

**Oxidative protein damage is associated with poor grip strength
among older women living in the community**

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Running Head: Oxidative protein damage and grip strength

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Abstract

Grip strength, an indicator of muscle strength, has been shown to be a predictor of poor outcomes among older adults. Protein carbonylation, an indicator of oxidative damage to proteins, leads to cellular dysfunction and a decline in tissue function. Oxidative stress has been implicated in the pathogenesis of sarcopenia. The objective was to determine whether serum protein carbonyl concentrations are associated with grip strength in older women living in the community. A cross-sectional study was conducted in 672 women, aged 65 and older, from the Women's Health and Aging Study (WHAS) I, the one-third most disabled women residing in the community in Baltimore, Maryland. Protein carbonyl and grip strength was measured in each patient. In a multivariate analysis adjusting for age, race, body mass index, and Mini-Mental Status Examination score, protein carbonyls (nmol/mg) were associated with grip strength (beta = -6.77, $P < 0.01$). The statistical association was unchanged after adjusting for hypertension, congestive heart failure, and depression. Ordered logistic regression models adjusted for the above factors showed that protein carbonyls are associated with increased odds of being in the lower quartiles of grip strength (Odds Ratio 8.74, 95% Confidence Interval 1.06 – 71.89, $P = 0.043$). These results suggest oxidative protein damage is independently associated with low grip strength among older women living in the community. Increased oxidative stress may be contributing to loss of muscle strength in older adults.

Keywords: aging, sarcopenia, interleukin-6, oxidative stress, protein carbonyls

Introduction

Muscle strength and muscle mass both deteriorate with age (15,17). The cause of this decrease in muscle strength and mass is not well characterized. In the human skeletal muscle, proteins, DNA and lipids are damaged by reactive oxygen species during oxidative stress. The levels of these damaged macromolecules and lipids increase with age (6,11,14,18). Protein carbonyls are known markers of oxidative stress and accumulate with aging (22). Protein carbonylation leads to cellular dysfunction and a decline in tissue function, and is involved in the pathogenesis of sarcopenia (5).

Hand grip strength is strongly correlated with other measures of muscle strength and therefore is often considered representative of total body muscle strength (21). In healthy, middle-aged men, grip strength at baseline was associated with incident disability and long-term mortality (19,20). Grip strength was also a predictor of cause specific and total mortality among older disabled women (21). In spite of this strong evidence, the mechanism that connects muscle strength and mortality is still a matter of speculation. We hypothesized that poor grip strength in older adults is associated with higher oxidative stress, as reflected by oxidative damage to proteins. To address this hypothesis, we measured serum protein carbonyl concentrations and grip strength among moderately to severely disabled older women living in the community in Baltimore, Maryland.

Materials and Methods

A cross-sectional study was conducted among 672 women, aged 65 and older, from the Women's Health and Aging Studies (WHAS) I, representative of the one-third most disabled women residing in the community in Baltimore, Maryland. Participants were recruited from an age-stratified random sample of women aged 65 years and older selected from Medicare enrollees residing in 12 contiguous zip code areas in Baltimore (7). Women were screened to identify self-reported physical disability that was categorized into four domains. The domains of disability were ascertained in a 20-30 minute home interview that included questions related to (1) mobility and exercise tolerance, i.e., walking for a quarter of a mile, walking up 10 steps without resting, getting in and out of bed or chairs, (2) upper extremity function, i.e., raising your arms up over your head, using your fingers to grasp or handle, lifting or carrying something as heavy as ten pounds, (3) higher functioning tasks (a subset of instrumental activities of daily living, not including heavy housework, i.e., using the telephone, doing light housework, preparing your own meals, shopping for personal items), and (4) basic self-care tasks (a subset of non-mobility dependent activities of daily living, i.e., bathing or showering, dressing, eating, using the toilet). WHAS I enrolled the one-third most disabled women ages 65 and older, those with disability in two or more domains. Of the 1409 women who met study eligibility criteria, 1002 agreed to participate in the study in 1992. There were no major differences in sociodemographic or reported health characteristics between eligible participants and those who declined to participate (7).

