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Selenium and impaired physical function in US and Spanish older adults

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ABSTRACT

Background: Selenium (Se) is a trace element with a narrow safety margin.
Objectives: To evaluate the cross-sectional and longitudinal dose-response association between Se exposure and measures of impaired physical function and disability in older adults.
Design: NHANES 2011–2014 cross-sectional (US, n = 1733, age ≥60 years) and Seniors-ENRICA-2 2017–2019 cross-sectional and longitudinal (Spain, n = 2548 and 1741, respectively, age ≥65 years) data were analyzed. Whole blood and serum Se levels were measured using inductively coupled plasma-mass spectrometry. Lower-extremity performance was assessed with the Short Physical Performance Battery, and muscle weakness with a dynamometer. Incident mobility and agility limitations, and disability in instrumental activities of daily living (IADL) were ascertained with standardized questionnaires. Analyses were adjusted for relevant confounders, including physical activity. Results across studies were pooled using random-effects meta-analysis.
Results: Meta-analyzed odds ratios (95% confidence interval) per log2 increase in whole blood Se were 0.54 (0.32; 0.76) for weakness, 0.59 (0.34; 0.83) for impaired lower-extremity performance, 0.48 (0.31; 0.68) for mobility limitations, 0.71 (0.45; 0.97) for agility limitations, and 0.34 (0.12; 0.56) for disability in at least one IADL. Analyses for serum Se in NHANES showed similar results. Findings suggest the inverse association with grip strength is progressive below 140 μg/L (p-value for non-linear trend in the Seniors-ENRICA-2 study = 0.13), and above 140 μg/L (p-value for non-linear trend in NHANES = 0.11). In the Seniors-ENRICA-2 cohort, with a 2.2 year follow-up period, a doubling in baseline Se levels were associated with a lower incidence of weakness [odds ratio (95% confidence interval): 0.45 (0.22; 0.91)], impaired lower-extremity performance [0.63 (0.32; 1.23)], mobility [0.43 (0.21; 0.91)] and agility [0.38 (0.18; 0.78)] limitations.
Discussion: In US and Spanish older adults, Se concentrations were inversely associated with physical function limitations. Further studies are needed to elucidate underlying mechanisms.

1. Introduction

Selenium (Se) is a trace element with beneficial effects on human health, mainly resulting from the antioxidant capacity of selenoenzymes [1]. However, the safe range of exposure to Se is generally narrow, and both Se deficiency and excess can be harmful to human health.

Clinical and epidemiological studies suggest that Se is a key nutrient

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for muscle health. Se deficiency due to prolonged parenteral nutrition has been associated with muscle pain and weakness, which are reversed with Se administration [2]. Cross-sectionally, Se deficiency has been associated with deleterious effects on muscle mass [3] and strength [4, 5]. For instance, in 676 older women from the Women's Health and Aging Study I (mean serum 117.7 μg/L), researchers found that low Se levels were associated with poor grip strength [4]. Similarly, among 327 individuals aged ≥65 years living in Taipei (mean serum Se 80.6 μg/L), the odds of low muscle mass decreased across increasing quartiles of serum Se [3]. In 891 participants aged ≥65 from the InCHIANTI study (mean plasma Se 75.0 μg/L), those in the lowest compared to the highest quartile of plasma Se had higher odds of poor hip, knee, and grip strength [5]. Among 628 participants in the Hertfordshire cohort study, higher Se intake was associated with shorter 3-m walk time in women [6]. However, previous studies had few participants at high Se levels and the shape of the dose-response association between Se exposure and measures of physical performance at serum Se levels above 100–120 μg/L is unclear. Moreover, there are no prospective studies evaluating the association between Se biomarkers and physical disability in older adults. Therefore, we aimed to: 1) Evaluate the cross-sectional association and the shape of the dose-response relationship between different biomarkers of Se exposure (namely, whole blood and serum Se), and physical performance and disability in two populations of older adults from the United States and Europe with a wide range of Se exposure levels; and 2) Evaluate the prospective association between whole blood Se and risk of impaired physical performance and disability in an older adult European population.

List of abbreviations:

BMI	Body Mass Index
CVD	Cardiovascular Disease
CATI	Computer-assisted telephone interview
DA	Deficit Accumulation
FNDDS	US Department of Agriculture's Food and Nutrient Database for Dietary Studies
IADL	Disability in Instrumental Activities of Daily living
LOD	Limit of Detection
MEDAS	Mediterranean diet adherence screener
PHQ-9	Patient Health Questionnaire
RDA	Recommended dietary allowance
Se	Selenium
SPPB	Short Physical Performance Battery

2. Methods

2.1. Study population and design NHANES

NHANES is an ongoing cross-sectional survey of a nationally representative U.S. population conducted by the CDC's National Center for Health Statistics [7]. NHANES includes an interview at home with a subsequent physical examination and additional interviews at mobile examination centers. In total, 3401 adults aged >60 years in NHANES 2011–2014 provided information in at least one physical function variable and 2280 (68.3%) had available determinations of Se in serum or whole blood. After exclusion of individuals with missing values on potential confounders (mainly Body Mass Index [BMI] or protein intake), data on 1733 (whole blood) and 736 (serum) participants was available for analyses. NHANES was approved by the National Center for Health Statistics Research Ethics Review Board, and written informed consent was obtained from all participants (Protocol #2011–17 and Continuation of Protocol #2011–17).

2.2. Seniors-ENRICA-2

Seniors-ENRICA-2 cohort was set up in 2017. In total, 3273 individuals were selected by sex- and district-stratified random sampling of all community-dwelling individuals aged ≥65 years holding a national healthcare card and living in the Madrid Autonomous Region. The study included a computer-assisted telephone interview (CATI) followed by two trained staff home visits. Information regarding lifestyles, self-rated health and morbidity was collected using CATI. During home visits, a dietary history and samples of blood were obtained, and a physical exam was performed. In 2019 participants were re-interviewed, and a new physical exam was performed.

From the initial sample of 3273 participants, 99.7% (3263) provided information of at least one physical function variable and 79.4% (2598) had available whole blood Se determinations. From these, a total of 2548 participants had data on all potentially important confounders and were included in cross-sectional analyses. In Seniors-ENRICA-2, prospective analyses were performed among 1140, 1121, and 780 older adults with no impaired lower extremity functional performance, mobility or agility limitations at baseline, respectively; as well as among participants with follow-up information on grip strength (n = 1221) or frailty (n = 1734).

Participants gave informed written consent, and the Clinical Research Ethics Committee of the *La Paz* University Hospital in Madrid approved the study (Protocol #HULP-PI 1793).

