Sports Medicine and Health Science 4 (2022) 95–100

Contents lists available at ScienceDirect

Sports Medicine and Health Science

journal homepage: www.keaipublishing.com/en/journals/sports-medicine-and-health-science/

Original Article

Are flexibility and muscle-strengthening activities associated with functional limitation?

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ARTICLE INFO

Keywords:
Functional capacity
Quality of life
Aging
Exercise
Independence
Health

ABSTRACT

This retrospective cohort study examined the relationship between self-reported participation in flexibility and muscular strengthening activities and the development of functional limitation (i.e., once an individual has difficulty with or becomes unable to perform activities of daily living). Data were obtained from 1318 adults (mean age 49.5 ± 9.7 years; 98.7% Caucasian; 14.9% female) enrolled in the Aerobics Center Longitudinal Study from 1979 to 2004 and free of functional limitation at baseline. Mail-back health surveys were used to prospectively determine incident functional limitation. Participation in muscle-strengthening and flexibility activities was assessed via self-report. Adjusted logistic regression analyses were used to determine the odds ratios (*OR*) and corresponding 95% confidence intervals for developing functional limitation during follow-up based on participation in general and specific categories of flexibility ('Stretching', 'Calisthenics', or 'Exercise Class') and muscle-strengthening activities ('Calisthenics', 'Free Weights', 'Weight Training Machines', or 'Other'). Overall, 42.6% of the sample reported incident functional limitation. After adjusting for potential confounders (e.g., age, sex, cardiometabolic risk factors), those who reported performing muscle-strengthening activities in general ($n = 685$) were at lower risk of developing functional limitation [$OR = 0.79$ (0.63–1.00)]. In addition, the specific flexibility activities of stretching ($n = 491$) and calisthenics ($n = 122$) were associated with 24% and 38% decreased odds of incident functional limitation, respectively. General muscle-strengthening, stretching, and calisthenics activities are prospectively associated with decreased risk of incident functional limitation in generally healthy, middle-aged and older adults. Thus, both public health and rehabilitation programs should highlight the importance of flexibility and muscle-strengthening activities during adulthood to help preserve functional capacity.

Introduction

Activities of daily living represent a domain of fundamental skills and routine tasks that are essential to independently care for oneself and include the following self-maintenance components: basic activities of daily living, mobility, and instrumental activities of daily living.[1] The inability of an individual to perform activities of daily living, deemed functional limitation,[2] contributes to higher morbidity and mortality risk in an aging population.[3,4] An individual's functional limitations (e.g., mobility impairments; difficulty performing recreational, household, daily, and personal care activities) are indicators of quality of life and may affect an individual's independence.[3–6] Mobility impairment and the loss of functional independence associated with aging may contribute to higher economic and societal costs.[7] Thus, to reduce the prevalence of functional limitation and to enhance public health efforts, there is a need to identify factors that play a significant role in the development of functional limitation.

In middle-aged and older adults, greater participation in physical activity and higher cardiorespiratory fitness (CRF) are associated with lower functional limitation.[2,8] Other constructs of physical fitness – including flexibility, muscular strength, and body composition – may also

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https://doi.org/10.1016/j.smhs.2022.03.001
Received 14 November 2021; Received in revised form 28 February 2022; Accepted 10 March 2022
Available online 18 March 2022

Abbreviations

ACLS	Aerobics Center Longitudinal Study
BMI	body mass index
CRF	cardiorespiratory fitness
CI	confidence interval
MET	metabolic equivalent
OR	odds ratio

be used to evaluate functional status.[9] These fitness constructs – particularly flexibility and strength – play a major role in everyday life, as limited balance and mobility are major risks for falls.[10–12]

