J Nutr Health Aging. 2019;23(10):949-957

PHYSICAL FUNCTION AND STRENGTH IN RELATION TO INFLAMMATION IN OLDER ADULTS WITH OBESITY AND INCREASED CARDIOMETABOLIC RISK

J. TAY[1,2], A.M. GOSS[1], J.L. LOCHER[3], J.D. ARD[4], B.A. GOWER[1]

1. Department of Nutrition Sciences, University of Alabama at Birmingham, Alabama, USA; 2. Singapore Institute of Clinical Sciences (SICS), Agency for Science, Technology and Research (A-STAR), Singapore; 3. Division of Gerontology, Geriatrics, and Palliative Care, UAB, Birmingham, Alabama, USA; 4. Department of Epidemiology and Prevention, Wake Forest University School of Medicine, Winston Salem, North Carolina, USA. Corresponding author: Jeannie Tay, Department of Nutrition Sciences, University of Alabama at Birmingham, 514 Webb Building, 1675 University Blvd, Birmingham, AL 35294-3360, USA. Email: jeantay@uab.edu

Abstract: *Background:* Inflammation is implicated in functional decline and the development of disability in aging. This study aimed to investigate the association of inflammation with physical function and muscle strength in older adults with obesity and increased cardiometabolic risk. *Design:* In baseline assessments from the CROSSROADS randomized controlled trial, serum interleukin-6 (IL-6), tumor necrosis factor-α (TNFα) and C-reactive protein (hs-CRP) were assayed in 163 older adults (37% males, 24% African American, BMI 34±3, age 70±5yrs) with hypertension, dyslipidemia and/or diabetes. Physical function was assessed by six-minute walk test (6MWT), chair sit-and-reach (CSR), hand-grip and knee-extension strength; specific-strength as muscle strength/mass ratio. Analyses included ANCOVA and multiple linear regression adjusted for thigh skeletal muscle (MRI), arm lean mass (DXA) and moderate-to-vigorous intensity physical activity (MVPA; accelerometry). *Results:* Higher hs-CRP ($p<0.01$) and IL-6 ($p=0.07$) were associated with lower 6MWT and CSR, respectively. A composite inflammation score combining all 3 inflammatory markers showed the strongest inverse association with 6MWT ($p<0.01$). MVPA moderated associations such that amongst participants who engaged in low MVPA, 6MWT distances and CSR scores were significantly lower in those with high IL-6 and TNFα ($p<0.05$), respectively. In participants with high MVPA, higher hs-CRP ($p<0.05$) and TNFα ($p=0.07$) were associated with poorer upper-extremity specific-strength. *Conclusions:* Chronic inflammation was associated with poorer physical function and specific strength in older adults with obesity and increased cardiometabolic risk. This association was strongest in participants with multiple elevated inflammatory markers. Physical activity levels below current recommendations mitigated the deleterious effects of inflammation on lower body mobility, underscoring the benefits of exercise for preserving physical function with age.

Key words: Inflammation, physical function, physical activity, obesity, cardiovascular disease risk factors.

Introduction

Aging is accompanied by "immunosenescence", the deterioration in immune function encompassing cytokine dysregulation and enhanced inflammation (1). This chronic, low-grade inflammation, characterized by abnormal cytokine production, increased acute-phase proteins, and activation of inflammatory signaling pathways, plays an important role in the pathophysiology of atherosclerosis, diabetes and other chronic diseases of aging (2, 3), which are highly prevalent in older adults and a major underlying cause of disability (4).

Sarcopenia, the age-related loss of skeletal muscle mass and strength, contributes to muscular weakness and impaired physical function (5). Adipose tissue is a key producer of inflammatory factors (6) and obesity may exacerbate the age-related decline in physical function through greater production of catabolic inflammatory cytokines which affect protein metabolism and induce skeletal muscle atrophy (7, 8). The concomitant loss of muscle and excess adiposity in "sarcopenic obesity"(9) therefore act synergistically to increase the propensity for frailty and disability in aging adults (10-12).

Several cross-sectional and epidemiological studies have suggested that inflammation predisposes physical function impairments in older persons (13-16). However, none have focused exclusively on individuals with obesity and cardiometabolic comorbidities. Previous studies also rarely considered objective measures of important covariates such as physical activity and skeletal muscle volume. This is despite evidence depicting the catabolic effect of inflammation on muscle (7, 16), and the role of lower muscle mass and quality in the development of physical frailty and dynapenia (10, 11, 17). Physical activity also suppresses muscle catabolism, reduces inflammation, and may delay the onset of immunosenescence (18-20).

To address these factors, this study aimed to investigate the associations between inflammatory markers interleukin-6 (IL-6), tumor necrosis factor-alpha (TNFα) and high-sensitivity C-reactive protein (hs-CRP), with objective assessments of physical function and muscle strength, taking into consideration the potential moderating effects of physical activity and muscle volume, in a biracial cohort of older adults with obesity and prevalent cardiovascular (CVD) risk factors.

Received May 4, 2019
Accepted for publication May 21, 2019
Published online September 26, 2019, http://dx.doi.org/10.1007/s12603-019-1260-4

Methods

Study Population

This paper reports baseline, cross-sectional data from 163 community-dwelling, older adults with independent function who participated in the Calorie restriction in overweight seniors: response of older adults to a dieting study (CROSSROADS) between May 2009 and October 2014 (https://clinicaltrials.gov/, Registration Number Identifier: NCT00955903). CROSSROADS was a prospective 1-year, randomized controlled trial (RCT) that compared the effects of altering diet composition, with/without calorie restriction, in older adults at increased CVD risk (21). The participants and study methodology were previously described (22). Key eligibility criteria were age ≥65 years, BMI 30-40 kg/m^2 (obese), weight stable and prescription of at least one medication for hypertension, hyperlipidemia or diabetes. Exclusion criteria included smoking, previous CVD event, use of corticosteroids, any concurrent treatments that would confound changes in body composition and weight, cognitive impairment, or any significant medical or physical limitations that precluded participation in assessments. The University of Alabama at Birmingham Institutional Review Board approved the study and all participants provided written informed consent.

Markers of Inflammation

Fasting blood samples were assessed for the following serum cytokines and acute-phase protein. Hs-CRP was measured on a SIRRIS analyzer (Stanbio Laboratory, Boerne, TX) by turbidometric methodology (Pointe Scientific, Canton, MI). TNFα and IL-6 were measured by electrochemiluminscence (Meso Scale Discovery, Rockville, MD). The minimum sensitivity of the assays were 0.05mg/L (hs-CRP), 0.5pg/ml (TNFα) and 0.25pg/ml (IL-6). Blind duplicate analyses yielded intra- and inter-assay coefficients of variation of 7.5% and 6.1% (hsCRP), 5.8% and 1.2% (TNFα), and 6.7% and 6.4% (IL-6), respectively.

Elevated levels of ≥two inflammatory markers likely represent a higher overall chronic inflammation status (14, 16). This approach of utilizing multiple cf. a single inflammatory marker has been superior in predicting CVD risk (23). Therefore, analyses were conducted on a composite inflammation score computed by summing the number of inflammatory markers above the median, as a more specific indicator of systemic inflammation. High or low overall inflammation status was defined as having ≥two or ≤one inflammatory marker(s) above the median, respectively.

