AGE (2016) 38: 1
DOI 10.1007/s11357-015-9864-z

Biomarkers of oxidative stress are associated with frailty: the Framingham Offspring Study

Christine K. Liu · Asya Lyass · Martin G. Larson · Joseph M. Massaro · Na Wang · Ralph B. D'Agostino Sr · Emelia J. Benjamin · Joanne M. Murabito

Received: 2 September 2015 / Accepted: 11 December 2015 / Published online: 23 December 2015

Abstract Cardiovascular disease and frailty frequently occur together. Both are associated with inflammation, which may be partially triggered by oxidative stress, especially in cardiovascular disease. We investigated whether inflammatory and oxidative stress biomarkers linked to cardiovascular disease were associated with frailty and the related outcome of gait speed. We report cross-sectional associations of biomarkers and frailty assessed at Framingham Offspring Study cycle eight. Participants ≥60 years were eligible if they had information on frailty and at least one of the following: C-reactive protein, interleukin-6, tumor necrosis factor receptor 2, 8-epi-FGFα isoprostanes (isoprostanes), lipoprotein phospholipase A2 (LpPLA2) mass or activity, osteoprotegerin, intracellular adhesion molecule-1, monocyte chemoattractant protein-1 or P-selectin. Stepwise logistic models were utilized for frailty and stepwise linear models for gait speed. Covariates included age, sex, body mass index, smoking, and co-morbidities. Odds ratios (ORs) and slope estimates (*B*) are reported per standard deviation increase of $\log_e$-transformed biomarker. Of the 1919 participants, 142 (7 %) were frail. In a stepwise model, frailty

Electronic supplementary material The online version of this article (doi:10.1007/s11357-015-9864-z) contains supplementary material, which is available to authorized users.

C. K. Liu (✉)
Section of Geriatrics, Department of Medicine, Boston University School of Medicine, 88 E Newton Street, Robinson 2, Boston, MA 02118, USA
e-mail: Christine.Liu@bmc.org

C. K. Liu
Jean Mayer USDA Human Nutrition Research Center on Aging, Tufts University, Boston, MA, USA

A. Lyass · M. G. Larson · J. M. Massaro · R. B. D'Agostino Sr, · E. J. Benjamin · J. M. Murabito (✉)
National Heart, Lung and Blood Institute's Framingham Heart Study, 73 Mount Wayte Ave, Suite 2, Framingham, MA 01702, USA
e-mail: murabito@bu.edu

A. Lyass · M. G. Larson · N. Wang · R. B. D'Agostino Sr,
Department of Mathematics and Statistics, Boston University, Boston, MA, USA

M. G. Larson · J. M. Massaro
Department of Biostatistics, Boston University School of Public Health, Boston, MA, USA

E. J. Benjamin
Department of Epidemiology, Boston University School of Public Health, Boston, MA, USA

E. J. Benjamin
Sections of Cardiovascular and Preventive Medicine, Department of Medicine, Boston University School of Medicine, Boston, MA, USA

J. M. Murabito
Section of General Internal Medicine, Department of Medicine, Boston University School of Medicine, Boston, MA, USA

odds increased with higher interleukin-6 (OR 1.90, 95 % CI 1.51, 2.38), isoprostanes (OR 1.46, 95 % CI 1.12, 1.92), and LpPLA2 mass (OR 1.29, 95 % CI 1.00, 1.65). Stepwise regression found that slower gait speeds were associated with interleukin-6 ($B = -0.025$ m/s, 95 % CI 0.04, −0.01), isoprostanes ($B = -0.019$, 95 % CI −0.03, −0.008), LpPLA2 mass ($B = -0.016$, 95 % CI −0.03, −0.004), and osteoprotegerin ($B = -0.015$, 95 % CI −0.03, −0.002, all $p < 0.05$). Interleukin-6, isoprostanes, and LpPLA2 mass were associated with greater frailty odds and slower gait speeds. Oxidative stress may be a mechanism contributing to frailty.

Keywords Frailty · Inflammation · Oxidative stress · Gait speed

Introduction

Frailty describes individuals with minimal injury resilience and little physiological reserve. In the definition of frailty by Fried et al., frail persons display three or more of the following characteristics: (1) unintentional weight loss, (2) weakness, (3) exhaustion, (4) low physical activity, and (5) slowness in motor performance (Fried et al. 2001). Prevalent in older adults, epidemiological cohorts have demonstrated that frail individuals are extremely vulnerable to disability (Xue et al. 2011; Ensrud et al. 2008), hospitalization (Fried et al. 2001), and death (Fried et al. 2001; Woods et al. 2005; Bandeen-Roche et al. 2006; Gill et al. 2010).

Excess inflammation has been postulated as a potential driver of frailty pathogenesis. Observational studies have shown that frailty is associated with several pro-inflammatory biomarkers, including interleukin-6 (Leng et al. 2007; Bandeen-Roche et al. 2009; Hubbard et al. 2009), C-reactive protein (CRP) (Hubbard et al. 2009; Walston et al. 2002; Puts et al. 2005), and tumor necrosis factor-α (Hubbard et al. 2009). While these studies have established a relation between frailty and systemic inflammation, the majority have only examined non-specific biomarkers of inflammation. The specific inflammatory pathways that are activated in frailty have not yet been well defined.

In animal models, mice displaying a frailty phenotype have greater vascular stiffness and cardiac dysfunction (Sikka et al. 2013). Epidemiologic studies have shown that cardiovascular disease is associated with higher rates of incident frailty (Woods et al. 2005). Recent work demonstrated that incident cardiovascular disease is predicted by hand grip strength, one of the components of frailty (Leong et al. 2015). Cardiovascular disease is caused by atherosclerosis, which has been shown to also increase inflammation (Legein et al. 2013; El Assar et al. 2013). One of the possible drivers of the relation between inflammation and cardiovascular disease is oxidative stress (Chen and Keaney 2012), which has also been thought to play a role in frailty (Mulero et al. 2011).

