Oxidative Protein Damage Is Associated With Severe Functional Dependence Among the Elderly Population: A Principal Component Analysis Approach

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Background. Studies of the role of oxidative stress in functional dependence among the aging population are limited. In this report, we address this situation through an analysis of a large panel of blood oxidative biomarkers in elderly population. Because the analysis of multiple biomarkers increases the complexity of data interpretation, this investigation has utilized both an analysis of single biomarkers in addition to employment of the statistical data reduction tool principal component analysis that might allow for a clearer description of redox status as compared with a single measure alone.

Methods. We studied three groups of participants older than 65 years based on their Barthel Index: an independent group (100-95), a moderately dependent group (94-60), and a severely dependent group (59-0).

Results. We observed a significant increase in circulating protein carbonyl levels in the severely dependent group as compared with the independent and moderately dependent groups. Using principal component analysis, we found at least three factors (an erythrocyte-related component, a protein damage–related component, and a plasma-related component) that could be used to assess the different oxidative parameters in our population. We discovered a significant association of higher levels of the protein damage–related component with the severely dependent group.

Conclusions. Protein damage levels could be assessed in clinical use as a biomarker of severe dependence. Furthermore, our results support the hypothesis that functional decline could be associated in part due to oxidative stress. Finally, we show that principal component analysis could be a useful statistical tool in the analysis of age-related decline.

Key Words: Functional dependence—Barthel index—Oxidative stress—Elderly population—Principal component analysis.

FUNCTIONAL dependence increases with aging (1,2). Low functional health status is associated with a worse quality of life, an increased risk of mortality, hospitalization and need for long-term care, and a higher health care cost (3–5). Several investigations have shown that oxidative stress is associated with an increased risk of disability, particularly among the elderly population (6–8); however, most studies have only investigated a few types of physical functions (eg, grip strength, knee strength, chair stand, walking time or walking speed), rather than a whole spectrum of functional characteristics (9). Additional data are needed to elucidate the relationship between oxidative stress and functional status in elderly individuals. For that reason, we have analyzed functional dependence using the Barthel Index (BI). BI is an assessment instrument used to determine a patient's level of independence in basic daily activities based on a large panel of several functional variables (10).

It is important to note that BI is a widespread diagnostic tool used to assess functional dependence by clinicians; therefore, an analysis of oxidative stress using BI could contribute to translational medicine by bringing the laboratory data and the clinician experience together to better understand the relationship between oxidative stress and functional status in the elderly population (11,12).

Accumulating evidence has demonstrated that an analysis of oxidative stress should ideally examine several oxidative parameters (13). As many factors can influence a single biochemical marker, cross-sectional measurements of one or two oxidative biomarkers measurements are not likely to reflect the true complexity of the process in vivo (14). An analysis of multiple oxidative biomarkers could provide a more reliable and more comprehensive view of redox status. To minimize the complexity of data interpretation of multiple biomarker analysis, the current investigation employed the

statistical data reduction tool principal component analysis (PCA) (15). PCA is a form of exploratory multivariate analysis that is used to reduce the number of variables and to detect relationships among the parameters applied. In the current study, the analysis of 11 oxidative parameters allows the creation of summary oxidative indexes that might provide a better description of the overall redox status compared with a single measure to provide a more clinically useful measure of oxidative stress. Application of this statistical tool presents an opportunity to determine whether functional dependence is more strongly associated with one or more oxidative variables rather than a single biomarker. The goal of the analysis is to try to identify summary factor(s) that underlie single variables (15).

Therefore, we conducted an observational cross-sectional study that evaluated the relationship between functional dependence and oxidative stress through the analysis of single oxidative parameters and summary oxidative indexes. The ultimate objective of the present study was to identify potential oxidative biomarkers of functional dependence in the aged population.

METHODS

Participants

Participants in this study consisted of 120 institutionalized individuals older than 65 years (90 women and 30 men; mean age 86 ± 7 years) selected from Santa Teresa nursing home (Oviedo, Spain). The participants were not selected based on their morbidity characteristics in order to study a sample set representative of the general population. Exclusion criteria were recent or current infection, malignant disease, and malnutrition. Experienced geriatricians carried out initial evaluations, which included both a medical and pharmacological exhaustive history review and a physical examination of each patient. The presence of disease is based on explicit diagnosis in the medical history. Diseases included in the current analysis were cognitive impairment, dementia, osteoporosis, hypertension, chronic obstructive pulmonary disease, depression, osteoarthritis, heart failure, ischemic heart disease, cancer, rheumatoid arthritis, dyslipidemia, and diabetes.

