

Sarcopenic Characteristics of Active Older Adults: A Cross-sectional Exploration.

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Research

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Abstract

Background: Ageing is associated with a decline in skeletal muscle mass and function (strength and power), known as sarcopenia. Inadequate dietary protein and inactivity have been shown to accelerate sarcopenia outcomes, occurring at different rates in males and females. Regardless, active older adults who often exceed the exercise guidelines still show signs of sarcopenia. This study aimed to explore the link between age, physical activity, protein intake, and biological sex with skeletal muscle mass, strength, power, and physical capacity/performance in active older adults.

Methods: Fifty-four active older adults were grouped according to age (middle aged: 50-59 years, and older age: ≥ 60 years), exercise volume (low: <150 min/week, moderate: >150 - 299 min/week, and high: >300 min/week), protein intake (low: <0.8 g/kg body mass (BM), moderate: ≥ 0.8 - 1.19 g/kgBM, and high: ≥ 1.2 g/kgBM), and biological sex (males and females). Skeletal muscle and fat mass (dual x-ray absorptiometry), strength (1-repetition maximum using leg press, chest press, lateral pull down, and hand grip), power (counter movement jump), and general fitness (cardiorespiratory capacity and gait speed) were assessed. Data were grouped based on variables, and a one-way ANOVA (or non-parametric equivalent) was applied to assess group differences.

Results: The middle-aged group had a 13%, 17%, and 12% higher leg press, chest press, and lateral pull down, respectively, compared to the older aged group ($P<0.05$). Participants that reported moderate and high training volumes had lower body fat mass compared to those that reported lower training volumes (26.1%, 25.1%, and 35.6%, respectively; $P<0.001$). Similarly, higher leg press (22% and 27%) and chest press (22% and 23%) was observed with moderate and high training volumes compared with lower. Higher protein intakes were associated with significantly less body fat mass ($P=0.019$), higher leg strength ($P=0.038$) and relative power (W/kg) ($P=0.048$) compared to the moderate and low protein intake groups. Significant differences based on biological sex were observed for all outcomes except for gait speed ($P=0.611$) and cardiorespiratory fitness ($P=0.147$).

Conclusion: Contributions of age, physical activity, daily protein intake, and biological sex can explain the individual variation in outcomes related to changes in body composition, strength, power, and/or cardiorespiratory fitness in a cohort of active older adults.

Background

The global population is ageing and older adults (i.e., middle-aged: ≥ 50 - 59 years; older: ≥ 60 years) are remaining active much later in life than in previous generations. This is evident in the growing participation rates of both recreational and competitive older adults in endurance events, such as marathons (1) and ultra- endurance marathons (2, 3). Many studies report that regular physical activity is imperative to mitigate age-related declines in skeletal muscle mass, strength, power, and general physical performance - known as sarcopenia (4, 5). For example, a systematic review and meta-analysis of 14

studies concluded that older adults engaging in regular physical activity reduced the odds of acquiring sarcopenia later in life (odds ratio [OR] =0.45; 95% confidence interval [CI] 0.37–0.55) (6). However, active older adults still show signs of declining physical function with increasing age despite often exceeding the physical activity guidelines and exercising significantly more than sedentary older adults (>150 min/week of moderate intensity or 75 min/week of vigorous intensity- or combination) (7, 8). For example, highly competitive Masters level athletes (≥ 35 years) show declines of decreased cardiorespiratory fitness (e.g., $\dot{V}O_2$) and peak power compared to younger trained athletes (i.e., 18-27 years) in a variety of sporting disciplines (4) (9). This decline in physical performance is most notable from the age of 50 years (middle-aged) until the age of 60-70 years (older) where it declines exponentially thereafter (10). Despite this, the results in recreationally active and Masters athletes regarding skeletal muscle mass (SMM) and function have been conflicting. Such differences are likely due to small sample sizes, few female athletes, limited outcome assessments (e.g., biochemistry markers), and not taking into consideration lifestyle factors (e.g., not assessing dietary intakes).

Though primary sarcopenia is considered to be age-related, the rates of decline in SMM and strength are often highly individual and been attributed to intrinsic (e.g., hormonal changes) and extrinsic (e.g., lifestyle choices and social influences) factors, in which physical activity habits, biological sex, and dietary habits play a key impacting role. Despite this, active older adults, while currently underrepresented in sarcopenia research, may provide the ideal cohort to study as they do not display the extrinsic factor of sedentary behaviour often seen in sarcopenia research (11). Therefore, understanding how age, habitual exercise, protein intakes, and biological sex may influence outcomes of body composition, strength, power, and physical performance (e.g., cardiorespiratory fitness and gait speed) in an active cohort may provide guidance for future intervention studies.

Ageing is associated with changes in body composition, characterised by a decrease in fat free mass (FFM) and an increase in fat mass (FM), in particularly a shift from subcutaneous FM to an increase in central adiposity (i.e., visceral fat) with no changes in body mass (BM) (12). For example, a 12-year longitudinal study observed a 12.5% decline in the cross-sectional area (CSA) of the thigh muscle of healthy older adults (initial age (mean \pm SD): 65.2 ± 4.2 years) (13). However, studies in recreationally active and Masters athletes in regards to SMM have been conflicting. One cross-sectional study found that strength- or power- trained Masters athletes (i.e, 68 ± 0.8 years) have greater muscle retention with age; whereas endurance trained older adults (i.e, 70 ± 0.7 years) had similar SMM as age-matched controls, and reduced SMM compared to younger sedentary (28 ± 0.1 years) or endurance trained individuals (14). In contrast, a study which observed forty high-level recreational Masters (i.e., 40-81 years) athletes who trained 4-5 times a week found, via magnetic resonance imaging (MRI), that mid-thigh muscle area and quadriceps area did not decline with progressing age (15). The inconsistencies between these results may be due to these studies not measuring any other lifestyle factors that may lead to declines in SMM.

The importance of maintaining strength throughout advancing age is highlighted by the recent shift in the European Working Group of Sarcopenia (EWOSP2) clinical diagnosis that placed low hand grip

strength at the forefront of their criteria (5). Strength is defined as the ability to generate force, usually through the application of concentric muscle action (16). Lower limb strength is associated with walking performance, the ability to get in and out of chair, step-climbing speed, and rate of falls (17). Additionally, the correlation between hand grip strength (HGS) and isometric knee extensor strength allows grip strength to be used as a measure of whole body strength in sedentary older adults (18). However, a study that looked at the ratio of HGS to quadriceps strength and found it to be significantly lower in the older group compared to the younger group indicating a greater age-related decline in quadriceps strength than HGS (19). It is unknown whether these findings would be seen in a cohort of active older adults. Given these research inconsistencies and confounding factors that have not been adequately controlled for in previous research, it would be suggested to use measure of upper and body strength outcomes for active older adults.