Given this evidence, we hypothesized that frailty and cardiovascular disease may share common biologic mechanisms. Using the Fried frailty criteria, we examined the cross-sectional associations of frailty with ten pro-inflammatory and oxidative stress biomarkers associated with cardiovascular disease. We chose biomarkers representing different phases of the inflammatory process. As a secondary aim, we also examined the cross-sectional associations of gait speed with our panel of inflammatory and oxidative stress biomarkers. Slow gait speeds have also been associated with inflammation (Verghese et al. 2012; Kositsawat et al. 2013; Ferrucci et al. 2002) and cardiovascular disease (Matsuzawa et al. 2013); additionally, slow gait speeds have been linked with greater morbidity and mortality (Studenski et al. 2011).

Methods

Study participants

The Framingham Offspring Study is a prospective, observational cohort study following the Offspring and Offspring spouses of the Framingham Heart Study Original cohort participants. The study's methods and sampling design details have been published previously (Feinleib et al. 1975). Established in 1971, the Framingham Offspring Study enrolled 5124 participants who have been examined every 4 to 8 years. At each examination, participants underwent a medical history, physical examination, and laboratory tests. Participants aged 60 years and older who attended examination cycle eight (2005–2008) were eligible ($n = 2328$) for this analysis. Participants were excluded if they had no biomarker information or had missing data regarding frailty or covariates. The final sample consisted of 1919 participants.

Inflammatory and oxidative stress biomarkers

Fasting blood and urine samples were centrifuged, frozen at −80 °C, and then thawed for assay. CRP was assayed using particle-enhanced immunonephelometry. Interleukin-6, tumor necrosis factor receptor 2 (TNFR2), lipoprotein-associated phospholipase A2 (LpPLA2) activity and mass, osteoprotegerin, intracellular adhesion molecule-1 (ICAM-1), monocyte chemoattractant protein-1 (MCP-1), and P-selectin were assayed using quantitative ELISA. Frozen urine was assayed for 8-epi-PGFα isoprostanes (isoprostanes) using quantitative ELISA. Details of assays have been published (Schnabel et al. 2008) or available in further detail online (Fontes et al. 2011). Mean intra-assay coefficients of variation were ≤7 %.

Frailty

We defined frailty as outlined by Fried et al. (Supplemental Table S1) (Fried et al. 2001). Participants were classified with unintentional weight loss if they answered "yes" to the question of unintentional loss greater than 10 lb in the past year. Exhaustion was defined as an answer of "Most or all of the time" to "Could not get going" or "I felt everything was an effort" from the Center for Epidemiologic Studies Depression Scale (Orme et al. 1986). Using the Framingham Physical Activity Index (Kannel et al. 1979), participants reported daily routine activities. Activities were assigned a value based on estimated oxygen consumption and multiplied by the average time spent on the activity within a 24-h period. The activity score was the sum of these values, ranging from 24 to 120. Scores in the lowest quintile for each sex were classified as low physical activity. To assess slowness, participants walked 4 meters at usual pace with time measured to the nearest hundredth of a second. The faster of the two trials was used in the analysis. Participants were classified with slowness using the gait speed criteria per Fried and colleagues. For weakness, participants were asked to squeeze a Jamar dynamometer (Lafayette Instrument Co, Lafayette, IN) as hard as possible, three times in each hand. Force generated was recorded in kilograms, and the highest value was used. Weakness was defined using the hand grip strength cutoffs previously defined (Fried et al. 2001). Individuals were considered frail if they fulfilled three or more criteria or pre-frail if they fulfilled one or two criteria.

Covariates

Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. History of cardiovascular disease was defined as coronary heart disease (myocardial infarction, angina pectoris, or coronary insufficiency), stroke or transient ischemic attack, intermittent claudication, or heart failure (Feinleib et al. 1975). Diabetes was defined as a fasting glucose more than 125 mg/dL, use of insulin, or use of oral hypoglycemic medications. Current smoking was smoking one or more cigarette per day in the prior year. History of cancer was defined using the World Health Organization International Classification of Diseases-O (Driver et al. 2012), excluding non-malignant neoplasms and non-melanoma skin cancers. Chronic kidney disease was defined by estimated glomerular filtration rate <60 mL/min/1.73 m^2 based on creatinine (Levey et al. 2009).

Statistical analyses

Biomarker values are reported as median value and 25th and 75th percentiles. For subsequent analyses, biomarkers were $\log_e$-transformed and standardized (mean 0, SD 1). Descriptive statistics are represented as mean and SD for continuous variables and frequencies for categorical variables.

For the frailty analyses, the comparison group was non-frail participants; pre-frail participants were excluded. Multivariate-adjusted logistic regression models were used to relate frailty (dependent variable) with individual biomarkers, adjusting for age, sex, body mass index, smoking, diabetes, cardiovascular disease, cancer, and chronic kidney disease. Since CRP and interleukin-6 were correlated ($r = 0.5$), CRP was withheld from the multi-biomarker models. Odds ratios for frailty are presented per one standard deviation increase in $\log_e$-transformed biomarker. Biomarkers individually associated with frailty ($p < 0.10$) were included in a backward elimination model ($p < 0.10$ to stay) with covariates forced in. Analyses were repeated for (i) participants 70 years or older and (ii) those free of cancer, as age and malignancy are potential modifiers of the association between inflammation and frailty (Singh and Newman 2011).

A similar set of analyses was performed for pre-frailty. The comparison group was non-frail participants only; frail participants were excluded from the analyses.

Multivariable-adjusted linear regression models were utilized to analyze gait speed with individual

biomarkers, adjusting for the covariates previously listed. Biomarkers individually associated with gait speed ($p < 0.10$) were included in a backward elimination ($p < 0.10$ to stay) with covariates forced into the model. Biomarker quartiles were calculated; multivariable-adjusted linear models were used to assess the relations between gait speed and biomarker quartiles. The highest quartile was used as the reference group. All statistical analyses were performed using SAS version 9.3 (SAS Institute, Cary, NC). A two-sided $p < 0.05$ was considered statistically significant.