The American College of Sports Medicine and American Heart Association recommend older adults perform muscular strengthening and flexibility activities at least two days per week for the maintenance of their physical independence and health.[13,14] In addition, flexibility has been touted as an important construct for individuals to successfully perform activities of daily living.[15] Previous studies indicate that decreased flexibility and mobility are not only associated with a greater risk of falling, but also difficulty performing and sustaining motor activities.[16,17] The resulting degenerative changes in soft-tissue may decrease neuromuscular function, emphasizing the need to further explore flexibility in an aging population.[18] Additionally, decreased muscular strength is related to an increased risk for developing functional limitation later in life.[5,19–21] While there is some evidence regarding general muscular strengthening activities and functional limitation,[5,19–21] the mode of strengthening activity remains under studied. Moreover, little is known about the effect of flexibility on functional limitation. Therefore, the purpose of this study is to determine the relationship between self-reported participation in flexibility and muscular strengthening activities and the development of functional limitation in a cohort of generally healthy, middle-aged and older adults.

Materials and methods

Participants were part of the Aerobics Center Longitudinal Study (ACLS), a prospective observational study at the Cooper Clinic in Dallas, TX, USA. Study details have been previously described.[22] Participants came to the clinic periodically for preventive health examinations, as well as counseling for physical activity, nutrition, and wellness. Participants were self- or employer-referred, and the majority of the cohort was Caucasian (> 95%), had a college education, and was from middle to upper socioeconomic strata. All participants underwent a thorough, physician-led physical examination, had anthropomorphic measurements taken, gave a blood sample for blood chemistry analyses, completed a detailed health history questionnaire, and performed a maximal graded treadmill test to assess CRF (measured as treadmill time in minutes). Additionally, health status and physical activity habits were assessed via self-report. From the physical activity questionnaire, total aerobic activity (min/week) from responses to 10 specific activities (walking, jogging, running, treadmill exercise, cycling, stationary cycling, swimming, racquet sports, aerobic dance, and other sports-related activities (e.g., basketball or soccer) was summed by multiplying the frequency and duration. The intensities of activities were estimated via activity-specific metabolic equivalent (MET) values from the Compendium of Physical Activities.[23] The MET value for a given activity was multiplied by the frequency and the duration, and then summed over all activities to determine total MET-min/week of aerobic activity. The detailed calculation was reported in a previous ACLS paper.[24] All participants provided written informed consent, and the ACLS received annual approval from the Cooper Institutional Review Board. The present investigation involved a de-identified dataset without any personal identifiable information, therefore is exempt from the Committee for the Protection of Human Subjects at the University of South Carolina.

In the current study, 1318 men and women completed a baseline medical examination between 1979 and 1995. Inclusion criteria for the current analysis required participants to have returned the 1995 mail-back survey for the baseline function limitation assessment and returned at least one additional health survey from 1999 to 2004 for determining functional limitation risk. Participants were excluded if they had baseline cardiovascular disease, cancer, or bone and joint problems including arthritis, gout, swollen/stiff joints, joint replacement, and chronic joint pain. Additionally, those with missing data on flexibility and muscle-strengthening exercises and those with baseline functional limitation were excluded.

Functional limitation was determined from responses to the mail-back health surveys in 1995, 1999, and 2004. The 1995 mail-back survey served as the baseline reference for functional limitation. The 1999 and 2004 surveys were used to prospectively determine incident functional limitation. The functional status questions encompassed the participants' ability to perform recreational activities (e.g., bicycling, fishing), household activities (e.g., cleaning, cooking), personal care activities (e.g., bathing, dressing), daily activities (e.g., bending, twisting), and activities requiring dexterity (e.g., writing, opening jars).[2,25] For each category of tasks, the participants were asked to rate the difficulty they had performing the tasks. The possible responses included: no difficulty, some difficulty, much difficulty, and cannot do. Subsequently, participants were identified as having a functional limitation if they reported having difficulty or not being able to perform at least one activity from the list of tasks.

