Protein intake is associated with lean mass and femur bone mass in individuals with rheumatic diseases from the NHANES cohort

Gabriel P. Esteves[1]; Paul Swinton[2]; Craig Sale[3]; Hamilton Roschel[1]; Bruno Gualano[1,4]; Eimear Dolan[1]

1. Applied Physiology and Nutrition Research Group - Center of Lifestyle Medicine, Faculdade de Medicina FMUSP, Universidade de São Paulo, São Paulo, SP, BR;

2. School of Health Sciences, Robert Gordon University, Aberdeen, UK;

3. Institute of Sport, Manchester Metropolitan University, Manchester, UK;

4. Food Research Centre, University of Sao Paulo, Sao Paulo, SP, BR.

Corresponding author: Gabriel P. Esteves:

Faculdade de Medicina FMUSP, Universidade de São Paulo, Av. Dr. Arnaldo, 455, São Paulo Brazil, 01246-903. Phone: +55 11 2648-1337; Fax: +55 11 30617490; E-mail: gabriel.perri.esteves@usp.br.

ORCID: 0000-0003-0740-0683.

Preprint – not yet peer reviewed.

Please cite as: Esteves et al. (2023) Protein intake is associated with lean mass and femur bone mass in individuals with rheumatic diseases from the NHANES cohort

Abstract

Background/Objectives: Strategies to protect musculoskeletal health in individuals with rheumatic diseases (RDs) are of utmost importance. Optimizing protein intake is one such potential strategy. The aim of this investigation was to explore the relationship between protein intake and muscle and bone masses in individuals with rheumatic diseases, using data from the NHANES database.

Method: Relevant data were extracted from six NHANES cycles, providing a total sample of 4,122 individuals with varying RDs (psoriatic arthritis, rheumatoid arthritis, osteoarthritis and gout). Potential confounding variables and their relationship to outcomes of interest were visualized using directed acyclic graphs. Outcomes of interest were lean and bone mass, measured at the whole-body, femur and lumbar spine by DXA. Multivariable regression models adjusted for potential confounding variables (body mass, sex, age, disease category and total caloric intake) were used.

Results: There was a small positive association between protein intake and muscle mass, both when protein was considered in absolute values (grams) (β=0.08 (95%CI 0.04-0.14), p-value=0.0002) or when relative to body mass (g/kgBM/d) (β=0.05 (95%CI 0.02-0.08), p-value=0.0036). A positive relationship was also shown between protein intake and femur BMD, but only when protein was considered in absolute values (β=0.08 (95%CI 0.03-0.14), p-value=0.0024).

Conclusion: Protein intake might have a relevant role in improving muscle, and potentially bone, mass in individuals with RD, although effects seem to be small. These findings pave the way for future randomized controlled trials to assess the role of increased protein intake on bone and muscle mass in patients with RD.

Introduction

Rheumatic diseases (RD) are chronic, inflammatory and/or auto-immune conditions that substantially impact quality of life due to symptoms such as chronic pain, fatigue, edema and musculoskeletal dysfunction (1). These conditions place a heavy burden on patients and health-care systems, and although cures do not currently exist, they can be managed using a range of pharmacological and non-pharmacological strategies. Frequently employed pharmacological strategies include pain medication; anti-inflammatory and immune-mediating drugs, such as glucocorticoids; and disease modifying anti-rheumatic drugs (DMARD) (2–4). Although effective, these treatments can lead to both acute and chronic adverse effects, such as adverse site reactions, nausea, vomiting and headaches (5), and also long-term repercussions, such as osteoporosis, myopathy, type 2 diabetes, dyslipidemia and hypertension (6–14). As such, co-adjuvant non-pharmacological treatment options, which usually revolve around physical activity, exercise training and nutritional intake (15,16) are a pertinent area of investigation due to their potential to attenuate many of the adverse consequences of these conditions, thus improving health and clinical outcomes (17–20). Despite this large clinical potential, investigation into the efficacy of these approaches is currently lacking, and substantial research is required to provide evidence-based recommendations to patients.

Strategies to protect musculoskeletal health are particularly relevant for RD patients, given the association between RD and adverse musculoskeletal outcomes (9,10,21,22). Although these conditions differ according to their specific characteristics, conditions such as gout (23), rheumatoid arthritis (24) and osteoarthritis (25,26) are associated with muscle and bone loss or muscle disability (27,28). Meta-analyses have identified that patients with rheumatoid arthritis have increased risk for bone fractures (24,29,30), which may be due to physical inactivity or to adverse musculoskeletal effects caused by medications, such as glucocorticoids(9,31–33), or to a combination of these factors. RD patients are also more likely to have sarcopenia – a condition defined by reduced muscle strength, mass and physical performance (34) – when compared to the general population (35,36). Sarcopenia increases the risk of

falls (37,38), which might further increase fracture risk given that these individuals also tend to have reduced bone mass.

Adequate protein intake may play an important role in protecting musculoskeletal health of RD patients (15). The importance of protein intake to muscle is well-recognised, and higher intakes than the recommended daily allowance (RDA) (*e.g.,* 1.5 versus 0.8 g/kgBM/d) have been suggested for other populations with a high risk of muscle loss and sarcopenia, such as older adults (39) and cancer patients (40). With regards to bone, protein was once considered harmful, with studies showing that higher protein intakes led to increased urinary calcium excretion (41). Subsequent investigations, however, showed a concomitant increase in intestinal calcium absorption, which compensated for increased urinary excretion, thus preventing increases in skeletal catabolism (42). Indeed, contemporary lines of evidence indicates that protein is likely beneficial to bone (42), with meta-analytic findings indicating a small, but statistically significant, positive effect of higher protein intakes on bone mineral density (BMD) (43,44) and reduced fracture risk (43,45). Despite these findings in the general population, there is limited empirical evidence on the association between protein intake and musculoskeletal health in individuals with RD (46,47). This is important, because it is currently unclear whether the state of chronic inflammation that is characteristic of rheumatic conditions, and/or frequent use of catabolic drugs, such as glucocorticoids, may impede the anabolic stimulus of increased protein intake. The National Health and Nutrition Examination Survey (NHANES) is a nationally representative survey of the US population, which includes demographic, dietary and health-related data on a large number of participants, and is a useful data source to explore relationships between key health and lifestyle-associated variables. As such, our aim was to use data from the NHANES database to explore associations between dietary protein intake and muscle and bone masses in individuals with RD.

Material and Methods

NHANES cycles and population of interest

We analysed data obtained from adult men and women without age restriction, including cycles from 2007 to 2018 of the continuous NHANES survey conducted by the USA Center for Disease Control and Prevention (CDC). Data from the "2017–2020 pre-pandemic" and 2019–2020 surveys were not included as they did not include dietary recall data, nor were they based on a nationally representative sample. Not all cycles included data for all bone imaging sites, which limited the total sample size in some cases (see Figure 1 and Supplementary Material 1 for a summary of data availability across cycles). Within the NHANES survey, data on medical conditions were collected, with assessed RDs within this questionnaire including rheumatoid arthritis, osteoarthritis, psoriatic arthritis and gout. We selected all participants who self-reported any of these conditions, since these patients are likely to share many of the risk factors associated with worsened musculoskeletal health, such as undergoing chronic glucocorticoid treatment and being predisposed to lower levels of physical activity and exercise due to disease-driven pain and disability (16). The original NHANES survey protocol was approved by the CDC and National Center for Health Statistics (NCHS) Ethics Review Board, and all participants provided written informed consent.

Main outcomes: muscle and bone mass

The NHANES uses DXA scans to provide measurements of bone and soft tissue in the total body, head, trunk, arms, and legs, with separate scans for the whole-body, femur and spine. For this investigation, we extracted all BMD measurements from the whole-body and at the femur and lumbar spine sites ($g \cdot cm^{-2}$). Total lean mass, as measured by DXA, was used to indicate muscle mass. Although this outcome includes all tissues apart from bone and fat mass, it is currently the preferred proxy method to assess skeletal muscle mass, given that this is the largest and most plastic component of lean mass (48). All DXA scans were conducted by certified radiology technologists, using Hologic densitometers (Hologic, Inc., Bedford, Massachusetts) coupled with APEX software (version 3.2). More detailed documentation of the DXA scanning process is available online (49).

Dietary assessment

Dietary data were derived from 24-hour dietary recall, which collects a list of all foods and beverages consumed by the participant within a 24-hour period and their respective amounts. Data were collected and inputted to the USDA AMPM instrument, and were later coded and linked to a database of foods and their nutrient composition, from which calculations of total daily nutrient intakes were derived. Dietary intake was assessed using two non-consecutive 24-hour dietary recalls, which were conducted via in-person interview by trained professionals and using standardised protocols that aimed to reduce occurrence of forgotten foods and to adequately estimate portion sizes using standardized measures. Further information on the protocols can be found at the NHANES protocol document (50). The mean of the two recalls was used and when 2 recalls were not available, the single available value was used.