3. Study variables

3.1. Blood selenium

In NHANES, serum and whole blood Se were measured using inductively coupled plasma-mass spectrometry (ELAN® DRC II) at the Division of Laboratory Sciences, National Center for Environmental Health, and Centers for Disease Control and Prevention (Atlanta, GA) [8]. In the Seniors-ENRICA-2, whole blood Se was measured using inductively coupled plasma-mass spectrometry (8900 ICP-QQQ) at the Department of Legal Medicine, Toxicology, and Physical Anthropology, School of Medicine, University of Granada (Spain). The limits of detection (LOD) were 4.5 μg/L for serum Se; 30.0 μg/L and 24.5 μg/L for whole blood Se in NHANES 2011–2012 and 2013–2014, respectively; and 0.3 μg/L for whole blood Se in the Seniors-ENRICA-2. No samples had levels below the LOD.

Intake of Selenium, vitamins, proteins and alcohol:

In NHANES, dietary intake of micro and macronutrients was measured via two 24-h dietary recall interviews. During the interview, participants provided details of food and beverages consumed during the 24-h period before the interview (midnight to midnight). Estimates of Se and vitamins intake from each food or beverage were calculated using the US Department of Agriculture's Food and Nutrient Database for Dietary Studies (FNDDS) [9]. Additionally, participants provided information on dietary supplements, and Se intake from each product was summed to estimate the total daily dose for each individual. Participants who self-reported consuming ≥1 alcoholic beverage per week during the preceding year were considered current drinkers, while occasional drinkers (i.e. those with a consumption below 1 alcoholic beverage per week) were classified as non-drinkers.

In the Seniors-ENRICA-2, habitual food consumption was assessed with a validated computerized diet history that collected information on 880 foods and 34 alcoholic beverages, and used photographs to allow classification in 7 portion sizes [10]. The intake of vitamins and proteins was estimated using standard food composition tables, including the US FNDDS [9,11,12]. Standard beverage composition tables were used to estimate alcohol content. Study participants were classified as non-drinkers (including also occasional drinkers with average alcohol intakes below 1 standard unit per week), ex-drinkers, or current drinkers.

In both studies, the recommended dietary allowance (RDA) was used as reference to categorize participants' intakes of vitamins and Se [13].A minimum intake of 1.2 gr of proteins per kg of weight/day was considered as adequate for our study population [14].

3.2. Physical performance and disability

Grip strength: Grip strength was measured with a Takei (NHANES) or a Jamar (Seniors- ENRICA-2) dynamometer. In the NHANES alternating hands were tested three times, while in the Seniors-ENRICA-2, grip strength was measured twice in the dominant hand. Combined grip strength was calculated as the sum of the largest reading from each hand in NHANES, and weakness was defined as the study-specific lowest quintile of grip strength according to sex and BMI quartiles in each study.

Lower extremity physical performance: In the NHANES, lower extremity function was evaluated with the question "Without using any special equipment, how much difficulty do you have standing up from an armless straight chair?" Individuals answering "some difficulty", "much difficulty" or "unable to do" were considered as having impaired function. In the Seniors-ENRICA-2, lower extremity function was assessed at baseline and follow-up with the Short Physical Performance Battery (SPPB), which includes three components: balance testing, walking speed and a *sit-to-stand* test [15]. Balance testing included a *side-by-side*, a *semitandem* and a *tandem* stand: 1) Participants were first asked to stand with their feet together. Those who were able to stand for 10 s in this position were then tested in the *semitandem* stand position, where the heel of one foot is placed to the side of the big toe of the other foot. Finally, those who were able to stand for 10 s in the *semitandem* were tested in the *full-tandem* stand, where the heel of one foot was placed in front of the toes of the other foot. A score of 0 in the balancing test indicates the inability to stand in any of the positions; a score of 4 indicates a full-tandem stand for 10 s 2) Time taken to walk 3 m was then estimated, and gait speed calculated as the distance in meters divided by the time in seconds. In this test, 0 points indicated the inability to perform the walk, and 4 being in the fastest quartile of walking speed according to sex and height. 3) The *sit-to stand* test consisted in standing up and sitting down from a chair five times repeatedly, with arms crossed across the chest. A score of 0 was given if a participant was unable to perform the five chair stands, while scores of 1, 2, 3, or 4 were assigned to participants who completed five chair stands in $\geq$16.7, 13.7–16.6, 11.2–13.6, and $\leq$11.1 s, respectively. The total SPPB score was calculated by the sum of the components, with a range from 0 to 12 (best performance). Participants with a score $\leq$9 were deemed to have a low SPPB.

Mobility disability: In both studies, mobility disability was evaluated with three questions from the *Rosow and Breslau* scale asked as follows: In NHANES: 1) "By yourself and without using any special equipment, how much difficulty do you have lifting or carrying something as heavy as 10 pounds like a sack of potatoes or rice?", 2) "By yourself and without using any special equipment, how much difficulty do you have walking for about 2 or 3 blocks?", 3) "By yourself and without using any special equipment, how much difficulty do you have walking up to 10 steps without resting?"; In the Seniors-ENRICA-2: 1) "How much difficulty do you experience picking up or carrying a shopping bag?", 2) "How much difficulty do you experience climbing one flight of stairs?", 3) "How much difficulty do you experience walking several city blocks (a few hundred meters)?"; Individuals who answered "some difficulty", "much difficulty", or "unable to do" to any of these three questions were considered as having mobility disability.

Agility disability: Impaired agility was evaluated with the question "By yourself and without using any special equipment, how much difficulty do you have stooping, crouching, or kneeling?" in NHANES; and the question "How much difficulty do you experience in bending or kneeling?" in the Seniors-ENRICA-2. Individuals who answered "some difficulty", "much difficulty", or "unable to do" were considered to have agility limitations.

Frailty: Based on the Rockwood's frailty index [16], a Deficit Accumulation (DA) Index was calculated using a total of 52 health deficits, including impairments in physical and cognitive functioning, self-reported health and vitality problems, mental health conditions, as well as morbidity, polypharmacy, and health services use [17]. The DA index summarizes age-related vulnerability, so the more health deficits (symptoms, signs, diseases, or disabilities) an individual has the higher the risk of death, institutionalization, health service use, or further deficit accumulation. Participants in the highest (worst) quintile of DA index were considered frail.

Disability in Instrumental Activities of Daily living (IADL): Complete information on instrumental activity disabilities was only available in the Seniors-ENRICA-2, where the Lawton-Brody index evaluated the individual's ability to use the telephone, go shopping, prepare meals, do the housework, do the laundry, use different means of transportation, take medication, and manage finances. Due to cultural issues, the questions on meal preparation, housework and laundry were excluded in men. Because the incidence of IADL disability was low (n = 1), prospective analyses using this outcome were not performed. In NHANES, information was only available for limitations doing housework, managing finances and preparing meals. In both studies, individuals with disability in 1 or more of the evaluated activities were considered as IADL disabled.

