PERSPECTIVE

Protein Intake and Human Health: Implications of Units of Protein Intake

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ABSTRACT

Understanding the health effects of protein intake is bedeviled by a number of factors, including protein quality and source. In addition, different units, including grams, grams per kilogram body weight (g/kg BW), and percent energy, may contribute to confusion about protein's effects on health, especially BW-based units in increasingly obese populations. We aimed to review the literature and to conduct a modeling demonstration of various units of protein intake in relation to markers of cardiometabolic health. Data from the Framingham Heart Study Offspring ($n = 1847$; 60.3 y; 62.5% women) and Third Generation ($n = 2548$; 46.2 y; 55.3% women) cohorts and the NHANES 2003–04 ($n = 1625$; 46.2 y; 49.7% women) and 2005–06 ($n = 1347$; 43.7 y; 49.5% women) cycles were used to model cross-sectional associations between 7 protein units (grams, percent energy, g/kg ideal BW, g/kg actual BW, BW-adjusted g/kg actual BW, g/kg lean BW, and g/kg fat-free BW) and 9 cardiometabolic outcomes (fasting glucose, systolic and diastolic blood pressure, total and HDL cholesterol, triglycerides, BMI, waist circumference, and estimated glomerular filtration rate). The literature review indicated the use of myriad units of protein intake, with differential results on cardiometabolic outcomes. The modeling demonstration showed units expressed in BW were confounded by BW, irrespective of outcome. Units expressed in grams, percent energy, and ideal BW showed similar results, with or without adjustment for body size. After adjusting for BW, results of units expressed in BW aligned with results of grams, percent energy, and ideal BW. In conclusion, protein intake in cardiometabolic health appears to depend on protein's unit of expression. Authors should be specific about the use of WHO (g/kg ideal BW) compared with US (g/kg actual BW) units, and ideally use gram or percent energy in observational studies. In populations where overweight/obesity are prevalent, intake based on actual BW should be reevaluated. *Adv Nutr* 2021;12:71–88.

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Introduction

US dietary recommendations for protein intake in adults have historically been expressed in grams per kilograms (g/kg) of body weight (BW). The current US RDA is 0.8 g/kg BW, with an acceptable macronutrient distribution range (AMDR) of 10–35% of energy intake (estimated to equate to 1.05–3.67 g/kg BW when based on reference BWs) (1, 2). The FAO of the UN/WHO recommendation is 0.83 g/kg BW, which was revised upward from 0.75 g/kg BW in 2002 (3, 4). In 2012, the European Food Safety Authority (EFSA) established its population reference intake for protein as 0.83 g/kg BW for all adults (5). Other European recommendations, including those of the Nordic countries (6), German-speaking (Germany, Austria, Switzerland) countries (7), and France (8), specify similar recommendations.

Brief history of WHO and US recommendations

In 1941, the RDA was expressed in grams with a reference BW of 70 and 56 kg for men and women, respectively (9). At these BWs, the daily recommended intakes were 70 g for men and 60 g for women, which translate into 1.00 and 1.07 g/kg BW, respectively (9). Protein requirements were not initially expressed in terms of BW, but largely have been since 1941 (**Supplemental Table 1**). In addition, they have shifted slightly over the decades, depending on the recommending body and new evidence, before settling at 0.8 g/kg BW in the United States some 20 y ago [rounded up from the FAO/WHO 1985 estimate of 0.75 g/kg BW (4)]. Exceptions to these recommendations have been indicated for select populations, notably active-duty military, who face extraordinary climates/environments and endure substantial physical training (10). There are a few

other groups with unique requirements, including those experiencing (childhood) growth, pregnancy, or lactation. Otherwise, recommended protein requirements in generally healthy adults do not vary; requirements are assumed to be largely independent of factors such as age, sex, body composition, and lifestyle, despite ongoing calls to reconsider needs, particularly for the preservation of muscle mass in those aging (2, 11, 12), in very active populations (13, 14), and in the context of acute or chronic kidney disease (15, 16). When the most recent protein DRIs were developed in 2005, there was insufficient evidence to support the revision of existing recommendations for protein for healthy adults (including older adults) (1). However, since then, evidence has accumulated that the DRIs should be reevaluated, particularly for aging adults and those with higher metabolic needs.