Results

Characteristics of study participants

Descriptive statistics are provided in Table 1, stratified by frailty category. The proportion of women was similar in the frail, pre-frail, and non-frail groups. Compared to non-frail, the frail group was older, had a higher mean body mass index, and has a greater burden of comorbidities. Of those who were frail, 31 % fulfilled four or five criteria. Among those who were pre-frail, 611 persons (71 %) fulfilled only one frailty criteria, while 253 (29 %) fulfilled two criteria. A total of 409 participants were excluded for missing data on frailty ($n = 121$), biomarkers ($n = 278$), or covariates ($n = 10$); excluded participants were similar in age and BMI to the rest of the sample (all $p > 0.25$) but were more likely to be smokers (10 % vs. 7 %, $p = 0.05$) and to have a history of diabetes (22 % vs. 15 %, $p = 0.002$).

Inflammatory and oxidative stress biomarkers with frailty: associations of biomarkers singly and as a group

Frailty was individually associated with elevated CRP, interleukin-6, isoprostanes, LpPLA2 mass, ICAM-1, and MCP-1 in single-marker models (all $p < 0.05$, Supplemental Table S2). In the stepwise model, three biomarkers were retained: interleukin-6, isoprostanes, and LpPLA2 mass (Table 2). One standard deviation of $\log_e$ interleukin-6 was associated with a 90 % increase in frailty odds ($p < 0.001$), and $\log_e$ isoprostanes were associated with a 46 % increase in frailty odds ($p = 0.006$). $\log_e$ LpPLA2 mass was also associated with a higher odds of frailty (OR 1.29, $p = 0.05$). Subsequently, analysis was limited to those aged 70 years of age or older. While the relation between isoprostanes and frailty became insignificant, the relation between interleukin-6 and frailty remained, although somewhat attenuated (OR 1.65, $p < 0.001$). The association of LpPLA2 mass with frailty in this subgroup remained significant and essentially unchanged (OR 1.42, $p = 0.02$). The associations of frailty with interleukin-6, isoprostanes, and LpPLA2 mass were unchanged and remained significant in individuals without cancer (all $p < 0.05$).

Inflammatory and oxidative stress biomarkers with pre-frailty: associations of biomarkers singly and as a group

Pre-frailty was individually associated with elevated levels of CRP, interleukin-6, TNFR2, LpPLA2 activity, ICAM-1, and MCP-1 (all $p < 0.05$, Supplemental Table S2). In the stepwise model where the biomarkers were examined as a group, the associations with interleukin-6, ICAM-1, and MCP-1 remained significant (all $p < 0.05$, Supplemental Table S3). There was little change in the strength of these associations in the subgroup of individuals free of cancer (all $p < 0.05$). In those 70 years or older, the association between pre-frailty and MCP-1 became non-significant but remained unchanged with interleukin-6 and ICAM-1 (both $p = 0.04$).

Associations of biomarkers with gait speed

Slower gait speeds were associated with higher levels of CRP, interleukin-6, isoprostanes, LpPLA2 mass and activity, and osteoprotegerin (all $p < 0.05$, Supplemental Table S4). In the stepwise model, interleukin-6, isoprostanes, LpPLA2 mass, and osteoprotegerin remained associated with gait speed (all $p < 0.05$, Table 3). Figure 1 displays the associations of gait speed with quartiles of interleukin-6, isoprostanes, LpPLA2 mass, and osteoprotegerin. Slower gait speeds were associated with the highest quartile of each biomarker.

Discussion

Principal findings

Our cross-sectional study of over a thousand community-dwelling older adults demonstrated several findings

Table 1 Characteristics of study sample ($n = 1919$)

	Frail ($n = 142$)	Pre-frail ($n = 864$)	Non-frail ($n = 913$)
Characteristic, mean ± SD or *n* (%)			
Age, years	77 ± 6	72 ± 7	69 ± 6
Women	77 (54)	495 (57)	463 (51)
Body mass index (kg/m^2)	29.6 ± 7.0	28.8 ± 5.3	27.3 ± 4.5
Current smoking	14 (9)	64 (7)	50 (5)
Diabetes	34 (24)	154 (18)	101 (11)
Cardiovascular disease	61 (43)	184 (21)	130 (14)
Cancer	34 (24)	140 (16)	129 (14)
Chronic kidney disease	45 (33)	132 (15)	103 (11)
Framingham Physical Activity Index score	29.9 ± 2.7	34.1 ± 4.6	37.0 ± 5.0
Gait speed (m/s)	0.80 ± 0.20	1.09 ± 0.26	1.28 ± 0.21
Hand grip strength (kg)	21.8 ± 8.50	27.5 ± 10.60	33.8 ± 10.70
Frailty criteria			
Slow (gait speed)	127 (89)	356 (41)	0
Weak (hand grip strength)	114 (80)	330 (38)	0
Low physical activity	113 (80)	227 (26)	0
Exhaustion	66 (46)	98 (11)	0
Weight loss	55 (39)	106 (12)	0
Biomarkers, median (25th percentile, 75th percentile)			
CRP (mg/L)	2.81 (1.26,5.93)	1.94 (0.90,3.83)	1.29 (0.72,2.65)
Interleukin-6 (pg/mL)	3.42 (2.15,6.48)	2.21 (1.46,3.28)	1.64 (1.17,2.56)
TNFR2 (pg/mL)	3151 (2506,4447)	2669 (2149,3443)	2385 (1963,2877)
Isoprostanes (mg/L)[a]	11.5 (8.50,15.40)	10.2 (7.60,14.30)	9.5 (7.1,12.8)
LpPLA2 mass (ng/mL)	210 (183,237)	199 (172,229)	199 (168,228)
LpPLA2 activity (nm/mL/min)	139 (119,166)	137 (115,160)	136 (114,159)
Osteoprotegerin (pm/L)	5.88 (4.82,7.41)	5.13 (4.23,6.13)	4.81 (4.01,5.59)
ICAM-1 (ng/mL)	307 (250,381)	293 (244,359)	270 (233,334)
MCP-1 (pg/mL)	415 (345,501)	384 (324,462)	364 (301,441)
P-selectin (ng/mL)	41 (32,51)	41 (33,49)	39 (32,47)

CRP C-reactive protein, *TNFR2* tumor necrosis factor receptor 2, *isoprostanes* 8-epi-FGFα isoprostanes, *LpPLA2* lipoprotein phospholipase A2, *ICAM-1* intracellular adhesion molecule-1, *MCP-1* monocyte chemoattractant protein-1

[a]Indexed to urinary creatinine

regarding the association of biomarkers of inflammation and oxidative stress with frailty and gait speed. We found that increased levels of isoprostanes and LpPLA2 mass were associated with greater odds of frailty. Higher mean concentrations of both these biomarkers and osteoprotegerin were associated with slower gait speeds. Our study confirmed that elevated levels of interleukin-6 were associated with frailty (Leng et al. 2007; Hubbard et al. 2009; Bandeen-Roche et al. 2009) and gait speed (Jenny et al. 2012). All associations were present in analyses that accounted for multiple biomarkers and potential confounders, including cardiovascular disease.