Data cleaning and statistical analysis

To investigate potential relationships between protein intake and lean and bone mass, multivariable linear regression models were used. BMD measurements at the whole-body, femur and lumbar spine imaging sites, alongside total body lean mass were considered as the dependent variables, while protein intake was considered as the independent variable. A directed acyclic graph (DAG) was constructed to identify variables of interest (Figure 2). DAGs are graphical tools that represent theories and assumptions underlying both the theoretical and statistical models applied in a given research question (51). Herein we attempted to identify and represent the most important variables related to lean and muscle mass and protein intake. In causal diagrams, such as DAGs, variables that affect both the exposure and the outcome of interest are recognized as a source of potential confounding. Within our models, we considered body mass, sex and age as potential confounding variables, and these were adjusted for in all models (*i.e.*, added as independent variables). Physical activity is another variable likely to influence these outcomes, however, a substantial amount of data from the NHANES questionnaire on physical activity was missing (>70% missing in some cases), and so this variable was not considered within the model. Physical activity monitor data (collected via accelerometers) also could not be included, as only two cycles within the six included reported these data. Total energy intake is considered to be an important mediator of the relationship between protein and other biological variables

(52), given that increased protein also increases energy availability, which may independently influence a range of factors, including muscle and bone. As such, it is important to adjust for total energy intake when aiming to estimate the influence of isolated macronutrients, such as protein. Including total energy intake as a model covariate, or using the residuals from a model regressing the nutrient exposure on total energy, are common strategies to adjust for energy intake in observational studies. However, a recent simulation study reported that a model that includes all energy sources (*i.e.*, all three macronutrients) as covariates within the model leads to less biased coefficient estimation compared to other approaches for total energy adjustment (53) and so this was the approach selected. Finally, since we included multiple different RD, a variable for disease type was also included within the model, to account for potential differences in the response for protein intake on the selected outcomes across conditions. The presented DAG illustrates these purported causal pathways (Figure 2). Note that additional pathways between some of the variables may exist, but for the sake of clarity, arrows that do not indicate potentially biasing pathways have been omitted. For a visualization of all pathways, see Supplementary Figures 3 and 4.

As recommended by the NHANES' analytical guidelines (54), sample weights were calculated using the dietary food recall sample weights divided by the number of cycles included, and utilized in the survey design of all models. After creating the sample design and prior to analysis, data with inadequate exam or food recall status (as classified by the NHANES database) were excluded from the analysis, as well as nutrient intake values that were considered compatible with measurement error (*i.e.*, extremely high values such as more than 8000 kilocalories per day (kcal/day) or 4.5 protein grams per kilogram of body mass per day (g/kgBM/d), or extremely small values such as less than 300 kcal/day and 10 grams per day (g/d) of any macronutrient). Unadjusted models used protein intake in absolute daily values (g/d) as the dependent variable, while adjusted models also used daily protein intake corrected by body mass (g/kgBM/d). Body mass, sex, age, energy intake (by inclusion of carbohydrate (g) and fat (g) intake) and disease category (rheumatoid arthritis, osteoarthritis, psoriatic arthritis and gout) were included as covariates in all adjusted models. Coefficients are presented in standardized format (Beta or β), with values representing the standard deviation change to the dependent variable per standard

deviation increase in the independent variable. Analyses were conducted using R and Rstudio software (R version 4.2.0, R Foundation for Statistical Computing, Vienna, Austria; Rstudio Build 492, PBC, Boston, MA), utilizing the 'survey' package (55), with sample weights and survey design accounted for using the 'svydesign' function and linear models ran using the 'svyglm' function. An *a priori* alpha of 0.05 was used as a decision rule to define compatibility/incompatibility between each hypothesis and the data (given the model used to generate each p value).

Results

Participant characteristics

Data for 52,336 participants were available within the complete NHANES databases, of which 5,926 remained after selecting for adult participants with RD. 1,804 of these were excluded due to inadequate DXA exam or food recall status, leaving data from 4,122 participants available for analysis. 1,078 participants had adequate data for the lean mass analysis. Regarding bone, participants with adequate data for whole body, femur and spine BMD were 1,049, 3,080 and 1,890 (see Figure 2 for a detailed description of data availability). Participant characteristics are described in Table 1. Participants with rheumatoid arthritis, osteoarthritis, psoriatic arthritis and gout were aged 58±13, 61±14,51±12 and 61±14 years. Most were women of Non-Hispanic White ethnicity, and with high BMI (30.52±7.06). Mean protein intake was above the current minimum recommendation (*i.e.*, 0.8 g/kgBM/d), being on average 0.92±0.45 g/kgBM/d. A graphical representation of the distribution of nutrient intake is available in Supplementary Material 2.

Muscle mass

Unadjusted models showed a positive association between lean mass and absolute daily protein intake (Beta = 0.42 [95%CI 0.33 to 0.52]; $p<0.0001$) (Table 2). Adjusted linear regression models showed a positive association between protein intake and lean mass, both for daily intake in grams (Beta = 0.08 [95% CI: 0.04 to 0.12]; $p=0.0003$) and g/kgBM/d (Beta = 0.05 [95% CI: 0.02; 0.09], p=0.0048).

Bone Mineral Density

Unadjusted linear regression models showed a positive association between protein intake and BMD measured at whole-body (Beta = 0.34 [95%CI 0.24 to 0.44]; p<0.0001), femur (Beta = 0.25 [95%CI 0.20 to 0.31]; p<0.0001), and lumbar spine (Beta = 0.13 [95%CI 0.07 to 0.18]; p<0.0001) (Table 2). Adjusted linear regression models showed a positive association between absolute daily protein intake (g) and femur BMD (Coefficient = 0.08 [95% CI: 0.03; 0.13], p=0.0028), but not for g/kgBM/d intake (Coefficient = 0.03 [95% CI: -0.02; 0.08], p=0.20) (see Figure 3 for partial regression plots of protein and all outcomes). No association was shown between whole body or lumbar spine BMD and protein intake, be it daily intake in grams or g/kgBM/d (all p>0.05).

201 **Table 1.** Participant's characteristics

Characteristic	**Overall**, N = 4,094	**Rheumatoid arthritis**, N = 1094	**Osteoarthritis**, N = 2,153	**Psoriatic arthritis**, N = 56	**Gout**, N = 791
Age (years)	59 (13)	61 (13)	60 (13)	50 (11)	58 (13)
Sex					
Male	1,853 (45%)	604 (76%)	787 (37%)	25 (45%)	437 (40%)
Female	2,241 (55%)	187 (24%)	1,366 (63%)	31 (55%)	657 (60%)
Race					
Mexican American	392 (10%)	57 (7%)	168 (8%)	3 (5%)	164 (15%)
Other Hispanic	320 (8%)	46 (6%)	154 (7%)	4 (7%)	116 (11%)
Non-Hispanic White	2,193 (54%)	390 (49%)	1,337 (62%)	34 (61%)	432 (39%)
Non-Hispanic Black	865 (21%)	206 (26%)	337 (16%)	10 (18%)	312 (29%)
Other	324 (8%)	92 (12%)	157 (7%)	5 (9%)	70 (6%)
Height (m)	166.5 (10.3)	170.8 (9.6)	165.7 (10.3)	166.7 (9.5)	165.1 (10.1)
Body mass (kg)	85.0 (21.6)	89.8 (20.2)	84.3 (22.1)	85.18 (18.6)	82.8 (21.3)

Characteristic	**Overall**, N = 4,094	**Rheumatoid arthritis**, N = 1094	**Osteoarthritis**, N = 2,153	**Psoriatic arthritis**, N = 56	**Gout**, N = 791
BMI (kg/m²)	30.5 (6.9)	30.6 (6.0)	30.6 (7.1)	30.6 (6.4)	30.2 (7.0)
Energy intake (kcal)	1,933 (774)	2,001 (790)	1,92 (755)	2,041 (808)	1,891 (795)
Carbohydrate intake (g)	231 (98)	233 (96)	231 (97)	238. (97)	229 (99)
Protein intake (g)	75 (33)	80 (34)	75 (32)	77 (32)	73 (33)
Protein intake (g/kgBM/d)	0.92 (0.43)	0.92 (0.42)	0.93 (0.42)	0.94 (0.40)	0.92 (0.47)
Fat intake (g)	75 (36)	77 (37)	75 (35)	81 (41)	73 (37)
Lean mass (kg)	54.1 (13.3)	60.2 (13.1)	53.4 (13.3)	52.0 (12.3)	52.1 (12.8)
Whole body BMD (g/cm²)	1.113 (0.121)	1.151 (0.116)	1.109 (0.119)	1.081 (0.112)	1.103 (0.124)
Femur BMD (g/cm²)	0.931 (0.169)	0.981 (0.167)	0.912 (0.166)	0.952 (0.143)	0.926 (0.169)
Femur BMD T-scores	-0.08 (1.30)	0.31 (1.23)	-0.23 (1.29)	0.07 (1.10)	-0.11 (1.31)

Characteristic	**Overall**, N = 4,094	**Rheumatoid arthritis**, N = 1094	**Osteoarthritis**, N = 2,153	**Psoriatic arthritis**, N = 56	**Gout**, N = 791
Spine BMD (g/cm²)	1.01 (0.17)	1.07 (0.17)	1.00 (0.17)	0.98 (0.14)	1.00 (0.17)
Spine BMD T-scores	-0.39 (1.50)	0.08 (1.50)	-0.51 (1.48)	-0.69 (1.22)	-0.48 (1.47)

Data are mean (SD) for continuous variables and n (%) for categorical variables.

202

203

204 **Table 2.** Unadjusted and adjusted linear regression models for all dependent variables (standardized coefficients)

	Unadjusted			Adjusted		
Term	β coefficient	95% CI	p-value	β coefficient	95% CI	p-value
Lean mass (n = 1,038)						
Protein intake (g)	0.42	0.33; 0.52	<0.0001	0.08	0.04; 0.12	0.0003
Protein intake (g/kgBM/d)	-	-	-	0.05	0.02; 0.09	0.0048
Whole body BMD (*n = 1,049)*						
Protein intake (g)	0.34	0.24; 0.44	<0.0001	0.11	-0.03; 0.25	0.1172
Protein intake (g/kgBM/d)	-	-	-	0.05	-0.09; 0.18	0.5147
Femur BMD (n = 3,061)						
Protein intake (g)	0.25	0.20; 0.31	<0.0001	0.08	0.03; 0.13	0.0028
Protein intake (g/kgBM/d)	-	-	-	0.03	-0.02; 0.08	0.2027
Spine BMD (n = 1,878)						
Protein intake (g)	0.13	0.07; 0.18	<0.0001	0.03	-0.05; 0.11	0.4220
Protein intake (g/kgBM/d)	-	-	-	0.00	-0.08; 0.08	0.9900

205

Discussion

Herein we examined associations between daily protein intake and both lean and muscle mass in individuals with RD using data from the NHANES survey. Following adjustment for confounding factors, we showed a positive relationship between daily protein intake and lean mass, when protein intake was considered in terms of absolute daily intakes (g/d) and when adjusted for body mass (g/kgBM/d), and between absolute daily protein intake (g/d) and femur BMD (although not for whole body or lumbar spine BMD). These results would seem to suggest that higher daily intakes of protein might be important for individuals with RD in order to help maintain lean and bone mass, although randomised controlled trials are required to confirm these exploratory findings, particularly in relation to the potential influence of protein on bone health.