The functional abilities of the participants were assessed using the BI (10). The BI is a 10-item scale of the following items: feeding, grooming, bathing, toilet use, dressing, walking, transfers, climbing stairs, fecal incontinence, and urinary incontinence. The highest score is 100 (independence) and the lowest is 0 (total dependence). The BI scores were used to divide the participants into three groups of functional dependence based on expert geriatrician criteria: an independent group = 100-95 ($n = 42$), a moderately dependent group = 94-60 ($n = 37$), and a severely dependent group = 59-0 ($n = 41$).

Each participant or participant's guardian received information about the purposes and objectives of the study and signed an informed consent term. The study was approved by the Hospital Central de Asturias (Oviedo, Spain) ethics committee.

Blood Collection

Blood samples were obtained by venipuncture after an overnight fast in the morning and a 15-minute rest. After centrifugation of blood, plasma was divided into aliquots and stored at $-20°C$ until further analysis. Erythrocytes were washed with ice-cold isotonic NaCl solution (0.9%) followed by centrifugation. Hemolysis of the washed packed cells was achieved by mixing them with cold distilled water, and these prepared hemolysates were stored at $-20°C$ until further analysis. Erythrocyte membranes were prepared according to the method of Dodge and colleagues (16) and stored at $-80°C$.

Biochemical Analysis

The erythrocyte hemoglobin concentration was measured by an automated hematology analyzer Sysmex SF-3000 (GMI Inc., MI). Plasma and erythrocyte membrane proteins were measured using the Bradford method (17).

Protein oxidative damage as measured by protein carbonyl levels was determined by the method developed by Levine and colleagues (18) with modifications as described by Coto-Montes and Hardeland (19). Data are presented as nmol protein carbonyl/mg protein for both plasma and erythrocyte membrane proteins (pPCO and ePCO). Lipid peroxidation was assessed by measurement of the reactive aldehydes malondialdehyde (MDA) and 4-hydroxy-2(E)-nonenal (4-HNE) content using the LPO Assay Kit from Calbiochem (No. 437634). Results are expressed as μmol (MDA+4-HNE)/g protein for plasma (pLPO) or μmol (MDA + 4-HNE)/g hemoglobin (Hb) for erythrocytes (eLPO).

To study erythrocyte resistance to H_2O_2 in vitro, we performed the erythrocyte hemolysis test (HT) using a modified version of the technique described by Farrell and colleagues (20,21). In this procedure, 300 μL aliquots of washed erythrocytes were pipetted into three disposable plastic tubes to conduct the HT. Next, 300 μL of 4% H_2O_2, freshly diluted in phosphate-buffered saline at pH 7.4, was added to the first tube containing erythrocyte suspensions. Also, 300 μL of phosphate-buffered saline was added to the second tube to study spontaneous hemolysis and distilled water to a volume of 3.8 mL was added to the last tube. All tubes were then gently mixed by inversion and incubated at 37°C for 3 hours. Tubes 1 and 2 were then diluted to a final volume of 4.1 mL with phosphate-buffered saline. All samples were centrifuged ($500g$, 3 minutes) and the absorbance of the supernatant was measured at 405 nm as an indication of the degree of hemolysis. Results are expressed as the percent of total hemoglobin released from cell suspensions that received distilled water.

Plasma total antioxidant activity (pTAA) was determined using the ABTS/H_2O_2/HRP method modified for plasma

samples (22). Results are expressed in equivalents of mg Trolox/mg protein.

Erythrocyte superoxide dismutase activity (eSOD; EC 1.15.1.1) was measured according to Martin and colleagues (23). Results are expressed as U/mg Hb. Catalase activity (eCAT; EC 1.11.1.6) was assayed according to Lubinsky and Bewley (24). Data are expressed as U/mg Hb·min. Glutathione peroxidase (eGPx; EC 1.11.1.9) and glutathione reductase activity levels were assayed according to Wheeler and colleagues (25). Data are expressed as U/g Hb·min. Glutathione-S-transferase activity (eGST; EC 2.5.1.18) was assayed according to Habig and colleagues (26), and data are expressed as U/g Hb·min.