In the context of the current literature

Isoprostanes, LpPLA2 mass, and osteoprotegerin are each associated with increased oxidative stress. Other studies with smaller sample sizes have shown that oxidative stress is associated with frailty. Malondialdehyde and protein carbonyls, which are upregulated during oxidative stress, were both cross-sectionally associated with frailty in 742 older adults (Ingles et al. 2014). Oxidized glutathione and 8-hydroxy-2′-deoxyguanosine, oxidative damage byproducts, were associated with frailty in two studies of elders (Wu et al. 2009; Serviddio et al. 2009), but these

Table 2 Odds ratios of frailty for multiple biomarkers in specified samples of participants

	Odds ratio (95 % CI)					
	Interleukin-6	*p* value	Isoprostanes	*p* value	LpPLA2 mass	*p* value
Primary sample: ≥60 years ($n = 937$)	1.90 (1.51, 2.38)	<0.001	1.46 (1.12, 1.92)	0.006	1.29 (1.00, 1.65)	0.05
Secondary sample: ≥70 years ($n = 418$)	1.65 (1.26, 2.17)	<0.001	1.33 (0.96, 1.84)	0.090	1.42 (1.07, 1.89)	0.02
Secondary sample: free of cancer ($n = 796$)	1.91 (1.48, 2.47)	<0.001	1.50 (1.11, 2.03)	0.008	1.35 (1.02, 1.79)	0.04

Per one standard deviation increase of $\log_e$-transformed biomarker. Mean ± SD of $\log_e$-transformed biomarkers for all persons included in primary sample are the following: IL-6 0.75 ± 0.73 pg/mL, isoprostanes 2.32 ± 0.48 mg/L, and LpPLA2 mass 5.27 ± 0.2 ng/mL. Comparison group is non-frail persons ($n = 913$, pre-frail individuals excluded). Biomarkers initially included in stepwise models if individual association was $p \leq 0.10$. Final models used backward selection with cutoff p value of 0.10. All models adjusted for age, sex, body mass index, current smoking, diabetes, cardiovascular disease, cancer, and chronic kidney disease

Isoprostanes 8-epi-FGFα isoprostanes, *LpPLA2* lipoprotein phospholipase A2

studies each had less than a hundred persons. While a cross-sectional study of 552 older adults reported that isoprostanes were not associated with frailty (Collerton et al. 2012), the disparity in findings with our results may be secondary to differences in sample size and isoprostane assessment. Our results showing that frailty is associated with isoprostanes and LpPLA2 in a larger sample support that there is a relation between oxidative stress and frailty.

Prior studies examining the relation between oxidative stress and gait speed have been conflicting. One study found that indicators of oxidative stress, such as carbonylated proteins, lipid peroxidation, and total antioxidant activity, were not associated with lower limb function (Caballero et al. 2014). In contrast, another study demonstrated that slower gait speeds in older women were associated with protein carbonyl levels (Semba et al. 2007). In persons with gait speeds less than 0.8 m/s, a gait speed associated with greater mortality (Studenski et al. 2011), in vitro induction of oxidative stress in whole blood cells generated more free radicals (Baptista et al. 2012). Of note, the prior two studies examined only a single oxidative stress biomarker, whereas we demonstrated that two oxidative stress biomarkers were simultaneously elevated in persons with slower gait speeds. Our results provide additional evidence for the relation between oxidative stress and gait speed.

Table 3 Associations of gait speed with biomarkers

Biomarker	Estimated beta (95 % CI)	*p* value
Gait speed in meters per second ($n = 1710$)[a]		
Interleukin-6	−0.025 (0.04, −0.010)	<0.001
Isoprostanes	−0.019 (−0.03, −0.008)	0.001
LpPLA2 mass	−0.016 (−0.03, −0.004)	0.008
Osteoprotegerin	−0.015 (−0.03, −0.002)	0.020

Regression estimates are per 1 SD of $\log_e$-transformed biomarker. Biomarkers initially included in stepwise models if individual association was $p \leq 0.10$. Final stepwise models used backward selection with cutoff p value of 0.10. All models adjusted for age, sex, body mass index, current smoking, diabetes, cardiovascular disease, cancer, and chronic kidney disease

Isoprostanes 8-epi-FGFα isoprostanes, *LpPLA2* lipoprotein phospholipase A2

[a] Sample includes all frail, pre-frail, and non-frail persons who had data on all ten biomarkers of interest

We also demonstrated an association between osteoprotegerin and slower gait speeds. Osteoprotegerin was originally described as a biomarker of bone remodeling, but emerging evidence shows it plays a role in the neuromuscular and cardiovascular systems as well. Regarding the neuromuscular system, in vivo experiments have shown that myoblasts produce osteoprotegerin; in a murine model of muscular dystrophy, exogenous osteoprotegerin increases skeletal muscle force (Cesari et al. 2015). A study of ten healthy young adult men found that quadriceps muscle damage increased osteoprotegerin production (Philippou et al. 2009). In terms of cardiovascular disease, osteoprotegerin is thought to contribute to atherosclerosis (Montecucco et al. 2007) and has been associated with multiple cardiovascular diseases (Montagnana et al. 2013), including peripheral arterial disease (Poulsen et al. 2011). Notably, peripheral arterial disease is also associated with slow gait speeds (McDermott et al. 2004).