Importantly, although the Institute of Medicine (IOM), the WHO, and other recommendations express intake in g/kg BW, they differ in their specificity about whether BW refers to an ideal or actual BW. In several places, the WHO 1985 and 2007 reports discussed protein intake in relation to body composition as well as ideal weight. Notably, the 1985 report suggests that body composition may be a determining factor in true requirements (Section 3.3); that the g/kg BW recommendation is for BWs "within the acceptable range" (Section 8.2.2); that intake should be modified so as to achieve a healthy weight; and that "the safe level of protein for those who are overweight should be based on the median acceptable weight for height and not on the actual weight" (Section 9.1.1) (4). In the WHO 2007 report, the language similarly points to the "safe" level of intake of 0.83g/kg BW as applicable to all adults "for all body weights within the acceptable range" (3). Meanwhile, recommendations for the German-speaking European countries are explicit that the recommended intake of protein (0.8 g/kg BW for adults <65 y old) applies only to those with BMIs <25 kg/m^2, and those with BMIs >25 kg/m^2 should use their "normal" BW to estimate requirements (7). Note also that there are yet other protein recommendations—for example, for critically ill patients, or those with diabetes (in Japan)—which outline protein (and energy) needs according to an ideal BW and, in doing so, distinguish needs of overweight and obese individuals from normal-weight individuals, while distinguishing protein from energy requirements in these populations (17, 18). Together, and including the data shown in **Supplemental Table 1**, it appears that protein recommendations from the WHO and select other groups are based on BWs within ideal ranges for height, which are defined (at least by the WHO) as BMI ranges of 20.1–25.0 for men and 18.7–23.8 for women [see Annex 2, Table C, in Joint FAO/WHO/United Nations University Expert Consultation on Energy and Protein Requirements (4)].

Interestingly, this distinction between ideal and actual BW by the WHO is not explicit in US protein recommendations. Although "reference body weights" exist in the IOM DRI report (1), as well as in the EFSA report (5), these seem to have been provided solely as a basis for the calculation of g/d values, rather than to indicate that protein intake should depend on an ideal weight or weight range.

Contextualizing units within the obesity epidemic

Meanwhile, trends of overweight and obesity continue to increase, with the recent prevalence estimates (2015–16) of obesity at 40% of the US adult population (19). Concurrently, age-adjusted protein intake as a proportion of energy has barely fluctuated (~1–2%) over the 1971 to 2010 time period, with mean adjusted absolute intake also fluctuating only slightly (81.6–84.4 g/d) (20).

Several fundamental problems arise with the confluence of protein needs, expressed in various units, and the context of the obesity epidemic. First, it is unclear the extent to which the g/kg BW measure is relevant in populations where 70% are overweight/obese; that is, where BW is dominated not by lean mass but by excess fat mass, if indeed intake is based on actual BW (as in US recommendations) rather than ideal BW (as in WHO or German-speaking countries' recommendations). This leads to a second potential problem: the use of the ratio measure (g/kg BW) in studies investigating associations between protein intake and health/disease outcomes is inherently confounded by BW, and possibly BMI, a risk factor for most disease outcomes. Third, as pointed out above, this confusion is evident in the literature. As a result of this, studies using different units to quantify protein intake

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Supplemental Tables 1–16, Supplemental Figures 1–4, and Supplementary Data (2 files) are available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/advances.
Data described in the manuscript are publicly available from the National Heart, Lung and Blood Institute Biologic Specimen and Data Repository Information Coordinating Center, with permission, and from the CDC, NHANES. The analytic code will be made available upon request.
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Abbreviations used: AMDR, acceptable macronutrient distribution range; BioLINCC, Biologic Specimen and Data Repository Information Coordinating Center; BW, body weight; CKD, chronic kidney disease; CVD, cardiovascular disease; DBP, diastolic blood pressure; EFSA, European Food Safety Authority; eGFR, estimated glomerular filtration rate; FHS, Framingham Heart Study; Gen3, FHS Third Generation cohort; HbA1c, glycated hemoglobin; IOM, Institute of Medicine; NCHS, National Center for Health Statistics; NHLBI, National Heart, Lung, and Blood Institute; RHF, renal hyperfiltration; SBP, systolic blood pressure; T2D, type 2 diabetes; WC, waist circumference.

will likely reach very different conclusions about protein's effects on health/disease, making summary statements of such effects exceedingly challenging. To our knowledge, this phenomenon has not yet been reviewed or demonstrated in the literature, representing a considerable gap in knowledge.