Standardized questionnaires were administered in the participant's home by trained interviewers. Mini-Mental Status Examination (MMSE) was recorded (7). Race was assessed in

a questionnaire as black, white, or other, current smoking as yes or no, and education as 0-8, 9-11, 12 years or more than 12 years as the highest level of formal education achieved. Two weeks later, a trained registered full-time study nurse conducted an examination of each study participant in her home, using a standardized protocol that included physical performance measures and a standardized physical examination. Approximately 75% of women also consented to phlebotomy performed during a separate visit by a trained phlebotomist who followed a standardized protocol. Further details on the methods and sampling design of the WHAS studies are published elsewhere (7).

There were 1002 women enrolled in the Women's Health and Aging Study I, of whom 672 women participated in the blood drawing and had serum protein carbonyl measurements at baseline. There were no significant differences in race or body mass index between those who did and did not participate in the blood drawing, but women who did and did not participate in the blood drawing were different by age (77.4 vs 80.7 years, respectively, $P < 0.0001$). Non-fasting blood samples were obtained by venipuncture between 9 AM and 2 PM. Processing, aliquoting, and freezing were carried out at the Core Genetics Laboratory of The Johns Hopkins University School of Medicine following a standardized protocol. Blood samples were delivered to Quest Diagnostics Laboratories (Teterboro, New Jersey) and in part stored continuously at -70°C until the time of analyses for serum protein carbonyls and serum interleukin (IL)-6.

Handgrip strength test was assessed by the nurse using a JAMAR hand dynamometer (Model BK-7498, Fred Sammons Inc., Brookfield, IL). Testing was done with the participant in a seated position and the elbow flexed at 90° . Three measurements were taken for each hand and the participant was encouraged to exhibit the best force possible. The handgrip strength test was completed by 919 of the 1002 women enrolled in the study. The reasons for not completing the

handgrip strength were as follows: systolic blood pressure of 180 mmHg or greater or diastolic pressure of 110 mmHg or greater (n = 55), pain (n = 10), tester or participant felt the test was unsafe (n = 11), participant refusal (n = 2), and other (n = 5). Women were excluded from the grip strength test if they had recent worsening of pain or arthritis in the wrist or hand, or tendonitis.

Serum protein carbonyls were measured using a commercial ELISA (Zentech PC Test, Protein Carbonyl Enzyme Immuno-Assay Kit, Biocell Corp, Papatoetoe, NZ). Protein carbonyls are stable under long term storage at -70°C (4). The assay has a minimum detectability of 0.02 nmol/mg protein, which is well below that range found in healthy human controls. Intra-assay and interassay CVs for protein carbonyl measurements were 10.1% and 18.2%. Serum IL-6 was measured using a commercial ELISA (Quantikine Human IL-6, R & D Systems, Minneapolis, MN).

Descriptive statistics were used to characterize the study population and to describe biochemical measurements of serum protein carbonyl and grip strength. Body mass index was categorized as underweight ($<18.5\text{ kg/m}^2$), normal range ($18.5\text{-}24.9\text{ kg/m}^2$), overweight ($\geq 25\text{-}29.9\text{ kg/m}^2$) and obese ($\geq 30\text{ kg/m}^2$) according to World Health Organization criteria (9). Linear regression analysis was used to examine the relationship between serum protein carbonyl and other factors with grip strength as a continuous outcome variable. Ordered logistic regression was used to examine the relationship between serum protein carbonyls and grip strength in quartiles, where the 25th, 50th, and 75th percentiles of grip strength were 14.8, 17.8, and 21.3 kg, respectively.

Results

Baseline demographic and disease characteristics of the 672 women from WHAS I participating in the study are described in Table 1. The mean age (SD) was 77.4 (7.9) years. The mean serum protein carbonyl concentration was 0.098 nmol/mg, and the mean grip strength was 18.1 (5.0) kg.