The finding of a positive relationship between protein intake and lean mass aligns with current evidence and recommendations for other populations (39,40,56). Protein is a macronutrient directly implicated in the development and maintenance of muscle mass, supplying essential amino acids for muscle protein synthesis (57,58). Reference values for minimum daily protein intake (*e.g.*, the protein RDA of 0.8 g/kgBM/d (59)) are based on nitrogen balance and protein metabolism studies, with the goal of avoiding net nitrogen losses across the average population (59). However, some populations have an increased risk for muscle loss, due to factors such anabolic resistance (*i.e.*, an impaired response to anabolic stimulus), as seen in the older and critically ill individuals (60,61); through an increased catabolic stimulus conveyed by a disease, as seen in cancer cachexia (62); or pharmacological treatment, such as glucocorticoids (10,15,63). In these situations, an increased protein intakes might be required, a case in point being the older adult population, wherein studies have shown an increased protein requirement (*i.e.*, 1.2–1.3 g/kgBM/day (64,65)). Our results suggest that protein intake is positively associated with lean mass in this population, and that higher protein intakes may also be warranted, given the aforementioned challenges to maintaining muscle mass for individuals with RD. Precise recommendations as to what these intakes should comprise are beyond the scope of the current

investigation, but intakes in line with those recommended for other populations at high risk of muscle and bone loss, *e.g.*, 1.0 – 1.5 g/kgBM/day, seem prudent.

We also showed a positive association between absolute protein intake and femur BMD. Protein has both structural and metabolic roles related to bone health: it comprises approximately half of bone volume and one third of its mass (66), and it stimulates the activity of anabolic hormones and growth factors, such as IGF-1 (67), which are important mediators of bone remodelling (42). Additionally, protein may exert an effect on bone by increasing lean mass, which is recognized as an important determinant of bone mass (42). In our analysis, only absolute protein intake was significantly associated with increased femur BMD. The impact of nutrition only on femur BMD has been previously reported, wherein different food clusters associated with bone mass at this site, but not across all sites (68). There is no clear explanation as to why the femur may be more susceptible to diet than other bone sites, although the higher presence of trabecular bone may be a potential factor to consider. The femur is also the most amenable loading site to physical activity and exercise, and as such, may have a higher remodelling rate, and be more amenable to the potential influence of protein intake (69). Alternatively, this could also be simply due to sample size for femur BMD being higher in our sample, thus increasing statistical power to detect the relatively small effects of protein intake on BMD.

Regarding the strength of the association, it is important to acknowledge that the coefficients reported herein (Lean mass $\beta = 0.08$ [95%CI 0.04; 0.12] and 0.05 [95%CI 0.02; 0.09]; femur BMD $\beta = 0.08$ [95%CI 0.03; 0.13]) can be considered of very small magnitude. This aligns with a recent meta-analysis, which showed that interventions to increase protein intake were only effective at increasing lean mass and strength when combined with resistance training or when focusing on sarcopenic/frail individuals (70). As seen here, increasing protein by itself is likely to only exert small effects, and based on current literature, coupling increased protein intake with exercise training interventions is more likely to improve musculoskeletal health and function further then increasing protein alone. It should be noted, however, that the long-term decline in muscle mass leading to sarcopenia corresponds to a 3 – 8% decrease in muscle mass per decade (71). If this progressive, albeit small decline could be partially

prevented by greater protein intakes, then even a small protective effect could prove clinically meaningful in the long term. However, the rate of muscle loss likely to occur in individuals with RD, and whether this is also amenable to protein, still remains to be determined.

Although both unadjusted and adjusted models showed a positive association between protein intake and bone or muscle masses, it is important to highlight that adjustment led to a large reduction in the magnitude of these associations. This suggests that the relationship between protein and bone and muscle masses can be severely biased if potential confounding variables are not considered. Herein, through the use of a DAG and current knowledge on the topic, we selected variables which likely contribute to confounding, such as body mass, sex, and age, and included them as covariates in our models, favouring more accurate estimates. Additionally, we adjusted for the role of energy intake by including all macronutrients as covariates in the model. Observational studies investigating the role of protein on bone do commonly adjust for energy, typically by adding total energy as a covariate or by using the residual method (72,73). Estimates based on these strategies may be biased, however, as suggested by Tomova et al. (53). Future observational studies should aim to adequately adjust for the role of energy intake, and other potential sources of confounding, if they aim to estimate the direct effect of protein on these outcomes.

Our approach has limitations. Given that any dietary exposure is likely to exert its effects in the long-term, and particularly for less plastic tissues such as bone, a single dietary assessment as is available within a cross-sectional study may not offer an accurate representation of longer-term nutrient intake. This may account, at least in part, for the larger and more consistent associations that were observed between protein intake and lean mass, compared to bone. Future studies using large databases of longitudinal studies are warranted to shed further light on this topic. Measurement error will always exist when estimating nutrient intakes from dietary recalls and other questionnaires, which can impact precision (74). The observational nature of the data hampers the establishment of causal links between protein intake and muscle and bone masses, since unaccounted confounding factors are likely to be present, including confounding by physical activity, which could not be controlled for in this analysis.

Additionally, only those RDs that were surveyed in the NHANES medical questionnaire, namely rheumatoid arthritis, osteoarthritis, psoriatic arthritis and gout, could be assessed. Carrying out such an analysis in other conditions that may face muscle and bone loss, such as Systemic Lupus Erythematosus and systemic sclerosis, is necessary before these findings can be extrapolated to other RDs.

In conclusion, protein intake positively associated with lean mass and femur BMD in individuals with RD. Although this relationship was not shown across all bone imaging sites, these associations are consistent with the body of literature from individuals without RD, aligning with recommendations to increase protein intake in populations facing, or at higher risk for, muscle and bone loss. The magnitude of all observed associations was, however, very small, and interventions focusing upon protein alone may have limited clinical benefit. Instead, recommendations to ensure adequate protein intake, but within the context of other lifestyle recommendations, such as increasing physical activity, may be most appropriate to protect against muscle and bone loss in this population. These findings provide the rationale for designing future randomized controlled trials focused on testing the efficacy and feasibility of high protein diets to manage muscle and bone mass in RD patients.

Data Availability Statement

All data used herein is available online in the original NHANES data repository (https://wwwn.cdc.gov/nchs/nhanes/). Statistical code utilized to analyse the data can be shared upon request.

References

1. Szekanecz Z, McInnes IB, Schett G, Szamosi S, Benkő S, Szűcs G. Autoinflammation and autoimmunity across rheumatic and musculoskeletal diseases. *Nat Rev Rheumatol* (2021) 17:585–595. doi: 10.1038/s41584-021-00652-9

2. Aletaha D, Smolen JS. Diagnosis and Management of Rheumatoid Arthritis. *JAMA* (2018) 320:1360. doi: 10.1001/jama.2018.13103

3. Fanouriakis A, Bertsias G. Changing paradigms in the treatment of systemic lupus erythematosus. *Lupus Sci Med* (2019) 6:e000310. doi: 10.1136/lupus-2018-000310

4. van der Goes MC, Jacobs JW, Bijlsma JW. The value of glucocorticoid co-therapy in different rheumatic diseases--positive and adverse effects. *Arthritis Res Ther* (2014) 16 Suppl 2:S2. doi: 10.1186/ar4686

5. Machado-Alba JE, Ruiz AF, Machado-Duque ME. Adverse drug reactions associated with the use of disease-modifying anti-rheumatic drugs in patients with rheumatoid arthritis. *Rev Panam Salud Publica* (2014) 36:396–401.