Along with declines in skeletal muscle size and strength, declines in skeletal muscle power and cardiorespiratory fitness have also been evident to occur with ageing (20, 21). In a cross-sectional study of one-hundred community-dwelling older adults (65-89 years), skeletal muscle power was reported to decline in men at a rate of 3.5% per year (22). Additionally, a study of Masters power athletes (i.e., sprinting, throwing, and jumping), aged 35-95 years, found skeletal muscle power dropped 1.2% per year, beginning from the age of 70 years (23). The events that involved mostly upper limbs (e.g., shot put and javelin throw), showed a highest rate of decline (1.4% per year), compared to lower limb events (e.g., long jump decreased 1.1%). Additionally, there has been an observed decrease in $\dot{V}O_2\text{max}$ of 2.2 ml/kg/min (5.5%) per decade in Masters endurance-trained athletes (24). However, this decline was significantly less than age-matched sedentary controls who had an observed decline of 3.3 ml/kg/min (12% per decade). It appears that regular physical activity during older age may play a role in being protective against outcomes related to sarcopenia in comparison to inactive older adults. Measures of muscle power and cardiorespiratory fitness may provide more reliable indicators of declines in muscle function in older populations that are considered 'active' (11). Moreover, another major limitation of many previous studies is the relatively few outcomes measured detecting intrinsic (e.g., inflammatory and anabolic resistance) (25, 26) and/or extrinsic (i.e., lifestyle factors) (27) factors, which makes it unclear as to whether these potential factors may contributing to the skeletal muscle functional decline, than ageing alone, in active older adults.

To our knowledge the potential single and combined associations between the influences of dietary intake (e.g., protein intake) and exercise status in relation to the outcomes of SMM, strength and power have not been investigated in a homogenous sample of 'active older adults'. Therefore, the present study aimed to explore the link between age, physical activity level, dietary protein intake, and biological sex with SMM, strength, power, and physical capacity/ performance in active older adults.

Subjects And Methods

Study population

In a cross-sectional design, fifty-four active older adults (mean \pm SD: age 58.9 ± 6.8 years, BM 74.2 ± 14.58 kg, height 1.72 ± 0.10 m, FM 27.9 ± 8.9 %) volunteered to participate in the study. Participants were eligible if they were ≥ 50 years, performed exercise training for recreational fitness and/or sports competitions ≥ 3 /week, for ≥ 90 min/week, had no functional limitations, were free from chronic disease/disorders, were not taking medications that could interfere with SMM structure and/or function (e.g., corticosteroids, testosterone replacement or anabolic drugs), were not undergoing immunosuppressive therapy or hormone replacement therapy, and were not adhering to structured resistance training program/s. Participants were grouped based on age; middle age (50-59 years) and older (≥ 60 years) and biological sex (males and females). All participants gave written informed consent. The study protocol obtained approval from the local ethics committee (Project number 12812). Data was collected during the period of September 2018 to January 2020, See **Figure 1** for participant flow.

Exercise volume

Prior to commencing any physical activity participants filled out a physical activity readiness questionnaire (PAR-Q). They self-reported their level of physical activity including exercise intensity and volume per week, and the modality of exercise. All participants were categorised into low (≤ 149 min/week), moderate (≥ 150 -299 min/week) or high (≥ 300 min/week) exercise volume [7].

Dietary assessment

Participants were educated and asked to complete a three days food-fluid diary prior to their baseline visit, as previously described (28, 29). Food-fluid diaries were analysed using FoodWorks v10.0 nutritional analysis software (Xyris Software, Brisbane, Australia, 2019) based on Australian food composition tables from Australian Food Composition Database (AFCD) 2019. Based on preliminary data participants were then categorised into low (< 0.8 g/kgBM/day), moderate (0.8-1.19 g/kgBM/day), and high (≥ 1.2 g/kgBM/day) protein intake to assess the effects that daily dietary protein intake alone has on outcomes of SMM, strength, power, and physical performance markers (30).

Experimental procedure and measurement of outcomes

For measurements of outcome variables, participants were required to attend the laboratory for the period between 07.00am to 09.00am in a fasted and euhydrated state (296 ± 5.6 mOsmol/kg; $53.4 \pm 19.9\%$ TBW; Seca 515 MBCA, Seca Group, Hamburg, Germany), and after avoiding strenuous exercise for a 24 h period. Height was assessed using a fixed stadiometer (Holtain, Crosswell, Crymych, UK). BM was measured (Seca 515 MBCA) to the nearest 0.1 kg, using standardised anthropometrical procedures. Total (kg) and relative (%) FM and FFM, and bone mineral content were assessed by a trained radiographer using a dual-energy X-ray absorptiometry (Prodigy, GE Lunar, Madison, WI; with analysis software 14.10). Appendicular lean mass (ALM) was determined by adding the total arm and trunk lean mass and then it

was adjusted for height (ASM/height²). Resting metabolic rate (RMR) was determined by indirect calorimeter (Vmax Encore Metabolic Cart; Carefusion, San Diego, CA) in temperate ambient conditions (22.2 ± 1.4°C), and in accordance with best practice guidelines (31). To comply with ethical procedures, prior to commencing the strength, power, and performance measures, participants were provided with a standardised breakfast (1.4 MJ, 15.3 g protein, 51.7 g carbohydrates, 6.8 g total fat). Physical assessment measures commenced ~30 min thereafter.

Blood collection and analysis

The remaining heparin whole blood samples were centrifuged at 4,000 rpm for 10 min within 15 min of sample collection. Aliquots of heparin plasma were placed in 1.5 ml microstorage tubes and frozen at -80°C until analysis, except 2 x µl plasma was used to determine P_{Osmol} in duplicate (CV 1.0%), using a freeze point osmometry (Osmomat 030; Gonotec, Berlin, Germany).

Circulating concentrations of cortisol (DiaMetra, Perugia, Italy), insulin-like growth factor-1 (IGF-1) (Crux Biolab, Scoresby, Australia), insulin (Crux Biolab, Scoresby, Australia), testosterone (17β-OH-4-androstene-3-one; DiaMetra, Perugia, Italy), estradiol (17β-Estradiol; DiaMetra, Perugia, Italy) were measured by enzyme-linked immunosorbent assay (ELISA). Plasma concentrations of interleukin (IL)-2, IL-6, IL-1β, tumor necrosis factor (TNF)-α, IL-8, and IL-10 were determined by high sensitivity multiplex ELISA (HCYTOMAG-28SK; EMD Millipore, Darmstadt, Germany). All assays were performed as per manufacturer's specifications, with standards and controls on each plate. The CV for analysed circulating biomarkers was ≤7.2%, and for systemic inflammatory cytokines was ≤13.5%.

