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Effects of a one-year physical activity program on serum C-terminal Agrin Fragment (CAF) concentrations among mobility-limited older adults

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Abstract

Objectives—C-terminal Agrin Fragment (CAF) has been proposed as a potential circulating biomarker for predicting changes in physical function among older adults. To determine the effect of a one-year PA intervention on changes in CAF concentrations and to evaluate baseline and longitudinal associations between CAF concentrations and indices of physical function.

Design—Ancillary study to the Lifestyle Interventions and Independence for Elders Pilot (LIFE-P), a multi-site randomized clinical trial designed to evaluate the effects of chronic exercise on the physical function of older adults at risk for mobility disability.

Setting—Four academic research centers within the U.S.

Participants—Three hundred thirty three older adults aged 70 to 89 with mild to moderate impairments in physical function.

Intervention—A 12-month intervention of either structured physical activity (PA) or health education promoting successful aging (SA).

Measurements—Serum CAF concentrations and objectives measures of physical function – i.e. gait speed and performance on the Short Physical Performance Battery (SPPB).

Results—The group*time interaction was not significant for serum CAF concentrations (p=0.265), indicating that the PA intervention did not significantly reduce serum CAF levels compared to SA. Baseline gait speed was significantly correlated with baseline CAF level (r = −0.151, p= 0.006), however the association between CAF and SPPB was not significant. Additionally, neither baseline nor the change in CAF concentrations strongly predicted the change in either performance measure following the PA intervention.

Conclusion—In summary, the present study shows that a one-year structured PA program did not reduce serum CAF levels among mobility-limited older adults. However, further study is needed to definitively determine the utility of CAF as a biomarker of physical function.

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INTRODUCTION

As the number of older men and women continues to rise worldwide, (1) the maintenance of physical independence is an important public health challenge (2,3). The capacity to perform basic physical tasks is a central tenet of health-related quality of life (4) and a key predictor of adverse health outcomes including hospitalization, post-surgical morbidity, and mortality (5–8). Interest has recently grown in discovering non-invasive biomarkers capable of predicting declines in physical function that ultimately lead to disability (9). The identification of such biomarkers may allow for improved identification of individuals at risk for becoming disabled and/or facilitate the development of novel therapeutic approaches to combat functional decline (10).

Recently, scientists have demonstrated increased interest in the contribution of the nervous system to age-related declines in function. The nervous system may play an important role in age-related functional decline due to its significant contributions to force production and movement. Some authors have proposed that declines in neuromuscular junction integrity may contribute to the development of sarcopenia and age-related functional decline (11). As such, indicators of neuromuscular junction (NMJ) integrity may serve as potential biomarkers for the progression of age-related functional decline.

One such potential biomarker is C-terminal Agrin Fragment (CAF). This 22 kDa protein is derived from the proteolytic cleavage of agrin by neurotrypsin (12). Agrin is a large heparin sulfate proteoglycan involved in the development and maintenance of the NMJ. Agrin also binds proteins on the extracellular surface of muscle such as dystroglycan complex and laminin, (13) making it an important component in maintaining proper musculoskeletal force transduction. Though relatively little is known regarding the precise mechanisms through which CAF may influence neuromuscular function, several studies have demonstrated that elevations in CAF may negatively impact NMJ and skeletal muscle integrity. For example, neurotrypsin overexpression in young mice was previously shown to reduce muscle fiber number and increased the proportion of type I fibers compared to control (14). Moreover, Hettwer et al. (15) also reported that older adults with low muscle mass and grip strength had significantly greater serum CAF concentrations than age-matched controls. These findings suggest that excessive cleavage of agrin results in NMJ fragmentation and fiber denervation and may contribute to skeletal muscle atrophy and loss of muscle strength. Given the importance of muscle mass and strength to maintenance of physical function in advanced age, it seems plausible that CAF concentrations may also be used as a biomarker of functional status among older adults. To our knowledge, however, no study to date has reported the association of CAF levels in older adults with objective performance measures of physical function.

Furthermore, given the importance of neuromuscular function to maintaining physical function, we speculate that CAF may also have utility in predicting function improvements in response to efficacious interventions. For instance, physical exercise is known to improve both neuromuscular and overall physical function – thus we hypothesize that CAF may predict functional responses to longer-term exercise interventions. However, data are

lacking regarding longitudinal changes in circulating CAF levels following physical exercise. The present work is an ancillary study to the Lifestyle Interventions and Independence for Elders Pilot (LIFE-P). LIFE-P was a multi-site clinical trial that tested the effects of a one year structured physical activity (PA) intervention relative to a successful aging (SA) health education program in older adults at risk for becoming physically disabled. The objectives of the present study were to determine the effect of a one-year PA intervention on changes in circulating CAF concentrations and to evaluate associations between CAF concentrations and indices of physical function.