Statistical Analysis

The statistical software package SPSS 15.0 for Windows (SPSS Inc., Chicago, IL) was used for all statistical analyses. Descriptive statistics were used to characterize the study population and to describe single oxidative biomarkers. Data are presented as frequencies (percentages) for categorical variables and means (standard deviation of mean) for continuous variables. Categorical variables were compared between groups using chi-square test. Continuous variables were compared between groups with analysis of variance. The normality of the data was analyzed by the Kolmogorov–Smirnov test. Assessments of ePCO, HT, and eGST levels were performed using log values to normally distribute these parameters. For clarity, the original values are used to describe the results.

We applied PCA to reduce the 11 oxidative parameters into a smaller set of principal components that account for most of the variance of the oxidative variables. eLPO, HT, and GST presented communality values (the proportion of the variance that can be explained by the factor model obtained) lower than 0.50; thus, these parameters were not taken into account for further analysis. The number of components retained was based on eigenvalues (the amount of the total variance that is explained by each component) of 1 or greater. A varimax rotation was used to obtain a set of independent and best interpretable components. Varimax rotation method minimizes the number of variables that has high saturations in each component. It simplifies the interpretation of the components by optimizing the solution. The components were interpreted based on the loadings that relate the parameter to the components. Loadings greater that 0.50 were used to identify the variables comprising a component, and this cut-off point also provided good separation of the components. Principal component scores were calculated for each participant and standardized to yield a sample mean equal to 0.

Single oxidative biomarkers and oxidative components were analyzed by analysis of variance. All data presented were adjusted with age as covariate. Variables are expressed as mean (standard deviation of mean). Differences were considered statistically significant when $p < .05$.

Table 1. Characteristics of the Sample by Dependence Level

	Independent	Moderately Dependent	Severely Dependent	p Value
Number	42	37	41	—
Barthel Index	100-95	94-60	59-0	—
Age (y)	84 (7)	86 (6)	89 (7)	.002
Female	31 (73.8)	29 (78.4)	30 (73.2)	.848
Total number of medical diagnoses per participant	2 (2)	2 (1)	2 (1)	1.000
Cognitive impairment	1 (2.4)	3 (8.1)	16 (39.0)	.000
Dementia	1 (2.4)	4 (10.8)	7 (17.1)	.081
Osteoporosis	11 (26.2)	9 (24.3)	7 (17.1)	.580
Hypertension	17 (40.5)	22 (59.5)	7 (17.1)	.001
Chronic obstructive pulmonary disease	5 (11.9)	5 (13.5)	5 (12.2)	.974
Osteoarthritis	11 (26.2)	9 (24.3)	8 (19.5)	.761
Depression	8 (19.0)	8 (21.6)	5 (12.2)	.521
Heart failure	3 (7.1)	1 (2.7)	2 (4.9)	.664
Ischemic heart disease	3 (7.1)	3 (8.1)	2 (4.9)	.840
Cancer	1 (2.4)	0 (0.0)	0 (0.0)	.392
Rheumatoid arthritis	0 (0.0)	0 (0.0)	2 (4.9)	.141
Dyslipidemia	6 (14.3)	9 (24.3)	5 (12.2)	.313
Diabetes	5 (11.9)	6 (16.2)	7 (17.1)	.780
Number of medications per participant	5 (3)	7 (2)	6 (3)	.024
Steroid anti-inflammatory drugs	0 (0.0)	2 (5.4)	1 (2.5)	.304
Nonsteroidal anti-inflammatory drugs	12 (27.9)	15 (40.5)	17 (42.5)	.325

Note: Values are presented as mean (standard deviation of mean) for continuous variables or as frequencies (percentage) for categorical variables.