3.3. Other variables

In both studies information from the following variables was also collected: age; sex; education (<high school, high school and > high school); smoking (never, ex-smoker, current smoker); moderate and vigorous physical activity; diet quality (measured with the 14-point Mediterranean diet adherence screener (MEDAS [18]) score in the Seniors-ENRICA and with a self-reported overall diet quality question in NHANES); and history of physician-diagnosed chronic conditions (i.e. hypertension, coronary heart disease, congestive heart failure, angina, cancer at any site, diabetes, osteoarthritis, and, in the Seniors-ENRICA-2, depression). In NHANES, depressive symptoms were screened by using the validated Patient Health Questionnaire (PHQ-9) [19]. A PHQ-9 score$\geq$10 was considered to indicate depressive symptoms. Cardiovascular disease (CVD) was defined as a self-reported diagnosis of coronary heart disease, congestive heart failure, heart attack or angina. Definition of hypertension was based on a self-reported physician diagnosis, current use of anti-hypertensive medication, or a clinical blood pressure reading 140/90 mmHg taken under standardized conditions. Definition of type 2 diabetes mellitus was based on a self-reported physician diagnosis, fasting glucose $\geq$126 mg/dL, or current use of anti-diabetic medication. Weight and height were measured according to standardized procedures, and the body mass index (BMI) calculated as measured weight in kg divided by squared height in m. Information on race/ethnicity was also registered in NHANES (Non-Hispanic White, Non-Hispanic Black, Mexican-American, and Other).

3.4. Statistical analyses

We first examined differences in the distribution of Se biomarkers by sociodemographic, lifestyle and clinical characteristics of study participants. In NHANES, all analyses were conducted taking into account the sampling design and all study statistics account for sampling weights. We assessed the cross-sectional (in both surveys) and prospective (in the Spanish cohort) associations between concentrations of Se biomarkers and measures of physical performance and disability (i.e. limitations in lower limb function, grip strength, mobility, agility, and IADL) using logistic (for dichotomous outcomes) or linear (for continuous outcomes) regression models.

Se concentrations were modeled as 1) continuous linear terms and scaled by the interquartile range; 2) quartiles; 3) log base 2-transformed;

Table 1
Geometric mean [95%CI] concentrations of whole blood and serum selenium by study participants' characteristics.

Characteristics	NHANES 2011–2014						Seniors-ENRICA-22017		
	Whole blood Se [μg/L]			Serum Se [μg/L]			Whole blood Se [μg/L]		
	n [weighted %]	Geometric mean [95%CI]	p-val	n [weighted %]	Geometric mean [95%CI]	p-val	n [weighted %]	Geometric mean [95%CI]	p-val
Overall*	1733 [100.0]	192.3 [189.4; 195.2]		736 [100.0]	129.7 [126.8; 132.8]		2548 [100.0]	113.4 [112.6; 114.3]	
Age,yr									
65-69	865 [49.9]	193.9 [190.7; 197.1]		365 [46.6]	129.8 [126.9; 132.7]		946 [37.1]	117.2 [115.9; 118.4]	
70-74	347 [20.0]	195.5 [191.5; 199.6]		171 [26.6]	129.7 [126.3; 133.2]		979 [38.4]	114.0 [112.8; 115.2]	
≥75	521 [30.1]	187.1 [182.1; 192.2]	0.004	200 [26.8]	129.8 [124.0; 135.9]	0.996	623 [24.5]	107.0 [105.5; 108.5]	0.000
Sex									
Men	841 [43.9]	196.5 [192.7; 200.5]		360 [47.5]	133.9 [128.7; 137.4]		1199 [47.1]	113.5 [112.5; 114.6]	
Women	892 [56.1]	189.1 [186.4; 191.7]	0.000	376 [52.5]	126.9 [123.8; 130.0]	0.016	1349 [52.9]	113.3 [112.2; 114.4]	0.768
Ethnicity									
Non-Hispanic white	872 [81.7]	192.1 [188.8; 195.5]		388 [46.9]	131.1 [125.5; 136.9]		2548 [100.0]	113.4 [112.6; 114.3]	
Non-Hispanic black	415 [7.6]	189.4 [185.5; 193.4]		170 [25.8]	125.4 [122.6; 128.3]				
Mexican-American	128 [2.8]	195.4 [190.1; 200.8]		55 [5.7]	124.2 [115.6; 133.5]				
Other & multi-racial	318 [7.9]	195.9 [190.7; 201.3]	0.058	123 [21.6]	133.6 [129.5; 137.8]	0.005			
Education									
<High School	445 [17.0]	189.9 [193.3; 197.0]		194 [36.9]	128.1 [122.1; 134.4]		1613 [63.3]	112.2 [111.3; 113.2]	
High School	408 [22.0]	188.4 [184.2; 192.6]		163 [17.5]	133.5 [126.4; 141.0]		481 [18.9]	113.9 [112.2; 115.7]	
>High School	880 [61.0]	194.4 [191.5; 197.3]	0.018	379 [45.6]	129.7 [126.2; 133.3]	0.329	454 [17.8]	117.0 [115.0; 119.0]	0.000
BMI, kg/m^2									
Under/normoweight, <25,	456 [27.2]	188.7 [185.7; 191.8]		192 [26.2]	131.1 [127.5; 134.7]		683 [26.8]	114.0 [112.4; 115.6]	
Overweight, 25–29.9,	606 [36.1]	195.9 [190.8; 201.2]		263 [37.1]	130.7 [124.9; 136.9]		1209 [47.1]	114.0 [113.0; 115.1]	
Obese, ≥30,	671 [36.7]	191.4 [188.9; 194.0]	0.004	281 [36.7]	127.8 [124.6; 131.2]	0.924	665 [26.1]	111.6 [110.1; 113.1]	0.020
Dietary quality†									
1st category [worst]	329 [15.4]	188.5 [183.4; 193.8]		139 [16.5]	125.0 [119.7; 130.6]		870 [34.1]	110.5 [108.9; 111.5]	
2nd category	734 [40.7]	190.7 [187.2; 194.3]		315 [42.4]	129.0 [126.0; 132.1]		1237 [48.6]	114.6 [113.6; 115.7]	
3rd category [best]	670 [43.9]	195.2 [192.0; 198.3]	0.003	282 [30.1]	132.5 [128.1; 137.9]	0.040	441 [17.3]	116.4 [114.4; 118.4]	0.091
RDA for selenium intake									
<55 μg/d	193 [9.5]	182.2 [177.3; 189.2]		71 [10.6]	119.5 [115.6; 123.5]		9 [0.4]	102.8 [84.2; 125.4]	
≥55 μg/d	1540 [90.5]	193.3 [177.3; 189.2]	0.007	665 [89.4]	131.0 [127.4; 134.8]	0.000	2539 [99.6]	113.4 [112.7; 114.2]	0.130
Nº RDA recommendations for vitamins									
<5	732 [42.2]	195.4 [192.1; 198.8]		286 [36.9]	133.8 [130.7; 137.0]		1636 [64.2]	115.3 [114.3; 116.3]	
≥5	1001 [57.8]	189.7 [189.4; 193.1]	0.002	450 [63.1]	127.5 [123.8; 131.3]	0.000	912 [35.8]	110.0 [108.8; 111.2]	0.000
Consumption of proteins ≥ 1.2 g/kg weight ‡									
No	1366 [78.0]	190.8 [187.4; 194.1]		568 [78.3]	128.5 [125.3; 131.7]		1134 [44.5]	111.2 [110.0; 112.3]	
Yes	367 [22.0]	197.8 [194.9; 200.9]	0.001	168 [21.7]	134.4 [129.2; 138.8]	0.063	1414 [55.5]	115.2 [114.2; 116.2]	0.000
Consumption of proteins [g/day]									
1st tertile	637 [33.4]	187.2 [183.0; 191.5]		246 [34.5]	123.8 [119.8; 127.9]		850 [33.4]	110.1 [108.7; 111.4]	
2nd tertile	555 [33.4]	191.9 [188.7; 195.2]		245 [35.5]	131.5 [127.0; 136.1]		849 [33.2]	114.1 [112.8; 115.4]	
3rd tertile	541 [33.2]	198.0 [194.5; 201.5]	0.020	245 [30.0]	134.8 [130.1; 139.7]	0.016	849 [33.2]	116.1 [114.8; 117.4]	0.000