Table 2 displays the univariate linear regression model used to examine the relationship between serum protein carbonyl levels, disease, and other risk factors associated with grip strength. Grip strength was significantly associated with age, race, smoking status, MMSE, serum protein carbonyls, hypertension, congestive heart failure, and depression. Grip strength was not significantly associated with education, coronary artery disease, peripheral artery disease, stroke, diabetes mellitus, chronic obstructive pulmonary disease, or cancer.

The multivariate analysis of the association between serum protein carbonyl and grip strength is displayed in Table 3. After adjusting for age, race, body mass index, and MMSE (Model 1), protein carbonyl levels were significantly associated with grip strength ($p < .01$). Addition of hypertension, congestive heart failure, and depression to the analysis (Model 2) produced similar results ($p < 0.05$).

The relationship between grip strength and serum protein carbonyls was also examined using ordered logistic regression (Table 4). The outcome was grip strength, expressed as an ordinal variable with four levels in order, created by quartile cut-off points of 14.8, 17.8 and 21.3 kg. In a multivariate analyses adjusting for age, race, IL-6, body mass index, and MMSE (Model 1), protein carbonyls were strongly associated with the lower quartiles of grip strength (O.R. 10.85, $P = 0.028$). Addition of hypertension, congestive heart failure, and depression to the

analysis (Model 2) produced similar results (O.R. 8.74, $P = 0.043$). The interpretation of the OR here is that one unit increase in protein carbonyls is associated with a higher odds of being in the lower quartiles compared with higher quartiles of grip strength.

Discussion

The present study shows that high serum protein carbonyls are independently associated with poor grip strength among moderately to severely disabled older women living in the community. These results are consistent with the idea that reactive oxygen species may contribute to a decline in muscle function with aging (10). Accumulation of reactive oxygen species throughout life are known to contribute to aging (8). The mechanism by which reactive oxygen species accumulate and contribute to the aging of muscles involves the insufficient function of the respiratory chain as well as mutations in mtDNA (13).

The oxidative capacity of human muscles has been characterized between younger and older adults (3), and is shown to decrease with age and muscle mass. In humans, elevated serum protein carbonyls have been described in the elderly compared with younger adults (2,12,16). Studies have shown that elevated serum protein carbonyls are associated with the age-induced decline in muscle performance (1). Grip strength, an indicator of muscle strength, has been associated with mortality and disability (19,20). Previous studies, however, have not characterized the relationship between grip strength and serum protein carbonyl levels. The association between protein oxidation and poor grip strength has clinical relevance, since there are modifiable risk factors that may affect oxidative stress such as smoking, dietary intake of carotenoids, ascorbate, selenium, plant polyphenols, and other antioxidants, and exercise.

The present study was conducted among older moderate to severely disabled women living in the community, and it is not clear whether serum protein carbonyls and grip strength will be significantly associated in younger populations, among less disabled older women, and among men. Further studies are needed to expand these investigations to other populations. In addition, oxidative protein damage was used as the only indicator of oxidative stress in the present study. Indicators of oxidative damage to lipids and oxidative damage to DNA could complement these investigations, and currently, such laboratory studies are in progress.

In summary, serum protein carbonyls were independently associated with grip strength, a finding which is consistent with the presumed biological mechanism by which the accumulation of reactive oxygen species can contribute to sarcopenia. These findings may contribute to the determination of the underlying mechanism of aging and the oxidative pathway.

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Table 1. Characteristics of women in the Women's Health and Aging Study I at baseline
(n = 672)

Characteristic¹	Mean (SD) or %
Age (years)	77.4 (7.9)
Race (white) (%)	72.77
Education < 12 years (%)	64.18
Current smokers (%)	10.42
Body mass index (kg/m ²)	
<18.5	3.44
18.5-24.9	23.61
25.0-29.9	36.39
≥30	36.56
MMSE score <24 (%)	16.82
Serum protein carbonyls (nmol/mg)	0.098
Grip strength (kg)	18.1 (5.0)
Hypertension (%)	56.78
Coronary heart disease (%)	23.21
Congestive heart failure (%)	10.57
Peripheral artery disease (%)	20.39
Stroke (%)	5.95
Osteoarthritis (%)	53.27

Diabetes mellitus (%)	16.96
Chronic obstructive pulmonary disease (%)	29.32
Depression (%)	16.96
Cancer (%)	11.31

¹Mean (SD) for continuous variables or percentages as noted.