METHODS

Participant recruitment

Details about specific study inclusion and exclusion criteria of LIFE-P have been reported previously (16, 17). Briefly, subjects were eligible for the study if they were aged between 70–89 years, were sedentary, had a SPPB score 9, and were able to walk 400 meters within 15 minutes. A total of 424 participants were randomized into the PA and SA arms at four sites (Cooper Institute, Stanford University, University of Pittsburgh, and Wake Forest University) and followed for at least 12 months. For the present study, stored blood samples were available at baseline and each follow-up timepoint from a total of 333 participants. All participants provided written informed consent and the study protocol was approved by the Institutional Review Boards of all participating institutions.

Successful aging intervention

The SA intervention was designed to provide attention and health education (16). Study participants randomly assigned to SA group attended weekly classes for the first 26 weeks and then monthly until the end of the trial. Workshops were held on various relevant health topics to older adults such as nutrition, medication use, foot care, and preventive medicine. Each class was concluded with a short instructor-led program of upper extremity stretching exercises and regular telephone contact was made to encourage further participation.

Physical Activity Intervention

Details of the PA intervention have been reported previously (17). Briefly, participants randomized to PA performed walking, strength, flexibility, and balance training in center- and home-based settings. Moderate-intensity exercise was promoted and assessed using the Borg scale (18). Participants were asked to walk at a target intensity of 13 (somewhat hard) and perform strength training at an intensity of 15 to 16 (hard). Staff monitored the volume and intensity of exercise throughout the study by recording the completed walking time and overall rating of perceived exertion (RPE) for each session.

Measures of Physical Function

The primary measures of physical function for this analysis were walking speed during a 400-m test and score on the SPPB. During the 400-m test, participants were asked to walk 10 laps of a 40-m course at their usual pace. The SPPB is based on timed measures of standing balance, short-duration walking (4 m), and ability to rise from a chair. Each task was scored on a scale from 0 to 4 depending on the ability and time needed to complete each

task. A summary score (range 0–12) was subsequently calculated by summing the three scores (19).

Blood Collection and CAF

Blood was collected and processed at each assessment visit (baseline, 6 months, and 12 months) according to standard clinical procedures. Serum samples were evaluated for CAF concentrations using a commercially available enzyme-linked immunoabsorbent assay (ELISA) kit (NTCAF Elisa Kit; Neurotune, Schlieren, Switzerland) as published previously (20). CAF values are expressed as log picomolar (pM) concentrations.

Statistical analysis

An *an priori* alpha level of 0.05 was set to determine statistical significance. Data were initially analyzed for normality and homogeneity of variance and descriptive statistics calculated. Baseline characteristics were compared using Student's t-test for continuous variables and the chi-square test for discrete variables. When continuous variables were non-normally distributed, the Mann-Whitney test was used. Longitudinal changes in serum CAF concentrations were evaluated using linear mixed effects modeling. Covariates included in the model were age, sex, baseline CAF concentration, and an index of co-morbidity as published previously (21, 22). The log transformation was applied to serum CAF concentrations because they were non-normally distributed.

Pearson's correlation coefficient (r) was determined to assess the association between baseline serum CAF concentration and baseline walking speed. Because SPPB was non-normally distributed, the association of baseline CAF concentrations of with baseline SPPB score was determined using Spearman's rank correlation (ρ). Because of the close relationship between age and baseline CAF levels, partial correlations controlling for the effect of age were performed as an a posteriori analysis. Linear regression models were created to evaluate the relationships between baseline CAF concentrations and longitudinal changes in CAF concentrations with changes in SPPB score and 400 m walking speed at 12 months. Regression models were created using the stepwise procedure as published previously (23, 24). Variables includes in the models include age, co-morbidity, baseline CAF concentrations, and change in CAF concentration at 12 months.

RESULTS

Data from a total of 333 persons were included in the present study. The mean age was 76.7 (±4.21) years, 68.2 % were women, and 23.7% were racial/ethnic minorities. Demographics characteristics are shown by intervention group in Table 1. Across groups, the main effect for time did not reach statistical significance (p =0.062). However, a significant age*time effect was observed (p=0.016) indicating that older age was associated with greater increases in serum CAF concentrations from baseline to 12 months. However, we did not observe a significant group*time interaction (p=0.265), indicating that a one-year structured physical activity program did not significantly reduce serum CAF levels compared to health education (Figure 1A). Likewise, the sex * time interaction did not suggest sex-related differences in the response to the intervention (p = 0.167).