(*continued on next page*)

Table 1 (*continued*)

Characteristics	NHANES 2011–2014						Seniors-ENRICA-22017		
	Whole blood Se [μg/L]			Serum Se [μg/L]			Whole blood Se [μg/L]		
	n [weighted %]	Geometric mean [95%CI]	p-val	n [weighted %]	Geometric mean [95%CI]	p-val	n [weighted %]	Geometric mean [95%CI]	p-val
Physical activity [METs-hours/week][§]									
1st tertile	617 [33.4]	189.7 [186.7; 192.8]		263 [35.7]	128.6 [124.5; 132.8]		850 [32.4]	111.8 [110.4; 113.2]	
2nd tertile	595 [33.8]	193.2 [189.6; 196.9]		263 [36.5]	131.0 [126.5; 135.5]		850 [33.4]	114.3 [113.0; 115.7]	
3rd tertile	521 [32.8]	194.9 [190.0; 198.1]	0.039	210 [27–8]	129.7 [125.8; 133.7]	0.345	848 [33.2]	114.1 [112.8; 115.3]	0.015
Smoking									
Never	860 [49.6]	192.7 [189.5; 195.9]		353 [47.7]	129.8 [126.4; 133.3]		1341 [52.6]	113.3 [112.2; 114.4]	
Ex-smoker	689 [39.8]	192.9 [189.3; 196.6]		314 [41.6]	130.6 [126.3; 135.2]		967 [38.0]	114.2 [112.9; 115.3]	
Current	184 [10.6]	187.3 [180.1; 194.9]	0.252	69 [10.7]	126.1 [121.1; 131.4]	0.325	240 [9.4]	110.6 [108.4; 112.8]	0.043
Alcohol consumption									
Not current	562 [28.7]	188.9 [185.1; 192.8]		238 [39.7]	127.5 [123.2; 132.0]		488 [19.2]	110.8 [108.9; 112.7]	
Current	1179 [71.3]	193.7 [190.1; 196.6]		497 [60.3]	131.3 [127.8; 134.9]		1896 [74.4]	189.6 [113.4; 115.1]	
Ex-drinkers			0.004			0.136	164 [6.4]	164.0 [108.4; 114.4]	0.000
Hypertension									
No	550 [36.9]	193.9 [189.5; 198.3]		242 [31.0]	132.7 [129.1; 136.1]		810 [31.8]	114.3 [112.8; 115.7]	
Yes	1183 [63.1]	191.4 [188.4; 194.4]	0.251	494 [69.0]	128.5 [124.9; 132.2]	0.096	1738 [68.2]	113.0 [112.1; 113.9]	0.121
Cardiovascular disease									
No	1359 [79.5]	193.1 [189.7; 196.5]		581 [78.9]	131.3 [128.1; 134.5]		2460 [96.6]	113.7 [112.9; 114.4]	
Yes	374 [20.5]	189.3 [185.9; 192.9]	0.077	155 [22.5]	124.7 [118.2; 131.5]	0.083	88 [3.4]	105.8 [100.8; 110.9]	0.000
Diabetes mellitus									
No	1438 [77.6]	192.4 [189.3; 195.6]		529 [71.3]	129.6 [126.8; 132.5]		2030 [76.7]	114.2 [113.3; 115.1]	
Yes	576 [22.4]	192.0 [188.1; 196.0]	0.833	207 [28.7]	130.1 [123.8; 136.7]	0.877	518 [20.3]	110.1 [108.6; 111.7]	0.000
Cancer									
No	1380 [75.1]	192.8 [189.9; 195.9]		588 [79.1]	129.6 [127.3; 132.0]		2471 [97.0]	113.5 [112.7; 114.3]	
Yes	353 [24.9]	190.7 [187.2; 194.3]	0.128	148 [20.9]	130.2 [122.8; 138.1]	0.858	77 [3.0]	110.6 [106.8; 114.5]	0.206
Osteoartritis									
No	876 [50.7]	192.2 [188.2; 196.3]		371 [47.6]	129.7 [126.5; 133.0]		1399 [54.9]	114.9 [113.8; 115.9]	
Yes	857 [49.3]	192.4 [189.4; 195.3]	0.954	365 [52.4]	129.8 [125.9; 133.8]	0.969	1149 [45.1]	111.6 [100.5; 112.7]	0.000
Depression									
No	1604 [94.9]	192.5 [189.3; 195.7]		680 [93.3]	130.5 [127.5; 133.7]		2337 [91.7]	113.6 [112.8; 114.4]	
Yes	129 [5.1]	188.7 [181.4; 196.2]	0.396	56 [6.7]	119.9 [109.1; 131.8]	0.093	211 [8.3]	110.8 [107.4; 114.2]	0.043

P-values correspond to Wald tests obtained from regression models on log-transformed selenium. *Based on participants with information in at least one functional variable. † The first, second and third categories correspond to participants with a fair or poor diet, a good diet, and an excellent or very good diet in the NHANES; or participants in the first, second, and third tertiles of the MEDAS score in the Seniors-ENRICA II. Cutoff values for the MEDAS score were <6 [tertile 1], 7–8 [tertile 2], and ≥9 [tertile 3].‡ Based on the subsample of participants with this information available. §Cutoff values for moderate and vigorous physical activity [METs-hours/week] were 1/24 in men and 1/20 in women in the NHANES; and 1.6/13.2 in men and 4/5.2 in women in the Seniors-ENRICA II.

and 4) restricted cubic splines with knots at the 10th, 50th, and 90th percentile for each cohort and biomarker to evaluate potential non-linear relationships. We conducted a test for trend in the association between increasing Se quartiles and measures of functional limitations by including Se medians corresponding to each quartile as continuous variables. Also, we evaluated departures from linearity in the restricted cubic spline models using the Wald test. Potential important confounders were included in models as follows: Model A adjusted for age, sex, and race/ethnicity (in NHANES); model B further adjusted for education, BMI, diet quality, protein intake, number of vitamins with intake above RDA, physical activity, smoking status, alcohol consumption, and prevalence of hypertension, CVD, diabetes, cancer, depression and/or osteoarthritis.