To assess muscle-strengthening and flexibility activities, participants were asked to provide answers to the following separate survey questions:

1) "Are you currently involved in a muscle-strengthening program?"
   a. "Can you specify the muscle-strengthening activity as 'Calisthenics,' 'Free Weights,' 'Weight Training Machines,' or 'Other'?"
2) "Are you currently involved in exercises to maintain or improve your joint flexibility?"
   a. "What type of exercises?"
      i. 'Stretching,' 'Calisthenics,' 'Exercise Class,' 'Yoga,' or 'Other'?"

We examined baseline differences using chi-square tests for categorical variables and *t*-tests for continuous variables across activity groups. Logistic regression analyses were used to estimate the odds ratios (*ORs*) and 95% confidence intervals (*CIs*) for incident functional limitation according to flexibility or muscle-strengthening activities including the following exposures: flexibility activities (yes/no), specific flexibility activity, muscle-strengthening activities (yes/no), and specific muscle-strengthening activity. In order to control for potential confounding factors, we included the following variables in the full model: baseline age (years), sex (male/female), total aerobic activity (MET-min/week), body mass index (BMI), current smoking (yes/no), hypertension (yes/no), diabetes (yes/no), hypercholesterolemia (yes/no), CRF (treadmill time duration in minutes), and flexibility activities (when muscle-strengthening was the exposure) or muscle-strengthening activities (when flexibility was the exposure). We set statistical significance for all analyses at $\alpha = 0.05$. All analyses were conducted using SAS statistical software (V.9.4, SAS Institute, Inc., Cary, North Carolina).

Results

Participants ($n = 1318$; 98.7% Caucasian; 14.9% female) had a mean age of 49.5 ± 9.7 years at baseline. Of the 1318 participants, 562 (42.6%) reported the development of one or more functional limitations. Of the 643 participants who reported performing flexibility activities (48.8% of the total sample), 259 reported incident functional limitation. Of the 685 participants who reported performing muscle-strengthening activities (52.0% of the total sample), 267 reported incident functional limitation.

Table 1
Baseline characteristics of participants by activity types.

Characteristic	Flexibility Activity			Muscle-strengthening Activity		
	No (675)	Yes (643)	*p* value	No (633)	Yes (685)	*p* value
Age (years)	**48.7 ± 9.4**	**50.3 ± 10.0**	**0.004**	49.2 ± 9.6	49.8 ± 9.8	0.62
Female (%)	6.9	8.0	0.15	7.6	7.3	0.36
Body Mass Index (kg/m^2)	**24.9 ± 3.1**	**24.3 ± 2.8**	**<0.0001**	**24.9 ± 3.1**	**24.3 ± 2.8**	**0.0004**
Aerobic activity (MET-min/wk)	**1281.8 ± 1161.1**	**1753.5 ± 1309.8**	**<0.0001**	**1226.4 ± 1082.4**	**1775.7 ± 1348.4**	**<0.0001**
Treadmill test duration (min)	**20.3 ± 4.9**	**21.8 ± 4.9**	**<0.0001**	**20.1 ± 5.0**	**21.9 ± 4.7**	**<0.0001**
Total cholesterol (mmol/L)	**5.39 ± 1.0**	**5.3 ± 1.0**	**0.03**	**5.4 ± 1.0**	**5.3 ± 0.9**	**0.0007**
Fasting blood glucose (mmol/L)	5.5 ± 1.0	5.4 ± 0.6	0.11	**5.5 ± 1.1**	**5.4 ± 0.5**	**0.0009**
Resting blood pressure (mmHg)						
Systolic	**120.0 ± 14.0**	**121.6 ± 14.0**	**0.04**	120.4 ± 13.4	121.2 ± 14.6	0.31
Diastolic	79.6 ± 9.4	79.2 ± 9.1	0.54	79.4 ± 9.4	79.4 ± 9.1	0.99
Current smoker (%)	3.3	2.4	0.27	3.1	2.6	0.24
Diabetes (%)	1.1	0.5	0.15	**1.2**	**0.4**	**0.009**
Hypertension (%)	11.4	11	0.89	10.6	11.8	0.82
Hypercholesterolemia (%)	**12.9**	**9.5**	**0.012**	**11.9**	**10.5**	**0.04**

Data shown as means ± *SD* unless specified otherwise. Significant differences are presented in bold and set at α = 0.05 level; MET, metabolic equivalent.