To establish whether the association of oxidative biomarkers and summary oxidative indexes on functional dependence could be influenced by morbid conditions, the analysis was repeated using age and total number of diseases per participant as covariates, including the prevalence of cognitive impairment, dementia, osteoporosis, hypertension, chronic obstructive pulmonary disease, depression, osteoarthritis, heart failure, ischemic heart disease, cancer, rheumatoid arthritis, dyslipidemia, and diabetes. Furthermore, other independent analysis was repeated after exclusion of persons with cognitive impairment or hypertension.

To corroborate the association between oxidative components and functional dependence, linear regression analysis for each oxidative component was fitted against BI after adjusting for age (Model 1). To establish whether the association of oxidative component levels and BI were influenced by morbid conditions, univariate linear regressions were also adjusted for age and the total number of diseases per participant (Model 2).

RESULTS

Characteristics of the Study Participants

Table 1 summarizes the demographic and clinical characteristics of the study groups. The mean age was significantly different between participants of the three groups. Analysis of the data showed that there were no significant differences

Table 2. Descriptive Analysis for Oxidative Biomarkers and Rotated Component Loadings

Variable	$M \pm SD$	Rotated Component Loadings		
		Component 1	Component 2	Component 3
pPCO (nmol/mg protein)	8.41 (2.17)	−0.037	0.839	0.092
pLPO (μmol/g protein)	0.12 (0.05)	−0.108	0.081	0.800
pTAA (mg Trolox/mg protein)	2.73 (1.20)	0.083	−0.056	0.742
ePCO (nmol/mg protein)	12.41 (3.55)	0.079	0.814	−0.071
eSOD (U/mg Hb)	16.12 (3.30)	0.788	0.029	−0.041
eCAT (U/mg Hb)	199.41 (45.81)	0.718	0.173	−0.098
eGPx (U/g Hb)	31.73 (5.78)	0.699	−0.108	−0.066
eGR (U/g Hb)	4.47 (1.81)	0.746	−0.009	0.226

Note: Values are presented as mean (standard deviation of mean). eCAT = erythrocyte catalase activity; eGPx = erythrocyte glutathione peroxidase activity; eGR = erythrocyte glutathione reductase activi; ePCO = erythrocyte protein carbonyl content; eSOD = erythrocyte superoxide dismutase activity; pLPO = plasma lipid peroxidation level; pPCO = plasma protein carbonyl content; pTAA = plasma total antioxidant activity.

between groups based on sex or total number of clinical diagnoses per participant. However, the severely dependent group presented an elevated prevalence of cognitive dependence as compared with independent and moderately dependent participants. In addition, the severely dependent group presented a lower prevalence of hypertension than the independent or moderately dependent groups. We showed statistical differences in the number of medications taken per participant between the study groups. There were no differences found in the intake of neither steroid anti-inflammatory drugs nor nonsteroidal anti-inflammatory drugs between groups.

Principal Component Analysis

PCA resulted in three eigenvalues greater than 1. The Kaiser–Meyer–Olkin measure of sampling adequacy tests was 0.643. Kaiser–Meyer–Olkin measure varies between 0 and 1. Kaiser–Meyer–Olkin values smaller than 0.5 indicate that factorial analysis should not be used with the sample

data are being analyzed because the analysis is weak. The observed significance level of Bartlett's test of sphericity was 0.000. The Bartlett's test of sphericity contrasts the null hypothesis that the matrix of correlations is an identity matrix, in which case no significant correlations exist between the variables and the factor model would not be relevant. The three components explained 61% of the total variance data (27% Component 1, 18% Component 2, and 16% Component 3). As shown in Table 2, four variables (eSOD, eCAT, eGPx, and eGR) loaded highest on the first component (Component 1 = "erythrocyte related"), two variables (pPCO and ePCO) loaded highest on the second component (Component 2 = "protein damage related"), and two variables (pLPO and pTAA) loaded highest on the third component (Component 3 = "plasma related").

