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Glyphosate exposure, muscular health and functional limitations in middle-aged and older adults

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ABSTRACT

Background: Glyphosate is the most widely used herbicide worldwide, both in domestic and industrial settings. Experimental research in animal models has demonstrated changes in muscle physiology and reduced contractile strength associated with glyphosate exposure, while epidemiological studies have shown associations between glyphosate exposure and adverse health outcomes in critical biological systems affecting muscle function.
Methods: This study used data from a nationally representative survey of the non-institutionalized U.S. general population (NHANES, n = 2132). Urine glyphosate concentrations were determined by ion chromatography with tandem mass spectrometry. Hand grip strength (HGS) was measured using a Takei Dynamometer, and relative strength estimated as the ratio between HGS in the dominant hand and the appendicular lean mass (ALM) to body mass index (ALMBMI) ratio. Low HGS and low relative HGS were defined as 1 sex-, age- and race-specific SD below the mean. Physical function limitations were identified as significant difficulty or incapacity in various activities.
Results: In fully-adjusted models, the Mean Differences (MD) and 95% confidence intervals [95%CI] per doubling increase in glyphosate concentrations were −0.55 [-1.09, −0.01] kg for HGS in the dominant hand, and −0.90 [-1.58. −0.21] kg for HGS/ALMBMI. The Odds Ratios (OR) [95% CI] for low HGS, low relative HGS and functional limitations by glyphosate concentrations were 1.27 [1.03, 1.57] for low HGS; 1.43 [1.05; 1.94] for low relative HGS; 1.33 [1.08, 1.63] for stooping, crouching or kneeling difficulty; 1.17 [0.91, 1.50] for lifting or carrying items weighting up to 10 pounds difficulty; 1.21 [1.01, 1.40] for standing up from armless chair difficulty; and 1.47 [1.05, 2.29] for ascending ten steps without pause difficulty.
Conclusions: Glyphosate exposure may be a risk factor for decreased grip strength and increased physical functional limitations. More studies investigating the influence of this and other environmental pollutants on functional aging are needed.

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1. Introduction

Skeletal muscle plays a critical role in maintaining physical function and metabolic health (Wolfe, 2006). In this regard, a growing number of studies reveal that, in both middle-aged and older adults, strength and muscle mass are inversely associated with increased risk of chronic health problems such as diabetes (Moon, 2014; Kunutsor et al., 2021a; Boonpor et al., 2021), cardiovascular disease (Tyrovolas et al., 2020; Lopez-Jaramillo et al., 2022; Peralta et al., 2023), cognitive impairments (Filardi et al., 2022; Tessier et al., 2022; Prokopidis et al., 2023), falls (Landi et al., 2012; Reijnierse et al., 2019), bone fractures (Kunutsor et al., 2021b), disability (Abay et al., 2022), as well as all-cause and

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cause-specific mortality (López-Bueno et al., 2022; Landi et al., 2022; Xiong et al., 2023). In general, muscle strength is a better predictor of adverse health outcomes than muscle mass (Cruz-Jentoft et al., 2019).

Muscle strength levels peak during early adulthood, are maintained during middle age and decrease thereafter (Dodds et al., 2014). This cycle is influenced by factors such as genetics (Pratt et al., 2019) and lifestyle (Oliveira et al., 2020; Azzolino et al., 2021; Pana et al., 2021; Li et al., 2023). Thus, it is well-known that resistance training, adequate nutrition or sufficient rest are vital for lifelong muscle well-being (Oliveira et al., 2020; Azzolino et al., 2021; Pana et al., 2021; Sabir et al., 2023; Li et al., 2023), while genetic predisposition seems to play a role in shaping the composition of muscle fibers, their distribution and their response to resistance training (Pratt et al., 2019), as well as neuronal maintenance and signal transduction (Fuku et al., 2019; Pratt et al., 2019).

Recent research findings from individual-level studies in European and North-American populations suggest a slight reduction in grip strength over the past few decades. However, it seems that this decline cannot be solely attributed to shifts in lifestyle factors (Steiber, 2015; Ahrenfeldt et al., 2018). Therefore, in order to obtain a comprehensive understanding of the determinants of muscle health throughout life, it is imperative to conduct studies on as yet unexplored factors that may contribute to a deterioration in muscle function. In this sense, and since there is convincing evidence of the role of environmental pollution in noncommunicable diseases and functional aging (García-Esquinas and Rodríguez-Artalejo, 2017), an increasing number of studies are evaluating the association between exposure to specific pollutants and muscle health. These suggest that exposure to air pollution (Zare Sakhvidi et al., 2022), secondhand smoke (Carrasco-Rios et al., 2019), metal (García-Esquinas et al., 2020, 2021; Wu et al., 2022; Chen et al., 2023), pyrethroids (Fang et al., 2023) or phthalates (Kim et al., 2016b) negatively influence muscular functioning.

Glyphosate is the most widely used herbicide worldwide in both domestic and industrial settings (Benbrook, 2016). Initially considered harmless to humans, recent studies have revealed that glyphosate exposure is associated with a broad spectrum of health-related outcomes in both non-occupationally (Geier and Geier, 2023; Eskenazi et al., 2023; Hsiao et al., 2023; Qi et al., 2023a; Yang et al., 2023) and occupationally (Zhang et al., 2023; Chang et al., 2023) exposed populations. Given that some of these outcomes [i.e., endocrine-disruption (Lesseur et al., 2021; Geier and Geier, 2023), insulin-resistance (Eskenazi et al., 2023; Qi et al., 2023b), bone mineral health (Wang et al., 2023), or neuroaxonal damage (Yang et al., 2023)] are intimately related to biological systems that influence muscle health, we hypothesized that glyphosate exposure could be a risk factor for impaired muscle strength and physical function limitations in the general population. Our hypothesis is supported by experimental evidence indicating that glyphosate-based herbicide (GBH) exposure during development causes muscle fibrosis and decreases levels of neuromuscular junctions in the soleus muscle of mouse pups (Barbosa et al., 2022). Additionally, chronic exposure to elevated levels of GBH in rats has been associated with the suppression of muscle levels and contractile strength (Nozdrenko et al., 2021). Furthermore, studies conducted on aquatic animals, commonly used in ecotoxicological research and considered "intermediate" in relation to potential effects on human exposures (Lacroix and Kurrasch, 2023), have shown alterations in muscular energy reserve (Menéndez-Helman et al., 2015), disruptions in normal muscle protein metabolism (dos Santos dosSantos Teixeira et al., 2018), increased lipid peroxidation (Sinhorin et al., 2014; dos Santos dosSantos Teixeira et al., 2018), and reduced acetylcholinesterase activity in the skeletal muscle (Modesto and Martinez, 2010; dos Santos dosSantos Teixeira et al., 2018) of populations exposed to glyphosate.