Covariates

Arm lean mass was measured with dual- energy X-ray absorptiometry (DXA; Lunar DPX-L, General Electric Corp., Madison, WI). Thigh skeletal muscle (tSM) was measured by magnetic resonance imaging (MRI; 3-3Tesla Philips Achieva system, Philips, Andover, MA) using previously described techniques (22). Physical activity levels were assessed with 7 consecutive days of triaxial accelerometry (ActiTrainer; Pensacola, FL) using previously defined and validated cut-points (24): moderate-to-vigorous intensity physical activity (MVPA) was defined as ≥1952 activity counts/min. Valid days had ≥600 minutes wear-time and participants were required to have >four valid days to be included in analyses. Ethnicity was determined by self-report.

Measures of physical function and strength

A comprehensive evaluation of overall physical function and frailty-associated deficits was obtained from a test battery that assessed strength, flexibility and gait (Table 1) (25-28).

Statistical Analysis

The association between each inflammation marker and physical function/muscle strength measure was graphed with a least squares regression line. Based on the observed relationship, one of three statistical approaches was used. When the graph suggested a non-linear, cubic association (e.g. hs-CRP and 6MWT), the point of inflection on the curve was calculated by solving for a derivative of the fitted regression, and used to dichotomize the data (high vs. low inflammation status). When the graph suggested a quadratic association, the inflammation marker was analyzed by median-stratified groups, given the non-normal distribution. Both these approaches used ANCOVA for final analyses. Otherwise, when the association was linear, the relationship was investigated with the marker as a continuous variable in multiple linear regression analyses. To ascertain the independent association of inflammation with physical function/strength, analyses controlled for tSM/arm lean mass, physical activity (MVPA), race and sex.

In all ANCOVA analyses, comparisons of regression slopes (test of interaction between grouping variable and covariates) were conducted to determine whether the assumption of homogeneity of regression slopes was met. Where interaction terms were significant, the Johnson-Neyman (J-N) procedure was used to identify the regions of significance along the observed range of the covariate where the group difference in outcome measure occurred (e.g.MVPA level where groups with high and low inflammation status differed in physical function) (29). For these variables (e.g.IL-6 and 6MWT), group means above and below the identified critical points on the covariate are presented.

Logarithm-transformed variables were used when residuals indicated significant deviations from normality. No significant multicollinearity (assessed through the tolerance statistic and variance inflation factor) was detected. Data are presented as (multivariate-adjusted) estimated marginal means and 95% confidence intervals, unless otherwise stated. Statistical tests were two-tailed with significance set at $p<0.05$ and performed using SPSS 23.0 (SPSS Inc., Chicago, IL).

Table 1
Measures of physical function and strength [a]

Physical function & strength	Description
Six Minute Walk Test (6MWT)	Assesses the self-paced distance walked over 6 minutes and provides an objective evaluation of submaximal aerobic and functional capacity (25). Participants walked around a 40m rectangular track within an enclosed corridor. As most activities-of- daily-living are performed at submaximal levels of exertion, the 6MWT better reflects the functional capacity for daily physical activities than other walk tests (26).
Chair Sit-and-Reach Test (CSR)	Provides a valid and reliable measure of hamstring flexibility in older adults (27). While sitted on a chair, participants would attempt to touch the toes of their leg (heel on floor and foot dorsiflexed), by slowly bending forward at the hip. A static position was held for 2s and the distance between the middle finger tip and middle of the toe (representing a "zero score") was measured. Reaches short of the toe were recorded as negative scores, while reaches beyond the toes were recorded as positive scores. The best score of 2 trials was recorded as the final score.
Short Physical Performance Battery (SPPB)	Comprising a battery of three tests, the SPPB provides an objective assessment of lower extremity function in older persons and has been shown to be a reliable predictor of disability, institutionalization and mortality in older persons (28). The standing balance test required participants to maintain a side-by-side, semi-tandem and tandem stance for 10s each. Repeated chair stands involved participants standing up from a chair and sitting down again, as quickly as possible, without the use of their arms, for five repetitions. Gait speed was assessed by the fastest time of two 3m usual-pace, walk attempts. Results of each test were scored on a 5-level categorical scale: zero represented inability to complete the test and 4 represented the highest level of performance. A summary performance SPPB score, reflecting the functional status of participants in this study, was created by summing the categorical rankings of performance on the 3 tests.
Muscle Strength	Upper extremity muscle strength was assessed by measuring maximum hand-grip using a hand-held dynamometer (T.K.K. 5401 Grip D; Takei Scientific, Nigata, Japan). Lower extremity muscle strength was assessed by measuring knee-extension strength using a universal shear beam load cell with digital transducer (LCC 500; Omega, Stanford, CT). Three trials were performed for both measurements and the maximum score achieved was recorded.
Specific Strength (muscle quality)	Upper extremity specific strength was assessed as the ratio of hand grip strength (kg) to arm lean mass (kg), determined by DXA. Lower extremity specific strength was calculated as the ratio of knee extension strength (kg) to tSM (L), determined by MRI.

Note: DXA, Dual energy x-ray absorptiometry; MRI, Magnetic resonance imaging; tSM, thigh skeletal muscle; a Standardized procedures and instructions were administered. Participants were asked to refrain from consuming caffeine, alcohol or a heavy meal at least 2h before testing and to avoid all strenuous physical exercise in the preceding 24h.

Results

Participant characteristics

The demographic and clinical characteristics of 163 older adults aged 65-84 years (BMI 29-41) who comprised the study population are reported in Table 2. Approximately two-thirds were women and one-quarter was African American. 98% of the women (but none of the men), met the definition for sarcopenia (appendicular skeletal muscle mass adjusted for BMI <0.789[men] and <0.512[women]) according to the Foundation for the National Institutes of Health (FNIH) criteria (30).

The majority of participants were on pharmacotherapy for multiple CVD risk factors. 89% were on anti-hypertensives, 69% on lipid-lowering medications and 21% on anti-glycemic agents. 44% of participants had impaired fasting glucose (fasting plasma glucose 100-125mg/dL (31)).

The median proinflammatory cytokine levels were IL-6 2.1pg/ml, hs-CRP 2.7mg/l and TNFα 4.7pg/ml. 42% of participants had IL-6 levels >2.5pg/ml, a level associated with an increased mobility disability risk in older persons (12). 35% of men and 52% of women had multiple elevated inflammatory markers and a high composite inflammation score.

Despite their age and comorbidities, participants were generally well-functioning without significant physical disabilities. Participants scored an average of 10 points on the SPPB (12 being the highest possible score) and 99% had hand-grip strengths above the cutoffs for muscular weakness (<26kg[men] and <16kg[women]) according to FNIH criteria (30). However, measures of mobility (gait speed and 6MWT) were below the normative values for healthy older adults (32). Women performed better in the CSR, whereas men had higher scores for 6MWT, gait speed, hand-grip and knee-extension strength. Men were more physically active, engaging in more than twice the duration of MVPA compared to women.

Association between inflammation and physical function

Associations between inflammation status and physical function are shown in Tables 3 (non-linear models) and 4 (linear models). The graph of hs-CRP against 6MWT suggested a non-linear, cubic association. Hs-CRP status was thus stratified by the point of inflection on the curve (hs-CRP 1.8 mg/L). In ANCOVA analyses, participants with a high hs-CRP>1.8 mg/L achieved a lower 6MWT distance compared to those with a low hs-CRP≤1.8 mg/L (458 vs. 496m, p=0.006, Table 3).

