C-TERMINAL AGRIN FRAGMENT IS INVERSELY RELATED TO NEUROMUSCULAR FATIGUE IN OLDER MEN

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ABSTRACT: Introduction: The aim of this study was to examine the relationship between serum C-terminal agrin fragment (CAF) concentrations and neuromuscular fatigue in older adults. Methods: Twenty-two healthy older men and women volunteered for this study. Resting fasted blood samples were collected and prepared for measurement of serum CAF concentration by a commercially available ELISA kit. The onset of neuromuscular fatigue was measured by monitoring electromyographic fatigue curves from the vastus lateralis muscle using the physical working capacity at fatigue threshold (PWCFT) test. Results: A significant inverse correlation for men was observed between CAF and PWCFT (r = -0.602; P = 0.05), but not for women (r = 0.208; P = 0.54). After controlling for age and body mass index, significant correlations (r = -0.69; P = 0.042) remained for men, but not for women (r = 0.12; P = 0.76). Conclusions: These data suggest that serum CAF concentrations were significantly related to the onset of neuromuscular fatigue independent of age and BMI in men only.


The loss of skeletal muscle mass and function by disease or aging (sarcopenia) may involve disassembly of the neuromuscular junction (NMJ), leading to motor unit remodeling. In healthy muscle, a synaptically located protein, agrin, is important for formation and maintenance of the NMJ. During the NMJ remodeling process, the enzyme neurtrotrypsin regulates the activity of agrin by cleaving and deactivating it into a 22-kDa C-terminal agrin fragment (CAF). Further, excessive neurtrotrypsin activity may result in NMJ degeneration and thus be a factor in the development of sarcopenia. Recently, Drey et al. reported that serum concentration of CAF may serve as a potential marker of NMJ degeneration. CAF concentrations were also related inversely to appendicular lean body mass (ALBM) in older men.

Fatigue may serve as an early indicator of the aging process, as it is a strong predictor of functional limitations in older adults. Recently, a significant (P < 0.05) relationship between the onset of neuromuscular fatigue, as determined by the physical working capacity at fatigue threshold test (PWCFT) and ALBM (r = 0.57) and sit-to-stand functionality test (0.44), was reported in a sample of older men and women (71 ± 6 years of age). This neuromuscular fatigue test possessed the discriminative ability to determine sarcopenic risk among older adults. If serum CAF is associated with NMJ degeneration, a corollary of motor unit remodeling, then it may be related to changes in myoelectric properties and recruitment strategies during exercise. The purpose of this study was to understand the relationship between the onset of neuromuscular fatigue and serum CAF concentrations in older adults.

METHODS

Subjects. Twenty-two healthy older men [age 67.9 ± 4.7 years; body mass index (BMI) 28.0 ± 4.9 kg/m²; n = 11] and women (age 72.4 ± 6.9 years; BMI 27.1 ± 4.5 kg/m²; n = 11) volunteered for this study. All participants were healthy and had not undergone major surgery in the 4 months preceding the investigation. The institutional review board at the University of Central Florida approved this study, and all subjects provided written informed consent.

Testing Procedures. Blood Sampling and Preparation. Resting blood samples were collected in the morning after an overnight fast. Blood was drawn from a forearm vein into serum separator tubes for sample preparation. The blood was allowed to clot for 30 minutes before centrifugation. Samples were centrifuged for 10 minutes at 1000g at −4°C and immediately aliquoted into designated preservative tubes. All samples were stored at −80°C until analysis.

Abbreviations: ALBM, appendicular lean body mass; BMI, body mass index; CAF, C-terminal agrin fragment; ELISA, enzyme-linked immunosorbent assay; EMG, electromyography; NMJ, neuromuscular junction; PWCFT, physical working capacity at fatigue threshold.

Key words: aging; electromyography; exercise; physiological adaptations; sarcopenia; serum biomarker.

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Serum CAF. Serum CAF was measured by a commercially available enzyme-linked immunoassay (ELISA) kit (Neurotune Catalog No. NT1001). The measuring range of this assay is 0.4–8 ng/ml. All samples were run in duplicate and thawed only once.

Electromyography Measurements. Electromyographic (EMG) measures were obtained using methodology from our laboratory, as detailed previously. The EMG signals were expressed as root-mean-square (rms) amplitude values (µVrms) and analyzed with custom-written software (LabVIEW, National Instruments Corp., Austin, Texas).

Determination of PWCFT. Determination of PWCFT values during a 2-minute, discontinuous incremental cycle ergometer test was determined using procedures described previously. PWCFT was defined as the average of the highest power output that resulted in a non-significant (P > 0.05, 1-tailed t-test) slope coefficient for the EMG amplitude vs. time relationship and the lowest power output that resulted in a significant (P < 0.05) positive slope coefficient. Reliability data from our laboratory for PWCFT values for 10 men and women similar to the cohort used in this study resulted in an intraclass correlation coefficient of 0.95 (SEM ±13.7 watts).

Statistical Analysis. Data were analyzed using PASW Statistics, version 18.0 (SPSS, Inc., Chicago, Illinois). Shapiro–Wilk tests were used to verify normal distribution of data. An independent t-test was used to assess mean differences for CAF and PWCFT between men and women. A Pearson product-moment correlation test was used to examine relationships between CAF and PWCFT. In addition, second-order partial correlations were used to examine the relationship between CAF and PWCFT covaried for age and BMI.

RESULTS

The mean CAF values for men (3.61 ± 1.3 pg/ml) and women (3.91 ± 1.1 pg/ml) did not differ significantly (P = 0.57). The PWCFT, however, was significantly different for men (73.6 ± 26.4 watts) compared with women (48.1 ± 14.5 watts). A significant inverse correlation for men (Fig. 1) was observed between CAF and PWCFT (r = -0.602; P < 0.05), but not for women (r = 0.208; P > 0.54). After controlling for age and BMI, significant correlations (r = -0.69; P < 0.042), remained for men, but not for women (r = 0.12; P = 0.76).

DISCUSSION

The primary finding is that serum CAF concentrations were related inversely (r = -0.602) to the onset of neuromuscular fatigue via PWCFT independent of age and BMI in men only. Our previous work has shown PWCFT to be sensitive for detecting sarcopenia risk as identified by ALBM. The current results also support published data showing CAF to be related inversely to ALBM in men, but not women, which suggests that the etiology of muscle loss with aging in men may be associated strongly with degeneration of the neuromuscular junction as measured by CAF, whereas, in women, sarcopenia may be more multifaceted. Future investigation is warranted to further establish the relationship between CAF and neuromuscular function during the aging process.

REFERENCES