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

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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) ($\beta$=0.08 (95%CI 0.04-0.14), p-value=0.0002) or when relative to body mass (g/kgBM/d) ($\beta$=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 ($\beta$=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·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).
### Table 1. Participant’s characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Overall, $N = 4,094$</th>
<th>Rheumatoid arthritis, $N = 1094$</th>
<th>Osteoarthritis, $N = 2,153$</th>
<th>Psoriatic arthritis, $N = 56$</th>
<th>Gout, $N = 791$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>59 (13)</td>
<td>61 (13)</td>
<td>60 (13)</td>
<td>50 (11)</td>
<td>58 (13)</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>1,853 (45%)</td>
<td>604 (76%)</td>
<td>787 (37%)</td>
<td>25 (45%)</td>
<td>437 (40%)</td>
</tr>
<tr>
<td>Female</td>
<td>2,241 (55%)</td>
<td>187 (24%)</td>
<td>1,366 (63%)</td>
<td>31 (55%)</td>
<td>657 (60%)</td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Mexican American</td>
<td>392 (10%)</td>
<td>57 (7%)</td>
<td>168 (8%)</td>
<td>3 (5%)</td>
<td>164 (15%)</td>
</tr>
<tr>
<td>Other Hispanic</td>
<td>320 (8%)</td>
<td>46 (6%)</td>
<td>154 (7%)</td>
<td>4 (7%)</td>
<td>116 (11%)</td>
</tr>
<tr>
<td>Non-Hispanic White</td>
<td>2,193 (54%)</td>
<td>390 (49%)</td>
<td>1,337 (62%)</td>
<td>34 (61%)</td>
<td>432 (39%)</td>
</tr>
<tr>
<td>Non-Hispanic Black</td>
<td>865 (21%)</td>
<td>206 (26%)</td>
<td>337 (16%)</td>
<td>10 (18%)</td>
<td>312 (29%)</td>
</tr>
<tr>
<td>Other</td>
<td>324 (8%)</td>
<td>92 (12%)</td>
<td>157 (7%)</td>
<td>5 (9%)</td>
<td>70 (6%)</td>
</tr>
<tr>
<td>Height (m)</td>
<td>166.5 (10.3)</td>
<td>170.8 (9.6)</td>
<td>165.7 (10.3)</td>
<td>166.7 (9.5)</td>
<td>165.1 (10.1)</td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td>85.0 (21.6)</td>
<td>89.8 (20.2)</td>
<td>84.3 (22.1)</td>
<td>85.18 (18.6)</td>
<td>82.8 (21.3)</td>
</tr>
<tr>
<td>Characteristic</td>
<td>Overall, N = 4,094</td>
<td>Rheumatoid arthritis, N = 1,094</td>
<td>Osteoarthritis, N = 2,153</td>
<td>Psoriatic arthritis, N = 56</td>
<td>Gout, N = 791</td>
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</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>30.5 (6.9)</td>
<td>30.6 (6.0)</td>
<td>30.6 (7.1)</td>
<td>30.6 (6.4)</td>
<td>30.2 (7.0)</td>
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<tr>
<td>Energy intake (kcal)</td>
<td>1,933 (774)</td>
<td>2,001 (790)</td>
<td>1,92 (755)</td>
<td>2,041 (808)</td>
<td>1,891 (795)</td>
</tr>
<tr>
<td>Carbohydrate intake (g)</td>
<td>231 (98)</td>
<td>233 (96)</td>
<td>231 (97)</td>
<td>238 (97)</td>
<td>229 (99)</td>
</tr>
<tr>
<td>Protein intake (g)</td>
<td>75 (33)</td>
<td>80 (34)</td>
<td>75 (32)</td>
<td>77 (32)</td>
<td>73 (33)</td>
</tr>
<tr>
<td>Protein intake (g/kgBM/d)</td>
<td>0.92 (0.43)</td>
<td>0.92 (0.42)</td>
<td>0.93 (0.42)</td>
<td>0.94 (0.40)</td>
<td>0.92 (0.47)</td>
</tr>
<tr>
<td>Fat intake (g)</td>
<td>75 (36)</td>
<td>77 (37)</td>
<td>75 (35)</td>
<td>81 (41)</td>
<td>73 (37)</td>
</tr>
<tr>
<td>Lean mass (kg)</td>
<td>54.1 (13.3)</td>
<td>60.2 (13.1)</td>
<td>53.4 (13.3)</td>
<td>52.0 (12.3)</td>
<td>52.1 (12.8)</td>
</tr>
<tr>
<td>Whole body BMD (g/cm²)</td>
<td>1.113 (0.121)</td>
<td>1.151 (0.116)</td>
<td>1.109 (0.119)</td>
<td>1.081 (0.112)</td>
<td>1.103 (0.124)</td>
</tr>
<tr>
<td>Femur BMD (g/cm²)</td>
<td>0.931 (0.169)</td>
<td>0.981 (0.167)</td>
<td>0.912 (0.166)</td>
<td>0.952 (0.143)</td>
<td>0.926 (0.169)</td>
</tr>
<tr>
<td>Femur BMD T-scores</td>
<td>-0.08 (1.30)</td>
<td>0.31 (1.23)</td>
<td>-0.23 (1.29)</td>
<td>0.07 (1.10)</td>
<td>-0.11 (1.31)</td>
</tr>
<tr>
<td>Characteristic</td>
<td>Overall, $N = 4,094$</td>
<td>Rheumatoid arthritis, $N = 1094$</td>
<td>Osteoarthritis, $N = 2,153$</td>
<td>Psoriatic arthritis, $N = 56$</td>
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<tr>
<td>Spine BMD (g/cm²)</td>
<td>1.01 (0.17)</td>
<td>1.07 (0.17)</td>
<td>1.00 (0.17)</td>
<td>0.98 (0.14)</td>
<td>1.00 (0.17)</td>
</tr>
<tr>
<td>Spine BMD T-scores</td>
<td>-0.39 (1.50)</td>
<td>0.08 (1.50)</td>
<td>-0.51 (1.48)</td>
<td>-0.69 (1.22)</td>
<td>-0.48 (1.47)</td>
</tr>
</tbody>
</table>