To test our hypothesis, we used data from a sample of the U.S. population aged 20 years and over, drawn from the National Health and Nutrition Examination Survey (NHANES) 2013–2014 and 2015–2016, where glyphosate measures were available.

2. Methods

The NHANES uses a multistage sampling design to obtain representative samples of the non-institutionalized US population on a biannual basis. It gathers sociodemographic and health information, conduct physical examinations, and collects biological samples in order to provide data on health issues such as prevalence of risk factors, disability and disease among the US residents (2015)· The overall participation rate was 68.5% for the years 2013–2014 and 58.7% for the years 2015–2016. For this study, data from all 2266 participants aged 20 years or older with data on urine glyphosate and at least one outcome of interest were initially included. After excluding participants with incomplete information on potential confounders (n = 134), the analytical sample consisted of 2132 participants. Among them, 1459 had information on HGS for at least the dominant hand, 856 data on muscle mass (and thus on relative HGS), and 1311 on at least one functional limitation.

3. Urine glyphosate

Urine glyphosate measurements were obtained form one-third of the study participants who consented to the use of their samples in future research. The samples were stored and the analyses conducted following strict Clinical Laboratory Improvement Amendments (CLIA) guidelines. Urine samples were frozen, shipped, and stored at the Centers for Disease Control (CDC) National Center for Environmental Health (NCEH) Environmental Health Laboratory (CDC, 2016) at −70 °C.

The laboratory employed the on-line 2D ion chromatography coupled to tandem mass spectrometry (IC- MS/MS) technique, with quantification using isotope dilution (Schütze et al., 2021). Each analytical urine run (200 μL) included low and high concentration quality control materials (QCMs) and reagent banks to ensure the validity and accuracy of the data. Low and high concentration QCMs were evaluated using standard probability statistical rules, obtaining an averaged low and high concentration for each analytical run (Caudill et al., 2008).

The concentration of glyphosate in urine ranged from 0.141 to 8.210 μg/L. A total of 745 observations were below the limit of detection (LOD), which was 0.2 μg/L, and were imputed as the limit of detection divided by the square root of 2 (Hornung and Reed, 1990). Urine glyphosate concentrations were divided by urine creatinine levels to account for urine dilution. Therefore, urine glyphosate levels were expressed as μg/g of creatinine.

4. Muscular health assessment

4.1. Hand grip strength

Isometric hand grip strength (HGS) was measured using a Takei Dynamometer Model T.K.K.5401 (Takei Scientific Instruments Co., Niigata, Japan). Participants were asked to squeeze the dynamometer as hard as possible with each of his/her hands in a standing position. In order to make accurate measurements, the test was performed three times in each hand, alternating hands with a 60-s rest between measurements. HGS in the dominant hand was expressed in kg, and, because normative values for HGS vary with sex (Lee et al., 2019; Landi et al., 2020; Ramírez-Vélez et al., 2021; Huemer et al., 2023; He et al., 2023), age (Lee et al., 2019; Landi et al., 2020; Ramírez-Vélez et al., 2021; He et al., 2023) and race (Duchowny et al., 2017), low HGS was defined as Z-scores <1 standard deviation (SD) for age, sex and race. The sum of the largest reading from each hand was used to calculate combined HGS (García-Esquinas et al., 2020).

4.2. Relative hand grip strength

Body composition was assessed by dual-energy X-ray absorptiometry

(DXA) in a subgroup of individuals aged 20–59 years. Scans were acquired using the Hologic Discovery model A densitometers (Hologic Inc., Bedford MA) using software version Apex 3.2, and analyzed with Hologic APEX version 4.0 software with NHANES BCA option. Appendicular lean mass (ALM) was estimated as the sum of right and left arm and leg mass, and, because muscle mass is correlated with body size, relative HGS (kg/body mass index-BMI) was calculated as the ratio between HGS in the dominant hand and the ALM to body mass index (ALMBMI) ratio (Chiles Shaffer et al., 2017, 2020; Kim et al., 2018; Andrews et al., 2022). Similar to what was done for HGS, low relative HGS was defined as 1 sex-, age- and race-specific SD below the mean for relative strength (Kim et al., 2016a).

5. Self-reported functional limitations

Physical function limitations were defined as significant difficulty or incapacity to complete activities like "bending, crouching, or kneeling", "lifting or transporting items weighing up to 10 pounds", "rising from a chair without armrests" and "ascending ten steps without pause".

6. Other variables

Information on age, sex, race/ethnicity, education level, lifestyles, prevalent diseases, fasting time and medication use was self-reported. Participants were categorized based on their smoking habits into current smokers (those who had smoked at least 100 cigarettes in their lifetime and reported smoking at the time of the interview), former smokers (those who had smoked at least 100 cigarettes in their lifetime but did not currently smoke), or never smokers. Regarding alcohol consumption, participants were classified as never drinkers, former drinkers, moderate drinkers (<2 drinks per day), and heavy drinkers (≥3 drinks per day). Former drinkers reported having consumed at least 12 drinks of alcoholic beverage in any one year, but drank<12 drinks/year, <1 drinks/month, or <0.03 drinks/day in the last year (García-Esquinas et al., 2020).

Participants also reported their daily television viewing time and completed a physical activity questionnaire based on the Global Physical Activity Questionnaire (World Health Organization), respectively. Work-related physical activity included both "paid and unpaid work", "studying or training", "household chores", and "yard work", while leisure-time physical activity encompassed "sports", "fitness", and "recreational activities". Proposed metabolic equivalent (MET) scores for vigorous and moderate physical activities, whether at work or during leisure time, were 8.0 and 4.0, respectively. MET-hours per week were calculated by multiplying the average number of hours spent in each activity by the respective MET score. The MET-hours per week for vigorous and moderate work-related activities were then combined, as were the MET-hours per week for vigorous and moderate leisure-time physical activities (García-Esquinas et al., 2020).

Weight and height were measured using standardized procedures, and the BMI was calculated as the measured weight in kg divided by squared height in m. Participants were classified into BMI-based categories: underweight (<18.5), normo-weight (18.5–25), overweight (25–30), and obese (≥30 kg/m2).

Data on food consumption was obtained through two 24-h dietary recalls. The first recall was conducted face-to-face by trained food recall data collectors, while the second was carried out over the phone 3–10 days later. Each food description was linked to a 8-digit food code, with the average intake of food groups 4, 6 and 7 (i.e. "Dry Beans, Peas, Other Legumes"; "Grain Products"; "Fruits and Vegetables") used for the present analyses. These food groups were chosen because, glyphosate and its metabolites have been primarily detected in grains (Soares et al., 2021), legumes (Bøhn et al., 2014) and vegetables (Chen et al., 2013; Soares et al., 2021). The total energy intake for each participant was calculated by summing the kilocalories from all reported foods and beverages. Vitamin intake from each food or beverage was computed using the Food and Nutrient Database for Dietary Studies provided by the US Department of Agriculture (FNDDS) (García-Esquinas et al., 2021).