Between-study heterogeneity was assessed using the chi square based Q statistic and quantified using the I^2 statistic. Given consistent results between NHANES and ENRICA-2 (I2<30%) [20], we used random-effects meta-analysis as implemented in STATA using the metan command to obtain pooled estimates when possible (similar outcome definitions).

To evaluate the consistency of the findings, we conducted analyses for log2 transformed Se and functional limitations stratified by the main participant's subgroups: age (<70, ≥70 years), sex (male, female), BMI

Table 2
Cross-sectional association between whole blood selenium and measures of impaired physical function and disability in older adults from the NHANES and Seniors-ENRICA II studies. Results are odds ratios [95% confidence interval].

	Weakness			Impaired lower-extremity performance			Mobility limitation			Agility limitation			Frailty			IADL disability		
	n/ total	Model A	Model B	n/total	Model A	Model B	n/total	Model A	Model B	n/total	Model A	Model B	n/ total	Model A	Model B	n/ total	Model A	Model B
NHANES																		
Per IQR [30.8]	340/ 1614	0.86 [0.70; 1.06]	0.85 [0.70; 1.02]	350/ 1730	0.77 [0.61; 0.97]	0.84 [0.67; 1.06]	619/ 1629	0.76 [0.62; 0.94]	0.81 [0.65; 1.00]	275/ 1703	0.77 [0.64; 0.92]	0.84 [0.65; 1.07]				325/ 1584	0.81 [0.60; 1.09]	0.85 [0.66; 1.10]
Quartiles																		
≤178.5	115/ 433	Ref.	Ref.	121/ 468	Ref.	Ref.	211/ 437	Ref.	Ref.	102/ 456	Ref.	Ref.				114/ 426	Ref.	Ref.
178.6–192.5	78/ 376	0.73 [0.52; 1.03]	0.81 [0.58; 1.14]	78/402	0.63 [0.41; 0.96]	0.78 [0.52; 1.18]	140/ 374	0.44 [0.30; 0.64]	0.45 [0.32; 0.63]	54/98	0.51 [0.32; 0.82]	0.59 [0.32; 1.08]				74/ 371	0.71 [0.49; 1.02]	0.84 [0.58; 1.21]
192.6–207.5	77/ 401	0.82 [0.82; 1.34]	0.88 [0.58; 1.33]	73/419	0.61 [0.42; 0.89]	0.80 [0.55; 1.18]	129/ 400	0.47 [0.30; 0.74]	0.56 [0.36; 0.86]	61/414	0.71 [0.48; 1.04]	0.92 [0.60; 1.41]				65/ 386	0.68 [0.37; 1.24]	0.80 [0.52; 1.24]
≥207.5	70/ 404	0.73 [0.40; 1.34]	0.71 [0.39; 1.28]	78/441	0.64 [0.36; 1.11]	0.71 [0.38; 1.33]	139/ 418	0.57 [0.35; 0.90]	0.62 [0.40; 0.96]	58/435	0.63 [0.39; 0.99]	0.69 [0.37; 1.30]				72/ 401	0.66 [0.39; 1.12]	0.75 [0.48; 1.15]
*p-trend**		0.33	0.26		0.09	0.28		0.01	0.05		0.09	0.43					0.16	0.21
Per log2	340/ 1614	0.52 [0.21; 1.29]	0.49 [0.21; 1.12]	350/ 1730	0.33 [0.12; 0.90]	0.51 [0.19; 1.36]	619/ 1629	0.27 [0.11; 0.66]	0.35 [0.14; 0.88]	275/ 1703	0.31 [0.14; 0.68]	0.46 [0.16; 1.35]				325/ 1584	0.35 [0.10; 1.19]	0.45 [0.16; 1.26]
Seniors-ENRICA II																		
Per IQR [23.7]	497/ 2540	0.77 [0.67; 0.88]	0.85 [0.74; 0.97]	676/ 2530	0.81 [0.72; 0.91]	0.89 [0.79; 1.00]	962/ 2520	0.79 [0.71; 0.87]	0.87 [0.78; 0.97]	1442/ 2520	0.87 [0.79; 0.96]	0.94 [0.85; 1.04]	504/ 2548	0.62 [0.55; 0.72]	0.70 [0.59; 0.82]	189/ 2534	0.64 [0.52; 0.79]	0.75 [0.60; 0.92]
Quartiles																		
≤117.8	165/ 636	Ref.	Ref.	226/ 631	Ref.	Ref.	290/ 628	Ref.	Ref.	397/ 628	Ref.	Ref.	194/ 637	Ref.	Ref.	88/ 637	Ref.	Ref.
117.9–128.4	136/ 634	0.94 [0.71; 1.22]	1.04 [0.78; 1.37]	173/ 631	0.79 [0.61; 1.01]	0.88 [0.68; 1.14]	248/ 629	0.83 [0.65; 1.05]	0.92 [0.71; 1.19]	370/ 629	0.93 [0.74; 1.18]	1.00 [0.78; 1.31]	133/ 637	0.67 [0.52; 0.87]	0.73 [0.54; 0.99]	39/ 632	0.53 [0.35; 0.80]	0.60 [0.38; 0.92]
128.5–144.6	109/ 637	0.76 [0.57; 1.01]	0.90 [0.67; 1.20]	149/ 636	0.68 [0.53; 0.88]	0.77 [0.59; 1.01]	226/ 630	0.71 [0.56; 0.91]	0.81 [0.62; 1.05]	355/ 630	0.87 [0.69; 1.11]	0.95 [0.73; 1.23]	103/ 637	0.51 [0.39; 0.68]	0.59 [0.43; 0.81]	33/ 633	0.48 [0.31; 0.75]	0.59 [0.37; 0.94]
≥144.7	87/ 633	0.61 [0.45; 0.83]	0.74 [0.54; 1.01]	129/ 633	0.59 [0.45; 0.76]	0.72 [0.54; 0.95]	198/ 633	0.56 [0.44; 0.72]	0.68 [0.52; 0.89]	320/ 633	0.67 [0.54; 0.85]	0.80 [0.62; 1.04]	74/ 637	0.36 [0.26; 0.48]	0.42 [0.29; 0.59]	31/ 632	0.48 [0.31; 0.75]	0.62 [0.38; 0.99]
*p-trend**		<0.01	0.05		<0.01	0.01		<0.01	<0.01		<0.01	0.07		<0.01	<0.01		<0.01	0.03
Per log2	497/ 2540	0.39 [0.26; 0.60]	0.55 [0.35; 0.85]	676/ 2530	0.44 [0.30; 0.65]	0.60 [0.40; 0.90]	962/ 2520	0.40 [0.28; 0.57]	0.54 [0.37; 0.80]	1442/ 2520	0.58 [0.41; 0.82]	0.77 [0.53; 1.12]	504/ 2548	0.19 [0.12; 0.29]	0.25 [0.15; 0.42]	189/ 2534	0.21 [0.11; 0.39]	0.32 [0.16; 0.63]
META-ANALYSIS (random-effects)																		
Per log2	837/ 4154	0.40 [0.24; 0.56]	0.54 [0.32; 0.76]	1026/ 4260	0.42 [0.26; 0.58]	0.59 [0.34; 0.82]	1581/ 4149	0.37 [0.24; 0.50]	0.48 [0.31; 0.68]	1717/ 4223	0.46 [0.20; 0.72]	0.71 [0.45; 0.97]	504/ 2548	0.19 [0.12; 0.29]	0.25 [0.15; 0.42]	514/ 4118	0.21 [0.08; 0.35]	0.34 [0.12; 0.56]