6. de Camargo MC, Barros BCA, Fulone I, Silva MT, Silveira MS do N, Camargo IA de, Barberato-Filho S, del Fiol F de S, Lopes LC. Adverse Events in Patients With Rheumatoid Arthritis and Psoriatic Arthritis Receiving Long-Term Biological Agents in a Real-Life Setting. *Front Pharmacol* (2019) 10: doi: 10.3389/fphar.2019.00965

7. Fardet L, Petersen I, Nazareth I. Risk of cardiovascular events in people prescribed glucocorticoids with iatrogenic Cushing's syndrome: cohort study. *BMJ* (2012) 345:e4928–e4928. doi: 10.1136/bmj.e4928

8. Fardet L, Cabane J, Kettaneh A, Lebbe C, Flahault A. Corticosteroid-induced lipodystrophy is associated with features of the metabolic syndrome. *Rheumatology* (2007) 46:1102–1106. doi: 10.1093/rheumatology/kem062

9. Compston J. Glucocorticoid-induced osteoporosis: an update. *Endocrine* (2018) 61:7–16. doi: 10.1007/s12020-018-1588-2

10. Pereira RMR, Freire de Carvalho J. Glucocorticoid-induced myopathy. *Joint Bone Spine* (2011) 78:41–44. doi: 10.1016/j.jbspin.2010.02.025

11. Nussinovitch U, Freire de Carvalho J, Maria R. Pereira R, Shoenfeld Y. Glucocorticoids and the Cardiovascular System: State of the Art. *Curr Pharm Des* (2010) 16:3574–3585. doi: 10.2174/138161210793797870

12. Pereira RMR, Perez MO, Paula AP, Moreira C, Castro CHM, Zerbini CAF, Domiciano DS, de Azevedo E, Mendonca LMC, Shinzato MM, et al. Guidelines for the prevention and treatment of glucocorticoid-induced osteoporosis: an update of Brazilian Society of Rheumatology (2020). *Arch Osteoporos* (2021) 16:49. doi: 10.1007/s11657-021-00902-z

13. Wilson JC, Sarsour K, Gale S, Pethö-Schramm A, Jick SS, Meier CR. Incidence and Risk of Glucocorticoid-Associated Adverse Effects in Patients With Rheumatoid Arthritis. *Arthritis Care Res (Hoboken)* (2019) 71:498–511. doi: 10.1002/acr.23611

14. Roubille C, Richer V, Starnino T, McCourt C, McFarlane A, Fleming P, Siu S, Kraft J, Lynde C, Pope J, et al. The effects of tumour necrosis factor inhibitors, methotrexate, non-steroidal anti-inflammatory drugs and corticosteroids on cardiovascular events in rheumatoid arthritis, psoriasis and psoriatic arthritis: a systematic review and meta-analysis. *Ann Rheum Dis* (2015) 74:480–489. doi: 10.1136/annrheumdis-2014-206624

15. Esteves GP, Mazzolani BC, Smaira FI, Mendes ES, de Oliveira GG, Roschel H, Gualano B, Pereira RMR, Dolan E. Nutritional recommendations for patients undergoing prolonged glucocorticoid therapy. *Rheumatol Adv Pract* (2022) doi: 10.1093/rap/rkac029

16. Rausch Osthoff A-K, Niedermann K, Braun J, Adams J, Brodin N, Dagfinrud H, Duruoz T, Esbensen BA, Günther K-P, Hurkmans E, et al. 2018 EULAR recommendations for physical activity in people with inflammatory arthritis and osteoarthritis. *Ann Rheum Dis* (2018) 77:1251–1260. doi: 10.1136/annrheumdis-2018-213585

17. Perandini L, Sales de Oliveira D, Mello S, Camara N, Benatti F, Lima F, Borba E, Bonfa E, Sa-Pinto A, Roschel H, et al. Exercise training can attenuate the inflammatory milieu in women with systemic lupus erythematosus. *J Appl Physiol* (2014) 117:639–47.

18. Benatti F, Miyake C, Dantas W, Zambelli V, Shinjo S, Pereira R, Silva M, Sa-Pinto A, Borba E, Bonfa E, et al. Exercise Increases Insulin Sensitivity and Skeletal Muscle AMPK Expression in Systemic Lupus Erythematosus: A Randomized Controlled Trial. *Front Immunol* (2018) 9:906.

19. Prado D, Benatti F, de Sa-Pinto A, Hayashi A, Gualano B, Pereira R, Sallum A, Bonfa E, Silva C, Roschel H. Exercise training in childhood-onset systemic lupus erythematosus: a controlled randomized trial. *Arthritis Res Ther* (2013) 15:R46.

20. Gualano B, Bonfa E, Pereira RMRR, Silva CA. Physical activity for paediatric rheumatic diseases: standing up against old paradigms. *Nat Rev Rheumatol* (2017) 13:368–379. doi: 10.1038/nrrheum.2017.75

21. Minetto MA, Qaisar R, Agoni V, Motta G, Longa E, Miotti D, Pellegrino MA, Bottinelli R. Quantitative and qualitative adaptations of muscle fibers to glucocorticoids. *Muscle Nerve* (2015) 52:631–639. doi: 10.1002/mus.24572

22. Yamada Y, Tada M, Mandai K, Hidaka N, Inui K, Nakamura H. Glucocorticoid use is an independent risk factor for developing sarcopenia in patients with rheumatoid arthritis: from the CHIKARA study. *Clin Rheumatol* (2020) 39:1757–1764. doi: 10.1007/s10067-020-04929-4

23. Dalbeth N, Petrie KJ, House M, Chong J, Leung W, Chegudi R, Horne A, Gamble G, McQueen FM, Taylor WJ. Illness perceptions in patients with gout and the relationship with progression of musculoskeletal disability. *Arthritis Care Res (Hoboken)* (2011) 63:1605–1612. doi: 10.1002/acr.20570

24. Kanis JA, Johansson H, Oden A, Johnell O, de Laet C, Melton LJ, Tenenhouse A, Reeve J, Silman AJ, Pols HA, et al. A Meta-Analysis of Prior Corticosteroid Use and Fracture Risk. *Journal of Bone and Mineral Research* (2004) 19:893–899. doi: 10.1359/JBMR.040134

25. Bultink IEM, Lems WF. Osteoarthritis and Osteoporosis: What Is the Overlap? *Curr Rheumatol Rep* (2013) 15:328. doi: 10.1007/s11926-013-0328-0

26. Liao C, Chen H, Kuo Y, Tsauo J, Huang S, Liou T. Effects of Muscle Strength Training on Muscle Mass Gain and Hypertrophy in Older Adults With Osteoarthritis: A Systematic Review and Meta-Analysis. *Arthritis Care Res (Hoboken)* (2020) 72:1703–1718. doi: 10.1002/acr.24097

27. Woolf AD. Global burden of osteoarthritis and musculoskeletal diseases. *BMC Musculoskelet Disord* (2015) 16:S3. doi: 10.1186/1471-2474-16-S1-S3

28. Woolf AD, Vos T, March L. How to measure the impact of musculoskeletal conditions. *Best Pract Res Clin Rheumatol* (2010) 24:723–732. doi: 10.1016/j.berh.2010.11.002

29. Jin S, Hsieh E, Peng L, Yu C, Wang Y, Wu C, Wang Q, Li M, Zeng X. Incidence of fractures among patients with rheumatoid arthritis: a systematic review and meta-analysis. *Osteoporosis International* (2018) 29:1263–1275. doi: 10.1007/s00198-018-4473-1

30. Xue A-L, Wu S-Y, Jiang L, Feng A-M, Guo H-F, Zhao P. Bone fracture risk in patients with rheumatoid arthritis. *Medicine* (2017) 96:e6983. doi: 10.1097/MD.0000000000006983

31. van Staa TP, Leufkens HGM, Cooper C. The epidemiology of corticosteroid-induced osteoporosis: A meta-analysis. *Osteoporosis International* (2002) 13:777–787. doi: 10.1007/s001980200108

32. LoCascio V, Bonucci E, Imbimbo B, Ballanti P, Adami S, Milani S, Tartarotti D, DellaRocca C. Bone loss in response to long-term glucocorticoid therapy. *Bone Miner* (1990) 8:39–51. doi: 10.1016/0169-6009(91)90139-Q

33. Chotiyarnwong P, McCloskey E v. Pathogenesis of glucocorticoid-induced osteoporosis and options for treatment. *Nat Rev Endocrinol* (2020) 16:437–447. doi: 10.1038/s41574-020-0341-0

34. Cruz-Jentoft AJ, Bahat G, Bauer J, Boirie Y, Bruyère O, Cederholm T, Cooper C, Landi F, Rolland Y, Sayer AA, et al. Sarcopenia: revised European consensus on definition and diagnosis. *Age Ageing* (2019) 48:16–31. doi: 10.1093/ageing/afy169

35. Torii M, Hashimoto M, Hanai A, Fujii T, Furu M, Ito H, Uozumi R, Hamaguchi M, Terao C, Yamamoto W, et al. Prevalence and factors associated with sarcopenia in patients with rheumatoid arthritis. *Mod Rheumatol* (2019) 29:589–595. doi: 10.1080/14397595.2018.1510565

36. Wiegmann S, Armbrecht G, Borucki D, Buehring B, Buttgereit F, Detzer C, Schaumburg D, Zeiner KN, Dietzel R. Association between sarcopenia, physical performance and falls in patients with rheumatoid arthritis: a 1-year prospective study. *BMC Musculoskelet Disord* (2021) 22:885. doi: 10.1186/s12891-021-04605-x

37. Stanmore EK, Oldham J, Skelton DA, O'Neill T, Pilling M, Campbell AJ, Todd C. Fall Incidence and Outcomes of Falls in a Prospective Study of Adults With Rheumatoid Arthritis. *Arthritis Care Res (Hoboken)* (2013) 65:737–744. doi: 10.1002/acr.21892

38. Brenton-Rule A, Dalbeth N, Bassett S, Menz HB, Rome K. The incidence and risk factors for falls in adults with rheumatoid arthritis: A systematic review. *Semin Arthritis Rheum* (2015) 44:389–398. doi: 10.1016/j.semarthrit.2014.08.001

39. Deutz NEP, Bauer JM, Barazzoni R, Biolo G, Boirie Y, Bosy-Westphal A, Cederholm T, Cruz-Jentoft A, Krznariç Z, Nair KS, et al. Protein intake and exercise for optimal muscle function with aging: Recommendations from the ESPEN Expert Group. *Clinical Nutrition* (2014) 33:929–936. doi: 10.1016/j.clnu.2014.04.007

40. Arends J, Bachmann P, Baracos V, Barthelemy N, Bertz H, Bozzetti F, Fearon K, Hütterer E, Isenring E, Kaasa S, et al. ESPEN guidelines on nutrition in cancer patients. *Clinical Nutrition* (2017) 36:11–48. doi: 10.1016/j.clnu.2016.07.015

