=sedentary young adults, SMA=sedentary middle-aged adults, AYA=active young adults, AMA=active middle-aged adults. *denotes significance from AYA, † denotes significance from AMA, ‡ denotes significance from SMA. All significance levels set at p<0.05.

Results

Participant demographics are displayed in Table 1. Following Mahalanobis Distance, one AYA was excluded due to very high leucine intake (17.87 g) and 97 participants were included in regression analysis.

There were significant age and activity-related group effects for protein intake per kg of body mass (p<0.001), energy intake per kg of body mass(p=0.04), KEPT (p=0.01), leucine intake (g·day^{-1}; p=0.01), CSAq (p=0.002), PFPT F(p=0.004), and DFPT (p=0.003). However, there was no significant age and activity-related group effect for KFPT (p=0.445). Figure 1 shows results from Bonferroni pairwise comparisons regarding energy intake. Active young adults consumed more kilocalories per kg of body mass than sedentary middle-aged adults (34.3 [30.0, 38.6] kcals·kg·day^{-1} vs. 26.8 [23.9, 29.6] kcals·kg·day^{-1}, p=0.039). Also, protein intake per kg of body mass was higher in active young adults (1.7 [1.5, 1.9] g·kg·day^{-1}) when compared to sedentary young (1.2 [1.0, 1.4] g·kg·day^{-1}, p=0.002) and middle-aged adults (1.1 [1.0, 1.3] g·kg·day^{-1}, p<0.001). In addition, the comparisons revealed that KEPT (201.1 [173.1, 229.1] Nm vs. 140.4 [117.0, 163.8] Nm, p=0.002) and CSAq (69.8 [62.4, 77.3] cm^2 vs. 52.5 [46.8, 58.2] cm^2, p<0.001) were significantly higher in young active adults than sedentary middle-aged adults, respectively (Figure 2). Figure 3 depicts lower PFPT in sedentary middle-aged adults (66.3 [55.1, 77.4] Nm) when compared to active young (92.1 [78.3, 105.9] Nm, p=0.012) and middle-aged adults (93.6 [81.1, 106.1]

	Protein (g·kg·day^{-1})		Energy (kcals·kg·day^{-1})		Leucine (g·day)	
	Estimate	R^2	Estimate	R^2	Estimate	R^2
CSAq	**7.30**	0.70	0.12	0.65	**1.25**	0.67
KEPT	**17.1**	0.62	-0.09	0.60	**6.36**	0.65
KFPT	5.66	0.50	-0.13	0.52	**3.28**	0.54
DFPT	**-4.38**	0.48	**-0.26**	0.48	0.08	0.45
PFPT	**11.9**	0.29	0.16	0.25	**2.39**	0.28

Note: Boldface indicates statistical significance (p<0.05). Estimates were adjusted for sex, age, and body mass index. CSAq=quadriceps cross sectional area, KEPT=knee extensors peak torque, KFPT=knee flexors peak torque, DFPT=dorsiflexors peak torque, PFPT=plantarflexors peak torque.

Table 2. Independent Associations between Nutritional Status on Muscle Size and Strength.

	Sedentary Behavior		LPA		MPA		VPA		MVPA	
	Estimate	R^2	Estimate	R^2	Estimate	R^2	Estimate	R^2	Estimate	R^2
CSAq	0.01	0.65	0.01	0.65	**0.20**	0.69	**0.78**	0.69	**0.21**	0.71
KEPT	-0.02	0.60	0.01	0.60	**0.63**	0.63	1.47	0.61	**0.61**	0.63
KFPT	0.01	0.52	-0.01	0.80	0.06	0.52	-0.42	0.52	0.03	0.52
DFPT	0.01	0.46	-0.01	0.46	**-0.24**	0.56	**-0.78**	0.52	**-0.25**	0.58
PFPT	0.01	0.25	0.01	0.25	**0.55**	0.34	**1.88**	0.31	**0.56**	0.36

Note: Boldface indicates statistical significance (p<0.05). Estimates were adjusted for sex, age, and body mass index. CSAq=quadriceps cross-sectional area, KEPT=knee extensors peak torque, KFPT=knee flexors peak torque, DFPT=dorsiflexors peak torque, PFPT=plantar flexors peak torque, LPA=light intensity physical activity, MPA=moderate intensity physical activity, VPA=vigorous intensity physical activity, MVPA=moderate to vigorous intensity physical activity.

Table 3. Independent Associations between Physical Activity on Muscle Size and Strength.

	Unstandardized β	SE	p-value
KEPT			
Constant	125.75	11.23	<0.01
Age	-1.04	0.30	<0.01
Leucine	11.31	2.47	<0.01
R-squared = .248; Adjusted R-squared = .232			
KFPT			
Constant	77.98	6.69	<0.01
Leucine	5.95	1.55	<0.01
R-squared = .134; Adjusted R-squared = .125			
CSAq			
Constant	50.46	2.99	<0.01
Age	-0.22	0.08	0.01
Leucine	2.63	0.67	<0.01
R-squared = .181; Adjusted R-squared = .163			

Note: n=97, KEPT=knee extensors peak torque, KFPT=knee flexors peak torque, CSAq=quadriceps cross sectional area.