IADL: Instrumental Activities of Daily Living.

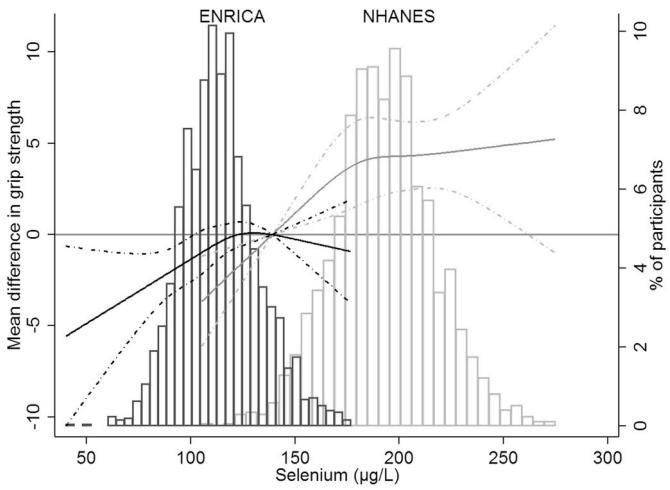

Fig. 1. Average differences in grip strength values by whole blood selenium concentrations. Curves represent average differences in grip strength [solid lines] and their 95%confidence intervals [dashed lines] based on restricted quadratic splines with knots at the 10, 50, and 90th percentiles of selenium distribution in participants from each study. The reference values were set at 140 µg/L, corresponding to the 90th percentile of Se distribution in the ENRICA population]. Results were obtained from linear regression models adjusted for age, sex [men or women], educational level [< high school, high school, or > high school], BMI [<25, 25–29.9, ≥30 kg/m2] race/ethnicity [only in NHANES], diet quality [continuous], protein intake [tertiles], number of vitamins with intake above RDA [continuous], moderate and vigorous physical activity [METs-hours/week in tertiles], smoking status [never, ex-smoker, current], alcohol consumption [never/ex-drinkers/current drinkers] diet quality, BMI [<25, 25–29.9, ≥30 kg/m2], and comorbidities [hypertension, cardiovascular disease, diabetes, cancer, depression and osteoarthritis].

(<30, ≥30 kg m^2), physical activity (tertiles METs hours/week), chronic morbidities, diet quality (tertiles), daily protein intake (tertiles g/kg/d), adequate daily protein intake (≤1.2, >1.2 g per kg of weight), RDA for selenium intake (≥55,<55 µg/day), and number of vitamins with intake below the RDA (≤5,>5). Effect-modification was tested with likelihood ratio tests that compared models with and without interaction terms. Additionally, we conducted interaction analyses according to participant's compliance with the RDA for each vitamin. As a sensitivity analysis, we adjusted ENRICA models for intake of animal and vegetable proteins, separately, yielding similar findings.

We performed all analyses with Stata version 14.1 (StataCorp LP, College Station, TX).

4. Results

Geometric means [GM] and their 95% confidence intervals [CI] for whole blood Se in NHANES and Seniors-ENRICA-2 were 192.3 µg/L [189.4; 195.2] and 113.4 µg/L [112.6; 114.3], respectively (Table 1). The GM for serum Se in NHANES was 129.7 µg/L [126.8; 132.8]. Among NHANES participants, the population weighted correlation between whole blood Se and serum Se, was 0.7.

Whole blood Se levels decreased with age, and were higher among participants with higher education and higher physical activity. Both whole blood and serum Se levels were lower in participants who met the RDA for ≥5 vitamins, with lower diet quality, whose daily Se and protein intake were below 55 µg or 1.2 g/kg, respectively, current smokers, not current drinkers, and participants with CVD and depression. In the NHANES, blood and serum Se levels where lower in Non-Hispanic black women compared to their counterparts (Table 1).

Physical function limitations were common. In NHANES, 20.3%, 40.0%, 16.1% and 20.5% participants showed impaired lower-extremity function, mobility disability, agility limitations, or disability in any of the 3 evaluated Lawton instrumental activities. In the ENRICA-2, 26.7%, 38.2%, 57.2%, and 7.5% had impaired lower-extremity function, mobility disability, agility limitations, or IADL disability, respectively (Table 2).

In multivariable analyses, the frequency of functional limitations was lower in individuals with higher whole blood Se concentrations (Table 2). Meta-analyzed odds ratios (95% confidence intervals) for a 2-fold increase in whole blood Se were 0.54 (0.32; 0.76) for weakness, 0.59 (0.34; 0.83) for impaired lower-extremity performance, 0.48 (0.31; 0.68) for mobility disability, 0.71 (0.45; 0.97) for agility limitations, and 0.34 (0.12; 0.56) for having at least one IADL disability. Additionally, in the Seniors-ENRICA-2, the odds ratio (95% confidence interval) of frailty for a 2-fold increase in Se concentrations was 0.25 (0.15; 0.42). Fig. 1 represents differences in grip strength as a smooth function of whole blood Se in both studies; to facilitate comparisons between them, the reference value was set at 140 µg/L, corresponding to the 90th percentile of Se distribution in the Seniors-ENRICA-2 population. Findings suggest that the inverse association is progressive below 140 µg/L (p-value for non-linear trend and for linear trend in the ENRICA study = 0.131 and 0.012, respectively), and thereafter (p-value for non-linear trend and for linear trend in NHANES = 0.112 and 0.005, respectively).