41. Sherman HC, Rose AR, Rose MS. CALCIUM REQUIREMENT OF MAINTENANCE IN MAN. *Journal of Biological Chemistry* (1920) 44:21–27. doi: 10.1016/S0021-9258(18)86268-8

42. Dolan E, Sale C. Protein and bone health across the lifespan. *Proceedings of the Nutrition Society* (2019) 78:45–55. doi: 10.1017/S0029665118001180

43. Wallace TCC, Frankenfeld CLL. Dietary Protein Intake above the Current RDA and Bone Health: A Systematic Review and Meta-Analysis. *J Am Coll Nutr* (2017) 36:481–496. doi: 10.1080/07315724.2017.1322924

44. Shams-White MM, Chung M, Du M, Fu Z, Insogna KL, Karlsen MC, LeBoff MS, Shapses SA, Sackey J, Wallace TC, et al. Dietary protein and bone health: a systematic review and meta-analysis from the National Osteoporosis Foundation. *Am J Clin Nutr* (2017)ajcn145110. doi: 10.3945/ajcn.116.145110

45. Wu A-M, Sun X-L, Lv Q-B, Zhou Y, Xia D-D, Xu H-Z, Huang Q-S, Chi Y-L. The Relationship between Dietary Protein Consumption and Risk of Fracture: a subgroup and dose-response meta-analysis of prospective cohort studies. *Sci Rep* (2015) 5:9151. doi: 10.1038/srep09151

46. Nunes EA, Colenso-Semple L, McKellar SR, Yau T, Ali MU, Fitzpatrick-Lewis D, Sherifali D, Gaudichon C, Tomé D, Atherton PJ, et al. Systematic review and meta-analysis of protein intake to support muscle mass and function in healthy adults. *J Cachexia Sarcopenia Muscle* (2022) 13:795–810. doi: 10.1002/jcsm.12922

47. Rizzoli R, Biver E, Bonjour J, Coxam V, Goltzman D, Kanis JA, Lappe J, Rejnmark L, Sahni S. Benefits and safety of dietary protein for bone health — an expert consensus paper endorsed by the European Society for Clinical and Economical Aspects of Osteoporororosis , Osteoarthritis , and Musculoskeletal Diseases and by the International Osteoporosis. *Osteoporosis International* (2018) 29:1933–48.

48. Buckinx F, Landi F, Cesari M, Fielding RA, Visser M, Engelke K, Maggi S, Dennison E, Al-Daghri NM, Allepaerts S, et al. Pitfalls in the measurement of muscle mass: a need for a reference standard. *J Cachexia Sarcopenia Muscle* (2018) 9:269–278. doi: 10.1002/jcsm.12268

49. Center for Disease Control. National Health and Nutrition Examination Survey (NHANES) Body Composition Procedures Manual. (2018) https://wwwn.cdc.gov/nchs/data/nhanes/2017-2018/manuals/Body_Composition_Procedures_Manual_2018.pdf

50. CDC/National Center for Health Statistics. NHANES Questionnaire Instruments. *NHANES Questionnaire Instruments* (2018) https://wwwn.cdc.gov/nchs/nhanes/continuousnhanes/questionnaires.aspx [Accessed September 18, 2022]

51. Tennant PWG, Murray EJ, Arnold KF, Berrie L, Fox MP, Gadd SC, Harrison WJ, Keeble C, Ranker LR, Textor J, et al. Use of directed acyclic graphs (DAGs) to identify confounders in applied health research: review and recommendations. *Int J Epidemiol* (2021) 50:620–632. doi: 10.1093/ije/dyaa213

462 52. Papageorgiou M, Martin D, Colgan H, Cooper S, Greeves JP, Tang JCY, Fraser WD, Elliott-Sale
463 KJ, Sale C. Bone metabolic responses to low energy availability achieved by diet or exercise in
464 active eumenorrheic women. *Bone* (2018) 114:181–188. doi: 10.1016/j.bone.2018.06.016

465 53. Tomova GD, Arnold KF, Gilthorpe MS, Tennant PWG. Adjustment for energy intake in
466 nutritional research: a causal inference perspective. *Am J Clin Nutr* (2022) 115:189–198. doi:
467 10.1093/ajcn/nqab266

468 54. CDC/National Center for Health Statistics. NHANES Survey Methods and Analytic Guidelines.
469 *https://wwwn.cdc.gov/nchs/nhanes/analyticguidelines.aspx* (2022)

470 55. Lumley T. Analysis of Complex Survey Samples. *J Stat Softw* (2004) 9: doi:
471 10.18637/jss.v009.i08

472 56. Phillips SM, Chevalier S, Leidy HJ. Protein "requirements" beyond the RDA: implications for
473 optimizing health. *Applied Physiology, Nutrition, and Metabolism* (2016) 41:565–572. doi:
474 10.1139/apnm-2015-0550

475 57. Tipton KD, Ferrando AA, Phillips SM, Doyle D, Wolfe RR. Postexercise net protein synthesis in
476 human muscle from orally administered amino acids. *American Journal of Physiology-*
477 *Endocrinology and Metabolism* (1999) 276:E628–E634. doi: 10.1152/ajpendo.1999.276.4.E628

478 58. Volpi E, Ferrando AA, Yeckel CW, Tipton KD, Wolfe RR. Exogenous amino acids stimulate net
479 muscle protein synthesis in the elderly. *Journal of Clinical Investigation* (1998) 101:2000–
480 2007. doi: 10.1172/JCI939

481 59. Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol,
482 Protein, and Amino Acids. Washington, D.C., D.C.: National Academies Press (2005). doi:
483 10.17226/10490

484 60. Burd NA, Gorissen SH, van Loon LJC. Anabolic Resistance of Muscle Protein Synthesis with
485 Aging. *Exerc Sport Sci Rev* (2013) 41:169–173. doi: 10.1097/JES.0b013e318292f3d5

486 61. Rennie MJ. Anabolic resistance in critically ill patients. *Crit Care Med* (2009) 37:S398–S399.
487 doi: 10.1097/CCM.0b013e3181b6ec1f

488 62. Durham WJ, Dillon EL, Sheffield-Moore M. Inflammatory burden and amino acid metabolism
489 in cancer cachexia. *Curr Opin Clin Nutr Metab Care* (2009) 12:72–77. doi:
490 10.1097/MCO.0b013e32831cef61

491 63. Troncoso R, Paredes F, Parra V, Gatica D, Vásquez-Trincado C, Quiroga C, Bravo-Sagua R,
492 Lopez-Crisosto C, Rodriguez AE, Oyarzuń AP, et al. Dexamethasone-induced autophagy
493 mediates muscle atrophy through mitochondrial clearance. *Cell Cycle* (2014) 13:2281–2295.
494 doi: 10.4161/cc.29272

495 64. Rafii M, Chapman K, Owens J, Elango R, Campbell WW, Ball RO, Pencharz PB, Courtney-Martin
496 G. Dietary Protein Requirement of Female Adults >65 Years Determined by the Indicator
497 Amino Acid Oxidation Technique Is Higher Than Current Recommendations. *J Nutr* (2015)
498 145:18–24. doi: 10.3945/jn.114.197517

65. Tang M, McCabe GP, Elango R, Pencharz PB, Ball RO, Campbell WW. Assessment of protein requirement in octogenarian women with use of the indicator amino acid oxidation technique. *Am J Clin Nutr* (2014) 99:891–898. doi: 10.3945/ajcn.112.042325

66. Heaney RP. Effects of protein on the calcium economy. *Int Congr Ser* (2007) 1297:191–197. doi: 10.1016/j.ics.2006.08.025

67. THISSEN J-P, KETELSLEGERS J-M, UNDERWOOD LE. Nutritional Regulation of the Insulin-Like Growth Factors*. *Endocr Rev* (1994) 15:80–101. doi: 10.1210/edrv-15-1-80

68. Mangano KM, Sahni S, Kiel DP, Tucker KL, Dufour AB, Hannan MT. Bone Mineral Density and Protein-Derived Food Clusters from the Framingham Offspring Study. *J Acad Nutr Diet* (2015) 115:1605-1613.e1. doi: 10.1016/j.jand.2015.04.001

69. Benedetti MG, Furlini G, Zati A, Letizia Mauro G. The Effectiveness of Physical Exercise on Bone Density in Osteoporotic Patients. *Biomed Res Int* (2018) 2018:1–10. doi: 10.1155/2018/4840531

70. Kirwan RP, Mazidi M, Rodríguez García C, Lane KE, Jafari A, Butler T, Perez de Heredia F, Davies IG. Protein interventions augment the effect of resistance exercise on appendicular lean mass and handgrip strength in older adults: a systematic review and meta-analysis of randomized controlled trials. *Am J Clin Nutr* (2022) 115:897–913. doi: 10.1093/ajcn/nqab355

71. Volpi E, Nazemi R, Fujita S. Muscle tissue changes with aging. *Curr Opin Clin Nutr Metab Care* (2004) 7:405–410. doi: 10.1097/01.mco.0000134362.76653.b2

72. Beasley JM, LaCroix AZ, Larson JC, Huang Y, Neuhouser ML, Tinker LF, Jackson R, Snetselaar L, Johnson KC, Eaton CB, et al. Biomarker-calibrated protein intake and bone health in the Women's Health Initiative clinical trials and observational study. *Am J Clin Nutr* (2014) 99:934–940. doi: 10.3945/ajcn.113.076786

73. Sahni S, Broe KE, Tucker KL, McLean RR, Kiel DP, Cupples LA, Hannan MT. Association of total protein intake with bone mineral density and bone loss in men and women from the Framingham Offspring Study. *Public Health Nutr* (2014) 17:2570–2576. doi: 10.1017/S1368980013002875

74. Gibson RS, Charrondiere UR, Bell W. Measurement Errors in Dietary Assessment Using Self-Reported 24-Hour Recalls in Low-Income Countries and Strategies for Their Prevention. *Advances in Nutrition: An International Review Journal* (2017) 8:980–991. doi: 10.3945/an.117.016980

Acknowledgements

The authors would like to express their gratitude towards Professor Rosa Maria R. Pereira (*In Memoriam*) for her invaluable contributions to this manuscript and the overall research program.