Table 2
Participant characteristics by sex[a]

Variable	Males	Females
Participant demographics		
Age (years)	70 (5)	70 (5)
Sex [n (%)]	61 (37)	102 (63)
Race [n (%)] *		
African American	6 (10)	33 (32)
Caucasian American	55 (90)	69 (68)
Medications		
Participants on medications [n (%)]		
Hypertension	54 (88.5)	91 (89.2)
Hyperlipidemia	42 (68.9)	71 (69.6)
Diabetes	15 (24.6)	19 (18.6)
Body weight and composition		
Weight (kg)	105.6 (12.0)	88.3 (11.0) ***
BMI (kg/m^2)	33.7 (3.2)	33.6 (3.0)
DXA measures [b]		
Total fat mass (kg)	40.6 (7.7)	42.0 (7.0)
Total fat mass (%)	38.7 (4.3)	48.2 (3.6)
Total fat free mass (kg)	63.9 (6.7)	44.9 (5.3) ***
Total fat free mass (%)	61.3 (4.3)	51.8 (3.6) ***
Total arm lean mass (kg)	7.6 (1.1)	4.7 (0.8) ***
MRI measures		
Thigh skeletal muscle [tSM] (cm^3) [c]	422.4 (56.8)	287.5 (42.3) ***
Inflammatory markers		
TNFα (pg/mL) [d]	5.2 (1.8)	5.0 (1.9)
IL-6 (pg/mL) [e]	3.0 (3.9)	2.8 (2.2)
hs-CRP (mg/L) [f]	2.7 (2.2)	3.7 (2.2) **
Physical function and strength		
Hand grip strength (kg) [d]	44.5 (7.5)	26.9 (5.2) ***
Isometric knee extension strength (kg) [g]	95.2 (34.5)	57.8 (22.4) ***
Chair sit and reach [CSR] (cm) [d, h]	-7.4 (11.7)	-0.8 (8.7) ***
Six- minute walk test [6MWT]: distance (m) [d]	505 (73)	433 (76) ***
SPPB- summary performance score [i]	10.2 (1.2)	10.0 (1.5)
SPPB- Balance score	3.9 (0.5)	3.8 (0.7)
SPPB- Repeated chair stands score	2.4 (1.0)	2.3 (1.2)
SPPB- Gait speed score	4.0 (0.2)	3.9 (0.4)
Gait speed (m/s)	1.10 (0.21)	1.03 (0.18) *
MVPA (min/day) [j]	18.3 (14.4)	7.2 (8.2) ***

Note: DXA, Dual energy x-ray absorptiometry; MRI, Magnetic resonance imaging; TNFα, tumor necrosis factor-α; IL-6, interleukin-6; hs-CRP, high sensitivity C-reactive protein; SPPB, Short physical performance battery score; MVPA, Moderate to vigorous intensity physical activity; The following variables were logarithmically transformed to attain normality before analyses: age, BMI, TNFα, IL-6, hs-CRP, CSR, knee extension strength and MVPA. Untransformed values are presented to facilitate interpretation; a. Differences between males and females by independent samples t-test (continuous variables) or $\chi 2$ tests (categorical variables). Data are unadjusted mean (SD), unless otherwise stated; b. Total analyzed n=161 (males: 61, females: 100) for total fat, fat free and arm lean mass; no DXA data was available for two females; c. Total analyzed n=155 (males: 57, females: 98) for thigh skeletal muscle (tSM); no thigh MRI images were available for four males and four females; d. Total analyzed n=162 (males: 61, females: 101) for TNFα, hand grip strength, 6MWT and CSR; the data was not available for one female; e Total analyzed n=161 (males: 61, females:100) for IL-6; the data was not available for two females; f. Total analyzed n=142 (males: 59, females: 83) for hs-CRP; 19 participants (males: two, females: 17) with CRP >10 mg/L were excluded from these analyses and the data was not available for two females; g. Total analyzed n=161 (males: 60, females:101) for knee extension strength; the data were not available for one female and one male; h. Lower score represents a poorer performance on the chair sit and reach test; i. Total analyzed n=154 (males: 58, females: 96) for SPPB; three males and six females that did not complete all three components of the SPPB were excluded from analyses; j. Total analyzed n=143 (males: 53, females: 90) for accelerometry data; ; the data were not available for three males and eight females; data from nine participants (males: five, females: four) that did not meet the validity criteria were excluded from these analyses; * p<0.05, ** p<0.01, *** p<0.001, significantly different between males and females.

The graph of IL-6 against CSR suggested a linear relationship. Multiple linear regression analysis showed participants with higher IL-6 levels tended to have poorer CSR scores after controlling for race, sex, tSM and MVPA (p=0.07, Table 4).

Based on the composite inflammation score, participants with a high overall inflammation status (multiple elevated inflammatory markers) had poorer physical function compared to those with a low overall inflammation status and levels below the median (Table 3). This association was strongest for 6MWT (p<0.01). None of the other associations were statistically significant after adjustment for covariates.

Accelerometer-measured physical activity moderated the association between inflammation and physical function

The graph of IL-6 against 6MWT distance displayed a non-linear, quadratic curve. ANCOVA analysis revealed a significant IL-6 group by physical activity (MVPA) interaction (p=0.02, Figure 1), indicating that the association between IL-6 and 6MWT distance differed with MVPA level. Application of the J-N procedure showed amongst participants who engaged in MVPA≤12min/day, those with a higher inflammatory status above the median (IL-6>2.1pg/mL) had poorer physical function. Specifically, those with high IL-6 achieved a shorter 6MWT distance (417m) than those with low IL-6 (461m, p=0.003). Conversely, among participants with higher MVPA>12 min/day, physical function did not differ with inflammation status (high IL-6 533m, low IL-6 513m; p=0.29).

The graph of TNFα versus CSR scores also suggested a non-linear, cubic association, with the point of inflection at 8.9pg/ml. There was a significant TNFα by MVPA interaction for CSR scores after adjusting for race, sex and tSM (p=0.03). Application of the J-N procedure showed amongst participants who undertook negligible MVPA<0.4min/day, CSR scores were significantly lower in participants with higher TNFα>8.9pg/ml, than lower TNFα ≤8.9pg/ml. In contrast, CSR scores were not significantly different between high versus low TNFα groups amongst participants who undertook at least some MVPA (≥0.4min/day). However, as only 4 participants had TNFα>8.9pg/ml, these analyses were deemed not meaningful and median-categorized data are reported in Table 3.