We analyzed the relationships between the three components and functional dependence (Table 3). The summary indexes differed in their associations with functional status. A higher protein damage–related component level was

Table 3. Single Oxidative Parameters and Summary Oxidative Indexes in the Study Groups

	Independent	Moderately Dependent	Severely Dependent
pPCO (nmol/mg protein)	7.69 (1.70)	7.82 (1.80)	9.72 (2.35)[bbb,ccc]
pLPO (μmol/g protein)	0.13 (0.05)	0.12 (0.05)	0.12 (0.05)
pTAA (mg Trolox/mg protein)	2.86 (1.09)	2.75 (1.38)	2.63 (1.15)
ePCO (nmol/mg protein)	10.10 (2.09)	10.76 (1.54)	16.29 (2.73)[bbb,ccc]
eLPO (μmol/g Hb)	4.72 (0.83)	4.86 (1.07)	5.27 (0.90)
HT (%)	15.71 (6.66)	20.21 (11.20)	23.53 (14.74)
eSOD (U/mg Hb)	14.85 (3.05)	17.04 (3.18)[a]	16.56 (3.37)
eCAT (U/mg Hb)	193.39 (43.13)	196.54 (49.63)	208.39 (45.29)
eGPx (U/g Hb)	31.00 (5.62)	32.81 (6.53)	31.52 (85.29)
eGR (U/g Hb)	4.03 (1.77)	4.76 (1.90)	4.64 (1.73)
eGST (U/g Hb)	3.94 (1.73)	3.66 (1.04)	3.11 (1.40)[b,c]
Erythrocyte-related component	−0.30 (0.88)	0.20 (1.11)	0.12 (0.96)
Protein damage-related component	−0.56 (0.65)	−0.42 (0.66)	0.97 (0.82)[bbb,ccc]
Plasma-related component	0.19 (0.89)	−0.01 (1.16)	−0.15 (0.91)

Notes: All data are adjusted by age. Data are expressed as mean (standard deviation of mean). eCAT = erythrocyte catalase activity; eGPx = erythrocyte glutathione peroxidase activity; eGR = erythrocyte glutathione reductase activity; eGST = erythrocyte glutathione-S-transferase activity; eLPO = erythrocyte lipid peroxidation level; ePCO = erythrocyte protein carbonyl content; eSOD = erythrocyte superoxide dismutase activity; HT = erythrocyte H_2O_2–induced hemolysis test; pLPO = plasma lipid peroxidation level; pPCO = plasma protein carbonyl content; pTAA = plasma total antioxidant activity.

Independent vs moderately dependent group: a: <.05; independent vs severely dependent group: b: <.05, bbb: <.001; moderately dependent vs severely dependent group: c: <.05, ccc: <.001.

Table 4. Associations Between Levels of Oxidative Components and BI Score (using BI as continuous outcome)

	BI			
	Model 1		Model 2	
	β	*p* Value	β	*p* Value
Erythrocyte-related component	−.115	.242	−.118	.231
Protein damage–related component	−.683	<.001	−.683	<.001
Plasma-related component	−.046	.641	−.043	.661

Notes: Model 1: adjusted for age. Model 2: adjusted for age and morbid conditions. β = standardized betas; BI = Barthel Index.

significantly associated with the severely dependent group but not with the independent or moderately dependent groups (independent vs severely dependent group: *p* < .001; moderately dependent vs severely dependent group: *p* < .001). No differences were detected between the erythrocyte-related and plasma-related components and functional dependence. After adjustment for morbid conditions, protein damage–related component retained statistical significance (data not shown). When participants with cognitive impairment or hypertension were excluded from the analysis, the associations of functional dependence and summary indexes remained similar to the association previously described (data not shown).

Table 4 displays the univariate linear regression models that were used to examine the relationship between oxidative components and BI using BI as continuous outcome. In univariate models that were adjusted by age, low BI score was directly associated with high levels of protein damage–related component. Because oxidative stress is associated with morbid conditions, an additional analysis was conducted to determine whether the prevalence of diseases altered the relationship between components and BI scores. When we applied univariate regressions that were adjusted by age and morbid conditions, association between protein damage–related component and BI remained statistically significant (data not shown). No associations were observed between erythrocyte-related and plasma-related components and BI.

Single Oxidative Parameters

As shown in Table 3, analysis of the data showed that pPCO and ePCO levels were significantly higher in the severely dependent participants than in the independent or moderately dependent participants (independent vs severely dependent group: *p* < .001; moderately dependent vs severely dependent group: *p* < .001).