Gait speed was the only functional indicator significantly correlated with baseline CAF level (r = −0.151, p= 0.006) (Figure 1B), indicating that higher baseline gait speeds were associated with lower serum CAF levels. Results were consistent when controlling for the effects of age as gait speed (r = −0.114, p = 0.038), but not SPPB (ρ = −0.047, p = 0.196), was significantly correlated with baseline CAF concentrations. Likewise, comorbidity index was correlated positively with total CAF level (r= 0.172, p= 0.002). Our findings suggest that SPPB score does not appear to be correlated with baseline serum CAF level (r=−0.086, p=0.115) (Figure 1C). In agreement with the findings of the correlation analyses, stepwise regression models indicated that comorbidity index and age were the only significant contributors to the final models as age and co-morbidity predicted change in gait speed while only contributed to the final model for SPPB.(Table 2a and 2b). Indeed, the standardized beta coefficients for both baseline concentrations of and changes in circulating CAF were small (range: −0.06 to 0.01) and thus did not significantly contribute to the final model.

DISCUSSION

Forty-two percent of the 37.3 million adults over the age of 65 years report having one or more physical limitations performing daily tasks that are essential for maintaining independence in the community (25). Preserving physical function has become a major public health priority as it would drastically reduce health care costs and improve quality of life for many older Americans and their families (26). In recent years, scientists have shown considerable interest in the discovery of non-invasive biomarkers capable of predicting declines in physical function that ultimately lead to disability (1, 7). The identification of such biomarkers may facilitate the development of novel therapeutic approaches to combat functional decline (10).

Recent findings indicate that declines in muscle strength occur more rapidly than declines in muscle mass and that the change in muscle size explains less than 10% of the between-subject variability in the change in muscle strength (27). As a result of these observations, interest has increased in understanding contributions of the nervous system to declines in muscle strength and overall physical function. Because they indicate neuromuscular integrity, circulating CAF concentrations have been proposed as such a biomarker for functional decline (28). The present study is the first to our knowledge to report on the association of CAF levels in older adults with objective performance measures of physical function as well as changes in serum concentrations of CAF following long-term exercise training.

Compared with health education, a one-year structured PA intervention did not reduce serum CAF levels among older adults at risk for becoming physically disabled. These data indicate that the change in serum CAF level was similar in the PA intervention group compared with that in the health education group. It should be noted, however, that prior studies have reported both increases and decreases in CAF concentrations in response to shorter term (12–20 weeks) exercise interventions. Drey et al. (28) previously that resistance exercise training (along with Vitamin D supplementation) was associated with significant decreases in CAF among 69 older adults – particularly among those with high initial CAF

concentrations. Conversely, Fragala et al. (29) noted that a 6-week resistance training intervention significantly increased CAF concentrations among 23 older adults. Several possibilities may explain the differences in results between these studies and those of the present study. First, Drey et al. utilized western blotting to quantify CAF while we and Fragala et al. utilized an immunoassay. Secondly, the prior studies both utilized resistance exercise modalities whereas the present study utilized a multimodal intervention which emphasized aerobic training as the primary modality. Thirdly, though our sample size was significantly larger, the longer duration of the present study could have influenced results as adherence was lower in the latter part of the trial.

Though we observed a significant bivariate correlation between gait speed and baseline CAF concentrations, the present study generally indicated that circulating CAF concentrations were not strongly associated with functional outcomes. These data suggest that CAF may not be the strongest biomarker for predicting functional decline in this population. These data are similar to findings from Drey et al. (28) indicating that gait speed and grip strength were not significantly associated with serum CAF concentrations. Interestingly, however, this group did report that CAF concentrations were negatively correlated with appendicular lean mass (aLM) among men, but not women. This is important to note given that the majority of participants in the present study were women. Future studies may be warranted which are specifically designed determine if CAF is a more useful biomarker for men relative women.

Major strengths of this study include the use of a simple and broadly applicable PA intervention, a large sample size, and relatively long period of intervention and follow-up. However, like any study, the present investigation is not without limitations. For instance, because prior studies have reported that older adults with sarcopenic characteristics had significantly greater serum CAF concentrations than age-matched controls, the addition of a direct measure of muscle mass or muscle strength would have strengthened this study's findings. Additionally, the inclusion of more than one outcome measure could have benefited the study, however few viable serum biomarkers have been proposed to date.