Data are mean (SD) for continuous variables and n (%) for categorical variables.
Table 2. Unadjusted and adjusted linear regression models for all dependent variables (standardized coefficients)

<table>
<thead>
<tr>
<th>Term</th>
<th>Unadjusted</th>
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<th>Adjusted</th>
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<tr>
<td></td>
<td></td>
<td>β coefficient</td>
<td>95% CI</td>
<td>p-value</td>
<td>β coefficient</td>
<td>95% CI</td>
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<tr>
<td>Lean mass (n = 1,038)</td>
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<td>Unadjusted</td>
<td></td>
<td>Adjusted</td>
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<tr>
<td>Protein intake (g)</td>
<td></td>
<td>0.42</td>
<td>0.33; 0.52</td>
<td>&lt;0.0001</td>
<td>0.08</td>
<td>0.04; 0.12</td>
</tr>
<tr>
<td>Protein intake (g/kgBM/d)</td>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.05</td>
<td>0.02; 0.09</td>
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<tr>
<td>Whole body BMD (n = 1,049)</td>
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<td>Unadjusted</td>
<td></td>
<td>Adjusted</td>
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<tr>
<td>Protein intake (g)</td>
<td></td>
<td>0.34</td>
<td>0.24; 0.44</td>
<td>&lt;0.0001</td>
<td>0.11</td>
<td>-0.03; 0.25</td>
</tr>
<tr>
<td>Protein intake (g/kgBM/d)</td>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.05</td>
<td>-0.09; 0.18</td>
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<tr>
<td>Femur BMD (n = 3,061)</td>
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<td>Unadjusted</td>
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<td>Adjusted</td>
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<tr>
<td>Protein intake (g)</td>
<td></td>
<td>0.25</td>
<td>0.20; 0.31</td>
<td>&lt;0.0001</td>
<td>0.08</td>
<td>0.03; 0.13</td>
</tr>
<tr>
<td>Protein intake (g/kgBM/d)</td>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.03</td>
<td>-0.02; 0.08</td>
</tr>
<tr>
<td>Spine BMD (n = 1,878)</td>
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<td>Unadjusted</td>
<td></td>
<td>Adjusted</td>
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<tr>
<td>Protein intake (g)</td>
<td></td>
<td>0.13</td>
<td>0.07; 0.18</td>
<td>&lt;0.0001</td>
<td>0.03</td>
<td>-0.05; 0.11</td>
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<tr>
<td>Protein intake (g/kgBM/d)</td>
<td></td>
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<td>-</td>
<td>-</td>
<td>0.00</td>
<td>-0.08; 0.08</td>
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</table>
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%
decline 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**


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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 Gaulano, Eimear Dolan; Supervision: Eimear Dolan, Paul Swinton, Craig Sale, Hamilton Roschel, Bruno Gaulano.

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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.
Figures

Total participants in selected surveys (2007–2018) (n = 59,842)

Excluded:
- Participants without rheumatic diseases (n = 53,245).

Adult participants with rheumatic disease (n = 6,597)

Excluded:
- Missing or inadequate DXA scan (n = 2,394)
- Missing or inadequate dietary recall (n = 109)

Adequate data and exam status selection

Analysed (n = 4,094)
- Lean mass (n = 1,038)
- Whole-body BMD (n = 1,049)
- Femur BMD (n = 3,061)
- Spine BMD (n = 1,878)

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. $\beta = \text{beta (standardized coefficient)}$. 
Fig 3B Partial regression plot showing the relationship between bone mineral density and protein intake (g or g/kgBM/d) for whole-body (A and B), femur (C and D) and spine (E and F) imaging sites, after adjusting for confounding variables. Dot size varies according to sample weights. 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. $\beta = \text{beta}$ (standardized coefficient)
Supplementary Figure S1 Density plots showing macronutrient and calorie intake distributions
Supplementary Figure S2 Scatterplots showing the unadjusted association between protein intake (g) and A) lean mass (g of body mass), B) whole-body BMD, C) femur BMD, and D) spine BMD. Beta coefficients and p-values resulting from the respective unadjusted model are also displayed. $\beta = \text{beta (standardized coefficient)}$
**Supplementary Figure S3** Directed acyclic graphs showing proposed causal pathways between variables for bone mineral density outcome. Main independent variable is shown in green. Biasing paths and variables that introduce confounding are shown in pink. BMD = bone mineral density. Made with dagitty.net
Supplementary Figure S4 Directed acyclic graphs showing proposed causal pathways between variables for lean mass outcome. Main independent variable is shown in green. Biasing paths and variables that introduce confounding are shown in pink. Made with dagitty.net