Investigation of antioxidant enzyme revealed an increase in eSOD activity in the moderately dependent group compared with the independent group (independent vs moderately dependent group: *p* < .050). Reduced eGST activity was associated with severe dependence when comparing the severely dependent group with the independent and moderately dependent groups (independent vs severely

dependent group: *p* < .050; moderate dependent vs severely dependent group: *p* < .050). There were no statistical differences in the other oxidative parameters among groups.

Because oxidative stress is associated with morbid conditions, additional analysis was conducted to determine whether the prevalence of diseases alters the relationship of biological markers and functional dependence. After adjustment for morbid conditions, pPCO and ePCO levels retained statistical significance. eGST levels were no longer found to be statistically different between groups. eLPO and HT showed elevated levels in the severely dependent group compared with the independent group (independent vs severely dependent group: *p* < .050). Finally, eSOD levels were significantly higher in the severely dependent participants than in the independent or moderately dependent participants (independent vs severely dependent group: *p* < .050; moderately dependent vs severely dependent group: *p* < .050; data not shown).

We observed an association between cognitive impairment and hypertension with functional dependence. Oxidative stress is associated with these diseases (27,28); thus, additional analysis was conducted to determine whether the prevalence of cognitive impairment and hypertension alters the relationship of single oxidative stress biomarkers and summary oxidative indexes with functional dependence. When participants with cognitive impairment were excluded from the analysis, the associations of functional dependence with the single oxidative parameter and summary indexes remained similar to the association previously described and remained statistically significant (data not shown). When participants with hypertension were excluded from the analysis, eGST levels were no longer found to be statistically different between groups; however, the other oxidative parameters studied remained statistically significant (data not shown).

DISCUSSION

From the large panel of single oxidative biomarkers and summary oxidative indexes studied, we report a statistically significant increase in pPCO and ePCO concentrations in the severely dependent group as compared with the independent and moderately dependent groups. Further indication of higher levels of oxidative protein damage in severely dependent participants was the significant association between participants exhibiting high protein damage–related component levels and the severely dependent group. Taken together, our results suggest that severe functional decline phenotype is intimately associated with increased levels of protein carbonyls in circulation. Our findings are consistent with similar epidemiological studies that showed that oxidative damage is elevated in more disabled elderly participants (7,29–31). Circulating protein carbonyls could constitute a useful clinical biomarker of severe functional dependence in the elderly population. Nevertheless, previous studies have investigated only a few types of physical functions rather

than a whole spectrum of functional characteristics; in the current investigation, this was achieved through analysis using the BI. BI is a commonly employed diagnostic tool used to assess functional dependence by clinicians. The analysis of functional status by the BI brings the experimental data and the clinician experience closer through a better understanding of the association between oxidative biomarkers and functional dependence, which has the potential to ultimately improve translational research.

Elevated carbonyl content has been associated with aging (32,33) and several diseases including Alzheimer's disease, rheumatoid arthritis, diabetes, sepsis, chronic renal failure, and respiratory distress syndrome (34). Although previous investigation showed that carbonyl content could be generated by oxidative as well as non-oxidative mechanisms (35), literature published on this topic generally reported carbonyl content as a relevant oxidative stress marker (28,31,34,36–38). Therefore, the current investigation shows that the older population that displayed severe functional dependence presented elevated oxidative stress levels. This connection between oxidative stress levels and functional limitation remains unclear. Whether the elevated levels of oxidative stress in more disabled participants that affect functional performance are the results of chronic diseases or indicators of chronic diseases is still controversial (4,29,31). Interestingly, we have observed an intimate association of elevated oxidative damage to protein with severe functional dependence, even when morbid conditions were adjusted for. Furthermore, we have observed statistically significant differences associated with the prevalence of cognitive impairment and hypertension between the groups analyzed; however, the associations between biomarkers with functional status remained unaltered after excluding the participants with cognitive impairment and hypertension. Levels of oxidative stress are positively correlated with severe functional dependence independent of chronic disease. One possible explanation is that elevated levels of oxidative stress reflect a biological deregulation due to aging, indicative of a redox imbalance that may directly affect physical dependence. It has been proposed that prolonged periods of bed rest, immobilization, or lack of physical activity (situations associated with the severely dependent group) result in a significant increase in oxidative stress in animal models and healthy and critically ill individuals (39–43). In fact, studies have shown that excessive oxidative stress is involved in the development of age-related sarcopenia and a subsequent decrease in muscle strength and mobility in animal models (44) and in the elderly population (45,46). Previous investigations reported elevated levels of oxidative stress in debilitating conditions that are often associated with functional decline in elderly population: anorexia, depression, cachexia, and anemia (47–49). Additional publications further corroborate these findings, having shown a direct relationship between increased oxidative stress and elevated levels of proinflammatory cytokines, which have been implicated in the pathogenesis of sarcopenia, anemia, and poor pulmonary function in older people (30,50–52). Both previous data and the evidence presented here support the hypothesis that functional decline could be associated in part to increased oxidative stress levels.