During physical examinations, participants were asked to report whether they had had any pain, aching or stiffness in their hands in the previous 7 days. Blood pressure was measured under standardized conditions, and hypertension was defined as mean systolic/diastolic blood pressure ≥140/90 mm Hg, a self-reported medical diagnosis, or the use of blood pressure-lowering medication. Diabetes was defined as non-fasting glucose ≥200 mg/dL, fasting glucose ≥126 mg/dL, a self-reported physician-based diagnosis, or the use of glucose-lowering medication. Total cholesterol and HDL cholesterol were measured enzymatically. Serum creatinine levels were determined using an end-point reaction method on the Roche/Hitachi Cobas 6000 chemistry analyzer. Finally, depressive symptoms were screened with the validated Patient Health Questionnaire (Kroenke et al., 2001).

7. Statistical methods

To address skewness, urine glyphosate levels were log-transformed for the analyses. Glyphosate concentrations were modeled as quartiles, with cutoffs determined from weighted distributions in the study sample, and as a base 2 log-linear term. Mean differences (MD) and 95% confidence intervals (95%CI) in HGS and relative HGS according to glyphosate levels were estimated using linear regression models; while Odds Ratios (OR) and 95%CI for low HGS, low relative HGS and prevalent functional limitations according to glyphosate levels were obtained from logistic regression levels. To address potential confounding, two sequential statistical models were employed. Model 1 included adjustments for sociodemographic variables: age and squared age (continuous, years), sex (men/women), ethnicity (non-Hispanic White/African-American/Mexican-American/other), education level (< high school/high school/> high school); as well as fasting time (continuous, hours), and recent hand pain (no/yes). Model 2 further adjusted for lifestyle-related risk factors: physical activity (METs in leisure time and work time), TV viewing time, smoking status (never/former/current), alcohol consumption (never/former drinker/moderate drinker heavy drinker/unknown) and BMI (underweight/normoweight/overweight/obese).

To explore the potential mediating effect of other health outcomes associated with glyphosate exposure, a third model (Model 3) was introduced. This model further adjusted for cardiovascular risk factors, including total cholesterol (continuous, mg/dL), HDL cholesterol (continuous, mg/dL), cholesterol lowering medication use (no/yes), hypertension (no/yes) and diabetes (no/yes); as well as for eGFR (continuous, mL/min/1.73 m^2), cardiovascular disease (no/yes), cancer (no/yes), respiratory disease (no/yes), osteoarticular disease (no/yes), and number of depressive symptoms.

Finally, we conducted several sensitivity analyses: 1) We adjusted the analyses for dietary information when available, taking into account factors such as food intake of groups 4, 6 and 7 (gr/day), sufficient protein intake per kg of body weight (>1.2 g/kg), the number of vitamins with intake above the Recommended Dietary Allowance (RDA), and total energy intake; 2) When available, we incorporated the poverty-to-income ratio into the models; 3) We excluded participants with current or longest occupations or industry groups in agriculture, fishing and forestry; 4) We adjusted the models for recent pesticide use; 5) We tested interaction terms for log-transformed creatinine-corrected urine glyphosate and indicator variables for specific subgroups of sex, age, education, race/ethnicity and BMI. P-values for the interaction terms were derived using the Wald test, including adjustment for the survey design; 6) We performed truncated regression imputation to handle glyphosate values below the LOD.

Statistical analyses were performed using STATA Software V.18 to accommodate the complex sampling design and derive appropriate standard errors. The type I significance level for hypothesis testing was

set at 0.05.

8. Results

In the final study subpopulation, the mean age (SE) was 51.62 (0.64) years, with 51.4% of participants identifying as women, and 69.3% self-identified as non-Hispanic White. In terms of educational attainment, 65% had completed higher education or above. The geometric mean of creatinine-corrected urine glyphosate was 0.56 (ranging from 0.04 to 0.76 μg/L). Glyphosate levels were higher among older participants, non-Hispanic White women, and those with chronic conditions such as diabetes, hypertension, cardiovascular disease, cancer or osteoarticular disease (Table 1). Moreover, participants who reported experiencing pain in the previous week and those with more depressive symptoms also exhibited higher glyphosate concentrations. Regarding dietary information, a positive association was observed with the consumption of fruits and vegetables, as well as with vitamin C and folate.

Figs. 1–3 exhibit an inverse linear dose-response correlation between urine glyphosate concentrations and grip strength measurements, revealing no significant departures from linearity (the p-values for non-linear trends for dominant HGS, combined HGS and relative HGS were 0.37, 0.66 and 0.35, respectively). Concurrently, Table 2 presents the MD [95% CI] in HGS for the dominant hand, combined HGS, and relative HGS according to creatinine-corrected urine glyphosate levels. In fully-adjusted models including dietary information, the MD comparing the second, third, and fourth quartiles to the first (lowest) quartile of glyphosate concentrations were as follows: 1.16 [−2.20, −0.11], −1.19 [−2.57, 0.20] and −1.70 [−3.27, −0.13] kg for HGS in the dominant hand (p-trend = 0.09); −2.99 [−5.40, −0.58], −2.18 [−4.77, −0.41] and −3.93 [−6.90, −0.95] kg for combined HGS (p-trend = 0.06); and −1.88 [−3.66, −0.10], −1.67 [−3.85, 0.52] and −3.30 [−5.30, −1.29] kg/BMI for relative HGS (p-trend<0.00). Similarly, MD per doubling increase in glyphosate concentrations were −0.55 [−1.09, −0.01] kg for HGS, −1.17 [−2.20, −0.15] kg for combined HGS, and −0.98 [−1.69. −0.28] kg for HGS/ALMBMI.

The OR [95% CI] for low HGS, low relative HGS and functional limitations by glyphosate concentrations are presented in Tables 3 and 4. In models adjusted for potential confounders, ORs for a doubling increase in urine glyphosate levels were 1.27 [1.03, 1.57] for low HGS; 1.43 [1.05; 1.94] for low relative HGS; 1.33 [1.08, 1.63] for stooping, crouching or kneeling difficulty; 1.17 [0.91, 1.50] for lifting or carrying items weighting up to 10 pounds difficulty; 1.21 [1.01, 1.40] for standing up from armless chair difficulty; and 1.47 [1.05, 2.06] for ascending ten steps without pause difficulty.