Strength Outcomes

Strength was assessed by performing a 1 repetition maximal strength (1-RM) in accordance with previously described procedures (32). During a familiarisation trial, proper lifting technique was demonstrated, then participants were familiarised with each resistance machine (Hammer strength; LifeFitness, Sydney, Australia) by performing 8-10 repetitions of a light load (~50% of predicted 1-RM). After the successful completion of a further five to six repetitions at a heavier weight selected by the instructor, the workload was increased incrementally until only one repetition with correct technique could be completed. Participants were given 3-5 min rest in-between attempts (33). The value indicative of 1-RM was the highest load that could be raised in one single repetitions using correct technique for leg press, *latissimus dorsi* (lat) pull down, and bench press. The 1-RMs were normalised by BM (1-RM/BM). Hand grip strength (HGS) was measured using a digital hand dynamometer (Jamar® Plus+ Digital hand dynamometer; Sammons Preston, Bolingbrook, IL, USA). HGS was measured in a standing position with the participants elbow by their side and flexed to a 90° angle and a neutral wrist position. Participants were asked to apply the maximum grip strength three times with both left and right hands, HGS was defined as the highest value for their dominant hand (34).

Submaximal incremental bike test

Submaximal aerobic fitness was determined using an incremental bike test using a cycle ergometer (*Corival*, Lode, Groningen, Netherlands) and a metabolic cart (Vmax Encore Metabolic Cart; Carefusion, San Diego, CA). The initial workload began at 1 watt (W) per kilogram of Fat free mass (W/kgFFM) and increased by 0.5 W/kgFFM every 3 min until participants could not maintain the speed at 60 RPM or higher or they reached a rating of perceived exertion (RPE) of 15-17 on the Borg scale (35). Heart rate (HR) (Polar Electro, Kempele, Finland), $\dot{V}O_{2max}$, respiratory quotient (RQ), and RPE were measured every 3 min in real-time. Cardiorespiratory fitness was expressed as Watts/RQ. Procedures were adjusted from standard fitness testing procedures (36).

Countermovement jump

A Force plate (400s+ Performance Force plate; Fitness Technology, Adelaide, Australia) was used to measure relative muscle power (W/kg), jump height (cm) and velocity (m/sec) during a countermovement jump test (CMJ). Participants were asked to start in a full erect standing position in the middle of the force plate, then instructed to dip to a self-selected depth and “jump for maximal height”. Hands were kept on the hips to minimize any influence of arm swing (37). Participants were asked to perform three attempts of a CMJ with 1 min rest in-between jumps. The Force plate was interfaced with computer software (Ballistic Measurement System; Fitness Technology, Adelaide, Australia), where the mean of three jumps was selected for further analysis.

Gait speed measurement

To assess gait speed, a walking course of 4 metres long was marked on the floor. The participant was instructed to walk from one end of the course to the other at their usual walking pace. The timer began as the participant started walking and the timer was stopped with the first footfall after the 4-metre line. The test was repeated twice and the average (of two scores) was determined. Gait speed was reported at seconds/meter.

Statistical analysis

Data in text and tables are presented as either mean \pm SD (descriptive experimental data) or mean and 95% confidence interval (CI) (primary and secondary variables), where indicated. Only participants with full data sets were used in analysis. All statistical analyses were performed using IBM SPSS statistics software (Verson 25.0, IBM Corp, Armonk, NY). Prior to analysis, assumptions of normality in the data were made using Shapiro-Wilk test and visualisations of normality plots. Variables with multiple groups were examined using a one-way repeated measures ANOVA or non-parametric Kruskal-Wallis H test, where appropriate. A Tukey's post-hoc test (or non-parametric equivalent) was applied to determine between

group differences. Significance was accepted at $P \leq 0.05$. Additionally, Cohen's d was applied to determine the magnitude of effect size for significance differences, with $d \geq 0.20$ for small, $d \geq 0.50$ for medium, and $d \geq 0.80$ for large effect size.

Results

Table 1 presents the participant characteristics. Of the fifty-four participants included in the data collection, 53 were included in the analysis, due to a missing food diary (**Figure 1**). Of the 53 participants 86% were Caucasian, 10% were Asian, and 4% were south-east Asian. The participants from this study came from a variety of sporting backgrounds that were composed of endurance runners and race walkers (61%), cyclists (9%), aerobic gym goers (16%), or a combination of multiple activities (14%). Based on the EWGOS clinical diagnosis 6% ($n=3$) had low HGS and were considered to have probable sarcopenia.

Table 1
Participant characteristics

| | |
|--|----------------------|
| Age, years | 58.8 (50 .0 to 74.1) |
| Height, m | 1.7 (1.5 to 1.9) |
| Body mass (BM), kg | 74.8 (47.0 to 107.1) |
| BMI, kg/m ² | 25.5 (18.5 to 39.0) |
| RMR, kCals/day | 2088 (1944 to 2232) |
| EWOSP category for sarcopenia | |
| Hand grip, (dominant), kg | 37.0 (33.8 to 40.1) |
| ADM/ht ² | 7.8 (7.1 to 8.5) |
| Gait speed, m/sec | 0.8 (0.8 to 0.9) |
| <i>n</i> = , considered sarcopenic | 0 |
| Exercise volume | |
| min/week | 228 (90 to 675) |
| iDXA measurements | |
| FFM, kg | 53.6 (33.1 to 70.0) |
| FM, % | 21.6 (6.2 to 40.0) |
| Arm lean mass, kg | 5.6 (10.9 to 29.3) |
| Leg lean mass, kg | 17.5 (2.9 to 9.0) |
| Strength, power, and physical performance | |
| Leg press, kg/BM | 1.9 (0.5 to 3.0) |
| Lateral pull down, kg/BM | 0.9 (0.4 to 1.0) |
| Chest press, kg/BM | 0.8 (0.2 to 1.3) |
| Vertical jump | |
| Jump height, cm | 17.3 (3.0 to 28.0) |