Therefore, our primary goal was to demonstrate that the association between dietary protein and health outcomes differs with the use of varying units of protein intake, which we show via a modeling demonstration of 9 different units of protein intake and 7 cardiometabolic outcomes across 4 population-based cohorts. Among our primary hypotheses are: *1*) the use of g/kg actual BW is an understudied measure in terms of its utility in populations that are dominated by overweight and obesity; and *2*) that this measure, as a ratio including actual BW, renders protein intake inherently correlated with and confounded by BW. Here, we focus exclusively on total dietary protein, and do not address other important factors that may affect associations between protein and human health, such as protein quality (e.g., amino acid content, digestibility), food source/origin, or source sustainability (21–23). Furthermore, our objective was not to make declarative statements about the effects of protein intake on human health, not least because the present study is a cross-sectional analysis; nor did we intend to estimate protein requirements in the context of excess fat compared with lean mass. Rather, we aimed to show that *1*) evidence for confusion about protein units is ubiquitous; *2*) results of associations differ by intake unit; and *3*) BW confounds associations when units themselves are based on actual BW.

Use of units in recent literature

Because ideal weight is implied or even explicit in WHO, German-speaking, and other recommendations, but not in IOM recommendations, it is creating confusion in the application of this standard in epidemiological studies, as well as trials. This confusion is evident across the literature, wherein both observational studies and trials of dietary protein—including very recent ones—use a variety of units to quantify or prescribe intake, including grams, g/kg actual or current BW, g/kg ideal BW, percent energy, and so on. For example, in the introduction to their 2017 paper describing their trial of protein intake at the US RDA and twice the US RDA in overweight participants, authors appeared to suggest that the WHO and IOM recommendations are the same (24). The authors are not alone in their confusion.

We nonsystematically scanned the dietary protein literature of the last 5 y (i.e., late 2019 in reverse chronological order to 2015) in relation to several cardiometabolic outcomes (obesity, dysglycemia, dyslipidemia, hypertension, kidney function, and relevant biomarkers), specifically looking for a variety of protein units and stopping when we had $\geq$1 example for a given outcome of each protein unit of interest. As the literature scan was not systematic, we did not explicitly exclude studies based on study characteristics (e.g., quality, sample size, language of publication). All studies referred to in the following sections are further detailed in **Supplemental Table 2**.

Protein units in studies of kidney function

In relation to kidney function, several papers have emerged on protein intake– and estimated glomerular filtration rate (eGFR)-based measures, including chronic kidney disease (CKD), declining eGFR, renal hyperfiltration, and so forth, with each study using different approaches to modeling protein intake (15, 16, 25–33). Esmeijer et al. (25) prospectively studied protein as g/kg ideal BW (assuming a BMI of 22.5 kg/m^2), grams, percent energy, and g/kg actual BW and declining eGFR. The first 3 units were modeled with adjustments for energy but not BMI, while the last unit was modeled with an adjustment for BMI but not energy. The authors noted that in their primary analyses they opted for ideal BW instead of actual BW, "since normalizing protein intake to actual body weight would result in erroneously high protein requirements in overweight and obese patients." They found that higher protein intake was related to declining eGFR, irrespective of unit, given their models. Lee et al. (16), in 2019, also used g/kg ideal BW (assuming a BMI of 22.5 kg/m^2) to cross-sectionally and prospectively study biomarker-based protein intake and eGFR and renal survival in patients with CKD. They found that higher protein intake was related to higher eGFR cross-sectionally and longer renal survival before, but not after, adjustments for BMI and other covariates. Meanwhile, Møller and colleagues (26), in 2018, studied diet record– and biomarker-based protein intake, presumably as g/kg actual BW, in relation to eGFR and other serum- and urine-based measures of kidney function in 308 overweight/obese individuals with prediabetes who had lost weight and were in weight maintenance. The authors reported no association of 1-y changes in diet record–based protein intake and changes in eGFR, either before or after adjusting for changes in BW, but found direct associations between changes in biomarker-based protein intake and changes in eGFR, an association which was nonsignificant after an additional adjustment for changes in BW. Enter a prospective study by Jhee et al. (34), which also presumably used g/kg actual BW, in 2 large Korean cohorts (the Korean Genome and Epidemiology Study and the Korean National Health and Nutrition Examination Survey), observing that higher protein intake was associated with a eGFR decline and higher odds of renal hyperfiltration (RHF) in analyses that were adjusted for both BMI and energy. Note that in an earlier, 2016 cross-sectional study of the Korean Genome and Epidemiology Study of protein intake and RHF (30), the authors did not report the units of protein intake (although these were presumably grams, and energy-adjusted) in their findings that total protein intake was not associated with RHF (no adjustment for body mass). A handful of other prospective and cross-sectional studies also recently investigated similar associations: Haring et al. (15) and Rebholz et al. (33) used grams in relation to incident CKD, Malhotra et al. (27) used percent energy, Herber-Gast et al. (32) used grams, Kaji et al. (35) used g/kg ideal BW