No direct association between pTAA and functional dependence was observed. Current data differed from some previous reports that have associated low-serum antioxidant content and adverse clinical outcomes in elderly participants. In the recent publications using The Third National Health and Nutrition Examination Survey data, low-serum carotenoid, α-carotene, and lycopene concentrations have been associated with increase risk of death (53,54). Ble and colleagues (55) showed an association between low circulating levels of vitamin E and the presence of frailty in elderly population. Additionally, Alipanah and colleagues (6) reported that total serum carotenoid and serum selenium levels were inversely associated with mean walking speed over 3 years of follow-up in moderately to severely disabled women aged 65 years or older, living in the community. A possible explanation of the differences with preceding investigations might relate to differences in the sample population. We have studied elderly men and women who were institutionalized with a wide range of age and functional status (68–105 years; BI score = 0–100). Other possible explanation might relate to the antioxidant detection method. Current study has measured pTAA as a marker of overall antioxidant capacity because it might give more biologically relevant information than that obtained from measuring concentrations of individual antioxidants (56). However, pTAA could not provide the adequate specificity or sensitivity required as potential oxidative marker of functional dependence in the aged population. Finally, it is important to note that investigations based on BI and oxidative stress are scarce. These data deserve a more detailed investigation.

We found at least three components (erythrocyte-related component, protein damage–related component, and plasma-related component) that could be used to summarize the 11 different oxidative parameters studied in our population of older adults. We have shown that eSOD, eCAT, eGPx, and eGR were placed in the erythrocyte-related component, pPCO and ePCO placed in the protein damage–related component, and pTAA and pLPO placed in the plasma-related component, indicating a conceivable relation between these single parameters within each component. To evaluate the construct validity of the components, we explored the relationships between the three components and functional dependence. We reported a close relation between high protein damage–related component level and low BI score in regression models. We also showed that a higher protein damage–related component level is found to be associated with the severely dependent group as compared with the independent and moderately dependent groups, even when morbid conditions were adjusted for,

whereas no differences were detected between the other two components and functional dependence. When we examined the contribution of the protein damage–related component measurements as compared with the individual oxidative measures (pPCO and ePCO) to dependence level, we found that the protein damage–related component did not show better association with severe dependence than the single biomarkers. The associations of pPCO and ePCO with severe functional dependence were extremely significant ($p < .001$); therefore, the calculation of summary variables using a principal component approach did not strengthen associations between oxidative damage and functional dependence. Nevertheless, our study is an initial attempt to examine clustering of oxidative biomarkers using PCA and how oxidative components relate to functional dependence. To our knowledge, no other study has used data reduction tools to identify summary oxidative measures in the older population and explored associations between these summary measures and functional dependence.

Several limitations of this study must be considered. First, a population sample more than 120 participants would have been desirable. Second, protein carbonyl levels can only be determined using experimental tools, as there is no clinical laboratory test that is used or available for clinical use for this purpose. Third, as this was an observational study, we have not been able to determine the causal directions of the observed association between protein oxidative damage and physical function. Finally, we have not found an oxidative biomarker that can be used to characterize moderate dependence.

Despite these limitations, the present study shows that high levels of circulating protein carbonyls are associated with severe functional dependence in older participants. This finding is consistent with the presumed biological mechanism by which oxidative stress is associated with functional decline. Furthermore, future research is necessary to further assess the use of PCA in the study of biomarkers of aging and age-related disease.

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