Similarly, the graphs of hs-CRP and TNFα versus upper extremity specific strength both showed non-linear, quadratic associations. There was a significant hs-CRP by MVPA interaction that persisted after controlling for race and sex (p=0.03). Application of the J-N procedure showed amongst participants who engaged in MVPA≥50min/day, those with higher hs-CRP>2.7mg/L had significantly poorer upper extremity specific strength compared to those with lower hs-CRP≤2.7mg/L. In contrast, upper extremity specific strength did not differ by hs-CRP status in participants who undertook MVPA<50min/day. However, as only 3 participants engaged in MVPA≥50 min/day, these analyses were deemed not meaningful and median-categorized data are reported in Table

Table 3

Multivariable-adjusted mean (95% CI) physical function scores, muscle strength and specific strength stratified according to low versus high inflammatory status (non-linear models)

Physical function & strength	Markers of inflammation	Inflammatory Status [a]		Mean Difference
		Low	High	
Six-minute walk test [6MWT]: distance (m)	hs-CRP (mg/ L) [b]	496 (474, 518)	458 (444, 473)	38 (11, 65) *
	Composite Inflammation Score [c]	488 (472, 504)	448 (430, 466)	40 (15, 64) *
Gait speed (m/s)	Composite Inflammation Score [c]	1.05 (1.01, 1.10)	1.04 (0.99, 1.09)	0.02 (-0.05, 0.08)
Chair sit and reach [CSR] (cm)	TNFα (pg/ ml)	-3.8 (-6.3, -1.3)	-4.2 (-6.8, -1.6)	0.4 (-3.3, 4.1)
	Composite Inflammation Score [c]	-3.4 (-6.0, -0.8)	-5.0 (-7.9, -2.1)	1.6 (-2.4, 5.6)
Knee extension strength (kg)	IL-6 (pg/ ml)	71.6 (64.8, 78.4)	75.5 (68.7, 82.4)	-3.9 (-13.8, 5.9)
	Composite Inflammation Score [c]	73.9 (66.7, 81.0)	78.8 (70.8, 86.8)	-5.0 (-15.9, 5.9)
Hand grip strength (kg)	IL-6 (pg/ ml)	33.8 (32.6, 35.1)	33.4 (32.1, 34.6)	0.5 (-1.3, 2.3)
	hs-CRP (mg/ L)	34.0 (32.6, 35.3)	34.1 (32.7, 35.5)	-0.1 (-2.1, 1.8)
	Composite Inflammation Score [c]	34.1 (32.8, 35.3)	34.0 (32.6, 35.5)	0.01(-1.9, 2.0)
Specific strength				
Upper extremity specific strength (kg/ kg) [d]	TNFα (pg/ ml)	5.8 (5.5, 6.0)	5.9 (5.7, 6.2)	-0.1 (-0.5, 0.2)
	hs-CRP (mg/ L)	5.8 (5.6, -6.1)	5.8 (5.6, -6.1)	-0.01(-0.4, 0.4)
	IL-6 (pg/ ml)	5.8 (5.6, 6.1)	5.8 (5.6, 6.1)	-0.01 (-0.4, 0.3)
	Composite Inflammation Score [c]	5.8 (5.6, 6.0)	5.9 (5.6, 6.1)	-0.10 (-0.4, 0.3)
Lower extremity specific strength (kg/ L) [e]	IL-6 (pg/ ml)	210.1 (190.1, 230.0)	229.5 (209.3, 249.6)	-19.4 (-48.1, 9.3)
	Composite Inflammation Score [c]	214.7 (193.6, 235.7)	233.5 (209.8, 257.1)	-18.8 (-51.1, 13.5)

Note: Only models with a non-linear association between the inflammatory marker and physical function, and analyzed by ANCOVA are shown in this table. Models for physical function and strength were adjusted for race, sex, moderate to vigorous intensity physical activity (MVPA), arm lean mass (hand grip strength) or thigh skeletal muscle (all other physical function scores), and age (gait speed). Models for specific strength were adjusted for race, sex and MVPA. With the exception of the model for gait speed, age was not included as a covariate as it did not improve the models' ability to explain the variance in physical function and was not significantly associated with physical function in these models. For the chair sit and reach test, lower scores indicate a poorer performance; IL-6, interleukin-6; hs-CRP, high sensitivity C-reactive protein; TNFα, tumor necrosis factor-α; a. Inflammatory status determined by median level of inflammatory marker, unless there was a significant inflammatory marker group by covariate interaction. IL-6: Low IL-6 ≤ 2.1 pg/ ml, High IL-6 > 2.1 pg/ ml; hs-CRP: Low hs-CRP ≤ 2.7 mg/L, High hs-CRP > 2.7 mg/L; TNFα: Low TNFα ≤ 4.7pg/ml, High TNFα > 4.7pg/ml; b. Non-linear association between hs-CRP and six minute walk test. Hs-CRP status delineated by point of inflection on curve: Low hs-CRP ≤ 1.8 mg/L, High hs-CRP > 1.8 mg/L; c. The composite inflammation score was computed by combining the 3 inflammatory markers. High overall inflammation status defined as having ≥ two inflammatory markers above the median (IL-6 > 2.1 pg/ ml, hs-CRP > 2.7 mg/L and/or TNFα > 4.7pg/ml). Low overall inflammation status defined as having ≤ one inflammatory marker above the median; d. Upper extremity specific strength calculated as the ratio of hand grip strength (kg) to arm lean mass (kg) by dual energy x-ray absorptiometry (DXA); e. Lower extremity specific strength calculated as the ratio of isometric knee extension strength (kg) to thigh skeletal muscle mass (L) by magnetic resonance imaging (MRI); * P< 0.01 significantly different by inflammatory status.

3. Further, the TNFα by MVPA interaction for upper extremity specific strength tended towards significance (p=0.05) although adjusting for race and sex attenuated the association (p=0.07). No other significant interactions were observed.

Discussion

The results of this study show that chronic inflammation, assessed by higher circulating IL-6, hs-CRP and TNFα levels, was associated with poorer physical function (6MWT and CSR) and upper extremity specific strength, independent of race, sex, physical activity, muscle volume and age, in older adults with obesity and increased CVD risk. The association was also strongest in individuals with multiple elevated inflammatory markers, underscoring its stronger prognostic significance. Importantly, these significant associations between all three inflammatory markers and various physical function measures persisted after controlling for objective measures of important covariates (physical activity and tSM), further supporting the relationship between chronic inflammation and impaired physical function in older adults.

Physical activity was an important moderator of the association between inflammation and physical function. In sedentary participants, those with a lower IL-6 inflammatory status walked an average of 44m further in the 6MWT compared to those with a higher IL-6 inflammatory status. However, this inverse association between inflammation and physical function was not significant in more physically active participants. Similarly, a higher TNFα inflammatory status was associated with poorer CSR scores only in sedentary but not active individuals. These results support a beneficial role of exercise in mitigating the deleterious effects of inflammation on mobility and flexibility.

Furthermore, the MVPA cutoffs determined for the significant interactions between MVPA and various inflammatory markers suggest that even modest increases in