Table 1 displays the distributions of baseline participant characteristics by activity types. Participants reporting flexibility activity were older, had lower BMI, higher aerobic activity participation, higher CRF, higher systolic blood pressure, lower total cholesterol, and reduced prevalence of hypercholesterolemia compared to those who did not report performing flexibility activity. The participants who reported muscle-strengthening activities had lower BMI, higher aerobic activity participation, higher CRF, lower total cholesterol, lower fasting blood glucose, and lower prevalence of diabetes and hypercholesterolemia than those who did not participate in muscle-strengthening activities. Table 2 displays the frequency of participants reporting overall and individual-level functional limitations by activity types.

Tables 3 and 4 display the independent associations between flexibility activities, muscle-strengthening activities, and incidence of functional limitation, respectively. As shown in Table 3, after adjusting for age and sex, those who reported performing flexibility activities in general ($n = 643$) were at lower risk of developing functional limitation compared to those who did not [*OR* = 0.75 (95%*CI*: 0.60–0.94), $p = 0.014$]. When the model was additionally adjusted for potential confounding factors including cardiometabolic risk factors, CRF and muscle-strengthening activities (Models 2, 3, and 4), the odds of developing functional limitation were no longer significantly different. Table 3 also presents specific types of flexibility activities and their association with the development of functional limitation. After adjusting for age and sex, those who reported stretching ($n = 491$) were at lower risk of developing functional limitation [*OR* = 0.70 (95%*CI*: 0.55–0.88), $p = 0.002$] than those who did not. Similar results were observed after additional adjustment for total aerobic activity, BMI, smoking status, hypertension, diabetes, and hypercholesterolemia (Model 2), and CRF (Model 3) with the *ORs* being slightly attenuated. When the model was further adjusted for muscle-strengthening activities, the odds of developing functional limitation were no longer significantly different (Model 4). Additionally, those who participated in calisthenics ($n = 122$) were at a lower risk of developing functional limitation in all models [adjusted for age, sex, total aerobic activity, BMI, smoking status, hypertension, diabetes, hypercholesterolemia, and CRF; Model 4 *OR* = 0.62 (95%*CI*: 0.41–0.93); $p = 0.022$].

We also examined the association between muscle-strengthening activities and functional limitation (Table 4). After adjusting for age and sex, those who reported performing muscle-strengthening activities in general ($n = 685$) were at lower risk of developing functional limitation compared to those who did not [*OR* = 0.71 (95%*CI*: 0.57–0.89), $p = 0.003$]. The *OR* remained significant yet slightly attenuated after further adjustment for total aerobic activity, BMI, smoking status, hypertension, diabetes, and hypercholesterolemia (Model 2). However, once CRF (Model 3) and flexibility activity (Model 4) were added to the model, the *OR* for developing functional limitation was no longer significantly lower than the odds for those who did not. When examining specific types of muscle-strengthening activities, those who reported using weight training machines ($n = 362$) were at a lower risk of developing functional limitation than those who did not [*OR* = 0.76 (95%*CI*: 0.59–0.99), $p = 0.038$] after adjusting for age and sex. When the model was additionally

Table 2
Frequency of participants reporting overall and individual-level functional limitations by activity types.