After a mean follow up period of 2.2 years in the Seniors-ENRICA-2 cohort, we identified 181, 260, 214, 238, 403, and 1 incident cases of weakness, SPPB ≤9, mobility disability, agility disability, and IADL disability, respectively (Table 3). In multivariable analyses, baseline whole blood Se concentrations were associated with a decreased incidence of weakness [odds ratio log2 (95% confidence interval): 0.45 (0.22; 0.91)], impaired lower extremity functional performance [0.63 (0.32; 1.23)], mobility disability [0.43 (0.21; 0.91)], agility limitations [0.38 (0.18; 0.78)], and frailty [0.42 (0.20; 0.90)].

Analyses for the association between serum Se and prevalence of functional limitations in the subset of NHANES participants with this information available (Table 4) showed no overall association with weakness [odds ratio log2 (95%CI): 1.05 (0.15; 7.16); n = 691 participants with serum Se)], but an inverse association with mobility disability [0.22 (0.05; 1.00); n = 695]. Inverse associations were also observed for standing difficulties, agility limitations and IADL limitations, but these were no longer significant after multivariate adjustment [0.87 (0.58; 1.29), n = 735; 0.12 (0.01; 1.30), n = 728; and 0.13 (0.01; 1.60), n = 676; respectively.].

In stratified analyses (**Supplementary Table S1** and S2), no consistent interactions were observed across functional variables and cohorts.

5. Discussion

In US and Spanish older adults, Se concentrations in whole blood were inversely associated with physical function limitations, with a very consistent magnitude of associations observed across studies. These associations were independent of protein intake or diet quality and were maintained after 2.2 years of follow-up in the Spanish cohort. In general, there was a good agreement between concentrations and determinants of whole blood and serum Se and, although most results for serum Se did not achieve statistical significance, findings for both Se biomarkers were consistent for most functional variables.

When nutritional adequacy is being assessed, functional biomarkers quickly responsive to changes in diet, such as selenoproteins, are the biomarkers of choice to evaluate exposure to Se. In this regard, samples of plasma and serum Se, which are virtually equivalent with respect to Se-containing components, are especially useful to monitor Se status in supplementation studies [21], even at doses above the tolerable upper intake level [22]. Whole blood Se, on the other hand, has been proposed as a better indicator of intermediate to long term average exposure [21], with slower responses to recent changes in dietary intake [23].

As noted in the introduction, some studies have already described consistent cross-sectional associations between Se biomarkers and muscular function [3–5]. Skeletal muscle is one of the major sites of Se

Table 3
Prospective association between whole blood selenium and risk of impaired physical function and disability in the Seniors-ENRICA II study. Results are odds ratios [95% confidence interval].

	Incident weakness (n = 1221)			Impaired lower-extremity performance (n = 1140)			Incident mobility limitation (n = 1121)			Incident agility limitation (n = 780)			Incident frailty (n = 1734)		
Serum Se [μg/L] NHANES	n/ total	Model A	Model B	n/ total	Model A	Model B	n/ total	Model A	Model B	n/ total	Model A	Model B	n/ totall	Model A	Model B
Per IQR [23.7]	181/ 1221	0.82 [0.67; 1.00]	0.80 [0.64; 0.97]	260/ 1140	0.83 [0.69; 0.99]	0.89 [0.73; 1.07]	214/ 1121	0.71 [0.58; 0.87]	0.80 [0.65; 0.99]	238/ 780	0.73 [0.60; 0.89]	0.75 [0.61; 0.93]	403/ 1734	0.78 [0.64; 0.95]	0.80 [0.64; 1.00]
Quartiles															
≤117.8	53/ 270	Ref.	Ref.	75/ 238	Ref.	Ref	56/ 228	Ref.	Ref.	55/ 157	Ref.	Ref.	126/ 398	Ref.	Ref.
117.9–128.4	47/ 301	0.81 [0.52; 1.25]	0.78 [0.50; 1.21]	62/ 285	0.64 [0.43; 0.97]	0.67 [0.43; 1.03]	57/ 267	0.86 [0.56; 1.33]	0.83 [0.52; 1.33]	69/ 185	1.18 [0.76; 1.86]	1.23 [0.76; 1.99]	114/ 434	1.13 [0.74; 1.73]	1.08 [0.69; 1.68]
128.5–144.6	39/ 319	0.64 [0.41; 1.01]	0.59 [0.37; 0.94]	60/ 293	0.61 [0.41:0.92]	0.68 [0.44; 1.06]	53/ 295	0.74 [0.47; 1.14]	0.80 [0.50; 1.28]	58/ 198	0.84 [0.53; 1.33]	0.77 [0.47; 1.26]	94/ 444	0.82 [0.53; 1.28]	0.67 [0.42; 1.07]
≥144.7	42/ 331	0.67 [0.43:1.06]	0.61 [0.38; 0.97]	63/ 324	0.63 [0.42; 0.94]	0.70 [0.45; 1.08]	48/ 331	0.50 [0.32; 0.79]	0.65 [0.40; 1.04]	56/ 240	0.60 [0.38; 0.94]	0.65 [0.40; 1.05]	69/ 458	0.62 [0.39; 0.98]	0.67 [0.41; 1.09]
*p-trend**		0.05	0.02		0.03	0.15		<0.01	0.08		<0.01	<0.02		0.02	0.03
Per log2	181/ 1221	0.51 [0.26; 1.02]	0.45 [0.22; 0.91]	260/ 1140	0.51 [0.27; 0.95]	0.63 [0.32; 1.23]	214/ 1121	0.29 [0.15; 0.58]	0.43 [0.21; 0.91]	238/ 780	0.34 [0.17; 0.68]	0.38 [0.18; 0.78]	403/ 1734	0.42 [0.22; 0.82]	0.42 [0.20; 0.90]

IADL: Instrumental Activities of Daily Living.
Model A is adjusted for age, sex. Model B is further adjusted for education, BMI, diet quality, protein intake, number of vitamins with intake above RDA, physical activity, smoking status, alcohol consumption, and comorbidities [hypertension, cardiovascular disease, diabetes, cancer, depression and osteoarthritis]. * p-values for linear trend were obtained by using an ordinal variable with the median selenium concentrations in each category.

Table 4
Cross-sectional association between serum selenium and measures of impaired physical function and disability in older adults from the NHANES. Results are odds ratios [95% confidence interval].