| | |
|---|---------------------|
| Relative power, W | 29.5 (27.9 to 31.1) |
| Velocity, m/sec | 1.9 (1.8 to 2.0) |
| Cardiorespiratory fitness | |
| Watts/RQ, | 111 (97 to 127) |
| Biochemistry | |
| BGL, mMol/L | 4.8 (4.6 to 5.1) |
| Insulin, uIU/ml | 7.1 (3.2 to 20.5) |
| IGF-1, pg/ml | 97 (33 to 160) |
| Testosterone, ng/ml | 1.4 (0.0 to 5.0) |
| Estradiol, pg/ml | 26 (0 to 273) |
| Cortisol, ng/ml | 366 (15 to 964) |
| WBC, pg/ml | 4.8 (2.3 to 7.1) |
| Neutrophils, pg/ml | 2.7 (1.1 to 4.5) |
| Lymphocytes, pg/ml | 2.1 (1.0 to 3.9) |
| Monocytes, pg/ml | 0.3 (0.1 to 1.1) |
| IL-2, pg/ml | 4.1 (0.3 to 10.8) |
| IL-6, pg/ml | 5.9 (0.8 to 36.8) |
| IL-8, pg/ml | 5.9 (0.2 to 28.1) |
| IL-10, pg/ml | 18.5 (1.0 to 50.1) |
| TNF- α , pg/ml | 2.3 (-0.4 to 5.6) |
| Abbreviations: ADM, appendicular muscle mass; BGL, blood glucose levels; BMI, Body mass index; FFM, fat free mass; FM, fat mass; IGF, insulin-like growth factor; IL, interleukin; RMR, resting metabolic rate; RQ; respiratory quotient TNF- α , tumour necrosis factor alpha; W, watts. | |

Body composition stratified by age, exercise volume, dietary protein intake, and biological sex

The middle-aged group had a higher average bone mineral density compared to the older group, with a moderate association on age ($d= 0.562$) (**Table 2A**). **Table 2B** shows between group differences of outcomes based on the level of reported exercise volume. There was a significant difference between

groups, with post-hoc analysis indicating the low exercise volume group having significantly higher FM compared to the moderate (10.5%, $P= 0.001$, $d= 1.27$) and high (9.5%, $P= 0.007$, $d= 1.30$) exercise volume group. There was no substantial difference between the moderate and high exercise volume groups ($P= 0.925$). A significant difference was observed between total daily protein intake with BM and FM (**Table 2C**). Mean BM for the high protein intake group was significantly lower compared to the low and moderate groups (-16.1 kg; $d= 0.386$). With no significant differences between the low and moderate protein intake groups. The high protein intake group had -9.9% ($P< 0.01$, $d= 0.435$) and -6.3% ($P= 0.078$, $d= 0.387$) lower FM compared to the moderate and low protein intake groups, respectively. There were no differences in FM comparing the low and moderate protein intake groups. **Table 2D** presents the differences in body composition based on biological sex. Male participants were 16 kg heavier than female participants ($d= 1.30$), had 28% higher FFM ($d= 2.34$), and showed higher arm ($d= 3.01$) and leg lean muscle mass ($d= 2.44$). Male participant relative SMM index was also significantly higher than female participants ($d= 2.85$). Female participants had significantly higher FM compared to male participants ($d= 0.921$).

Table 2

Body composition of participants based on age (A), exercise volume (B), protein intake (C), biological sex (D).

| | BM (kg) | FFM (kg) | FM (%) | Arm lean mass (kg) | Leg lean mass (kg) | BMD | ADM/ht ² |
|-----------------|----------------|----------------|-----------------------------|--------------------|--------------------|--------------|---------------------|
| A. | | | | | | | |
| Middle | 74.7 | 55.3 | 27.0 | 5.6 | 18.0 | 1.3 | 7.8 |
| n=27 | (68.5 to 80.9) | (51.3 to 59.2) | (23.5 to 30.6) | (5.0 to 6.3) | (16.4 to 19.7) | (1.2 to 1.3) | (7.3 to 8.4) |
| Older | 74.5 | 51.9 | 30.0 | 5.7 | 17.7 | 1.2 | 7.6 |
| n=27 | (69.2 to 80.3) | (47.3 to 56.6) | (26.2 to 22.6) | (5.1 to 6.4) | (16.1 to 19.2) | (1.1 to 1.2) | (7.0 to 8.1) |
| P-value | 0.936 | 0.573 | 0.250 | 0.822 | 0.459 | 0.040 | 0.451 |
| B. | | | | | | | |
| Low | 78.0 | 51.8 | 35.6 | 5.5 | 16.9 | 1.1 | 7.7 |
| n=16 | (69.9 to 86.1) | (45.4 to 58.3) | (31.2 to 39.9) | (4.5 to 6.5) | (14.7 to 19.1) | (1.1 to 1.3) | (6.9 to 8.6) |
| Moderate | 75.8 | 56.4 | 25.1 | 5.8 | 18.6 | 1.3 | 7.8 |
| n=24 | (70.0 to 82.0) | (52.0 to 60.7) | (45.8 to 58.3) ^a | (5.2 to 6.5) | (16.7 to 20.3) | (1.2 to 1.3) | (7.3 to 8.4) |
| High | 69.5 | 50.8 | 26.1 | 5.4 | 16.7 | 1.2 | 7.5 |
| n=14 | (60.3 to 78.9) | (44.7 to 56.7) | (21.8 to 30.9) ^a | (4.4 to 6.3) | (14.2 to 19.1) | (1.1 to 1.3) | (6.7 to 8.3) |
| P-value | 0.285 | 0.247 | <i>P</i> <0.001 | 0.710 | 0.259 | 0.751 | 0.710 |
| C. | | | | | | | |
| Low | 86.1 | 57.1 | 35.2 | 6.7 | 19.7 | 1.2 | 8.6 |
| n=6 | | | | (4.4 to 9.0) | | | |