Author Contribution Statement

Conceptualization: Gabriel Perri Esteves, Eimear Dolan; Methodology: Gabriel Perri Esteves, Paul Swinton; Formal analysis and investigation: Gabriel Perri Esteves, Paul Swinton, Eimear Dolan; Writing - original draft preparation: Gabriel Perri Esteves, Eimear Dolan; Writing - review and editing: Gabriel Perri Esteves, Paul Swinton, Craig Sale, Hamilton Roschel, Bruno Gualano, Eimear Dolan; Supervision: Eimear Dolan, Paul Swinton, Craig Sale, Hamilton Roschel, Bruno Gaulano.

Funding

G.P.E., B.G. and E.D. are supported by research grants from the São Paulo Research Foundation [FAPESP grant #2020/07860-9, 2017/13552-2, 2019/05616-6; 2019/26899-6].

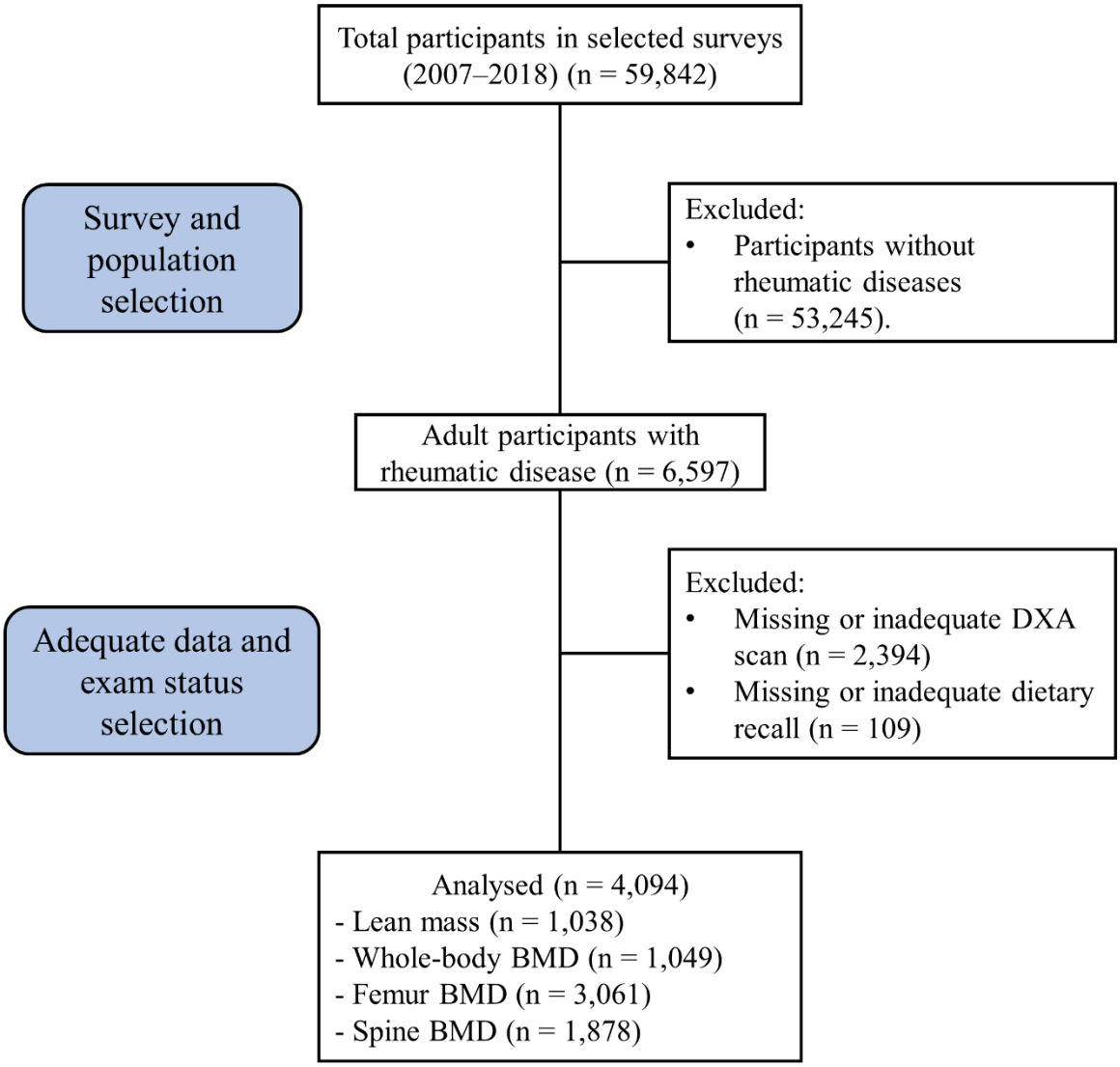
Ethical Approval

The original NHANES survey protocol was approved by the CDC and National Center for Health Statistics (NCHS) Ethics Review Board, and all participants provided written informed consent.

Competing Interests

The authors declare no competing interests.

552 **Figures**



553

554 **Fig 1** Study flowchart

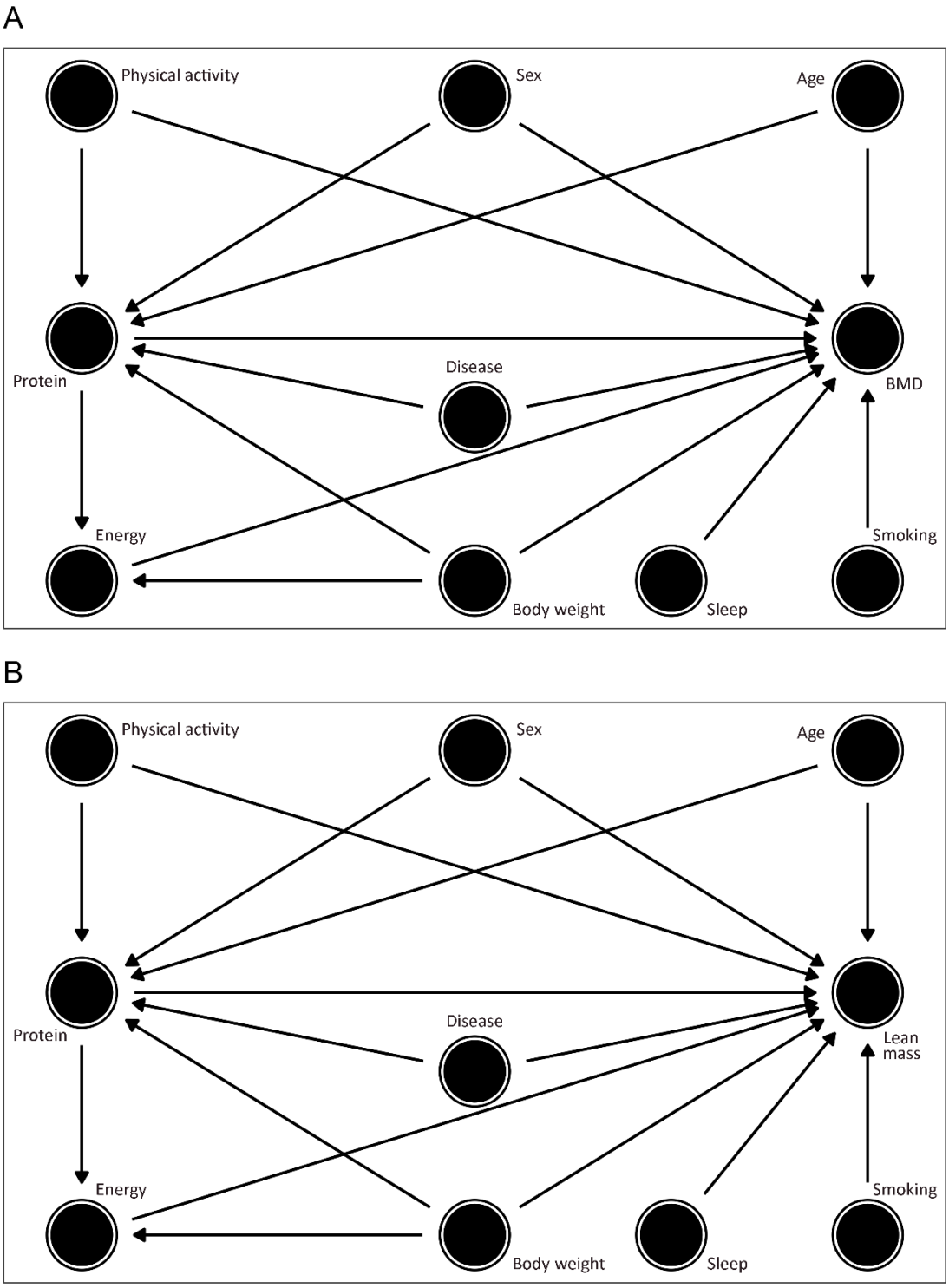


Fig 2 Directed acyclic graph showing potential causal paths between variables of interest related to A) bone mineral density (BMD) and B) lean mass