Table 4

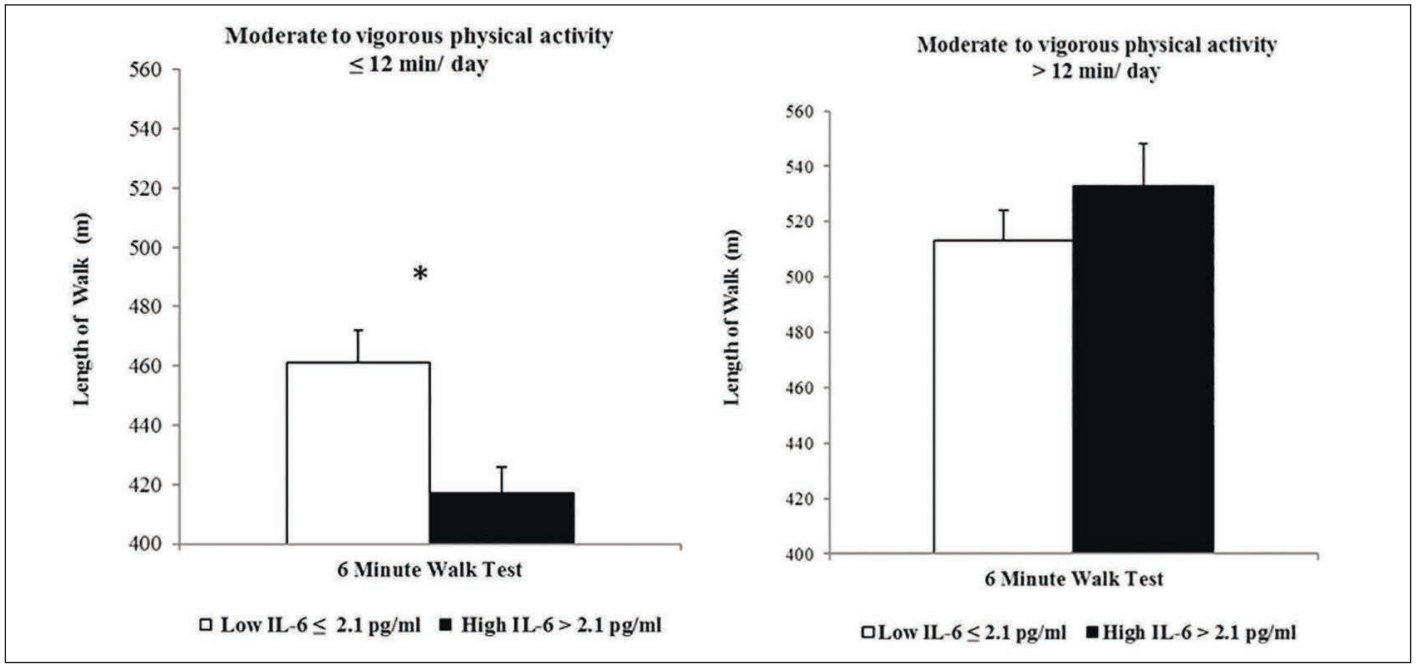
Multiple linear regression models of inflammation versus physical function, muscle strength and specific strength (linear models)

Physical function & strength	Markers of inflammation	β (SEE)	Standardized β	P value for β	R^2
Six-minute walk test [6MWT]: distance (m)	TNFα (pg/ml)	-2.74 (2.98)	- 0.064	0.36	42 %
Gait speed (m/s)	TNFα (pg/ ml)	0.01 (0.01)	0.10	0.25	22%
	hs-CRP (mg/ L)	-0.01 (0.01)	- 0.06	0.49	20%
	IL-6 (pg/ ml)	-0.001 (0.01)	-0.01	0.87	21%
Chair sit and reach [CSR] (cm)	hs-CRP (mg/ L)	-0.43 (0.47)	- 0.086	0.36	13 %
	IL-6 (pg/ ml)	-2.74 (1.50)	- 0.160	0.07	14 %
Knee extension strength (kg)	TNFα (pg/ ml)	0.04 (1.25)	0.002	0.97	36 %
	hs-CRP (mg/ L)	1.82 (1.28)	0.120	0.16	36 %
Hand grip strength (kg)	TNFα (pg/ ml)	0.10 (0.23)	0.018	0.68	77 %
Specific strength					
Lower extremity specific strength (kg/ L) [a]	TNFα (pg/ ml)	0.72 (3.75)	0.02	0.85	9%
	hs-CRP (mg/ L)	4.84 (3.79)	0.12	0.20	10%

Note: Only models with a linear association between the inflammatory marker and physical function, and analyzed by multiple linear regression are shown in this table. Models for physical function and strength were adjusted for race, sex, moderate to vigorous intensity physical activity (MVPA), arm lean mass (hand grip strength) or thigh skeletal muscle (all other physical function scores), and age (gait speed). The model for specific strength was adjusted for race, sex and MVPA. With the exception of the model for gait speed, age was not included as a covariate as it did not improve the models' ability to explain the variance in physical function and was not significantly associated with physical function in these models. IL-6 was logarithmically transformed to attain normality. For the chair sit and reach test, lower scores indicate a poorer performance; SEE, Standard error of the estimate; TNFα, tumor necrosis factor-α; hs-CRP, high sensitivity C-reactive protein: IL-6, interleukin-6; a. Lower extremity specific strength assessed as the ratio of isometric knee extension strength (kg) to thigh skeletal muscle mass (L) by magnetic resonance imaging (MRI).

Figure 1

Significant IL-6 inflammation status by physical activity (moderate-to-vigorous intensity physical activity, MVPA) interaction on physical function (6-minute walk test, 6MWT), IL-6 group x MVPA, p=0.02



White bars, participants with a low IL-6 level below the median (IL-6 ≤ 2.1 pg/ml); black bars, participants with a high IL-6 level above the median (IL-6 > 2.1 pg/ml). Values are estimated marginal means adjusted for race, sex and thigh skeletal muscle (SEM) by ANCOVA. The association between IL-6 and 6MWT distance differed with MVPA level. Length of walk achieved in the 6MWT for participants with (A) MVPA ≤ 12 min/ day (Low IL-6 group, n= 39; High IL-6 group, n= 51) and (B) MVPA > 12 min/ day (Low IL-6 group, n=28; High IL-6 group, n= 15). * p<0.05, significantly different between IL-6 groups.

MVPA, below current guidelines (33, 34) were associated with better physical function, regardless of inflammation status. Disability affects quality of life and is frequently a more significant concern to older adults than extending lifespan. Therefore, this lower MVPA level which may be more achievable for the majority of older adults has important relevance to those typically seen in clinical practice for ambulatory medical care who represent a target group where preventative intervention could be most beneficial. The findings add to the growing evidence that activity levels below current recommendations are associated with significant health benefits and risk reductions, and different physical activity guidelines may be justified for different health outcomes (35, 36). If intervention trials confirm that replacing sedentary behavior with low levels of MVPA can mitigate the deleterious effects of inflammation on physical function, the results will be important for informing physical activity guidelines, given that few older adults meet the current recommendations of at least 150 minutes of moderate-intensity or 75 minutes of vigorous-intensity aerobic activity each week (33, 34).

These findings are also corroborated by the results of an 18-month study of obese, older adults with osteoarthritis where participants randomized to a combined aerobic walking and strength training program had greater improvements in mobility (6MWT) compared to those randomized to a diet-induced weight loss intervention (37). This occurred although participants in the diet group had greater reductions in weight and IL-6. Moreover, longitudinal cohort studies in older adults have shown a direct association between physical inactivity and the subsequent development of reduced physical function (38). The benefits of regular physical activity for improving physical function in the older adults therefore extend beyond its anti-inflammatory effects to include improved mobility, flexibility, strength preservation, and protection against chronic diseases that result in impaired function (39), factors considered in the present study. In addition, the Women's Health study showed that elevated CRP accounted for 33% of the increased CVD risk attributed to physical inactivity, highlighting the role of inflammation, secondary to physical inactivity in influencing CVD (40). Furthermore, while anti-inflammatory agents are a possible strategy for alleviating inflammation, these pharmacotherapies are associated with side effects (41). Meta-analyses showed Tocilizumab, a monoclonal antibody that targets IL-6 receptors and commonly prescribed in chronic inflammatory diseases (e.g. rheumatoid arthritis) produced a proatherogenic lipid profile (42). Collectively, these findings highlight the advantages of lifestyle interventions such as exercise, which have multiple health benefits besides improving immunological and physical function, as an ideal anti-inflammatory treatment strategy in older adults.