In relation to the potential mediating effect of other health outcomes, our findings indicate that the association between glyphosate exposure and most functional limitations can be largely attributed to an increased prevalence of chronic conditions in individuals with higher glyphosate exposure. Specifically, substantial changes in estimations were observed from model 2 to model 3, with percentage changes of 42.4% for difficulty in stooping, crouching or kneeling; 41.2% for difficulty in lifting or carrying items weighting up to 10 pounds; and 42.9% for difficulty standing up from armless chair difficulty (Table 4). Conversely, changes in estimates for the other studied outcomes suggested a considerably lower contribution of these factors, which percentage changes consistently below 15% (Tables 2–4).

In sensitivity analyses, consistent results were obtained when adjusting for variables related to diet and poverty-to-income ratio in the subsample of participants with this information available, as well as when excluding workers in herbicide-related industries, and when adjusting for recent pesticide use (Supplementary Table 1). Furthermore, no significant interactions were found for any of the variables studied (Supplementary Table 2). Finally, the results remained robust when imputing glyphosate values below the LOD (Supplementary Figure 1 and Supplementary Table 3).

Table 1
Baseline characteristics of study participants by creatinine-adjusted urine glyphosate tertiles, n = 2132.

	Quartiles (μg/g creatinine)				
	Q1 (≤0.25)	Q2 (0.25–0.41)	Q3 (0.44–0.70)	Q4 (≥0.70)	p-val
n	532	551	514	535	
Age	47.89 (17.59)	51.05 (18.44)	51.41 (16.38)	56.15 (15.78)	<0.01
Sex, female	211 (40.37)	277 (50.78)	284 (53.37)	315 (61.04)	<0.01
Education					
<High school	102 (11.97)	123 (14.34)	118 (14.19)	119 (13.44)	
High school	122 (20.51)	130 (23.92)	106 (19.62)	120 (20.48)	
>High school	308 (67.53)	298 (61.74)	290 (66.19)	296 (66.09)	0.67
Race					
Non-Hispanic White	196 (64.14)	238 (66.96)	234 (70.41)	269 (75.89)	
Non-Hispanic Black	140 (14.04)	96 (8.90)	92 (9.33)	71 (6.02)	
Hispanic	72 (8.763)	84 (9.21)	75 (7.29)	68 (5.41)	
Other	124 (13.06)	133 (14.93)	113 (12.97)	127 (12.69)	<0.01
Smoking					
Never	254 (48.56)	288 (54.97)	262 (53.51)	288 (52.60)	
Former	140 (27.83)	144 (27.21)	135 (24.95)	139 (27.52)	
Current	138 (23.60)	119 (17.82)	117 (21.54)	108 (19.87)	0.59
Alcohol intake					
Never	74 (14.07)	83 (13.54)	73 (11.01)	97 (13.49)	
Former	115 (18.32)	130 (19.93)	116 (20.52)	141 (24.32)	
Moderate drinker	135 (26.78)	140 (30.48)	126 (30.32)	95 (22.04)	
Heavy drinker	65 (16.46)	70 (16.44)	62 (13.84)	68 (17.10)	
Unknown	143 (24.36)	128 (19.61)	137 (24.30)	134 (23.05)	0.46
BMI	30.07 (7.25)	29.33 (6.75)	29.98 (7.24)	28.86 (6.71)	0.28
Kcal/day	2367 (972.9)	2314 (895.5)	2314 (895.5)	2314 (795.2)	0.19
Recreational PA, Mets-h/week	13.21 (22.82)	15.59 (28.00)	15.6 (31.38)	11.58 (20.47)	0.17
Work-related PA, Mets-h/week	41.7 (99.34)	36.3 (85.76)	41.31 (99.58)	22.86 (60.19)	0.02
Total cholesterol, mg/dL	192.2 (40.34)	190.5 (41.54)	192.0 (41.20)	192.4 (52.80)	0.89
HDL cholesterol, mg/dL	52.44 (17.97)	53.98 (17.53)	53.9 (17.39)	56.15 (18.00)	0.26
Lipid-lowering treatment	101 (20.47)	139 (22.47)	126 (24.88)	159 (28.94)	0.15
Diabetes	78 (10.33)	82 (12.13)	111 (17.13)	129 (17.97)	0.01
Hypertension	201 (32.46)	234 (40.23)	221 (38.48)	261 (42.35)	0.04
CVD	57 (8.82)	66 (11.08)	71 (12.54)	103 (15.23)	0.13
Cancer	50 (11.21)	48 (9.32)	60 (14.40)	87 (19.17)	<0.01
Osteoarticular Disease	156 (28.33)	166 (31.19)	180 (36.20)	222 (44.93)	<0.01
Recent pain	69 (14.97)	78 (20.29)	89 (22.22)	102 (27.53)	0.00
Respiratory Disease	94 (17.74)	105 (18.75)	102 (21.26)	100 (18.86)	0.58
CKD	52 (8.85)	63 (11.48)	48 (9.10)	68 (9.39)	0.56

(continued on next page)

Table 1 (*continued*)

	Quartiles (μg/g creatinine)				
	Q1 (≤0.25)	Q2 (0.25–0.41)	Q3 (0.44–0.70)	Q4 (≥0.70)	p-val
Nº depressive symptoms	1.74 (3.29)	1.96 (3.50)	2.20 (3.66)	2.46 (4.27)	<0.01
Fasting time, hours	7.99 (5.35)	7.77 (5.50)	6.91 (5.21)	6.21 (5.64)	<0.01
Subsample with dietary information, n	443	466	425	454	
Food groups					
Legumes, nuts, seeds, gr/d	42.91 (90.81)	60.93 (108.5)	50.38 (104.7)	44.67 (76.94)	0.20
Grain products, gr/d	317.5 (272.8)	332.1 (228.1)	322.9 (202.3)	330.5 (216.1)	0.91
Fruits, gr/d	185.8 (251.2)	179.4 (206.8)	156.2 (180.7)	236.2 (292.5)	0.01
Vegetables, gr/d	188.2 (158.6)	210.4 (204.2)	206.5 (168.5)	241.9 (202.4)	0.02
Nutrients					
Proteins/kg	1.02 (0.51)	1.06 (0.54)	1.03 (0.45)	1.02 (0.48)	0.87
Selenium	119.10 (57.21)	114.40 (53.75)	118.6 (48.56)	111.1 (48.63)	0.20
Vitamin A, mg/d	721.0 (850.5)	628.1 (427.7)	634.2 (418.1)	698.8 (530.9)	0.23
Vitamin B1, mg/d	1.59 (0.79)	1.57 (0.75)	1.66 (0.73)	1.67 (0.75)	0.45
Vitamin B2, mg/d	2.17 (1.30)	2.19 (1.15)	2.25 (1.13)	2.13 (0.87)	0.82
Niacin, mg/d	25.60 (14.00)	25.70 (12.79)	26.42 (12.12)	24.38 (10.87)	0.42
Vitamin B6, mg/d	2.08 (1.35)	2.21 (1.43)	2.10 (1.15)	2.07 (0.99)	0.57
Vitamin B12, mg/d	5.51 (8.49)	4.91 (3.61)	4.68 (3.45)	4.54 (3.48)	0.35
Vitamin C, mg/d	80.60 (74.51)	80.14 (70.48)	72.64 (61.06)	92.93 (79.60)	0.05
Vitamin D, mg/d	4.95 (4.86)	4.67 (4.34)	4.70 (5.05)	5.19 (5.44)	0.78
Vitamin E, mg/d	9.27 (6.86)	9.52 (6.77)	9.45 (5.65)	9.30 (5.35)	0.97
Folate, mg/d	363.6 (189.4)	406.4 (230.0)	393.1 (185.4)	404.1 (199.9)	0.02