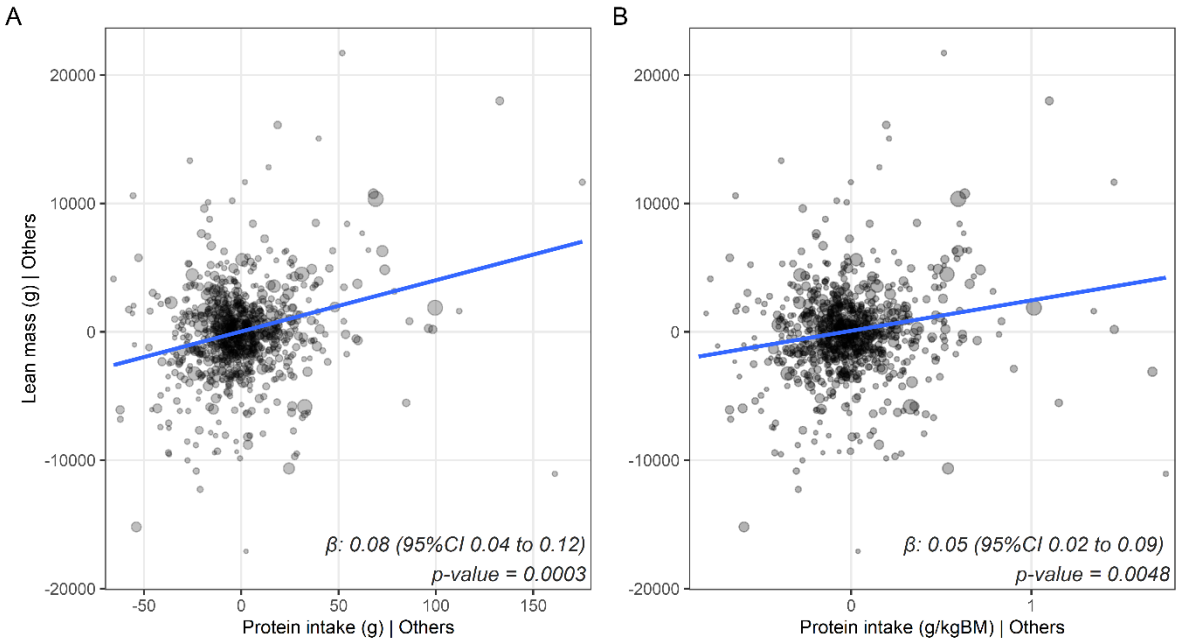
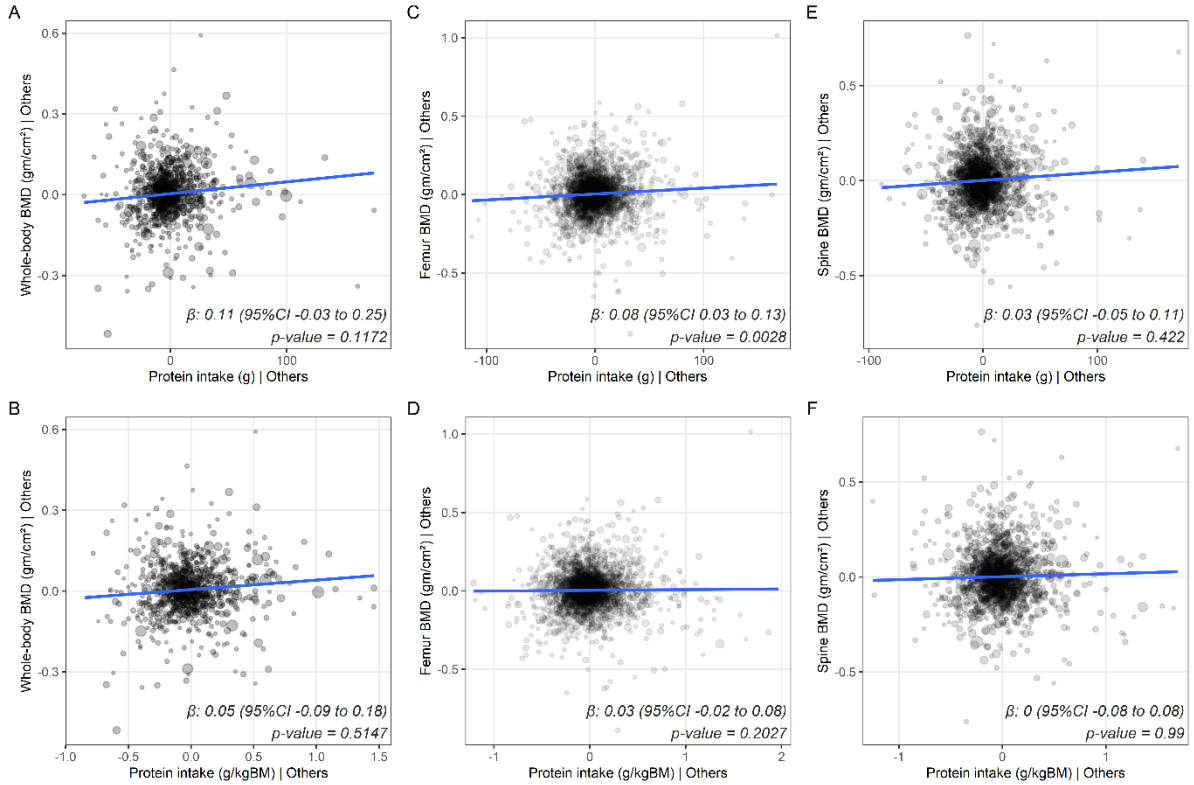


Fig 3A Partial regression plot showing the relationship between lean means and protein intake (g or g/kgBM/d, A and B) after adjusting for confounding variables. Dot size varies according to sample weights (bigger means a higher weight in the model). The blue line indicates the linear regression line fitted through the data. Beta coefficients and p-values resulting from the respective adjusted multivariable models are also displayed. β = beta (standardized coefficient).

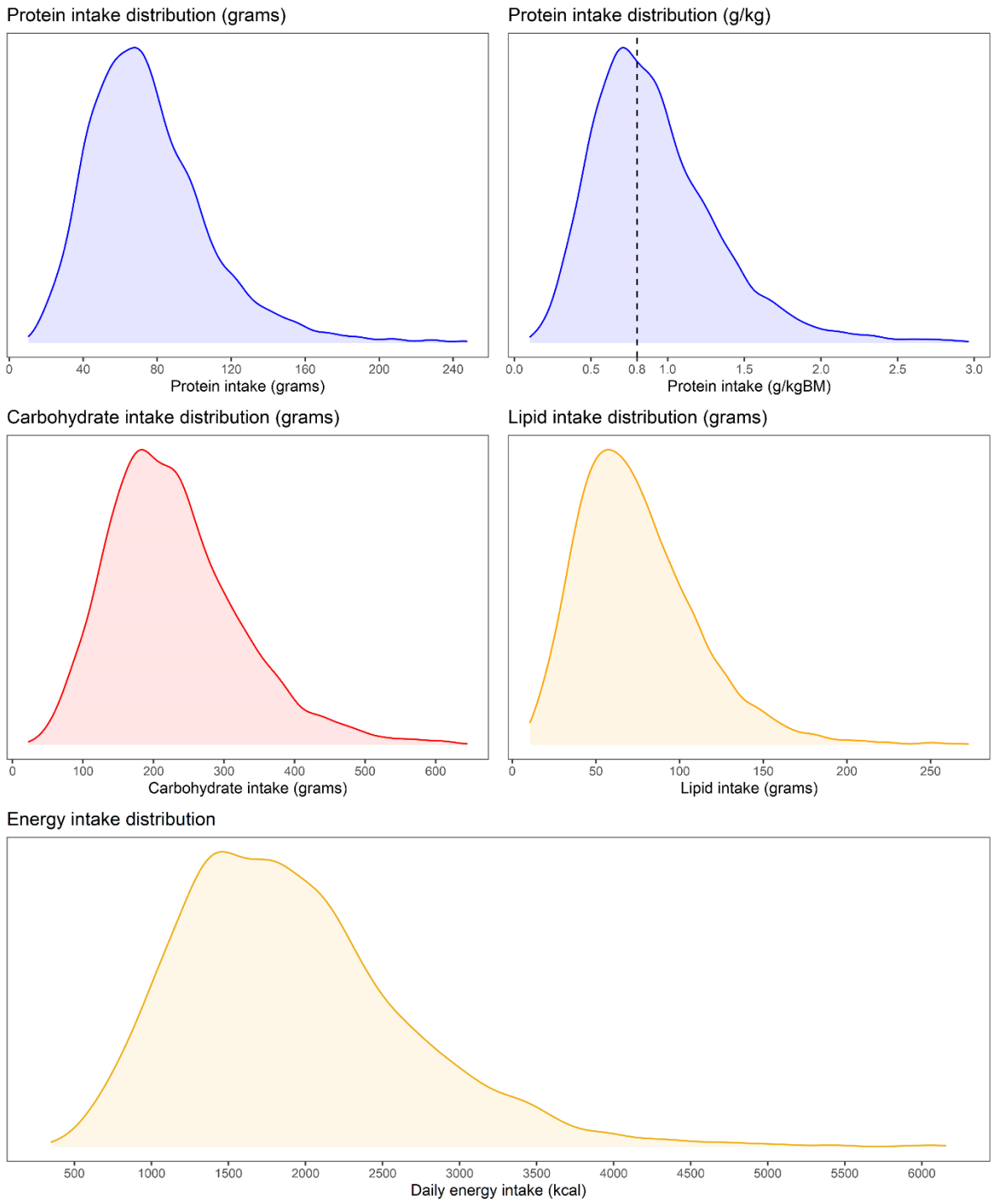


564

565 **Fig 3B** Partial regression plot showing the relationship between bone mineral density and protein
566 intake (g or g/kgBM/d) for whole-body (A and B), femur (C and D) and spine (E and F) imaging sites,
567 after adjusting for confounding variables. Dot size varies according to sample weights. The blue line
568 indicates the linear regression line fitted through the data. Beta coefficients and p-values resulting
569 from the respective adjusted multivariable models are also displayed. β = beta (standardized
570 coefficient)