Our study confirmed the associations between interleukin-6, frailty, and gait speed. The cross-sectional relation between elevated interleukin-6 and frailty has

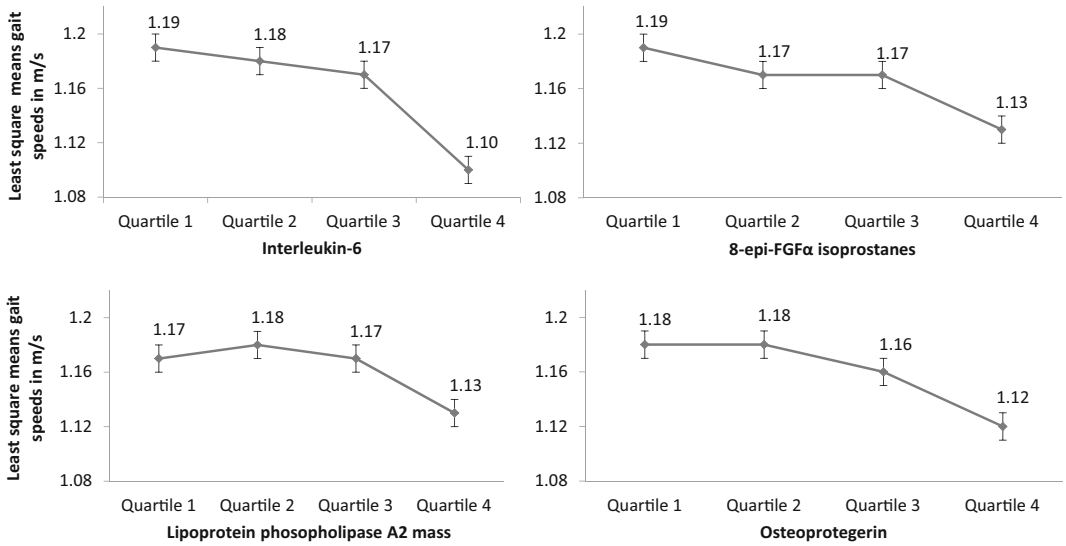

Fig. 1 Associations of quartiles of individual biomarkers with gait speed in covariate-adjusted regression models ($n = 1710$), adjusted for age, sex, body mass index, smoking, diabetes, cardiovascular disease, cancer, and chronic kidney disease; *bars* represent standard error. Biomarker values of 25th percentile, 50th percentile, and 75th percentile: IL-6 in picograms per milliliter (0.15, 1.3, 2.0), isoprostanes in milligrams per liter (10, 567, 927), and LpPLA2 mass in nanograms per milliliter (38, 171, 200)

been demonstrated in multiple cohorts of older adults (Leng et al. 2007; Bandeen-Roche et al. 2009; Hubbard et al. 2009). In regard to gait speed, our study confirmed investigators' findings that interleukin-6 is associated with slower gait speeds (Ferrucci et al. 2002; Boxer et al. 2008; Jenny et al. 2012).

Potential mechanisms

Oxidative stress likely causes musculoskeletal system damage, which facilitates the development of frailty and slow gait speeds. Potential pathways include increased intracellular calcium, which is associated with oxidative stress; studies in mice have shown that intracellular calcium promotes proteasomal activity and accelerates muscle breakdown (Boittin et al. 2006). Reactive oxygen species have been reported to trigger apoptosis of murine skeletal muscle (Adhihetty et al. 2007). In elderly rats, chronic-free radical exposure decreases myoblast proliferation (Derbre et al. 2014). In addition, the remaining muscle is likely impaired; murine models have demonstrated that changes in LpPLA2 affect muscle contractility and endurance (Gong et al. 2006). In a murine model of frailty, adenosine triphosphate production in skeletal muscle, which fuels muscle contraction, is reduced in comparison to skeletal muscle from wild-type mice (Akki et al. 2014). The product of all these mechanisms is dysfunctional muscle tissue in reduced amounts.

Strengths and limitations of study

Strengths of our study include the large sample size. We investigated multiple biomarkers representing diverse inflammatory pathways; our analyses accounted for simultaneous activation of multiple pathways through stepwise models of multiple biomarkers. Additionally, we examined the association of these biomarkers with gait speed, reinforcing our primary findings. Several limitations also merit comment. The lack of ethnic diversity in our study sample may limit the generalizability of the results. We suspect that the relation between oxidative stress and frailty is bi-directional, with the presence of one condition increasing the risk of the other condition. However, the study design was cross-sectional, limiting our ability to determine causality or temporality of this association. There are multiple definitions of frailty; while the Fried criteria are utilized the most frequently, it does limit the relevance of our findings to other frailty definitions. Finally, while the panel of biomarkers examined was relatively large, it was not comprehensive and may have excluded the contribution of pathways such as those related to autoimmunity (Leng et al. 2011).

Clinical implications

Reduction of oxidative stress may be a potential target for frailty treatment; one promising intervention is physical activity (Liu and Fielding 2011). In pilot studies, regimens of regular physical activity decreased biomarkers of oxidative stress (de Oliveira et al. 2012) and reduced the expression of oxidative stress genes (Gano et al. 2011). In clinical trials, frailty prevalence was reduced by 14.7 % using a year-long multidisciplinary intervention that included physical activity (Cameron et al. 2013); similarly, frailty prevalence was reduced from 23 to 10 % with a program of just physical activity (Cesari et al. 2015). Studies have shown that physical activity interventions are feasible even in those who are functionally limited (Pahor et al. 2014). Future studies should continue to investigate the effects of physical activity on oxidative stress, inflammation, and frailty.

Conclusions

In community-dwelling older adults, biomarkers of oxidative stress were associated with frailty and gait speed. Elevated levels of isoprostanes and LpPLA2 mass were cross-sectionally associated with increased odds of frailty and slower gait speeds. Osteoprotegerin was associated with slower gait speed. Our findings with these biomarkers add to the growing body of evidence that oxidative stress contributes to frailty in older adults. Reduction of oxidative stress may be a potential target for frailty treatment. Future research should explore whether frailty can be prevented or treated by interventions that reduce oxidative stress.