Values are expressed as percentages (standard deviations) except when their units are indicated, in which case they are expressed as means (standard deviations). Abbreviations: BMI = Body mass index; CKD = Chronic kidney disease; CVD = Cardiovascular disease; eGFR = Estimated glomerular filtration rate; PA= Physical activity.

9. Discussion

In this observational study, urine glyphosate was cross-sectionally associated with decreased muscle strength and higher prevalence of physical function limitations in middle-aged and older adults from the US population. These associations were consistent across subgroups defined by age, sex, race and anthropometric characteristics.

Although direct comparison of urinary concentrations between studies is challenging due to differences in analytical methods, urine sampling, age distribution, and data reporting, findings from both Europe and the US suggest a prevalent (Ospina et al., 2022; Andersen et al., 2022) and increasing exposure to glyphosate (Conrad et al., 2017). For example, the German Environmental Specimen Bank study detected a continuous increase in the fraction of samples with detectable concentrations in the period 2001–2013 (from 10% in 2001 to 57.5% in 2012 and 56.4% in 2013) (Conrad et al., 2017). In another, in this case non-peer-reviewed, report on glyphosate residues in 182 urine samples from 18 different European countries, commissioned by the European Community in 2013, glyphosate was detected in 44% of the samples, with a mean concentration ranging from 0.09 to 0.47 μg/L (Hoppe HW, 2013). In our dataset, with data from 2013 to 2016, approximately 71% of participants showed detectable glyphosate levels in their urine, with concentrations very similar to that of other non-occupational studies from the US (Ospina et al., 2022), and somewhat higher of the ranges reported in Europe.

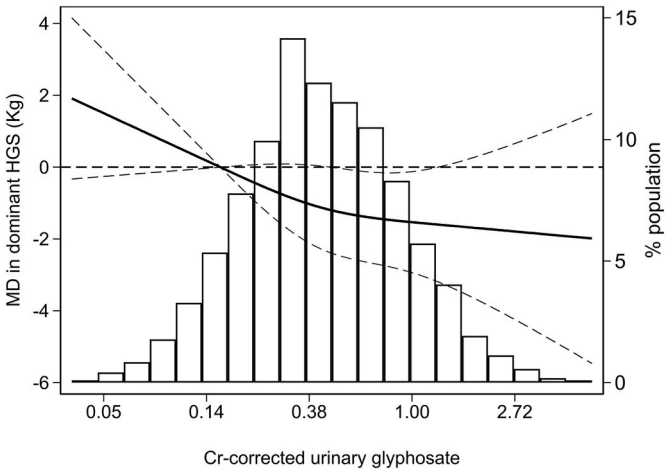

Fig. 1. Mean Differences (MD) for hand grip strength (HGS) in the dominant hand by creatinine-corrected urine glyphosate concentrations Lines represent mean differences (continuous line) and 95% confidence intervals (dotted lines) for HGS based on restricted cubic splines for log-transformed creatinine-corrected urine glyphosate concentrations with knots at the 10th, 50th and 90th percentiles. The reference was set at the 10th percentile of creatinine-corrected urine glyphosate distribution. Models were adjusted for age, sex, ethnicity, education level, fasting time, recent hand pain, physical activity, TV viewing time, smoking status, alcohol consumption and BMI.

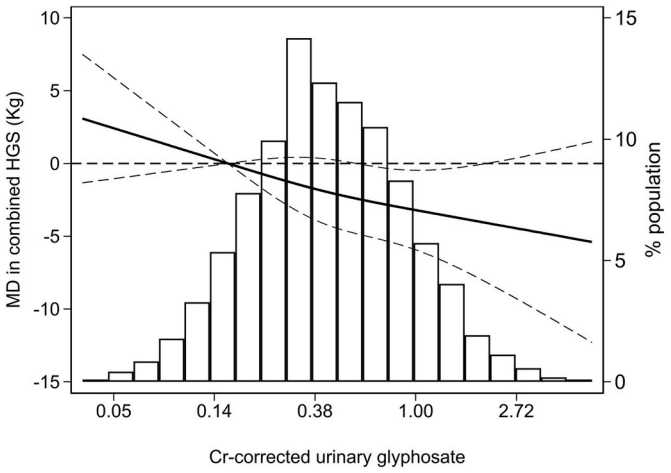

Fig. 2. Mean Differences (MD) for combined hand grip strength (HGS) by creatinine-corrected urine glyphosate concentrations.

While there is a lack of epidemiological studies on the relationship between glyphosate exposure and muscle health or functional limitations, the associations found are biologically plausible and finds support in both in vitro and experimental animal research (Nozdrenko et al., 2021; Barbosa et al., 2022), as well as in human studies showing the alteration of biological systems critical for muscle function in individuals exposed to this herbicide. For instance, analyses of the US NHANES 2013–2016 population revealed an inverse association between urinary glyphosate concentrations and total bone mineral density in adults aged 20 years and older (Wang et al., 2023). This is noteworthy, as it is reasonable to speculate that an alteration in bone physiology may also affect muscle function. Indeed, bone metabolism plays a critical role in providing structural support, mechanical load and

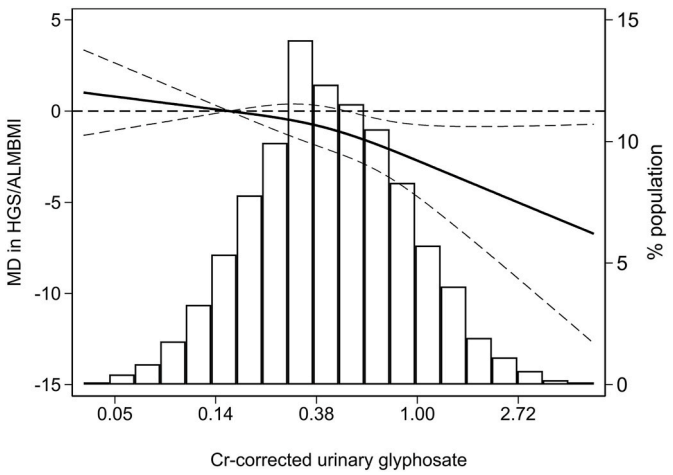

Fig. 3. Mean Differences (MD) for combined relative hand grip strength (HGS divided by the ALMBMI ratio) by creatinine-corrected urine glyphosate concentrations Lines represent mean differences (continuous line) and 95% confidence intervals (dotted lines) for relative HGS based on restricted cubic splines for log-transformed creatinine-corrected urine glyphosate concentrations with knots at the 10th, 50th and 90th percentiles. The reference was set at the 10th percentile of creatinine-corrected urine glyphosate distribution. Models were adjusted for age, sex, ethnicity, education level, fasting time, recent hand pain, physical activity, TV viewing time, smoking status and alcohol consumption.

mineral reserves (such as calcium and phosphorus), and in the production of red blood cells and local hormones essential for muscle function.