| | | | | | | | |
|--|-------------------|----------------|-------------------|--------------|----------------|--------------|--------------|
| | (70.1 to 102.1) | (46.8 to 67.6) | (27.8 to 39.0) | | (15.5 to 24.0) | (1.2 to 1.3) | (7.3 to 9.9) |
| Moderate | 80.6 | 57.1 | 31.6 | 5.4 | 17.6 | 1.3 | 7.6 |
| n =15 | (72.6 to 92.0) | (50.7 to 63.5) | (25.9 to 37.3) | (4.6 to 6.3) | (14.6 to 20.6) | (1.1 to 1.4) | (6.6 to 8.6) |
| High | 70.0 ^a | 51.8 | 25.3 ^a | 5.4 | 17.1 | 1.2 | 7.6 |
| n =30 | (65.8 to 70.7) | (47.9 to 55.8) | (22.2 to 28.3) | (4.8 to 5.9) | (15.7 to 18.4) | (1.1 to 1.3) | (7.1 to 7.9) |
| P-value | 0.005 | 0.237 | 0.006 | 0.237 | 0.355 | 0.435 | 0.143 |
| D. | | | | | | | |
| Males | 80.2 | 59.2 | 25.8 | 6.5 | 19.8 | 1.3 | 8.4 |
| n =36 | (75.5 to 84.9) | (56.3 to 59.4) | (23.1 to 28.4) | (6.2 to 6.9) | (19.7 to 20.8) | (1.2 to 1.3) | (7.9 to 8.7) |
| Females | 64.1 | 42.4 | 34.1 | 3.8 | 13.4 | 1.1 | 6.5 |
| n =18 | (54.8 to 62.0) | (39.5 to 45.3) | (29.2 to 38.5) | (3.5 to 4.1) | (12.6 to 14.2) | (1.0 to 1.1) | (6.1 to 6.9) |
| P-value | < 0.001 | < 0.001 | < 0.001 | < 0.00 | < 0.001 | < 0.001 | < 0.001 |
| Mean (95% CI). (A) Age: middle-age (50-59 years) and older (≥ 60 years); (B) Exercise volume: low (≤ 140 -90 mins); moderate (≥ 150 -299 mins); High (≥ 300 mins); (C) Protein intake; low (≤ 0.8 g/kgBM/day); Moderate (≥ 0.8 -1.19 g/kgBM/ day); High (≥ 1.2 g/kgBM/day); (D) Males and females; Between group differences ^a significance vs low; ^b significance vs moderate; $P < 0.05$. | | | | | | | |
| Abbreviations: ADM, Appendicular muscle mass, BM, body mass; FFM, fat free mass; FM, Fat mass; BMD, Bone mineral density. | | | | | | | |

Strength outcomes stratified by age, exercise volume, dietary protein intake, and biological sex

Significantly higher leg press ($d= 0.758$), lat pull down ($d= 0.532$), and chest press ($d= 0.600$) 1-RMs were observed in middle-aged compared with and older participants (**Table 3A**). There was no substantial difference between HGS based on age. Based on exercise volume there was a significant mean difference for 1-RM leg press for low vs. high ($P= 0.012$, $d= 0.902$) and low vs. moderate $P= 0.018$, $d= 1.06$) exercise groups (**Table 3B**). Additionally, the low exercise group had a significantly lower 1-RM chest press compared to the moderate exercise group ($P=0.049$, $d= 0.89$), but there was no significant difference in chest press between the low and high exercise group ($P=0.115$) or the moderate and high exercise group and ($P=0.993$). High protein intake presented a significantly higher 1-RM leg press compared with moderate protein intake ($d= 0.682$) (**Table 3C**). Male participants presented significantly higher 1-RM assessment compared with female participants **Table 3D**. Leg press 1-RM was 20% greater in male compared with female participants ($d=0.708$). Male participants showed 30-33% higher chest press ($d=1.42$) and lat pull down ($d=0.4$), and HGS ($d=2.39$), compared with female participants.

Table 3

Strength outcomes stratified by age (A), Exercise volume (B), Protein intake (C) and biological sex (D)

| | Leg press (kg/BM) | Chest press (kg/BM) | Lat pull down (kg/BM) | Hand grip, dominant, kg |
|--------------------------|----------------------------------|-------------------------------------|----------------------------------|--------------------------------|
| A. | | | | |
| Middle n=27 | 2.2 (2.0 to 2.3) | 0.817 (0.78 to 0.97) | 1.0 (0.93 to 1.3) | 38.7 (34.1 to 43.0) |
| Older n=27 | 1.8 (1.5 to 2.0) | 0.73 (0.63 to 0.83) | 0.9 (0.82 to 1.0) | 37.3 (33.5 to 41.0) |
| P-value | 0.008 | 0.042 | 0.047 | 0.264 |
| B. | | | | |
| Low n=16 | 1.6 (1.3 to 1.95) | 0.67 (0.53 to 0.81) | 0.90 (0.7 to 1.0) | 34.4 (27.7 to 41.0) |
| Moderate n=24 | 2.1 ^a (1.9 to 2.4) | 0.86 ^a (0.76 to 0.96) | 1.0 (0.92 to 1.0) | 40.5 (36.3 to 44.6) |
| High n=14 | 2.2 ^a (1.9 to 2.4) | 0.85 (0.72 to 1.0) | 1.0 (0.89 to 1.1) | 37.0 (28.3 to 39.7) |
| P-value | 0.006 | 0.044 | 0.363 | 0.112 |
| C. | | | | |

| | | | | |
|--|----------------------------------|------------------------|------------------------|------------------------|
| Low n=6 | 1.8 (1.4 to 2.1) | 0.72 (0.61 to 0.92) | 0.92 (0.81 to 1.1) | 44.3 (35.8 to 52.8) |
| Moderate n=15 | 1.7 (1.4 to 2.1) | 0.82 (0.62 to 1.0) | 0.93 (0.70 to 1.0) | 36.5 (29.4 to 43.7) |
| High n=30 | 2.1 ^b (1.9 to 2.3) | 0.82 (0.70 to 0.92) | 1.0 (0.91 to 1.1) | 36.4 (32.7 to 40.1) |
| P-value | 0.053 | 0.147 | 0.219 | 0.204 |
| D. | | | | |
| Males n=36 | 2.1 (1.9 to 2.2) | 0.90 (0.83 to 0.93) | 1.1 (1.0 to 1.1) | 42.6 (40.0 to 45.3) |
| Females n=18 | 1.7 (1.4 to 2.0) | 0.60 (0.51 to 0.70) | 0.77 (0.68 to 0.87) | 25.8 (22.8 to 28.8) |
| P-value | 0.011 | P<0.001 | P<0.001 | P<0.001 |
| Mean (95% CI). (A) Age: middle-age (50-59 years) and older (≥ 60 years); (B) Exercise volume: low (≤ 140 -90 mins); moderate (≥ 150 -299 mins); High (≥ 300 mins); (C) Protein intake; low (≤ 0.8 g/kgBM/day); Moderate (≥ 0.8 -1.19 g/kgBM/ day); High (≥ 1.2 g/kgBM/day); (D) Males and females; Between group differences ^a significance vs low; ^b significance vs moderate; $P < 0.05$. | | | | |
| Abbreviations: BM, body mass. | | | | |