Acknowledgments We thank Emily Abrams for her assistance. The investigators are indebted to Framingham Offspring Study participants and staff. Lipoprotein-associated phospholipase A2 activity measurements were provided by GlaxoSmithKline and mass measurements by diaDexus at no cost. This was presented at the Annual Meeting of the American Geriatrics Society in Grapevine, TX, May 3–5, 2013. Correspondence should be addressed to Dr. Christine Liu, Boston Medical Center, 88 East Newton Street, Robinson 2, Boston, MA, 02118 or Christine.Liu@bmc.org.

Compliance with ethical standards

Funding This work was supported by the National Institute on Aging (grant number R01-AG029451 to JM Murabito, 1R01-AG028321 to EJB) and by the National Heart, Lung and Blood Institute (grant number 1RO1-HL64753, R01-HL076784 to EJB), both at the National Institutes of Health. The Framingham Heart Study is supported by the National Heart, Lung, and Blood Institute in collaboration with Boston University (contract number N01-HC-25195). CKL was supported by the American Federation for Aging Research, the John A. Hartford Foundation, and the U.S. Department of Agriculture, under Agreement no. 1950-51000-068-01S.

References

Adhihetty PJ, O'Leary MF, Chabi B, Wicks KL, Hood DA (2007) Effect of denervation on mitochondrially mediated apoptosis in skeletal muscle. J Appl Physiol 1985, 20061122

Akki A, Yang H, Gupta A, Chacko VP, Yano T, Leppo MK, Steenbergen C, Walston J, Weiss RG (2014) Skeletal muscle ATP kinetics are impaired in frail mice. Age (Dordr) 36:21–30. doi:10.1007/s11357-013-9540-0, 20130522

Bandeen-Roche K, Xue QL, Ferrucci L, Walston J, Guralnik JM, Chaves P, Zeger SL, Fried LP (2006) Phenotype of frailty: characterization in the women's health and aging studies. J Gerontol A Biol Sci Med Sci 61:262–266

Bandeen-Roche K, Walston JD, Huang Y, Semba RD, Ferrucci L (2009) Measuring systemic inflammatory regulation in older adults: evidence and utility. Rejuvenation Res 12:403–410. doi:10.1089/rej.2009.0883

Baptista G, Dupuy AM, Jaussent A, Durant R, Ventura E, Sauguet P, Picot MC, Jeandel C, Cristol JP (2012) Low-grade chronic inflammation and superoxide anion production by NADPH oxidase are the main determinants of physical frailty in older adults. Free Radic Res 46:1108–1114. doi:10.3109/10715762.2012.692784, 20120529

Boittin FX, Petermann O, Hirn C, Mittaud P, Dorchies OM, Roulet E, Ruegg UT (2006) Ca^{2+}-independent phospholipase A2 enhances store-operated Ca^{2+} entry in dystrophic skeletal muscle fibers. J Cell Sci 119:3733–3742. doi:10.1242/jcs.03184, 20060822

Boxer RS, Dauser DA, Walsh SJ, Hager WD, Kenny AM (2008) The association between vitamin D and inflammation with the 6-minute walk and frailty in patients with heart failure. J Am Geriatr Soc 56:454–461. doi:10.1111/j.1532-5415.2007.01601.x, 20080105

Caballero B, Rubio-Gonzalez A, Potes Y, Martinez-Reig M, Sanchez-Jurado PM, Romero L, Solano JJ, Abizanda P, Coto-Montes A (2014) Associations of the antioxidant capacity and hemoglobin levels with functional physical performance of the upper and lower body limbs. Age (Dordr) 36:851–867. doi:10.1007/s11357-013-9607-y, 20140103

Cameron ID, Fairhall N, Langron C, Lockwood K, Monaghan N, Aggar C, Sherrington C, Lord SR, Kurrle SE (2013) A multifactorial interdisciplinary intervention reduces frailty in older people: randomized trial. BMC Med 11:65. doi:10.1186/1741-7015-11-65, 20130311

Cesari M, Vellas B, Hsu FC, Newman AB, Doss H, King AC, Manini TM, Church T, Gill TM, Miller ME, Pahor M, LIFE Study Group (2015) A physical activity intervention to treat

the frailty syndrome in older persons—results from the LIFE-P study. J Gerontol A Biol Sci Med Sci 70:216–222. doi:10.1093/gerona/glu099, 20141111

Chen K, Keaney JF Jr (2012) Evolving concepts of oxidative stress and reactive oxygen species in cardiovascular disease. Curr Atheroscler Rep 14:476–483. doi:10.1007/s11883-012-0266-8

Collerton J, Martin-Ruiz C, Davies K, Hilkens CM, Isaacs J, Kolenda C, Parker C, Dunn M, Catt M, Jagger C, von Zglinicki T, Kirkwood TB (2012) Frailty and the role of inflammation, immunosenescence and cellular ageing in the very old: cross-sectional findings from the Newcastle 85+ Study. Mech Ageing Dev 133:456–466. doi:10.1016/j.mad.2012.05.005, 20120601

de Oliveira VN, Bessa A, Jorge ML, Oliveira RJ, de Mello MT, De Agostini GG, Jorge PT, Espindola FS (2012) The effect of different training programs on antioxidant status, oxidative stress, and metabolic control in type 2 diabetes. Appl Physiol Nutr Metab 37:334–344. doi:10.1139/h2012-004, 20120329

Derbre F, Gratas-Delamarche A, Gomez-Cabrera MC, Vina J (2014) Inactivity-induced oxidative stress: a central role in age-related sarcopenia? Eur J Sports Sci 14(Suppl 1):S98–S108. doi:10.1080/17461391.2011.654268, 20120301

Driver JA, Beiser A, Au R, Kreger BE, Splansky GL, Kurth T, Kiel DP, Lu KP, Seshadri S, Wolf PA (2012) Inverse association between cancer and Alzheimer's disease: results from the Framingham Heart Study. BMJ 344, e1442. doi:10.1136/bmj.e1442, 20120312

El Assar M, Angulo J, Rodriguez-Manas L (2013) Oxidative stress and vascular inflammation in aging. Free Radic Biol Med 65:380–401. doi:10.1016/j.freeradbiomed.2013.07.003, 20130710