Table 2. Univariate linear regression models of serum protein carbonyls and other risk factors with grip strength (n = 672)

Characteristic	Beta	SE	P
Age (years)	-0.235	0.023	<0.0001
Race (white)	-2.061	0.426	<0.0001
Education <12 years	-0.375	0.403	0.35
Current smokers (%)	1.421	0.629	0.024
Body mass index (kg/m ²)			
MMSE	-1.719	0.512	0.0008
Serum protein carbonyls (nmol/mg)	-6.068	2.841	0.03
Hypertension	0.913	0.339	0.02
Coronary heart disease	-0.239	0.457	0.61
Congestive heart failure	-1.940	0.623	0.002
Peripheral artery disease	-0.363	0.478	0.45
Stroke	-1.240	0.815	0.13
Diabetes mellitus	0.679	0.514	0.19
Chronic obstructive pulmonary disease	0.170	0.424	0.689
Depression	-1.772	0.510	0.0005
Cancer	-0.770	0.610	0.21
Osteoarthritis	0.142	0.387	0.71

Table 3. Multivariate linear regression models of serum protein carbonyls and other risk factors with grip strength (n = 672)

Characteristic	Beta	SE	P
<u>Model 1</u>			
Protein carbonyls (nmol/mg)	-6.77	2.58	0.0089
Age (years)	-0.19	0.02	<0.0001
Race (white)	-1.70	0.40	<0.0001
Body mass index (kg/m ²)			
<18.5	-1.89	1.04	0.069
25.0-29.9	1.26	0.44	0.004
≥30	1.53	0.45	0.0007
MMSE	-0.89	0.49	0.07
<u>Model 2</u>			
Protein carbonyls (nmol/mg)	-5.83	2.57	0.024
Age (years)	-0.18	0.02	<0.0001
Race (white)	-1.53	0.41	0.0002
Current smoking	0.91	0.58	0.12
Body mass index (kg/m ²)			
<18.5	-1.95	1.03	0.06

25.0-29.9	1.21	0.44	0.006
≥30	1.59	0.45	0.0005
MMSE	-0.68	0.49	0.16
Hypertension	0.22	0.36	0.54
Congestive heart failure	-1.33	0.57	0.02
Depression	-1.38	0.47	0.003

Table 4. Multivariate ordered logistic regression models of serum protein carbonyls and other risk factors with grip strength (n = 672)

Variable	OR¹	95% CI	<i>p</i>
<u>Model 1</u>			
Protein carbonyls (nmol/mg)	10.85	1.30-91.00	0.028
IL-6 (pg/mL)	1.06	1.00-1.13	0.053
Age (years)	1.09	1.07-1.11	<0.0001
Race (white)	1.80	1.30-2.49	0.0004
Body mass index (kg/m ²)			
<18.5	1.57	0.67-3.69	0.30
25.0-29.9	0.64	0.45-0.90	0.01
≥30	0.56	0.39-0.80	0.002
MMSE	1.21	0.82-1.80	0.34
<u>Model 2</u>			
Protein carbonyls (nmol/mg)	8.74	1.06-71.89	0.043
IL-6 (pg/mL)	1.06	1.00-1.13	0.052
Age (years)	1.09	1.06-1.11	<0.0001
Race (white)	1.72	1.24-2.39	0.001
Current smoking	0.74	0.46-1.19	0.22
Body mass index (kg/m ²)			

<18.5	1.63	0.69-3.88	0.27
25.0-29.9	0.63	0.44-0.90	0.01
≥ 30	0.53	0.37-0.76	0.001
MMSE	1.14	0.76-1.69	0.53
Hypertension	1.01	0.75-1.35	0.95
Congestive heart failure	1.46	0.92-2.34	0.11
Depression	1.79	1.22-2.63	0.003