Table 4. Stepwise Regression Determinants for Predicting Muscle Strength and Size of the Upper Leg.

	Unstandardized β	SE	p-value
DFPT			
Constant	37.17	3.20	<0.01
MVPA	-0.25	.06	<0.01
Protein	-7.71	2.72	<0.01
Leucine	2.35	.63	<0.01
R-squared = .314; Adjusted R-squared = .292			
PFPT			
Constant	52.02	8.13	<0.01
MVPA	0.41	0.16	0.01
Leucine	3.78	1.36	0.01
R-squared = .139; Adjusted R-squared = .120			
Note: n=97, DFPT=dorsiflexors peak torque, PFPT=plantar flexors peak torque.			

Table 5. Stepwise regression determinants for predicting muscle strength of the lower leg.

Nm, p=0.008). Figure 3 also shows lower DFPT in active middle-aged adults (21.1 [16.6, 25.7] Nm) when compared to sedentary adults of similar age (30.2 [25.3, 35.1] Nm, p=0.039) and sedentary young individuals (30.7 [26.8, 34.6] Nm, p=0.023).

The results of regression analysis of muscular fitness and dietary intake and PA on CSAq, KEPT, KFPT, DFPT, and PFPT showed several important relationships. Dietary protein was moderately associated with CSAq, KEPT, and DFPT (p<0.05; Table 2). Energy intake was moderately associated with DFPT (p<0.05; Table 2). Daily leucine intake was moderately associated with CSAq, KEPT, and KFPT (p<0.05; Table 2). In addition, there were moderate associations between MPA and CSAq and KEPT (p<0.05; Table 3). VPA was moderately associated with CSAq and MVPA with CSAq and KEPT (p<0.05; Table 3). Age and leucine intake significantly predicted 25% of the variance in KEPT (p<0.01) and 18% of the variance in CSAq (p<0.01) with only leucine intake being predictive for KFPT (p<0.01; Table 4). MVPA and protein intake had an inverse relationship with DFPT while leucine intake remained positive (p<0.01), and leucine intake and MVPA predicted 14% of the variance in PFPT (p<0.01, Table 5).

Discussion

This study aimed to evaluate dietary and PA contributors to lower limb skeletal muscle size and strength in sedentary and active middle aged and young adults. The main findings were that age and leucine intake significantly predicted 25% of the variance in KEPT and 18% of the variance in CSAq, leucine predicted 13% of the variance in for KFPT, and leucine and MVPA predicted 14% of the variance in PFPT. Interestingly, MVPA and protein per kg of body weight had an inverse relationship with DFPT while leucine remained positive. Additionally, AYA consumed 25% more kilocalories than SMA and 34-42% more protein ($g \cdot kg \cdot day^{-1}$) than SYA and SMA, respectively. AYA also had 33% higher KEPT, 27% higher CSAq, and 30% higher PFPT than SMA. AMA had 34% greater PFPT than their sedentary age-matched peers. Unexpectedly, DFPT was 35-37% lower in AMA than SMA and SYA adults, respectively. No significant differences were observed for KFPT between groups.

Adequate nutrient and energy intake from consumed foods are essential in the maintenance of muscle mass, and physical function as insufficient intakes lead to catabolism[27,28]. Furthermore, it has been suggested that the current recommendation of 0.8 $g \cdot kg \cdot day^{-1}$ is not optimal for older adults[9,28–30]. Leucine had protective effects on whole body lean mass, whole body fat mass, body fat percentage, and KEPT but not DFPT following disuse in adults aged 45-60 years[31]. Additionally, a leucine-enriched bolus of ~21 g of whey protein and 3 g of leucine has shown to increase muscle protein synthesis in sarcopenic men with no difference from healthy men[32]. Our results are in line with the previous findings as leucine contributed significantly to KEPT, KFPT, PFPT, DFPT, and CSAq.

Though muscle groups of the lower extremity are associated with high individual variability particularly regarding age, height and body mass, they were examined in this study because they are most related to functional activities[33–35]. These associations were apparent in the knee extensors and flexors, but not the ankle extensors and flexors[36]. Additionally, strength losses of 24-30% over 12 years have been reported in the knee extensors and flexors while the elbow flexors and extensors ranged from 16-19%,

respectively[37]. Furthermore, it has been suggested that the decrease in muscle quality associated with age-related decreases in strength are greater in the lower limbs when compared to the upper limbs[33]. Age-related reductions in muscle size as measured by cross-sectional area have also been reported with the greatest losses reflected in the quadriceps (~16%)[37]. Though the results regarding dorsiflexion were not as expected and the results of the plantar flexors show promise, the most commonly examined muscle groups of the leg are the knee extensors and flexors[33].