In summary, the present study shows that a one-year structured physical activity program did not reduce serum CAF levels among mobility-limited older adults. These findings add to prior literature which has indicated the need for circulating biomarkers related to changes in physical function among older adults. This study adds to a relative lack of clarity in the literature regarding the utility of CAF as a biomarker of physical function is mixed. Thus, further study is needed to definitively determine the utility of CAF as a biomarker of physical function. Future studies are particularly needed which specifically evaluate the effects of sex and exercise modality on longitudinal changes in CAF concentrations and potential biologic mediators of these responses.

Acknowledgments

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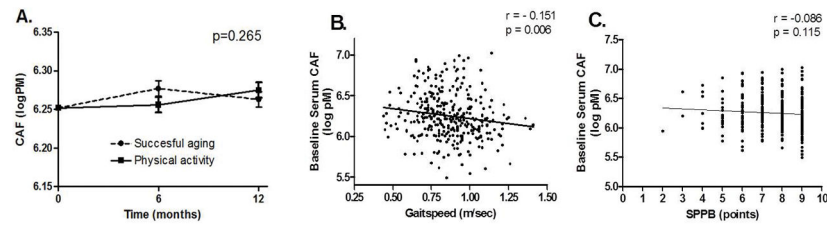
Figure 1.
Figure 1A. Serum CAF levels according to randomized groups at baseline and during follow-up. Means estimated from repeated measures analysis of covariance adjusted for gender, intervention assignment and visit. p-value indicates that for the group*time interaction.
Figure 1B. Scatterplot demonstrating the linear relationships between baseline serum CAF levels and self-selected gait speed. Correlation coefficient indicates bivariate associations.
Figure 1C. Scatterplot demonstrating the linear relationships between baseline serum CAF levels and score on the Short Physical Performance Battery. Correlation coefficient indicates bivariate associations.

Table 1

Baseline characteristics of study participants by randomized group.

Characteristics	PA (n = 166)	SA (n = 167)	P-value
Age, years	77.0 ± 4.3	76.3.0 ± 4.	0.156
Body mass index, kg/m^2	29.6 ± 5.4	31.0 ± 5.9	0.045
Systolic blood pressure, mm Hg	133.0 ± 17.8	132.0 ± 17.7	0.604
Diastolic blood pressure, mm Hg	69.9 ± 10.6	68.6 ± 10.7	0.252
Women	112 (67.5)	115 (68.9)	0.785
Fair/Poor health, self-rated	37 (22.3)	24 (14.4)	0.062
Co-morbidity index, score[*]	1.6± 1.1	1.7 ± 1.1	0.337
Prevalent Health Conditions			
Myocardial infarction	11 (6.6)	18 (10.8)	0.179
Congestive heart failure	9 (5.4)	9 (5.4)	0.990
Lung disease	23 (13.8)	25 (15.1)	0.738
Cancer	26 (15.6)	28 (16.9)	0.748
Diabetes	43 (25.7)	25 (15.1)	0.016
Stroke	7 (4.2))	9 (5.4)	0.600
Osteoarthritis	36 (21.6)	36 (21.6)	0.977

Values are expressed as means ± S.D. or n (%). PA = Physical Activity; SA = Successful Aging Health Education.

[*] Calculated as a composite score based on the presence/absence of 10 prevalent comorbidities: hypertension, heart attack, heart failure, stroke, cancer, diabetes, broken hip, arthritis, liver disease, and lung disease. A higher score indicates greater co-morbidity burden.

Table 2a

Results of the stepwise regression model for the change in gait speed following the exercise intervention

	Beta[a]	T	Prob(t)	95%CI	Partial R^2	Model R^2	Probability>F
Included variables						0.045	0.002
Co-morbidity index	−0.152	−2.561	0.011	−0.03, −0.04	0.033		
Age	−0.151	−2.536	0.012	0.00, 0.00	0.023		
Excluded variables							
Gender	−0.15	−0.258	0.797				
Baseline CAF	−0.061	−1.013	0.312				
CAF*	0.012	0.196	0.845				

[a]Standardized β;

CAF = C-terminal Agrin Fragment.

*indicates change from baseline CAF concentration at 12 month

Table 2b

Results of the stepwise regression model for the change in SPPB following the exercise intervention

	Beta[a]	T	Prob(t)	95%CI	Partial R²	Model R²	Probability >F
Included variables						0.030	0.004
Age	−0.174	−2.922	0.004	−0.13, −0.02	0.030		
Excluded variables							
Co-morbidity index	−0.067	−1.119	0.264				
Gender	0.002	0.042	0.967				
BaselineCAF	−0.058	−0.967	0.334				
CAF*	−0.026	−0.425	0.671				

[a]Standardized β;

CAF = C-terminal Agrin Fragment.

*indicates change from baseline CAF concentration at 12 months