Power and physical performance outcomes stratified by age, exercise volume, dietary protein intake, and biological

sex

Comparing age to physical performance and power outcomes (**Table 4A**), the middle-aged group presented significantly higher relative power (W/kg) ($d= 0.900$) and velocity ($d= 0.755$) compared to the older aged group. Additionally, the middle-aged group, showed 30% higher average watts/RQ, reflecting a higher cardiorespiratory fitness, compared to the older aged group ($d= 0.822$). Moreover, the middle-aged group jumped on average 6 cm higher compared to the older group ($d=1.39$). Exercise volume did not influence any substantial difference on jump height or gait speed (**Table 4B**). Moderate exercise volume showed a significantly higher relative power compared to low exercise volume ($P= 0.009$, $d=1.02$) and a trend towards significance compared to the high exercise volume ($P= 0.052$, $d= 0.875$). Moderate exercise volume also showed significant higher velocity compared to low ($P= 0.009$, $d= 0.83$), but not high ($P= 0.495$), exercise volume. Comparing groups based on relative dietary protein intake, there was no significant difference between groups (**Table 4C**). The comparison of power and physical performance outcomes based on biological sex (**Table 4D**), indicated that male participants jumped 6 cm higher ($d= 0.802$), had 11% higher relative power ($d= 0.72$), and 13% higher velocity ($d= 1.11$) compared to female participants. There was no statistical significant difference between biological sex for gait speed and cardiorespiratory fitness.

Table 4

Performance and power outcomes stratified by age (A), Training volume (B), Protein intake (C) and biological sex(D)

| | Jump height, cm | Relative power (W/kg) | Velocity (m/s) | Gait speed (m/s) | Cardiorespiratory fitness, Watts/RQ |
|--------------------------------|------------------------|-------------------------------------|----------------------------------|------------------------|-------------------------------------|
| A. | | | | | |
| | 20.3 | 31.8 | 2.0 | 0.82 | 133.7 |
| Middle <i>n=27</i> | (18.5 to 22.1) | (30.0 to 33.6) | (0.0 to 2.0) | (0.72 to 0.84) | (115.6 to 151.8) |
| Older <i>n=27</i> | 14.5 (12.5 to 12.5) | 26.4 (23.9 to 28.9) | 1.8 (1.6 to 1.9) | 0.82 (0.72 to 0.85) | 93.3 (72.17 to 114.4) |
| P-value | <i>P</i> <0.001 | 0.002 | 0.006 | 0.249 | 0.004 |
| B. | | | | | |
| Low <i>n=16</i> | 14.9 (11.5 to 18.4) | 25.9 (22.7 to 29.1) | 1.7 (1.6 to 1.9) | 0.9 (0.8 to 0.9) | 89.5 (64.4 to 117.7) |
| Moderate <i>n=24</i> | 18.8 (16.7 to 21.0) | 31.3 ^a (28.8 to 33.7) | 2.0 ^a (1.9 to 2.1) | 0.8 (0.7 to 0.9) | 129.0 (107.2 to 150.8) |
| High <i>n=14</i> | 17.3 (14.4 to 20.3) | 29.2 ^b (27.6 to 30.9) | 1.9 (1.7 to 2.0) | 0.83 (0.7 to 0.9) | 114.0 (82.1 to 145.9) |
| P-value | 0.102 | 0.010 | 0.011 | 0.351 | 0.053 |

| | | | | | |
|---|------------------------|------------------------|-----------------------|---------------------|--------------------------|
| C. | | | | | |
| Low n =6 | 16.3 (11.2 to 21.3) | 27.3 (23.0 to 31.6) | 1.9 (1.6 to 2.1) | 0.8 (0.7 to 0.9) | 106.7 (64.7 to 148.7) |
| Moderate n =15 | 17.8 (13.7 to 22.0) | 26.0 (19.5 to 32.6) | 1.7 (1.4 to 2.1) | 0.8 (0.7 to 0.8) | 117.1 (97.7 to 137.9) |
| High n =30 | 17.6 (15.9 to 19.1) | 31.2 (29.6 to 32.8) | 2.0 (1.9 to 2.1) | 0.8 (0.8 to 0.9) | 117 (100 to 129) |
| P-value | 0.729 | 0.056 | 0.057 | 0.330 | 0.536 |
| D. | | | | | |
| Males n =36 | 19.0 (17.1 to 21.0) | 30.4 (28.4 to 32.5) | 2.01 (1.92 to 2.1) | 0.8 (0.7 to 0.8) | 20.0 (17.5 to 22.5) |
| Females n =18 | 14.1 (11.5 to 16.7) | 26.9 (24.0 to 30.0) | 1.75 (1.6 to 1.88) | 0.8 (0.8 to 0.9) | 19.1 (14.6 to 23.5) |
| P-value | 0.003 | 0.048 | 0.001 | 0.611 | 0.147 |
| <p>Mean (95% CI). (A) Age: middle-age (50-59 years) and older (≥ 60 years); (B) Exercise volume: low (≤ 140-90 mins); moderate (≥ 150-299 mins); High (≥ 300 mins); (C) Protein intake; low (≤ 0.8 g/kgBM/day); Moderate (≥ 0.8-1.19 g/kgBM/ day); High (≥ 1.2 g/kgBM/day); (D) Males and females; Between group differences ^a significance vs low; ^b significance vs moderate; $P < 0.05$.</p> <p>Abbreviations: RQ, respiratory quotient; W, watts.</p> | | | | | |

Discussion

This study aimed to assess the link between self-reported and objective measures of age, physical activity, habitual dietary protein intake, and biological sex as they relate to measures of body composition, strength, power, and physical performance outcomes in a population of active older adults. The main findings were: 1) middle-aged and older adults had no significant differences in body composition, but did display differences in strength, power, and performance; 2) higher exercise volumes (≥ 150 min/week) in active older adults may explain differences in FM, strength, and power outcomes; 3) higher dietary protein intakes (≥ 1.2 g/kgBM/day) was linked with lower FM and higher strength; and, 4) significant differences in body composition, strength, and power outcomes exist between male and female active older participants. There are many cross-sectional studies that examine some of these outcome measures in either community dwellers or frail and institutionalised older adults (38). However, the current study is the first to comprehensively explore these prospective relationships in a cohort of active older adults. Overall, this study demonstrated that the contributions of age, physical activity, daily dietary protein intake, and biological sex can help explain individual variation in outcomes related to changes in body composition, strength, power, and physical performance.