	All ($n = 1318$)	Participants who reported flexibility activity ($n = 643$)	Participants who reported muscle-strengthening activity ($n = 685$)
Overall functional limitation	562 (42.6%)	259 (40.3%)	267 (39.0%)
Moderate recreational activities limitation	33 (2.5%)	14 (2.2%)	12 (1.8%)
Strenuous recreational activities limitation	487 (37.0%)	228 (35.5%)	30 (33.6%)
Light household activities limitation	3 (0.2%)	1 (0.2%)	2 (0.3%)
Moderate household activities limitation	1 (0.1%)	0	1 (0.2%)
Strenuous household activities limitation	135 (10.2%)	62 (9.6%)	58 (8.5%)
Light daily activities limitation	30 (2.3%)	12 (1.9%)	13 (1.9%)
Moderate daily activities limitation	107 (8.1%)	50 (7.8%)	50 (7.3%)
Strenuous daily activities limitation	86 (6.5%)	39 (6.1%)	38 (5.6%)
Activities requiring dexterity limitation	49 (3.7%)	27 (4.2%)	28 (4.1%)
Moderate personal care activities limitation	5 (0.4%)	3 (0.5%)	3 (0.4%)

Data shown as *n* (%) for each column.

Table 3
Odds ratios (*ORs*) and 95% confidence intervals (*CIs*) for functional limitation by flexibility activities.

	Proportion Reporting Activity (*n*/total sample)	Proportion Reporting Functional Limitation per Activity Subgroup (*n*/row total)	Model 1[a] *OR* (95%*CI*)	Model 2[b] *OR* (95%*CI*)	Model 3[c] *OR* (95% *CI*)	Model 4[d] *OR* (95%*CI*)
General Flexibility						
No	675/1318	303/675	1.00	1.00	1.00	1.00
Yes	643/1318	259/643	**0.75 (0.60–0.94)**	0.82 (0.65–1.03)	0.85 (0.67–1.07)	0.90 (0.70–1.15)
p value			**0.014**	0.09	0.17	0.39
Stretching						
No	827/1318	375/827	1.00	1.00	1.00	1.00
Yes	491/1318	187/491	**0.70 (0.55–0.88)**	**0.74 (0.58–0.94)**	**0.76 (0.60–0.97)**	0.79 (0.62–1.01)
p value			**0.002**	**0.014**	**0.026**	0.06
Calisthenics						
No	1196/1318	520/1196	1.00	1.00	1.00	1.00
Yes	122/1318	42/122	**0.59 (0.39–0.87)**	**0.58 (0.39–0.87)**	**0.58 (0.39–0.88)**	**0.62 (0.41–0.93)**
p value			**0.009**	**0.008**	**0.009**	**0.022**
Exercise Class						
No	1255/1318	536/1255	1.00	1.00	1.00	1.00
Yes	63/1318	26/63	0.77 (0.44–1.33)	0.86 (0.49–1.51)	0.84 (0.47–1.47)	0.88 (0.50–1.55)
p value			0.34	0.60	0.53	0.65

Significant differences are presented in bold and set at $\alpha = 0.05$ level.
[a] Adjusted for age, and sex.
[b] Adjusted for variables in Model 1 plus total aerobics activity (MET-min/week), body mass index, current smoking (yes/no), hypertension (present/not), diabetes (present/not), and hypercholesterolemia (yes/no).
[c] Adjusted for variables in Model 2 plus cardiorespiratory fitness (treadmill time duration in minutes).
[d] Adjusted for variables in Model 3 plus strengthening activity.

Table 4
Odds ratios (*ORs*) and 95% confidence intervals (*CIs*) for functional limitation by muscle-strengthening activities.