Ensrud KE, Ewing SK, Taylor BC, Fink HA, Cawthon PM, Stone KL, Hillier TA, Cauley JA, Hochberg MC, Rodondi N, Tracy JK, Cummings SR (2008) Comparison of 2 frailty indexes for prediction of falls, disability, fractures, and death in older women. Arch Intern Med 168:382–389. doi:10.1001/archinternmed.2007.113

Feinleib M, Kannel WB, Garrison RJ, McNamara PM, Castelli WP (1975) The Framingham Offspring Study. Design and preliminary data. Prev Med 4:518–525

Ferrucci L, Penninx BW, Volpato S, Harris TB, Bandeen-Roche K, Balfour J, Leveille SG, Fried LP, Md JM (2002) Change in muscle strength explains accelerated decline of physical function in older women with high interleukin-6 serum levels. J Am Geriatr Soc 50:1947–1954

Fontes J, Pryde M, Ingram C, Sutherland P, Larson M, Benjamin E (2011) Framingham Heart Study inflammatory markers manual Offspring exam 8 and Omni 1(3). Available from: www.framinghamheartstudy.org/share/vascularprotocols.html

Fried LP, Tangen CM, Walston J, Newman AB, Hirsch C, Gottdiener J, Seeman T, Tracy R, Kop WJ, Burke G, McBurnie MA, Cardiovascular Health Study Collaborative Research Group (2001) Frailty in older adults: evidence for a phenotype. J Gerontol A Biol Sci Med Sci 56:M146–M156

Gano LB, Donato AJ, Pierce GL, Pasha HM, Magerko KA, Roeca C, Seals DR (2011) Increased proinflammatory and oxidant gene expression in circulating mononuclear cells in older adults: amelioration by habitual exercise. Physiol Genomics 43:895–902. doi:10.1152/physiolgenomics.00204.2010, 20110524

Gill TM, Gahbauer EA, Han L, Allore HG (2010) Trajectories of disability in the last year of life. N Engl J Med 362:1173–1180. doi:10.1056/NEJMoa0909087

Gong MC, Arbogast S, Guo Z, Mathenia J, Su W, Reid MB (2006) Calcium-independent phospholipase A2 modulates cytosolic oxidant activity and contractile function in murine skeletal muscle cells. J Appl Physiol 100:399–405. doi:10.1152/japplphysiol.00873.2005, 20050915

Hubbard RE, O'Mahony MS, Savva GM, Calver BL, Woodhouse KW (2009) Inflammation and frailty measures in older people. J Cell Mol Med 13:3103–3109. doi:10.1111/j.1582-4934.2009.00733.x, 20090306

Ingles M, Gambini J, Carnicero JA, Garcia-Garcia FJ, Rodriguez-Manas L, Olaso-Gonzalez G, Dromant M, Borras C, Vina J (2014) Oxidative stress is related to frailty, not to age or sex, in a geriatric population: lipid and protein oxidation as biomarkers of frailty. J Am Geriatr Soc 62:1324–1328. doi:10.1111/jgs.12876, 20140624

Jenny NS, French B, Arnold AM, Strotmeyer ES, Cushman M, Chaves PH, Ding J, Fried LP, Kritchevsky SB, Rifkin DE, Sarnak MJ, Newman AB (2012) Long-term assessment of inflammation and healthy aging in late life: the cardiovascular health study all stars. J Gerontol A Biol Sci Med Sci 67:970–976. doi:10.1093/gerona/glr261, 20120224

Kannel WB, Feinleib M, McNamara PM, Garrison RJ, Castelli WP (1979) An investigation of coronary heart disease in families. The Framingham Offspring Study. Am J Epidemiol 110:281–290

Kositsawat J, Barry LC, Kuchel GA (2013) C-reactive protein, vitamin D deficiency, and slow gait speed. J Am Geriatr Soc 61:1574–1579. doi:10.1111/jgs.12403, 20130808

Legein B, Temmerman L, Biessen EA, Lutgens E (2013) Inflammation and immune system interactions in atherosclerosis. Cell Mol Life Sci 70:3847–3869. doi:10.1007/s00018-013-1289-1, 20130221

Leng SX, Xue QL, Tian J, Walston JD, Fried LP (2007) Inflammation and frailty in older women. J Am Geriatr Soc 55:864–871. doi:10.1111/j.1532-5415.2007.01186.x

Leng SX, Tian X, Matteini A, Li H, Hughes J, Jain A, Walston JD, Fedarko NS (2011) IL-6-independent association of elevated serum neopterin levels with prevalent frailty in community-dwelling older adults. Age Ageing 40:475–481. doi:10.1093/ageing/afr047, 20110517

Leong DP, Teo KK, Rangarajan S, Lopez-Jaramillo P, Avezum A Jr, Orlandini A, Seron P, Ahmed SH, Rosengren A, Kelishadi R, Rahman O, Swaminathan S, Iqbal R, Gupta R, Lear SA, Oguz A, Yusoff K, Zatonska K, Chifamba J, Igumbor E, Mohan V, Anjana RM, Gu H, Li W, Yusuf S, Prospective Urban Rural Epidemiology (PURE) Study investigators (2015) Prognostic value of grip strength: findings from the prospective urban rural epidemiology (PURE) study. Lancet 386:266–273. doi:10.1016/S0140-6736(14)62000-6, 20150513

Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF 3rd, Feldman HI, Kusek JW, Eggers P, Van Lente F, Greene T, Coresh J, CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration) (2009) A new equation to estimate glomerular filtration rate. Ann Intern Med 150:604–612

Liu CK, Fielding RA (2011) Exercise as an intervention for frailty. Clin Geriatr Med 27:101–110. doi:10.1016/j.cger.2010.08.001

Matsuzawa Y, Konishi M, Akiyama E, Suzuki H, Nakayama N, Kiyokuni M, Sumita S, Ebina T, Kosuge M, Hibi K, Tsukahara K, Iwahashi N, Endo M, Maejima N, Saka K, Hashiba K, Okada K, Taguri M, Morita S, Sugiyama S, Ogawa H, Sashika H, Umemura S, Kimura K (2013) Association between gait speed as a measure of frailty and risk of cardiovascular events after myocardial infarction. J Am Coll Cardiol 61:1964–1972. doi:10.1016/j.jacc.2013.02.020, 20130314