Based on the EWGSOP2 clinical diagnosis criteria, 6% of the participants had low HGS and were considered to have “probable” sarcopenia. However, when analysed further these participants were not confirmed to have sarcopenia based on muscle quantity (5). This prevalence for sarcopenia is substantially lower than the 1-29% reported rates for community dwelling adults over the age of 50 years (39). A similar study by Fien, Climstein (40) found that in a cohort of 156 Master’s athletes, 3.8% were considered to be below the sarcopenic HGS cut-off points. The difference in results is likely due to the larger sample size and the higher level of training of the Masters’ athletes compared to the more recreationally active older adults used in the current study. Considering that the prevalence of sarcopenia in aged-care facilities have been reported as high as 41% (41), our data suggests that active older adults levels of SMM, skeletal muscular strength, and physical function place them at very low risk of adverse health effects caused by sarcopenia and ageing, and that even higher levels of training may mitigate this (42).

Ageing is often associated with a decline in FFM, strength and physical performance that is accelerated with sedentary behaviour (5). The effects on ageing alone on these outcomes in Masters or recreationally active adults, is less defined. In the current study, there was no observed differences in body composition based on age. However, for strength, power, and physical performance outcomes, the middle-aged group had higher strength (i.e., 1-RM leg press, chest press, and lateral pull down), CMJ, and cardiorespiratory fitness compared to the older-aged group. The largest effect size for age was observed for jump height ($d=1.39$), relative power ($d=0.900$) and cardiorespiratory fitness ($d= 0.822$). The decline in muscle power with ageing has been indicated to decline more rapidly than SMM and muscular strength, and therefore muscle power may be more of a substantial indicator of physical function with ageing, especially in the active population (22). A cross-sectional study by Pearson, Young (21) found that peak power (W) declined with increasing age at a similar rate between elite Masters weight lifters and controls (1.2% and

1.3% per year); while muscular strength declined at a similar but lower rate (0.6% and 0.5% per year). The cause of reduced force- and power-producing capabilities relative to muscle size with ageing appears to be attributed to a reduction in type II fast-twitch muscle fibre size. A original study by Lexell, Henriksson-Larsén (43) observed from cross-sections of the *Vastus Lateralis* in 30 male cadavers that older adults (71 ± 1 years) had on average 18% less muscle fibre size, and 25% decline in total muscle fibres compared to younger adults (30 ± 6 years). The difference in total muscle size was purported to be accounted by a marked reduction in the number of myofibrils in the older muscle (478,000 vs 364,000). In support of this, a more recent study by Nilwik et al. (2013) (44) found that type II muscle fibres were substantially smaller (29%) in older (71 ± 1 years) versus a young (23 ± 1 years) population. The decrease in individual muscle fibres, in particularly type II, could potentially explain the observed lower muscle strength and lower body power in the older-aged group compared with the middle-aged group in the current study. Additionally, in relation to age, there was a significant difference in cardiorespiratory fitness with the average fitness levels being 30% less in the older group than the middle-aged group ($d= 0.822$). There have been numerous cross-sectional and longitudinal studies reporting a decrease in $\dot{V}O_2$ max with age, irrespective of training status (24, 45). These studies have reported a rate of decline in sedentary individuals to be approximately 10% per decade (20, 46, 47), whilst even highly active individuals have a decline of ~5% (48-50). A study by Stathokostas et al. (20), in a 10-year longitudinal study in healthy older adults (73.5 ± 6.4 yr; 28 women, 72.1 ± 5.3 yr) observed a 14% and 7% decline in $\dot{V}O_{2max}$ in men and women, respectively. Additional analysis showed that age-related changes in $\dot{V}O_{2max}$ were not significantly related to physical activity (20). Irrespective of the mechanisms, this indicates that decreasing muscle power and strength and cardiorespiratory fitness in older age may be cause for concern and it is clear that activity alone may not mitigate these changes occurring with age. Additionally, the maintenance of muscle power and cardiorespiratory fitness in older adults may be an important target for intervention, given its implications for ambulation and physical function in older adults.

The current recommendations based on the WHO suggest that all adults should engage in at least 150 minutes of moderate activity throughout the week to support bone, joint and muscle health, which in turn may reduce functional limitations prevent falls and promote independent living (7). The results of this study observed that individuals who achieved the recommended exercise volume (≥ 150 -299 mins per week) or higher (>300 mins per week), when compared to the lower training group (≤ 150 -90mins), had significantly lower fat mass (-10.5% and -9.5%), and significantly higher strength and power outcomes. However, considering that the low training group had the highest proportion of females (50%, **Supplementary material 2**), and females typically present with ~10% higher body fat, are generally produce less power and strength compared to men, this could explain the differences between the groups, more than training alone (51, 52). Another limitation for this study is the lack of comparison made with a non-athletic control group. Therefore, despite there being a difference in body fat percentage between the exercise groups, it is not known if these are any better than the general population and if the larger proportion of females were the cause of the significant difference.

This study found that higher intakes of protein (≥ 1.2 g/kgBM per day) were associated with lower body weight, lower fat mass and higher 1RM leg press compared to the moderate and low protein intake group. There was a moderate effect size when comparing the low intake to the high intake group for FM ($d=0.785$), weight ($d=0.646$) and leg press ($d=0.628$). While the effect size comparing the low and moderate intake was small for the same variables ($d < 0.5$). Our findings partially support the assumption that physically active older adults may require higher amounts of protein (≥ 1.2 g/kgBM per day) than the current recommendations (0.8 g/kgBM per day) and may be a key factor in preventing the decline in muscle strength in older adults (53, 54). There have been numerous observational studies that have correlated protein intake in relation to sarcopenia to explore diet- muscle health relationships (55). However, the majority of these studies are conducted in free-living community dwellers, with limited studies in physically active older adults. One of the few studies in active older adults compared protein intakes and physical function in active (150 mins per week of moderate-to-vigorous activity) older (67.5 ± 1.8 years) women and found a significant difference in self-reported physical function - as measured using hand grip strength and short physical performance battery (SPPB) - in women with higher protein intakes (>1.1 g/kgBM per day) compared to low protein intakes (0.8g/kgBM per day) (56). Additionally, a recent systematic review and meta-analysis of observational studies assessing protein intake with various physical performance outcomes found that reasonably high (1.0g/kgBM per day) and very high protein intakes (>1.2 g/kgBM per day) were associated with more favourable lower-limb physical performance ($P<0.05$) and lower limb strength ($P=0.05$) when compared to low protein (<0.8 g/kgBM per day) in community dwelling older adults. It is widely accepted that physical activity increases the sensitivity of skeletal muscle to the anabolic properties of protein consumption, and the active older population, which exercises at higher amounts than the general population, requires more protein. However, total protein intake *per say* may not be sufficient enough to support active ageing. Numerous studies have indicated that distribution, timing and quality of protein are important to consider (57). Therefore, future studies should focus on these factors alongside total protein intake in an active older population.