Below, we outline some of the possible mechanisms through which glyphosate could affect musculoskeletal physiology.

9.1. Hormonal disruption

Hormonal activity plays a crucial role in the regulation of muscle trophism, where Insulin-like Growth Factor-1 (IGF-1), testosterone, and thyroid hormones are instrumental in promoting protein synthesis, cellular growth, and metabolic activity.

At the cellular level, glyphosate has the potential to disrupt crucial hormonal pathways necessary for proper muscle function. In vitro, glyphosate exposure can increase insulin resistance in skeletal muscle by modulating IRS-1/PI3K/Akt mediated mechanisms (Jayaraman et al., 2023); while in rats, it stimulates the NFkB signaling pathway in the liver, leading to decreased expression of insulin receptor and glucose transporter-2 (Prasad et al., 2022). Studies in human cells also indicate disruptions in androgen receptor transcription (Gasnier et al., 2009) and inhibition of aromatase enzymes (Defarge et al., 2016) following exposure to GBHs, while in vivo studies consistently indicate that GBHs alter estrogen- and androgen-signaling pathways (de Araújo-Ramos et al., 2021). A recent meta-analysis also demonstrated an inverse relationship between increased glyphosate exposure and decreased testosterone and luteinizing hormone concentrations in animal models (Mohammadi et al., 2022). Consistent with this experimental evidence, urinary glyphosate was inversely associated with total blood estradiol and testosterone, as well as the fraction of active sex hormones, in the US NHANES 2013–2016 population (Geier and Geier, 2023). Finally, as for alterations in thyroid metabolism, in male Wistar rats, low levels of GBHs alter hypothalamus signaling and thyroid morphometry, leading to increased serum T4 levels and target organ changes (Oliveira et al., 2023); whereas in humans, evidence from the Agricultural Health Study suggests that glyphosate exposure is a risk factor for hypothyroidism (Shrestha et al., 2018).

9.2. Nervous system damage

Glyphosate exposure has been associated with damage to the nervous system, whose regulation is also essential for proper muscle function and locomotion. In vitro, human neuroblastoma SH-SY5Y cells exposed to glyphosate show altered proliferation (Hao et al., 2019), whereas glyphosate alone induces a negative regulation in the expression of genes responsible for the synthesis of neuronal cytoskeleton proteins (TUBB3) and axonal growth cones (GAP43), increased expression of genes regulating the proliferation and differentiation of neural stem cells during neurogenesis (Wnt3a, Wnt5a, Wnt7a), as well as higher mRNA expression of calcium-calmodulin-dependent protein kinase 2 isoforms (Martínez et al., 2020). Likewise, in vitro exposure of brain rat tissues to GBH inhibits the activity of mitochondrial respiratory chain enzymes (Neto da Silva et al., 2020), with increased oxidative stress (Astiz et al., 2009). Additionally, a study on murine embryonic dorsal root ganglia cultures showed that administration of GBH at the start of myelination interferes with myelination in a dose-response manner, while exposure to GBH cultures with pre-existing myelin

Table 2
Mean differences (MD) and 95% confidence intervals [95% CI] in hand grip strength (HGS, kg) for the dominant hand, combined HGS (kg), and relative muscle strength (kg/m 2 and kg/BMI) according to creatinine-corrected urine glyphosate levels.

		Quartiles (μg/g creatinine)					
	n	Q1 (≤0.24)	Q2 (0.25–0.41)	Q3 (0.44–0.70)	Q4 (>0.70)	†P-trend	Per doubling
HGS dominant hand (kg)							
Model 1	1459	Ref.	−1.21 [-2.37,-0.04]	−1.01 [-2.45, −0.04]	−1.88 [-3.48,-0.28]	0.06	−0.57 [-1.12,-0.02]
Model 2	1459	Ref.	−1.18 [-2.24,-0.11]	−1.09 [-2.52, 0.33]	−1.71 [-3.30,-0.12]	0.09	−0.55 [-1.10,-0.00]
Model 3	1459	Ref.	−1.33 [-2.36,-0.30]	−0.95 [-2.40, 0.51]	−1.56 [-3.01,-0.09]	0.13	−0.47 [-0.96, 0.01]
Combined HGS (kg)							
Model 1	1436	Ref.	−3.06 [-5.72,-0.39]	−1.98 [-4.61, 0.66]	−4.30 [-7.35,-1.26]	0.04	−1.23 [-2.26, −0.20]
Model 2	1436	Ref.	−2.99 [-5.40,-0.58]	−2.18 [-4.77, 0.41]	−3.93 [-6.90,-0.95]	0.06	−1.17 [-2.20, −0.15]
Model 3	1436	Ref.	−3.32 [-5.63,-1.00]	−1.87 [-4.51, 0.77]	−3.59 [-6.36,-0.82]	0.09	−1.01 [-1.93, −0.08]
HGS/ALMBMI (kg/BMI)							
Model 1	856	Ref.	−2.21 [-3.97, −0.45]	−2.01 [-4.29, 0.27]	−3.73 [-5.44,-2.01]	0.00	−1.13 [-1.80,-0.47]
Model 2	856	Ref.	−1.88 [-3.66,-0.10]	−1.67 [-3.85, 0.52]	−3.30 [-5.30,-1.29]	0.00	−0.98 [-1.69,-0.28]
Model 3	856	Ref.	−1.93 [-3.66,-0.19]	−1.36 [-3.61, 0.89]	−3.14 [-5.19,-1.10]	0.00	−0.90 [-1.58,-0.21]

ALMBMI: Appendicular Lean Mass to Body Mass Index ratio; BMI: Body Mass Index; HGS: Hand Grip Strength; Q: Quartiles.
†P-values for trend were calculated using the quartile median values.
Model 1 adjusted for age and squared age (continuous, years), sex (men/women), ethnicity (non-Hispanic White, African-American, Mexican-American, other), education level (< high school, high school, > high school), fasting time (continuous, hours), and recent pain in the hands.
Model 2: As model 1 + further adjusted for lifestyle-related risk factors: physical activity (METs in leisure time and work time), time watching TV, smoking status (never, former, current), alcohol consumption (never, former, moderate, heavy drinker, unknown) and BMI.
Model 3: As model 2 + further adjusted for total cholesterol (continuous, mg/dL), HDL cholesterol (continuous, mg/dL), cholesterol lowering medication use (no, yes), hypertension (no, yes), diabetes (no, yes), eGFR, cardiovascular disease and cancer (no, yes), respiratory disease (no, yes), osteoarticular disease (no, yes), and number of depressive symptoms.