However, higher MVPA levels did not negate the association between poorer upper extremity specific strength and higher hs-CRP and TNFα. While these findings are limited by modest sample sizes and marginal statistical significance, they suggest exercise modality may affect the association between inflammation and physical function. In concordance with previous studies that found significant associations between inflammatory markers and mobility measures, this study found the most consistent association with the 6MWT (13, 15). The MVPA data in this cohort were measured by accelerometry, raising the likelihood that MVPA reflected ambulatory activity (e.g. walking). This may explain the significant effect of MVPA on the association between inflammation and the 6MWT or CSR, both assessments of lower body mobility and flexibility. In contrast, walking may be less effective for building upper extremity specific strength, potentially leaving this measure vulnerable to inflammation-induced weakness. Differences in the type, intensity and duration of exercise may have varying effects on inflammation (43). Existing RCTs have predominantly studied the effects of aerobic exercise. However, resistance exercise reduced the production of catabolic cytokines (TNFα), which is associated with accelerated protein degradation in rheumatoid arthritis, an inflammatory condition resembling the decline in lean mass and disordered cytokine production during aging (7). Further research on the exercise modality that is most effective at reducing chronic inflammation and preserving physical function involving different muscles in older adults is thus required.

In obesity, adipocyte hypertrophy due to impaired differentiation and macrophage infiltration of adipose tissue leads to greater releases of inflammatory cytokines to create a pro-inflammatory milieu that promotes insulin resistance and endothelial dysfunction, important precursors in the development of metabolic diseases (3). In addition to obesity, participants in the present study had multiple cardiometabolic comorbidities. This increased CVD risk was reflected by their relatively higher median inflammatory marker levels that were equivalent or greater than that reported in other cohorts with pre-existing CVD and mobility limitations (14, 44). While there is evidence that actively contracting muscle can synthesize and release IL-6, a myokine, after prolonged physical activity, independent of adiposity (45), participants in the present study had been asked to refrain from exercise at least 24h before their blood draws. Further, IL-6 levels have been shown to reach 22 pg/mL following strenuous exercise (46). Therefore, the higher IL-6 concentrations observed in this study more likely reflected an elevated, chronic circulating level rather than an acute increase following a bout of physical activity.

In contrast to previous studies (13, 15), this study enrolled a relatively homogeneous cohort with obesity and CVD risk factors. Individuals with potentially confounding conditions related to inflammation or an increased likelihood of disability (e.g. smoking, cancer or any significant respiratory or CVD), were excluded. The extent of inflammation and frailty in such individuals would be conceivably greater than that observed in this study and results may not be generalizable to all community-dwelling, obese elderly. Nevertheless, this exclusion reduces the likelihood that inflammation was a proxy

for disease burden that is associated with, but not instrumental, in the development of impaired physical function. Moreover, as all participants were obese, the likelihood of differences in adiposity confounding associations (lower inflammation in more physically active individuals due to a lower fat mass), was minimized. The participants were well-functioning older adults and the narrow variation in physical function scores, especially apparent from the SPPB and gait speed scores, may have obscured the potential to detect associations between inflammation and some physical function measures. Further, while exercise- mediated induction of the peroxisome proliferator- activated receptor γ co-activator 1 α (PGC-1α) gene expression in skeletal muscle has been proposed as a possible mechanism by which exercise suppresses chronic inflammation (20) and short-term studies have showed a beneficial effect of exercise on skeletal muscle gene expression (47, 48), exercise has not been consistently associated with reduced systemic inflammation in longer-term intervention studies (21, 49). It is possible that the functional benefits of exercise may be partially mediated through local anti-inflammatory effects within skeletal muscle with no effect on cytokine levels in systemic circulation (48). This may also partly explain the lack of associations observed for some physical function measures in this study. Future intervention studies should therefore assess both muscle protein and systemic inflammation to provide greater mechanistic insight into the role of physical activity in modulating inflammation and physical function in older adults. The cross-sectional nature of the analyses also precludes confirmation of causality. In this regard, longer-term RCTs (≥1 year) showed a combination of exercise and weight loss produced the greatest improvements in physical function, which was associated with lower inflammation (21, 37, 49). Dose-response reductions in inflammatory markers with weight loss have been reported (37). These findings highlight the significant contribution of obesity to systemic inflammation and underscore the advantages of weight loss in obese individuals. Collectively, the research suggests exercise can potentiate the alleviation of chronic inflammation when combined with weight (fat) loss, and this joint strategy may be most effective in ameliorating frailty in obese, older adults (50).

Conclusions

Chronic, systemic inflammation reflected by increased inflammatory marker levels was associated with poorer physical function and specific strength in well-functioning older adults with obesity and increased CVD risk. This association was strongest in participants with multiple elevated inflammatory markers. Physical activity mitigated the deleterious effects of inflammation on lower body mobility, highlighting its importance in the armamentarium of strategies for physical function preservation with age. Future controlled intervention trials should investigate the effects of physical activity levels below current recommendations on both muscle protein and systemic inflammation, and the association with physical function. The results will be important for informing physical activity guidelines for older adults.

Acknowledgments: The authors thank the volunteers for their participation and gratefully acknowledge the staff of the UAB Metabolism Core Laboratory (Nutrition Obesity Research Center, Diabetes Research and Training Center, Center for Clinical and Translational Science) for their involvement in this study.

Author Contributions: JT and BAG had full access to all data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: All authors. Analysis and interpretation of data: JT and BAG. Drafting of the manuscript: JT and BAG. Critical revision the manuscript for intellectual content: All authors. Read and approved the final manuscript: All authors. Obtained funding: JLL, JDA and BAG.

Conflict of interest: The authors declared no conflicts of interest.

Funding: This study was supported by the National Institute on Aging at the National Institutes of Health (R01 AG033094), UAB Nutrition Obesity Research Center (P30 DK56336) and Diabetes Research Center (P30DK079626). JT was supported by a postgraduate research scholarship from the Agency for Science, Technology and Research (A-STAR), Singapore. JLL and JT were supported by a Career Academic Leadership Award from NIA (K07 AG043588). Funding sources had no role in the design, conduct, or reporting of the study or the decision to submit the manuscript for publication.

Ethical Standards: The University of Alabama at Birmingham Institutional Review Board approved the study and all participants provided written informed consent.