	Proportion Reporting Activity (*n*/total sample)	Proportion Reporting Functional Limitation per Activity Subgroup (*n*/row total)	Model 1[a] *OR* (95% *CI*)	Model 2[b] *OR* (95% *CI*)	Model 3[c] *OR* (95% *CI*)	Model 4[d] *OR* (95% *CI*)
General Muscle-strengthening						
No	633/1318	295/633	1.00	1.00	1.00	1.00
Yes	685/1318	267/685	**0.71 (0.57–0.89)**	**0.79 (0.63–1.00)**	0.81 (0.64–1.03)	0.84 (0.66–1.08)
p value			**0.003**	**0.046**	0.08	0.17
Calisthenics						
No	1136/1318	490/1136	1.00	1.00	1.00	1.00
Yes	182/1318	72/182	0.81 (0.58–1.12)	0.85 (0.61–1.18)	0.87 (0.63–1.22)	0.93 (0.66–1.32)
p value			0.20	0.33	0.43	0.68
Free Weights						
No	1002/1318	438/1002	1.00	1.00	1.00	1.00
Yes	316/1318	124/316	0.85 (0.65–1.11)	0.95 (0.72–1.25)	0.97 (0.74–1.28)	1.00 (0.76–1.33)
p value			0.22	0.72	0.83	0.99
Weight Training Machines						
No	956/1318	427/956	1.00	1.00	1.00	1.00
Yes	362/1318	135/362	**0.76 (0.59–0.99)**	0.85 (0.66–1.11)	0.85 (0.66–1.11)	0.87 (0.67–1.13)
p value			**0.038**	0.23	0.23	0.30
Other						
No	1197/1318	511/1197	1.00	1.00	1.00	1.00
Yes	121/1318	51/121	0.91 (0.62–1.34)	0.98 (0.66–1.45)	1.00 (0.67–1.48)	1.03 (0.69–1.53)
p value			0.63	0.92	0.99	0.90

Significant differences are presented in bold and set at $\alpha = 0.05$ level.
[a] Adjusted for age, and sex.
[b] Adjusted for variables in Model 1 plus total aerobics activity (MET-min/week), body mass index, current smoking (yes/no), hypertension (present/not), diabetes (present/not), and hypercholesterolemia (yes/no).
[c] Adjusted for variables in Model 2 plus cardiorespiratory fitness (treadmill time duration in minutes).
[d] Adjusted for variables in Model 3 plus flexibility activity.

adjusted for other potential confounders including cardiometabolic risk factors, CRF, and flexibility activities, the odds of developing functional limitation were no longer significantly different.

Discussion

This is among the first longitudinal studies to assess the association between flexibility and muscle-strengthening activities and risk of developing functional limitation in generally healthy middle-aged and older adults – a cohort reflective of the natural aging process. Those who participated in muscle-strengthening activity were 21% less likely to develop functional limitation, after adjusting for age, sex, and traditional cardiometabolic risk factors (Models 1 and 2). Those who participated in either stretching or calisthenics for flexibility were 24% and 38% less likely to develop functional limitation, respectively, independent from age, sex, cardiometabolic risk factors, and CRF (Models 1 to 3). Furthermore, calisthenics for flexibility was the only activity that decreased an individual's likelihood of developing functional limitation when adjusted for muscle-strengthening activities (Model 4). Taken together, our findings that both muscular strengthening and flexibility activities reduce the risk of developing functional limitation further support the American College of Sports Medicine and American Heart Association's recommendations to perform these types of activities to preserve physical independence and maintain health.[13]

