C-terminal Agrin Fragment is Inversely Related to Neuromuscular Fatigue in Older Men


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ABSTRACT

Recently it has been postulated that C-terminal agrin fragment (CAF) is a serum biomarker of neuromuscular junction (NMJ) degeneration that may serve as a viable method for identifying patients at risk for skeletal muscle mass and function loss. The physical working capacity at fatigue threshold (PWC_{FT}) is a measure of neuromuscular efficiency and resistance to fatigue which has been associated with the health and functional capacity of older adults.

METHODS CONTINUED

The physical working capacity at fatigue threshold (PWC_{FT}) is a measure of neuromuscular efficiency and resistance to fatigue which has been associated with the health and functional capacity of older adults.

RESULTS

The mean CAF values for men (3.61±1.39 pg/mL) and women (3.78±1.87 pg/mL) were significantly different. The PWC_{FT}, however, was significantly (p=0.008) different for men (73.6±28.4 watts) vs. women (48.1±14.5 watts). A significant inverse correlation for the men was observed (r=-0.54, p=0.02). For the women (r=0.39, p=0.02), the correlation for age and BMI, significant correlations (r=-0.69, p=0.042), remained for the men but not for the women (r=0.12, p=0.76).

INTRODUCTION

The loss of skeletal muscle mass and function by disease or aging (sarcopenia) may involve the dysfunction of the neuromuscular junction leading to motor unit (MU) remodeling. Recently, Drey et al. reported that serum concentrations of C-terminal agrin fragment (CAF) may serve as a potential marker of neuromuscular junction (NMJ) degeneration. CAF concentrations were also inversely related with 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, Buschmann et al. demonstrated a significant (p<0.05) relationship between the onset of neuromuscular fatigue as determined by the physical working capacity at fatigue threshold test (PWC_{FT}) and age-related immunosenescence (n=577) in a representative sample of older adults (n=577). Further, this neuromuscular fatigue test possessed the discriminative ability to determine sarcopenic risk among a sample of older adults. If serum CAF is associated with NMJ degeneration, a correlative of NMU remodeling, then it may be related to changes in myofilamental properties and recruitment strategies during exercise. The purpose of this study, is to examine the relationship between the onset of neuromuscular fatigue and serum CAF concentrations in older men and women.

Participants:

Twenty-two healthy older men (age: 67.9±4.7 y; BMI: 28.6±4.9 kg/m\(^2\); n=11) and women (age: 72.4±6.9 y; BMI: 27.1±4.9 kg/m\(^2\); n=11) volunteered for this study. All participants were healthy and had not undergone major surgery in the 4 months preceding the study.

Blood Sampling and Preparation

Blood samples were collected in the morning following an overnight fast. Blood was drawn from a forearm vein into serum separator tubes for serum sample preparation. Serum tubes were allowed to clot for 30 minutes prior to centrifugation. Serum samples were then separated by centrifugation for 10 minutes at 1000g at 4°C. Serum was immediately aliquotted into designated preservation bottles and samples were stored at -80°C until analysis. Prior to analysis, samples were thawed only once, centrifuged to remove particulates, and mixed completely by vortex.

Serum CAF

Serum CAF was measured by commercially available ELISA kit (Method 1). Samples were prepared by incubating 50 µL of serum with 50 µL of incubator buffer in Deepwell plates for 1 hour at 25°C. Following centrifugation, samples were diluted 10-fold and 100 µL of diluted sample was transferred to the pre-coated ELISA plate and incubated for 1 hour at room temperature. The ELISA plate was washed and 100 µL of CAF detector antibody was added to each well for a 30 minute incubation at room temperature. The ELISA plate was washed again and 100 µL of SA-poly-HRP solution was added to each well for another 30 minute incubation at room temperature. A TMB solution was then added to each well to initiate color formation. The enzyme-substrate reaction was terminated by the addition of a stop and the color change was measured spectrophotometrically at a wavelength of 450 nm. The concentration of CAF was determined using the standard curve generated from the samples to the standard curve. The measuring range of this assay is 0.4 to 8 ng/mL. All samples were run in duplicate and thawed only once.

Electromyography (EMG) Measurements

A bipolar (4.6 cm center-to-center) surface electrode (Quinton Quick-Prep silver-silver chloride) arrangement was placed over the vastus lateralis muscle of the right thigh. Prior to electrode placement, the skin at each site was shaved and cleaned with alcohol. The EMG electrodes were placed based on the recommendations from the SENIAM project for EMG electrode placement (Hermens et al. 1999). Inter-electrode impedance was kept below 5.000 ohms with abrasion of the skin both before and after the electrodes. The raw EMG signal was sampled at 1 kHz, differentially amplified (EMG 100x, bandwidth = 10-500Hz, gain: x1000; MP150 BIOPAC Systems, Inc., Santa Barbara, CA), and digitally bandpass filtered (zero-phase shift fourth-order Butterworth) at 10-50 Hz. The signals were recorded and stored on a personal computer (Dell Latitude E6530; Dell Inc., Round Rock, TX) for off-line analysis. The EMG signals were expressed as root mean square (rms) amplitudes (µVrms) and were calculated and analyzed with custom-written software (LabView, National Instruments Corporation, Austin, TX).

Sample of EMG signal obtained during incremental cycle ergometer test

Determination of PWC_{FT}:

PWC_{FT} was determined during a discontinuous incremental cycle ergometer test using the procedures adapted from deVries et al. (1987, 1989). The initial work rate was set at 30 watts and increased 15 watts each stage for the men and women. The subjects pedaled at 50 revolutions per minute (rpm) for each two-minute stage of the test on an electronically-cycled cycle ergometer (Lode, Excalibur Sport, Groningen, the Netherlands). Top clips were utilized for each subject. Following each stage, two needle electromyographic (EMG) needle electrodes were inserted from the vastus lateralis to the rectus muscle. The EMG amplitude values for each of the 10 s epochs were plotted across time for each power output of the test. The PWC_{FT} was defined as the test power at which result in a non-significant (p>0.05; single-tailed t-test) slope coefficient for the EMG amplitudes versus time threshold, and the lowest power output that resulted in a significant (p<0.05) positive slope coefficient. The reliability of the PWC_{FT} values for ten men and women similar to the ones used in this study resulted in ICC=0.95 with a SEM =13.7 watts.

Statistical Analysis

Data were analyzed using PASW statistics, version 18 (SPSS, Inc., Chicago, IL). Shapiro-Wilk tests were used to verify normal distribution of data. An independent t-test was used to examine mean differences for CAF and PWC_{FT} between men and women. A Pearson product-moment correlation test was used to examine relationships between CAF and PWC_{FT}.


REFERENCES