	Weakness (n = 691)			Impaired lower-extremity performance (n = 735)			Mobility limitation (n = 695)			Agility limitation (n = 728)			IADL disability (n = 676)		
Serum Se [μg/L] NHANES	n/ total	Model A	Model B	n/ total	Model A	Model B	n/ total	Model A	Model B	n/ total	Model A	Model B	n/ totall	Model A	Model B
Per IQR [22.9]	144/ 691	0.99 [0.66; 1.49]	0.97 [0.60; 1.57]	140/ 735	0.64 [0.41; 1.00]	0.87 [0.44; 1.75]	251/ 695	0.59 [0.41; 0.84]	0.71 [0.48; 1.07]	112/ 728	0.49 [0.24; 0.99]	0.60 [0.33; 1.11]	135/ 676	0.51 [0.24; 1.09]	0.63 [0.33; 1.21]
Quartiles															
≤118.3	36/ 154	Ref.	Ref.	49/ 165	Ref.	Ref	83/ 158	Ref.	Ref.	39/ 162	Ref.	Ref.	39/ 145	Ref.	Ref.
118.4–129.0	32/ 180	0.92 [0.34; 2.50]	1.03 [0.35; 3.02]	32/ 187	0.39 [0.16; 0.91]	0.56 [0.20; 1.57]	55/ 168	0.39 [0.17; 0.87]	0.39 [0.14; 1.06]	24/ 187	0.20 [0.09; 0.47]	0.23 [0.09; 0.62]	35/ 178	0.45 [0.15; 1.31]	0.72 [0.21; 2.51]
129.1–144.8	48/ 204	2.00 [0.70; 6.82]	3.08 [0.91; 10.4]	40/ 223	0.88 [0.35; 1.33]	0.78 [0.41; 1.50]	68/ 214	0.34 [0.19; 0.63]	0.35 [0.15; 0.79]	36/ 222	0.38 [0.15; 1.00]	0.33 [0.09; 1.22]	41/ 207	0.42 [0.16; 1.11]	0.43 [0.11; 1.70]
≥144.9	28/ 153	0.82 [0.27:2.47]	0.76 [0.21; 2.79]	19/ 160	0.39 [0.14; 1.09]	0.56 [0.12; 2.51]	45/ 155	0.34 [0.15; 0.77]	0.43 [0.18; 1.01]	13/ 157	0.23 [0.09; 0.63]	0.27 [0.09; 0.80]	20/ 146	0.37 [0.11; 1.24]	0.53 [0.16; 1.71]
*p-trend**		0.95	0.87		0.07	0.79		<0.01	0.04		0.06	0.07		0.12	0.22
Per log2	144/ 691	1.03 [0.21; 5.17]	1.05 [0.15; 7.16]	140/ 735	0.15 [0.03; 0.77]	0.87 [0.58; 1.29]	251/ 695	0.10 [0.03; 0.39]	0.22 [0.05; 1.00]	112/ 728	0.05 [0.00; 0.72]	0.12 [0.01; 1.30]	135/ 676	0.06 [0.00; 0.92]	0.13 [0.01; 1.60]

IADL: Instrumental Activities of Daily Living.

storage (approximately between 30 and 45% of the total pool) and several selenoproteins have been involved in muscular function. The most well-known is selenoprotein N, whose deficiency, caused by mutations in the human gene, has been associated with several neuromuscular disorders [34]. In vivo, mice supplementation with Se show increases in selenoprotein N synthesis and expression, which translate into better calcium release from the sarcoplasmic reticulum [35,36]. Other selenoproteins like selenoprotein K or selenoprotein W influence the normal physiology of skeletal muscle, but their precise functions has yet to be elucidated [2,37,38]. Potential increases in circulating and intracellular selenoprotein expression at the observed intake levels are compatible with a protective effect of Se on muscular function brought about by selenoenzymes.

Previous epidemiological research has found evidence of other potential health benefits of Se status in older populations, such as a reduced risk of immune dysfunction [24,25], cognitive decline [26], cardiovascular disease [27], certain tumors [25], or overall mortality [28–32]. In all these processes, Se is probably contributing to the alleviation of reduced reactive oxygen species-mediated inflammation, reduced DNA damage or prolonged telomere length [33]. Still, there is concern that some of these effects may revert at high levels of exposure.

Our results should be interpreted with caution. Although analyses in participants with follow-up information confirmed our cross-sectional findings, the duration of follow-up was short and reverse causation cannot be ruled out. Concentrations of circulating micronutrient biomarkers in older institutionalized adults can be altered in the presence of inflammation [39], a central mechanism in age-related function impairments [41]. According to this, inflammation could mediate redistribution of Se from plasma to the liver, with reductions in blood concentrations that may not necessarily reflect true micronutrient deficiency [39]. Unfortunately, we could not address this hypothesis, although a bidirectional relationship likely exists.

This work has several strengths. First, it is the largest to date evaluation of association between biomarkers of Se exposure and functional outcomes in humans. We made an effort to ensure the replicability of the results in two different populations, including a representative sample of the US population. Despite these populations showing very different exposures to Se, mainly attributed to differences in Se soil content and use of supplements [24], results showed consistent findings. Second, we adjusted our models and addressed interactions by a great number of variables, including important determinants of muscular function like protein and vitamin intake, or diet quality [42–44]. And third, we used different biomarkers of Se exposure and a number of validated measures of physical function, and we applied different methods to model the potential non-linear dose-response relationship between Se and functional variables.

Our results provide evidence for a link between Se and functional limitations in older adults. Experimental studies as well as long-term follow-up studies with biological biomarkers of inflammation are needed to understand the physio-pathological mechanisms underlying the observed associations.

Author contributions

EGE is responsible for study design, statistical analysis and writing of the first draft. EGE, RO, JRB and FRA collected data. MCR, RO, MSP, BPG, EGG, JRB, RQ, PO, FG, MTP, ANA, RPB and FRA reviewed the manuscript for important intellectual content. All authors had full access to the data (including statistical reports and tables) in the study and can take responsibility for the integrity of the data and the accuracy of the data analysis.

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Data accessibility

Data from the Seniors-ENRICA-2 can be accessed upon request to the authors. Data from the NHANES study are already publicly available.

Declaration of competing interest

The authors have no conflicts of interest to declare.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.redox.2020.101819.

Model A is adjusted for age, sex, and ethnicity [in NHANES]. Model B is further adjusted for education, BMI, diet quality, protein intake, number of vitamins with intake above RDA, physical activity, smoking status, alcohol drinking, and comorbidities [hypertension, cardiovascular disease, diabetes, cancer, depression and osteoarthritis]. * p-values for linear trend were obtained by using an ordinal variable with the median selenium concentrations in each category.

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