McDermott MM, Liu K, Greenland P, Guralnik JM, Criqui MH, Chan C, Pearce WH, Schneider JR, Ferrucci L, Celic L, Taylor LM, Vonesh E, Martin GJ, Clark E (2004) Functional decline in peripheral arterial disease: associations with the ankle brachial index and leg symptoms. JAMA 292:453–461. doi:10.1001/jama.292.4.453

Montagnana M, Lippi G, Danese E, Guidi GC (2013) The role of osteoprotegerin in cardiovascular disease. Ann Med 45:254–264. doi:10.3109/07853890.2012.727019, 20121030

Montecucco F, Steffens S, Mach F (2007) The immune response is involved in atherosclerotic plaque calcification: could the RANKL/RANK/OPG system be a marker of plaque instability? Clin Dev Immunol 2007, 75805. doi:10.1155/2007/75805

Mulero J, Zafrilla P, Martinez-Cacha A (2011) Oxidative stress, frailty and cognitive decline. J Nutr Health Aging 15:756–760

Orme JG, Reis J, Herz EJ (1986) Factorial and discriminant validity of the center for epidemiological studies depression (CES-D) scale. J Clin Psychol 42:28–33

Pahor M, Guralnik JM, Ambrosius WT, Blair S, Bonds DE, Church TS, Espeland MA, Fielding RA, Gill TM, Groessl EJ, King AC, Kritchevsky SB, Manini TM, McDermott MM, Miller ME, Newman AB, Rejeski WJ, Sink KM, Williamson JD, LIFE study investigators (2014) Effect of structured physical activity on prevention of major mobility disability in older adults: the LIFE study randomized clinical trial. JAMA 311:2387–2396. doi:10.1001/jama.2014.5616

Philippou A, Bogdanis G, Maridaki M, Halapas A, Sourla A, Koutsilieris M (2009) Systemic cytokine response following exercise-induced muscle damage in humans. Clin Chem Lab Med 47:777–782. doi:10.1515/CCLM.2009.163

Poulsen MK, Nybo M, Dahl J, Hosbond S, Poulsen TS, Johansen A, Hoilund-Carlsen PF, Beck-Nielsen H, Rasmussen LM, Henriksen JE (2011) Plasma osteoprotegerin is related to carotid and peripheral arterial disease, but not to myocardial ischemia in type 2 diabetes mellitus. Cardiovasc Diabetol 10:76. doi:10.1186/1475-2840-10-76, 20110812

Puts MT, Visser M, Twisk JW, Deeg DJ, Lips P (2005) Endocrine and inflammatory markers as predictors of frailty. Clin Endocrinol 63:403–411. doi:10.1111/j.1365-2265.2005.02355.x

Schnabel R, Larson MG, Dupuis J, Lunetta KL, Lipinska I, Meigs JB, Yin X, Rong J, Vita JA, Newton-Cheh C, Levy D, Keaney JF Jr, Vasan RS, Mitchell GF, Benjamin EJ (2008) Relations of inflammatory biomarkers and common genetic variants with arterial stiffness and wave reflection. Hypertension 51:1651–1657. doi:10.1161/HYPERTENSIONAHA.107.105668, 20080421

Semba RD, Ferrucci L, Sun K, Walston J, Varadhan R, Guralnik JM, Fried LP (2007) Oxidative stress and severe walking disability among older women. Am J Med 120:1084–1089. doi:10.1016/j.amjmed.2007.07.028

Serviddio G, Romano AD, Greco A, Rollo T, Bellanti F, Altomare E, Vendemiale G (2009) Frailty syndrome is associated with altered circulating redox balance and increased markers of oxidative stress. Int J Immunopathol Pharmacol 22:819–827

Sikka G, Miller KL, Steppan J, Pandey D, Jung SM, Fraser CD 3rd, Ellis C, Ross D, Vandegaer K, Bedja D, Gabrielson K, Walston JD, Berkowitz DE, Barouch LA (2013) Interleukin 10 knockout frail mice develop cardiac and vascular dysfunction with increased age. Exp Gerontol 48:128–135. doi:10.1016/j.exger.2012.11.001, 20121113

Singh T, Newman AB (2011) Inflammatory markers in population studies of aging. Ageing Res Rev 10:319–329. doi:10.1016/j.arr.2010.11.002, 20101208

Studenski S, Perera S, Patel K, Rosano C, Faulkner K, Inzitari M, Brach J, Chandler J, Cawthon P, Connor EB, Nevitt M, Visser M, Kritchevsky S, Badinelli S, Harris T, Newman AB, Cauley J, Ferrucci L, Guralnik J (2011) Gait speed and survival in older adults. JAMA 305:50–58. doi:10.1001/jama.2010.1923

Verghese J, Holtzer R, Lipton RB, Wang C (2012) High-sensitivity C-reactive protein and mobility disability in older adults. Age Ageing 41:541–545. doi:10.1093/ageing/afs038, 20120314

Walston J, McBurnie MA, Newman A, Tracy RP, Kop WJ, Hirsch CH, Gottdiener J, Fried LP, Cardiovascular Health Study (2002) Frailty and activation of the inflammation and coagulation systems with and without clinical comorbidities: results from the cardiovascular health study. Arch Intern Med 162:2333–2341

Woods NF, LaCroix AZ, Gray SL, Aragaki A, Cochrane BB, Brunner RL, Masaki K, Murray A, Newman AB, Women's Health Initiative (2005) J Am Geriatr Soc 53:1321–1330. doi:10.1111/j.1532-5415.2005.53405.x

Wu IC, Shiesh SC, Kuo PH, Lin XZ (2009) High oxidative stress is correlated with frailty in elderly Chinese. J Am Geriatr Soc 57:1666–1671. doi:10.1111/j.1532-5415.2009.02392.x, 20090721

Xue QL, Walston JD, Fried LP, Beamer BA (2011) Prediction of risk of falling, physical disability, and frailty by rate of decline in grip strength: the women's health and aging study. Arch Intern Med 171:1119–1121. doi:10.1001/archinternmed.2011.252