An obvious beneficial relationship between PA and muscle health in those over the age of 60 years is evident, however, it appears that the type of PA (e.g., leisure time, structured exercise, and occupational) is of little importance[10,11]. Furthermore, physical inactivity and disuse can negatively affect muscle protein synthesis contributing to anabolic resistance that has been observed in older adults[9]. Typical physical activity levels (PAL) change from sedentary, to moderate, and back to sedentary from childhood to the eight decade of life[38]. Peak fat free mass (pFFM) seems to mirror this trend initially with higher PAL resulting in higher pFFM by age 20, but the relationship fails to remain later in adulthood[38]. These findings suggest that managing energy intake and adopting PA early in life may be the key to maintaining muscle mass and strength and reducing the chance of sarcopenic obesity, a highly prevalent combination of sarcopenia and obesity, in aging[38]. Nevertheless, physical activity remains an important variable as it has been associated with a 26% reduction in risk for developing functional impairments[12].

In our study, AMA had greater PFPT than their sedentary peers, regardless of age and MVPA. Previous research showed that individuals who consumed animal based proteins which are high in leucine and other nutrients when compared to plant proteins had significantly higher skeletal muscle mass regardless of PA[12,39]. However, it is important to weigh the risks and benefits of consuming a diet high in animal based proteins as they require more land, water, and energy to produce[39]. These requirements may contribute to environmental issues like nutrient depletion of soil, deforestation, environmental pollution, and increased greenhouse gas emissions[39]. Unsurprisingly, those who were more active and consumed more protein had the highest percentages of skeletal muscle mass indicating a symbiotic relationship between protein intake and PA[12]. After 12 weeks of walk training combined with 31 g of casein protein or placebo supplementation body composition, muscle contractility and function, and VO_{2max} significantly improved in all 114 physically active sexagenarians with adequate protein intakes[40]. Interaction effects were observed for lean body mass and fat mass only indicating a contribution of PA in body composition, muscular health, cardiovascular health, and physical function with the potential of additional benefits from increased protein[40]. Similar improvements were observed in 60-89 year olds following 16 weeks of concurrent training, however, no additional benefits were observed from supplementing leucine-rich whey protein thrice per day[41]. Interestingly, ten Haaf et al. showed additional improvements regarding body composition in older adults who were consuming just over the RDA for protein at baseline (~0.89 $g \cdot kg \cdot day^{-1}$)[40]. At the same time, Kirk et al., did not observe additional benefits in those consuming >1.0 $g \cdot kg \cdot day^{-1}$ of dietary protein at baseline[41].

Though PA intensity and duration was determined by acclerometry, concurrent activity was determined by self-reported amounts and types of resistance training. In addition, though the wrist accelerometery accounts for rhythmic aerobic activity it may not include stationary activities, water activities, and some resistive exercises. Nutrient intakes were also self-reported as participants were asked to estimate their food amounts using supplemental handouts equating portion sizes to the hands and common household items. Nevertheless, this study was a significant contribution to research on sarcopenia by contributing to the literature on a cross-sectional glance at muscle quality among middle-aged compared to young adults. Muscle strength has been observed to peak in early adulthood and start declining during midlife in many populations regardless of overall health[42,43]. Sarcopenia has been reported in individuals aged 20 and older with prevalence ranging from 19.2-42.3%[44]. Regardless, most sarcopenia research has been performed in individuals 60 years of age or older. The need for studies that directly compare young adults and middle-aged adults such as this one has been specifically expressed[21]. Additionally, this study contributes to the literature on aging adults by examining nutrition and PA together as contributors to muscle health in specific points of the human lifespan.

Conclusion

This study contributes significantly to the literature regarding symptoms of sarcopenia in middle age. Specifically, the results suggest that factors including protein intake, leucine intake, PA, and concurrent training interact to contribute to muscle health with aging. Additionally, these findings indicate that muscle strength can be preserved with concurrent activity regardless of age and that additional benefits may occur with adequate protein and leucine intakes.

Ethics approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.The study was approved by the North Dakota State University (NDSU) and Sanford Health (Fargo, ND) Institutional Review Boards.

Consent to participate

Informed consent was obtained by all individual participants included in the study.

Authors' contributions

Formal Analysis, Investigation, Writing-Original Draft, Visualization [Kara A. Stone]; Investigation, Writing-Original Draft [Allison M. Barry]; Investigation, Writing- Review & Editing [Christopher J. Kotarsky]; Investigation, Writing-Review & Editing [Nathan D. Dicks]; Methodology, Formal Analysis, Writing-Review & Editing Supervision, Project Administration, Funding Acquisition [Sherri N. Stastny]; Methodology, Formal Analysis, Writing- Review & Editing, Supervision [Wonwoo Byun]; Formal Analysis, Writing-Review & Editing, Supervision, Project Administration, Funding Acquisition [Steven Mitchell]; Formal Analysis, Writing-Review & Editing [Ryan McGrath]; Conceptualization, Methodology, Writing- Review & Editing, Supervision, Project Administration, Funding Acquisition [Kyle J. Hackney]

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