The results of this study based on gender found that males had significantly more FMM, ADM and regional muscle mass (arms and legs). The findings of this study strengthen the already abundant results of previous studies that have reported significant sex differences in lean muscle mass, as estimated by DEXA, MRI and CT (51, 58, 59) On average FFM was 28% higher in males than in women with a large effect size ($d=2.34$). This gender difference remained after adjusting for height (ADM^2) where men had 25% more SMM compared to women ($d=2.86$). The muscle distribution between genders showed that women had 41% less arm SMM and 31% less leg SMM. These findings support previous works by Gallagher and Heymsfield (59) , which reported that females have a larger proportion of their appendicular muscle mass in their lower extremities in comparison to males (as estimated by DEXA). Additionally, Janssen *et al* (58) reported similar gender differences in upper (40%) and lower body (33%) muscle mass based on MRI images. This difference in muscle distribution when adjusting for differences in total body mass is the likely cause for the observed differences in strength outcomes. For example the observed gender differences in lower body strength ($\sim 30\%$) which are smaller than those

observed for upper body strength (~50%). The differences in skeletal muscle mass, strength and power between the genders is most likely due to the greater capacity for muscular hypertrophy as a result of higher levels of circulating testosterone seen in males (60). Additionally, considering the clear physiological differences between genders, future intervention studies should consider differentiating between gender.

Limitations

The observations of this study are tempered by the limitations inherent to cross-sectional studies. In addition, our data set included participants who self-reported their food intake and exercise frequency. This creates its own limitations, as food diary accuracy and exercise under- and over-reporting are common limitations in nutritional studies (61). Additionally, the population size was smaller than similar studies, and therefore makes it difficult to assess the causal relationship between body composition, strength, and performance outcomes.

Conclusion

This study showed that the contributions of age, physical activity, daily protein intake, and gender can help explain individual variation in outcomes related to changes in body composition, strength, power, and performance in a cohort of active older adults. Further comparisons indicated that this cohort is at a low risk of adverse outcomes caused by sarcopenia. Strength and power outcomes were influenced by age, training status, protein intake, and gender. It is likely that differences in muscle fibre distribution, hormonal status and level of physical intensity may contribute to these differences.

Declarations

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Conflicts of interest declaration

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Ethics, consent and permissions

All participants gave written informed consent to take part in the study, which included permission to publish. The study protocol obtained approval from the local ethics committee (Project number 12812).

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Figures

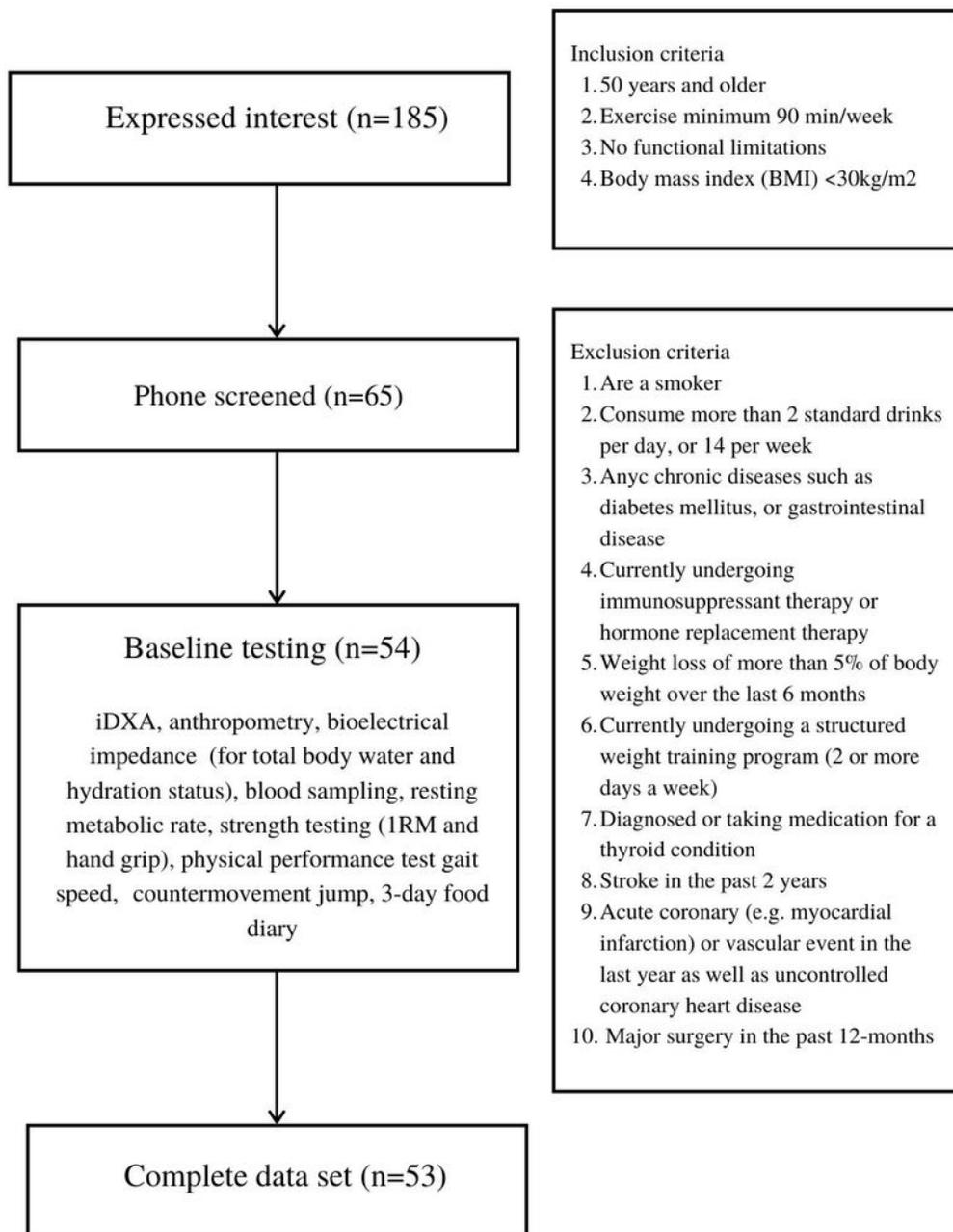


Figure 1

Flow diagram for the identifications, screening, eligibility and participant completion.

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