571

572

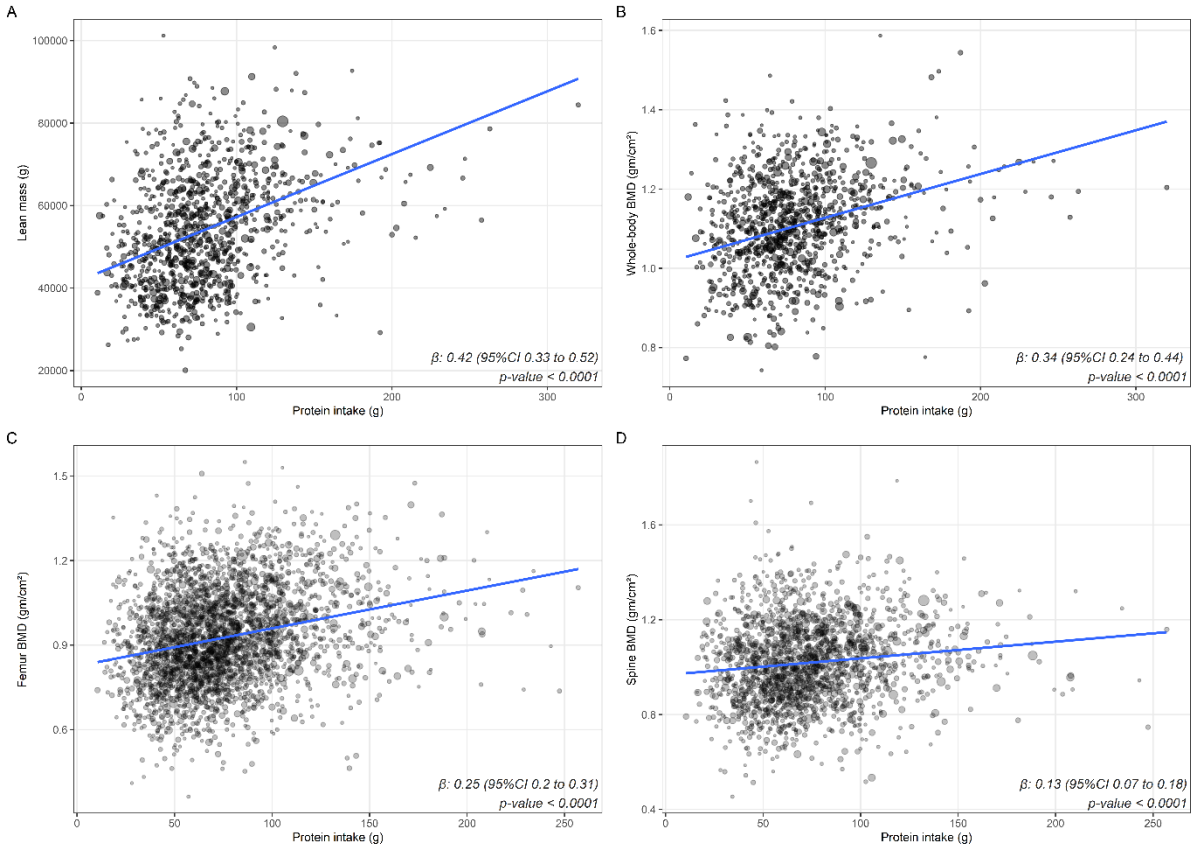


573

574 **Supplementary Figure S1** Density plots showing macronutrient and calorie intake distributions

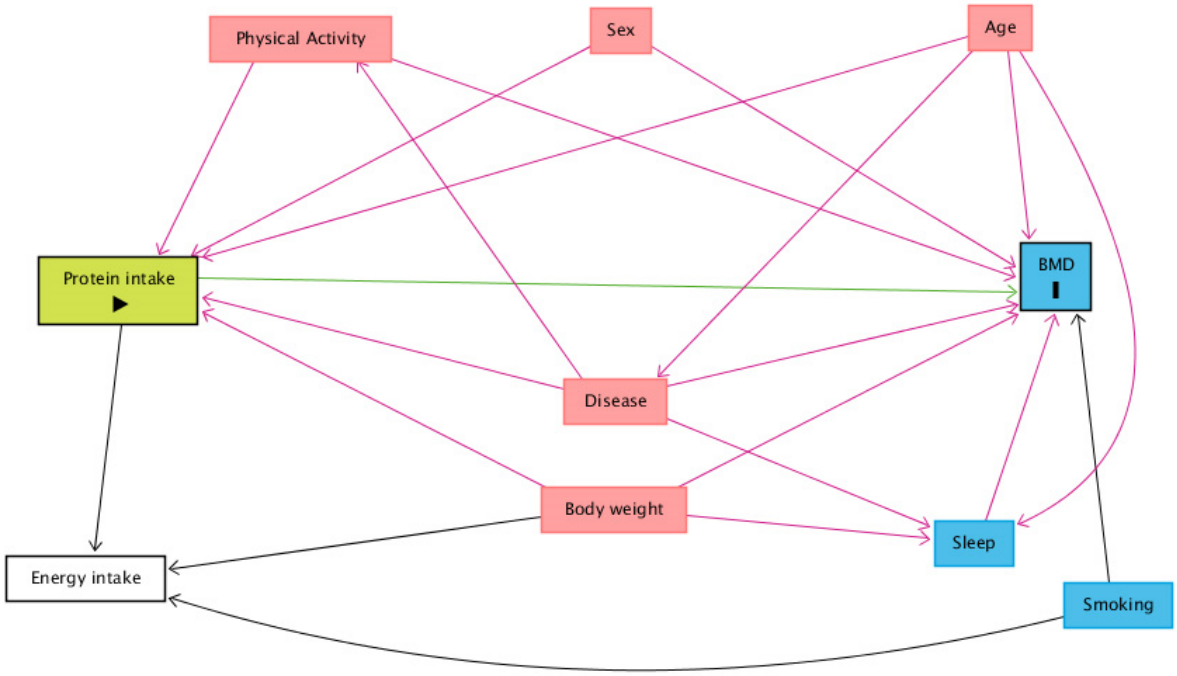
575

576



577

578 **Supplementary Figure S2** Scatterplots showing the unadjusted association between protein intake (g)
579 and A) lean mass (g of body mass), B) whole-body BMD, C) femur BMD, and D) spine BMD. Beta
580 coefficients and p-values resulting from the respective unadjusted model are also displayed. β = beta
581 (standardized coefficient)

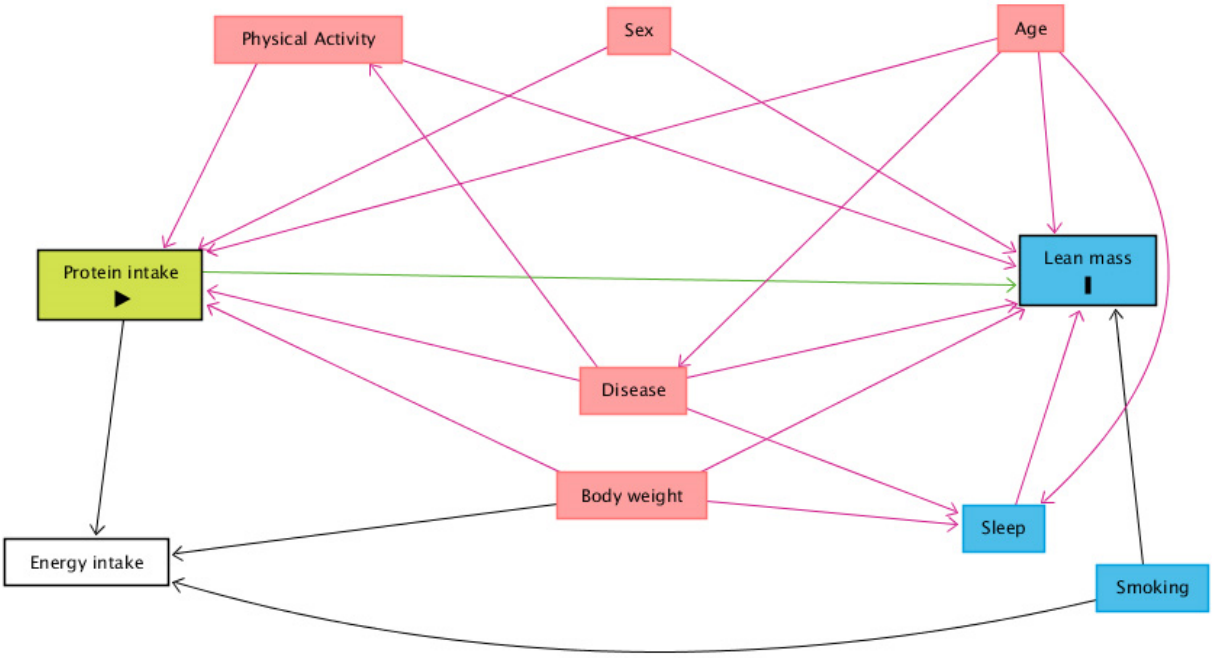


582

583 **Supplementary Figure S3** Directed acyclic graphs showing proposed causal pathways between
584 variables for bone mineral density outcome. Main independent variable is shown in green. Biasing paths
585 and variables that introduce confounding are shown in pink. BMD = bone mineral density. Made with
586 dagitty.net

587

588



589

590 **Supplementary Figure S4** Directed acyclic graphs showing proposed causal pathways between
591 variables for lean mass outcome. Main independent variable is shown in green. Biasing paths and
592 variables that introduce confounding are shown in pink. Made with dagitty.net