References

1. Alvarez-Rodriguez L, Lopez-Hoyos M, Munoz-Cacho P, Martinez-Taboada VM. Aging is associated with circulating cytokine dysregulation. Cell Immunol. 2012;273(2):124-32. Epub 2012/02/10. doi: 10.1016/j.cellimm.2012.01.001. PubMed PMID: 22316526.
2. Pawelec G, Goldeck D, Derhovanessian E. Inflammation, ageing and chronic disease. Curr Opin Immunol. 2014;29:23-8. Epub 2014/04/26. doi: 10.1016/j.coi.2014.03.007. PubMed PMID: 24762450.
3. Hotamisligil GS. Inflammation and metabolic disorders. Nature. 2006;444(7121):860-7. Epub 2006/12/15. doi: 10.1038/nature05485. PubMed PMID: 17167474.
4. Fried LP, Guralnik JM. Disability in older adults: evidence regarding significance, etiology, and risk. J Am Geriatr Soc. 1997;45(1):92-100. Epub 1997/01/01. PubMed PMID: 8994496.
5. Dutta C, Hadley EC. The significance of sarcopenia in old age. J Gerontol A Biol Sci Med Sci. 1995;50 Spec No:1-4. Epub 1995/11/01. PubMed PMID: 7493199.
6. Makki K, Froguel P, Wolowczuk I. Adipose tissue in obesity-related inflammation and insulin resistance: cells, cytokines, and chemokines. ISRN Inflamm. 2013;2013:139239. Epub 2014/01/24. doi: 10.1155/2013/139239. PubMed PMID: 24455420; PubMed Central PMCID: PMCPMC3881510.
7. Rall LC, Rosen CJ, Dolnikowski G, et al. Protein metabolism in rheumatoid arthritis and aging. Effects of muscle strength training and tumor necrosis factor alpha. Arthritis Rheum. 1996;39(7):1115-24. Epub 1996/07/01. PubMed PMID: 8670319.
8. Haddad F, Zaldivar F, Cooper DM, Adams GR. IL-6-induced skeletal muscle atrophy. J Appl Physiol (1985). 2005;98(3):911-7. Epub 2004/11/16. doi: 10.1152/japplphysiol.01026.2004. PubMed PMID: 15542570.
9. Roubenoff R. Sarcopenic obesity: the confluence of two epidemics. Obes Res. 2004;12(6):887-8. doi: 10.1038/oby.2004.107. PubMed PMID: 15229325.
10. Villareal DT, Banks M, Siener C, Sinacore DR, Klein S. Physical frailty and body composition in obese elderly men and women. Obes Res. 2004;12(6):913-20. Epub 2004/07/02. doi: 10.1038/oby.2004.111. PubMed PMID: 15229329.
11. Newman AB, Haggerty CL, Goodpaster B, et al. Strength and muscle quality in a well-functioning cohort of older adults: the Health, Aging and Body Composition Study. J Am Geriatr Soc. 2003;51(3):323-30. Epub 2003/02/18. PubMed PMID: 12588575.
12. Ferrucci L, Harris TB, Guralnik JM, et al. Serum IL-6 level and the development of disability in older persons. J Am Geriatr Soc. 1999;47(6):639-46. Epub 1999/06/12. PubMed PMID: 10366160.
13. Taaffe DR, Harris TB, Ferrucci L, Rowe J, Seeman TE. Cross-sectional and prospective relationships of interleukin-6 and C-reactive protein with physical performance in elderly persons: MacArthur studies of successful aging. J Gerontol A Biol Sci Med Sci. 2000;55(12):M709-15. Epub 2000/12/29. PubMed PMID: 11129392.
14. Penninx BW, Kritchevsky SB, Newman AB, et al. Inflammatory markers and incident mobility limitation in the elderly. J Am Geriatr Soc. 2004;52(7):1105-13. Epub 2004/06/24. doi: 10.1111/j.1532-5415.2004.52308.x. PubMed PMID: 15209648.
15. Brinkley TE, Leng X, Miller ME, et al. Chronic inflammation is associated with low physical function in older adults across multiple comorbidities. J Gerontol A Biol Sci