Table 3
Odds Ratios (OR) and 95% confidence intervals [95% CI] for low hand grip strength (HGS) and low muscle mass according to creatinine-corrected urine glyphosate levels.

	Quartiles (μg/g creatinine)					
	Q1 (≤0.24)	**Q2 (0.25–0.41)**	**Q3 (0.44–0.70)**	**Q4 (>0.70)**	**P-trend†**	**Per doubling**
Low HGS						
n/total	37/351	43/358	64/371	72/379		216/1459
sub n model 4*/subtotal	25/294	38/301	50/308	60/330		173/1233
Model 1	Ref.	1.51 [0.83,2.74]	1.86 [1.24,2.79]	2.16 [1.04,4.50]	0.08	1.26 [1.01,1.57]
Model 2	Ref.	1.68 [0.92,3.08]	2.10 [1.34,3.31]	2.32 [1.13,4.77]	0.07	1.27 [1.03,1.57]
Model 3	Ref.	1.90 [1.04,3.48]	2.14 [1.33,3.43]	2.50 [1.27,4.92]	0.05	1.28 [1.05,1.56]
Low HGS/ALMBMI						
n/total	31/239	30/231	31/210	39/176		131/856
sub n model 4*/subtotal	25/197	28/196	26/176	35/158		114/727
Model 1	Ref.	1.17 [0.41,3.33]	1.19 [0.41,3.46]	2.25 [1.11,4.54]	0.05	1.42 [1.07, 1.89]
Model 2	Ref.	1.10 [0.40,3.06]	1.21 [0.43,3.35]	2.31 [1.09,4.88]	0.06	1.43 [1.05,1.94]
Model 3	Ref.	1.11 [0.40,3.08]	1.11 [0.41,3.01]	2.19 [1.08,4.41]	0.07	1.38 [1.02, 1.88]

ALMBMI: Appendicular Lean Mass to Body Mass Index ratio; HGS: Hand Grip Strength; Q: Quartiles.
†P-values for trend were calculated using the quartile median values.
Model 1 adjusted for age and squared age (continuous, years), sex (men/women), ethnicity (non-Hispanic White, African-American, Mexican-American, other), education level (< high school, high school, > high school), fasting time (continuous, hours), and recent pain in the hands.
Model 2: As model 1 + further adjusted for lifestyle-related risk factors: physical activity (METs in leisure time and work time), time watching TV, smoking status (never, former, current), alcohol consumption (never, former, moderate, heavy drinker, unknown) and BMI.
Model 3: As model 2 + further adjusted for total cholesterol (continuous, mg/dL), HDL cholesterol (continuous, mg/dL), cholesterol lowering medication use (no, yes), hypertension (no, yes), diabetes (no, yes), eGFR, cardiovascular disease and cancer (no, yes), respiratory disease (no, yes), osteoarticular disease (no, yes), and number of depressive symptoms.

Table 4
Odds Ratios (OR) and 95% confidence intervals [95% CI] for functional limitations according to creatinine-corrected urine glyphosate levels.

Functional impairments	Quartiles [μg/g creatinine]					
	Q1 [≤0.24]	Q2 [0.25–0.41]	Q3 [0.44–0.70]	Q4 [>0.70]	†P-trend	Per doubling
Stooping, crouching, kneeling rowhead						
n/total	45/301	71/322	68/303	103/375		287/1301
Model 1	Ref.	1.86 [1.19, 2.91]	1.36 [0.81, 2.27]	2.52 [1.39, 4.59]	0.04	1.25 [1.04, 1.50]
Model 2	Ref.	1.88 [1.23, 2.87]	1.34 [0.76, 2.36]	2.82 [1.50, 5.34]	0.02	1.33 [1.08, 1.63]
Model 3	Ref.	1.58 [0.99, 2.52]	1.01 [0.58, 1.76]	2.04 [1.00, 4.19]	0.09	1.19 [0.98, 1.47]
Lifting/carrying items >10 lb.rowhead						
n/total	24/302	32/319	43/302	51/368		150/1291
Model 1	Ref.	0.94 [0.41, 2.17]	1.69 [0.81, 3.51]	1.51 [0.75, 3.04]	0.09	1.16 [0.92, 1.47]
Model 2	Ref.	0.96 [0.41, 2.25]	1.81 [0.82, 4.03]	1.52 [0.69, 3.33]	0.14	1.17 [0.91, 1.50]
Model 3	Ref.	0.82 [0.34, 1.97]	1.49 [0.65, 3.41]	1.23 [0.58, 2.61]	0.31	1.10 [0.87, 1.38]
Standing from armless chairrowhead						
n/total	14/304	20/326	22/303	35/378		91/1311
Model 1	Ref.	0.76 [0.39, 1.49]	1.90 [0.43, 2.73]	1.83 [1.03, 3.26]	0.01	1.20 [1.02, 1.40]
Model 2	Ref.	0.78 [0.42, 1.43]	1.15 [0.43, 3.12]	1.89 [1.05, 3.41]	0.01	1.21 [1.01, 1.40]
Model 3	Ref.	0.63 [0.32, 1.23]	0.95 [0.35, 2.56]	1.46 [0.72, 2.96]	0.06	1.12 [0.89, 1.40]
Ascending ten steps without pauserrowhead						
n/total	5/253	10/269	6/222	16/290		37/1434
Model 1	Ref.	4.85 [1.54, 15.29]	1.09 [0.28, 4.19]	6.34 [2.09, 19, 24]	0.04	1.37 [1.00, 1,90]
Model 2	Ref.	5.98 [1.52, 23.43]	1.00 [0.23, 4.31]	8.46 [2.19, 32.56]	0.03	1.47 [1.05, 2.06]
Model 3	Ref.	5.70 [1.08, 30.15]	0.63 [0.08, 5.14]	8.06 [2.08, 31.17]	0.04	1.48 [0.95, 2.29]