Previous work from the ACLS found that individuals with moderate to high CRF have decreased odds for developing functional limitation compared to those with low CRF.[25] Since muscular strength and flexibility are constructs of physical fitness, our current findings expand the previous work and strengthen the case that physical fitness is associated with the incidence of functional limitation in middle-aged and older adults. When we analyzed participation in specific modes of flexibility activities (Table 3), participation in either stretching or calisthenics demonstrated significantly lower odds of developing functional limitation. Although the benefits of performing flexibility activities are not yet well supported,[26] our results demonstrate that these activities, especially stretching and calisthenics, may decrease an individual's risk for developing functional limitations. Recommendations regarding physical activity for adults should include the performance of flexibility activities regularly, similar to other modes of exercise.[13,14,27] Additionally, the current study provides evidence that for those who may be unable to perform resistance training, flexibility training may provide some benefit for their continued functional ability.[28] Although our study did not reveal any significant associations between specific modes of muscle-strengthening activities and incident functional limitation after adjustment, those who reported general participation in muscular strengthening activities demonstrated significantly lower risk of developing functional limitation (Table 4). This is supported by previous literature that shows muscular strengthening and resistance exercise activities are beneficial for the reduction of risk for functional limitation.[13,14,26] In older adults, an individual's muscular strength is related to their risk of falling and may have implications on the development of functional limitation.[10,16] Falls in an aging population may lead to injury, which in turn may also lead to fear and avoidance behaviors resulting in a negative effect on an individual's function and independence.[29] Thus, maintaining muscular strength in an aging population is vital to help prevent falls leading to injury and limitation.[11,12]

A limitation of the study is that the main outcome measure of the development of functional limitation relied upon self-report measures. However, self-reported functional status has been shown to be relatively accurate when compared with performance measures in older adults (aged 60 and older).[30] In addition, we controlled for the presence of major diseases and musculoskeletal problems at baseline, thus the results from the current analyses are unlikely to be caused by pre-existing functional complications, which can significantly influence the development of functional limitation.[19,31] Another limitation is that participation in flexibility and muscle-strengthening activities was also self-reported. Participants reported whether they currently participated in the activities; however, we are unaware of specific muscle groups targeted, how frequently they participated in the activities, and potential sub-types of activity. As the sample for this study was predominantly Caucasian, male, white-collar workers with a middle to upper socioeconomic status, this sample may not allow for a comparison of the exposures of flexibility and muscle-strengthening activities to the general public.

To the best of our knowledge, this study is the first to examine the relationship between the performance of flexibility activities and functional limitation. Other strengths of this study include the sample size, long-term follow-up, and thorough baseline physical examination from the ACLS. The physical examination allowed formal and systematic evaluation of the presence of medical conditions at baseline.

Conclusion

In generally healthy, middle-aged and older adults, general participation in muscle-strengthening activities is associated with a lower risk of developing functional limitation, encompassing a range of activities including recreational, household, daily, and personal care activities. Additionally, participation in stretching or calisthenics for flexibility is protective against incident functional limitation. Thus, both public health efforts and rehabilitation programs should highlight the importance of flexibility and muscle-strengthening activities during adulthood to help preserve the ability to independently perform activities of daily living.

Ethics approval statement

Participants were part of the Aerobics Center Longitudinal Study (ACLS), a prospective observational study at the Cooper Clinic in Dallas, TX, USA. All participants provided written informed consent, and the ACLS received annual approval from the Cooper Institutional Review Board. The present investigation involved a de-identified dataset without any personal identifiable information, and therefore is exempt from the Committee for the Protection of Human Subjects at the University of South Carolina.

Authors’ Contributions

SNB acquired funding. CEP and XS conceived of the study. XS managed the database. CEP and LMR performed statistical analyses. CEP, LMR, SRW, and XS interpreted results. CEP, LMR, and SRW drafted the manuscript. All authors provided critical reviews and manuscript edits before approval of the final version.

Submission Statement

The manuscript has not been published and is not under consideration for publication elsewhere. If accepted, this manuscript will not be published elsewhere including electronically in the same form or in any other language, without the written consent of the copyright-holder. All authors approve publication of this work.

Fundings

The Aerobics Center Longitudinal Study was supported by multiple grants from the National Institutes of Health (NIH), including grants from the NHLBI (HL62508), NIDDK (DK088195), and NIA (AG06945). The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.

Data availability statement

Data belong to a third party and may not be legally shared by the authors. Data may be requested from The Cooper Institute (https://www.cooperinstitute.org/ccls/).

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We thank the Cooper Clinic physicians and technicians for collecting the data and the staff at the Cooper Institute for data entry and data management.

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