Med Sci. 2009;64(4):455-61. Epub 2009/02/07. doi: 10.1093/gerona/gln038. PubMed PMID: 19196644; PubMed Central PMCID: PMCPMC2657165.
16. Visser M, Pahor M, Taaffe DR, et al. Relationship of interleukin-6 and tumor necrosis factor-alpha with muscle mass and muscle strength in elderly men and women: the Health ABC Study. J Gerontol A Biol Sci Med Sci. 2002;57(5):M326-32. PubMed PMID: 11983728.
17. Hollmann W, Struder HK, Tagarakis CV, King G. Physical activity and the elderly. Eur J Cardiovasc Prev Rehabil. 2007;14(6):730-9. Epub 2007/11/29. doi: 10.1097/HJR.0b013e32828622f9. PubMed PMID: 18043292.
18. Simpson RJ, Lowder TW, Spielmann G, Bigley AB, LaVoy EC, Kunz H. Exercise and the aging immune system. Ageing Res Rev. 2012;11(3):404-20. Epub 2012/04/03. doi: 10.1016/j.arr.2012.03.003. PubMed PMID: 22465452.
19. Gleeson M. Immune function in sport and exercise. J Appl Physiol (1985). 2007;103(2):693-9. Epub 2007/02/17. doi: 10.1152/japplphysiol.00008.2007. PubMed PMID: 17303714.
20. Handschin C, Spiegelman BM. The role of exercise and PGC1alpha in inflammation and chronic disease. Nature. 2008;454(7203):463-9. Epub 2008/07/25. doi: 10.1038/nature07206. PubMed PMID: 18650917; PubMed Central PMCID: PMCPMC2587487.
21. Ard JD, Gower B, Hunter G, et al. Effects of Calorie Restriction in Obese Older Adults: The CROSSROADS Randomized Controlled Trial. J Gerontol A Biol Sci Med Sci. 2016. doi: 10.1093/gerona/glw237. PubMed PMID: 28003374.
22. Haas MC, Bodner EV, Brown CJ, et al. Calorie restriction in overweight seniors: response of older adults to a dieting study: the CROSSROADS randomized controlled clinical trial. J Nutr Gerontol Geriatr. 2014;33(4):376-400. Epub 2014/11/27. doi: 10.1080/21551197.2014.965993. PubMed PMID: 25424512; PubMed Central PMCID: PMCPMC4248142.
23. Tziakas DN, Chalikias GK, Kaski JC, et al. Inflammatory and anti-inflammatory variable clusters and risk prediction in acute coronary syndrome patients: a factor analysis approach. Atherosclerosis. 2007;193(1):196-203. Epub 2006/07/22. doi: 10.1016/j.atherosclerosis.2006.06.016. PubMed PMID: 16857204.
24. Freedson PS, Melanson E, Sirard J. Calibration of the Computer Science and Applications, Inc. accelerometer. Medicine and science in sports and exercise. 1998;30(5):777-81. Epub 1998/05/20. PubMed PMID: 9588623.
25. A. T. S. Committee on Proficiency Standards for Clinical Pulmonary Function Laboratories. ATS statement: guidelines for the six-minute walk test. American journal of respiratory and critical care medicine. 2002;166(1):111-7. Epub 2002/07/02. doi: 10.1164/ajrccm.166.1.at1102. PubMed PMID: 12091180.
26. Solway S, Brooks D, Lacasse Y, Thomas S. A qualitative systematic overview of the measurement properties of functional walk tests used in the cardiorespiratory domain. Chest. 2001;119(1):256-70. Epub 2001/02/07. PubMed PMID: 11157613.
27. Jones CJ, Rikli RE, Max J, Noffal G. The reliability and validity of a chair sit-and-reach test as a measure of hamstring flexibility in older adults. Res Q Exerc Sport. 1998;69(4):338-43. Epub 1998/12/29. doi: 10.1080/02701367.1998.10607708. PubMed PMID: 9864752.
28. Guralnik JM, Ferrucci L, Pieper CF, et al. Lower extremity function and subsequent disability: consistency across studies, predictive models, and value of gait speed alone compared with the short physical performance battery. J Gerontol A Biol Sci Med Sci. 2000;55(4):M221-31. Epub 2000/05/16. PubMed PMID: 10811152.
29. Preacher KJ, Curran PJ, Bauer DJ. Computational tools for probing interaction effects in multiple linear regression, multilevel modeling, and latent curve analysis. Journal of Educational and Behavioural Statistics. 2006;31:437- 48.
30. Studenski SA, Peters KW, Alley DE, et al. The FNIH sarcopenia project: rationale, study description, conference recommendations, and final estimates. J Gerontol A Biol Sci Med Sci. 2014;69(5):547-58. Epub 2014/04/17. doi: 10.1093/gerona/glu010. PubMed PMID: 24737557; PubMed Central PMCID: PMCPMC3991146.
31. American Diabetes Association. 2. Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes-2018. Diabetes Care. 2018;41(Suppl 1):S13-S27. Epub 2017/12/10. doi: 10.2337/dc18-S002. PubMed PMID: 29222373.
32. Enright PL, Sherrill DL. Reference equations for the six-minute walk in healthy adults. American journal of respiratory and critical care medicine. 1998;158(5 Pt 1):1384-7. Epub 1998/11/17. doi: 10.1164/ajrccm.158.5.9710086. PubMed PMID: 9817683.
33. World Health Organization. Global Recommendations on Physical Activity for Health. Geneva, Switzerland: World Health Organization; 2010.
34. Nelson ME, Rejeski WJ, Blair SN, et al. Physical activity and public health in older adults: recommendation from the American College of Sports Medicine and the American Heart Association. Medicine and science in sports and exercise. 2007;39(8):1435-45. Epub 2007/09/01. doi: 10.1249/mss.0b013e3180616aa2. PubMed PMID: 17762378.
35. Nicklas BJ. No Expiration Date on the Association Between Physical Activity and Mortality. J Am Geriatr Soc. 2018;66(5):850-2. Epub 2018/01/11. doi: 10.1111/jgs.15243. PubMed PMID: 29319876.
36. LaMonte MJ, Buchner DM, Rillamas-Sun E, et al. Accelerometer-Measured Physical Activity and Mortality in Women Aged 63 to 99. J Am Geriatr Soc. 2018;66(5):886-94. Epub 2017/11/17. doi: 10.1111/jgs.15201. PubMed PMID: 29143320; PubMed Central PMCID: PMCPMC5955801.
37. Messier SP, Mihalko SL, Legault C, et al. Effects of intensive diet and exercise on knee joint loads, inflammation, and clinical outcomes among overweight and obese adults with knee osteoarthritis: the IDEA randomized clinical trial. JAMA. 2013;310(12):1263-73. doi: 10.1001/jama.2013.277669. PubMed PMID: 24065013; PubMed Central PMCID: PMCPMC4450354.
38. Stenholm S, Koster A, Valkeinen H, et al. Association of Physical Activity History With Physical Function and Mortality in Old Age. J Gerontol A Biol Sci Med Sci. 2016;71(4):496-501. Epub 2015/08/21. doi: 10.1093/gerona/glv111. PubMed PMID: 26290538; PubMed Central PMCID: PMCPMC4834834.
39. Bouaziz W, Vogel T, Schmitt E, Kaltenbach G, Geny B, Lang PO. Health benefits of aerobic training programs in adults aged 70 and over: a systematic review. Arch Gerontol Geriatr. 2017;69:110-27. Epub 2016/12/03. doi: 10.1016/j.archger.2016.10.012. PubMed PMID: 27912156.
40. Mora S, Cook N, Buring JE, Ridker PM, Lee IM. Physical activity and reduced risk of cardiovascular events: potential mediating mechanisms. Circulation. 2007;116(19):2110-8. Epub 2007/10/31. doi: 10.1161/CIRCULATIONAHA.107.729939. PubMed PMID: 17967770; PubMed Central PMCID: PMCPMC2117381.
41. Boekholdt SM, Stroes ES. The interleukin-6 pathway and atherosclerosis. Lancet. 2012;379(9822):1176-8. Epub 2012/03/17. doi: 10.1016/S0140-6736(12)60361-4. PubMed PMID: 22421338.
42. Interleukin-6 Receptor Mendelian Randomisation Analysis Consortium, Swerdlow DI, Holmes MV, et al. The interleukin-6 receptor as a target for prevention of coronary heart disease: a mendelian randomisation analysis. Lancet. 2012;379(9822):1214-24. Epub 2012/03/17. doi: 10.1016/S0140-6736(12)60110-X. PubMed PMID: 22421340; PubMed Central PMCID: PMCPMC3316968.
43. Beavers KM, Brinkley TE, Nicklas BJ. Effect of exercise training on chronic inflammation. Clin Chim Acta. 2010;411(11-12):785-93. Epub 2010/03/02. doi: 10.1016/j.cca.2010.02.069. PubMed PMID: 20188719; PubMed Central PMCID: PMCPMC3629815.
44. Cesari M, Penninx BW, Newman AB, et al. Inflammatory markers and onset of cardiovascular events: results from the Health ABC study. Circulation. 2003;108(19):2317-22. Epub 2003/10/22. doi: 10.1161/01.CIR.0000097109.90783.FC. PubMed PMID: 14568895.
45. Munoz-Canoves P, Scheele C, Pedersen BK, Serrano AL. Interleukin-6 myokine signaling in skeletal muscle: a double-edged sword? FEBS J. 2013;280(17):4131-48. Epub 2013/05/15. doi: 10.1111/febs.12338. PubMed PMID: 23663276; PubMed Central PMCID: PMCPMC4163639.
46. Steensberg A, Febbraio MA, Osada T, et al. Interleukin-6 production in contracting human skeletal muscle is influenced by pre-exercise muscle glycogen content. J Physiol. 2001;537(Pt 2):633-9. Epub 2001/12/04. PubMed PMID: 11731593; PubMed Central PMCID: PMCPMC2278951.
47. Lambert CP, Wright NR, Finck BN, Villareal DT. Exercise but not diet-induced weight loss decreases skeletal muscle inflammatory gene expression in frail obese elderly persons. J Appl Physiol (1985). 2008;105(2):473-8. Epub 2008/06/07. doi: 10.1152/japplphysiol.00006.2008. PubMed PMID: 18535122; PubMed Central PMCID: PMCPMC2519937.
48. Greiwe JS, Cheng B, Rubin DC, Yarasheski KE, Semenkovich CF. Resistance exercise decreases skeletal muscle tumor necrosis factor alpha in frail elderly humans. FASEB J. 2001;15(2):475-82. Epub 2001/02/07. doi: 10.1096/fj.00-0274com. PubMed PMID: 11156963.
49. Beavers KM, Ambrosius WT, Nicklas BJ, Rejeski WJ. Independent and combined effects of physical activity and weight loss on inflammatory biomarkers in overweight and obese older adults. J Am Geriatr Soc. 2013;61(7):1089-94. doi: 10.1111/jgs.12321. PubMed PMID: 23772804; PubMed Central PMCID: PMCPMC3714323.
50. Villareal DT, Aguirre L, Gurney AB, et al. Aerobic or Resistance Exercise, or Both, in Dieting Obese Older Adults. N Engl J Med. 2017;376(20):1943-55. Epub 2017/05/18. doi: 10.1056/NEJMoa1616338. PubMed PMID: 28514618; PubMed Central PMCID: PMCPMC5552187.