†P-values for trend were calculated using the quartile median values.
Model 1 adjusted for age and squared age (continuous, years), sex (men/women), ethnicity (non-Hispanic White, African-American, Mexican-American, other), education level (< high school, high school, > high school), fasting time (continuous, hours), and recent pain in the hands.
Model 2: As model 1 + further adjusted for lifestyle-related risk factors: physical activity (METs in leisure time and work time), time watching TV, smoking status (never, former, current), alcohol consumption (never, former, moderate, heavy drinker, unknown) and BMI.
Model 3: As model 2 + further adjusted for total cholesterol (continuous, mg/dL), HDL cholesterol (continuous, mg/dL), cholesterol lowering medication use (no, yes), hypertension (no, yes), diabetes (no, yes), eGFR, prevalence of cardiovascular disease and cancer (no, yes), respiratory disease (no, yes), osteoarticular disease (no, yes), and number of depressive symptoms.

contributes to demyelination (Szepanowski et al., 2018). In vivo, most studies have shown effects on the central nervous system after glyphosate exposure, with evidence in rats that it can infiltrate the brain barrier (Winstone et al., 2022), induce inflammation (Winstone et al., 2022), and alter neurotransmission (Costas-Ferreira et al., 2022). In a study by Nozdrenko and cols., chronic glyphosate intoxicated rats showed a significant increase in the time of muscle response after nerve stimulation, an important parameter of the kinetics of skeletal muscle contraction (Nozdrenko et al., 2021). Also, in the study by Barbose et al. mouse offspring exposed to glyphosate during pregnancy and lactation showed a reduction in neuromuscular junctions secondary to the alteration of cholinesterase enzyme activity and subsequent hyperstimulation of the postsynaptic compartment (Barbosa et al., 2022). Finally, a case report of a 70-year old man showed that glyphosate exposure induced a vasculitic neuropathy and mild weakness of the lower limbs (Kawagashira et al., 2017); although the most suggestive evidence of a potential association between chronic low-dose glyphosate exposure and neurotoxic effects in humans is the recently published NHANES 2013–2014 study,

in which a positive correlation was observed between urinary glyphosate and serum neurofilament light levels (Yang et al., 2023), a biomarker of axonal damage associated with neurologic disorders, low grip strength and sarcopenia (Pratt et al., 2022).

9.3. Mitochondrial disruption

Mitochondrial dysfunction plays a key role in sarcopenia signaling, and while specific studies on glyphosate's impact on skeletal muscle mitochondria are lacking, research in other tissues indicates significant alterations in bioenergetics and increased oxidative stress after glyphosate exposure. For example, in liver hepatocytes, glyphosate competes with succinate, influencing lactate dehydrogenase and reducing mitochondrial respiratory activity (Strilbyska et al., 2022). Also, glyphosate alters activities of glucose-6-phosphate dehydrogenases and malate dehydrogenases in various organs, impacting processes related to energy production, redox balance, and metabolic intermediates (Daruich et al., 2001). The herbicide also alters mitochondrial permeability, membrane potential, and inhibits the respiratory chain in rat liver mitochondria (Strilbyska et al., 2022), brain (Pereira et al., 2018) and liver (Szarek et al., 2000) tissues from different fish species, or the immortalized human epidermal cell line HaCaT (Heu et al., 2012). Despite this evidence, the association between glyphosate exposure and mitochondrial disruption in humans is scarce, with data from 209 participants in the Flemish Environment and Health Study showing no correlation between mitochondrial DNA concentrations and levels of urinary glyphosate or its primary biodegradation metabolite, aminomethylphosphonic acid (AMPA). Moreover, in this same study, a doubling in urinary AMPA concentrations was associated with increased leukocyte telomere length (Cosemans et al., 2022).

9.4. Alteration of the gut microbiota-muscle axis

Studies on animal models consistently reveal a significant connection between gut microbiota and both muscle mass and muscle fiber structure (Ticinesi et al., 2019), while a few human studies have shown positive correlations between microbiota composition and grip strength (Zhao et al., 2023), leg muscle function (Iwasaka et al., 2023) or muscle mass (Yamamoto et al., 2022).

Glyphosate exposure in different animal species has been correlated with alterations in the gut microbiome (Mao et al., 2018; Del Castilo et al., 2022; Lehman et al., 2023), and in the intestinal and urine metabolome (Mesnage et al., 2021; Hu et al., 2021). Specifically, glyphosate appears to alter the gut microbiome of Sprague–Dawley rats through inhibition of the shikimate pathway (Mesnage et al., 2021), while gestational and early-life exposure to low doses of glyphosate or GBHs would alter urine metabolomic biomarkers involved in one-carbon metabolism, including homocysteine, methionine and N-glyphosate (Hu et al., 2021). In humans the evidence is even sparser, with a recent study suggesting that glyphosate exposure may be associated with an overall increase in bacterial species richness, as well as fatty acid metabolites and phosphate levels (Mesnage et al., 2022).

9.5. Strengths and limitations of this study

The strengths include its sampling design, high quality laboratory methods, thorough evaluation of potential confounding variables, as well as the consistency of the various sensitivity analyses performed. Also, the consistency of results between muscle strength measures and functional outcomes enhances its plausibility and reinforces their potential relevance. Regarding its limitations, NHANES employs a cross-sectional design, preventing the assessment of temporal associations. However, we have no evidence to suggest that individuals with lower strength or physical impairments are more likely to be exposed to glyphosate, making reverse causation unlikely. Additionally, NHANES relies on a single spot urine sample for glyphosate measurement, introducing the possibility of non-differential measurement error. Furthermore, NHANES did not measure levels of AMPA, and, although urine is the most frequently employed biomarker to measure population exposure to glyphosate (Faniband et al., 2021; Ferreira et al., 2021; Lemke et al., 2021; Connolly and Koch, 2023), its short half-life, typically between 9 and 18 h (Connolly et al., 2019; Zoller et al., 2020; Kohsuwan et al., 2022), and uncertainty about its bioaccumulation in the human body, may limit its use as a biomarker of chronic exposure. Concerning the outcome, physical performance was based on self-reports, and the prevalence of certain impairments was low, constraining our ability to detect associations. Finally, the study design does not allow determine the extent to which glyphosate in urine serves as a biomarker of exposure to GBHs, which may contain other potentially toxic compounds for muscles (de Araújo-Ramos et al., 2021).

10. Conclusions

Our results suggest that glyphosate exposure may be a risk factor for decreased grip strength and increased physical functional limitations. More studies investigating the influence of this and other environmental pollutants on functional aging are needed.

CRediT authorship contribution statement

Sara Jauregui-Zunzunegui: Writing – original draft. **Fernando Rodríguez-Artalejo:** Writing – review & editing. **María Tellez-Plaza:** Writing – review & editing, Data curation. **Esther García-Esquinas:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.envres.2024.118547.

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