in relation to change in eGFR, and Lew et al. (28) used grams, percent energy, and g/kg actual BW in relation to incident end-stage renal disease. Each study adjusted for both BMI and energy intake. Haring et al. (15), Rebholz et al. (33), and Lew et al. (28) reported nonsignificant associations of higher total protein intake and elevated disease risk, Malhotra et al. (27) reported greater decline in eGFR among participants with diabetes only, and Herber-Gast et al. (32) reported no associations of total protein and changes in eGFR. Berryman et al. (31) used g/kg actual BW, adjusted for BMI but not energy, and found no cross-sectional association between total protein and eGFR in healthy US adults. Finally, Oosterwijk et al. (29) recently studied protein intake, as grams, and (cross-sectional) prevalence of low eGFR in a sample of adults with type 2 diabetes (T2D), adjusted for BMI and energy (residual method only), and found no association with total protein intake. Other recent protein-related papers using only biomarker-based measures (e.g., urine urea nitrogen) have also variously estimated protein intake, including as g/kg actual BW (36), g/kg ideal BW (36, 37), and g/24-hr (38). Notably, differential associations observed in the above-referenced papers may be due not only to the protein unit, but also to the different adjustment models (in observational studies), as well as to the different outcomes (e.g., eGFR, change in eGFR, incident CKD, etc.) and populations.

Protein units in studies of diabetes and diabetes-related traits

The dietary protein-kidney function research is not the only domain to suffer from a multiplicity of protein units. The observational and experimental literature on T2D and related factors [e.g., fasting glucose, insulin, glycated hemoglobin (HbA1c)] suffers from similar problems. In the last 4 y, ≥4 systematic reviews and meta-analyses (39–42) of ≤21 prospective cohorts in ≤12 papers concluded that higher total protein intake was related to a higher T2D risk. The studies included in these meta-analyses expressed protein intake in either energy-adjusted grams or percent energy. Additional observational (cross-sectional or prospective cohorts, or secondary analyses of trials analyzed as cohorts) papers we scanned relating protein to either diabetes and glucose metabolism biomarkers (fasting glucose, insulin, HOMA-IR, HbA1c, etc.) since 2015 used grams (43–47), g/kg actual BW (31, 48–50), and/or percent energy (39, 49, 51–55), and all inconsistently adjusted for either a measure of body size and/or energy. Of particular relevance among these was a study by Sluik et al. (49), who analyzed protein intake as both g/kg actual BW and percent energy in relation to incident diabetes or prediabetes in 4 cohorts. Their g/kg actual BW final model was adjusted for waist circumference (WC), while that of percent energy was adjusted for BMI and WC. Notably, prior to adjusting for anthropometric factors, results for g/kg and percent energy models were significant, but opposite of each other (with percent energy showing higher risk, and g/kg actual BW showing lower risk). Significant results remained only for WC-adjusted g/kg BW: a lower incidence ratio of diabetes per 1 SD g/kg BW higher intake (49).

Of the 6 trials we located investigating protein in relation to biomarkers of glucose metabolism, 4 used percent energy (56–59), while 2 used g/kg actual BW (60, 61). In arms comparing 35% energy from protein with 18% energy from protein (fat at 30% energy) over 6 mo in overweight/obese participants with T2D, Marco-Benedi et al. (56) reported significant, more favorable changes for glucose, HOMA-IR, and insulin, but not HbA1c, in the context of energy restriction. Also in the context of energy restriction, Mateo-Gallego et al. (57) observed no differences between groups of overweight/obese women (20%, 27%, or 35% energy from protein; fat held at 30% of energy) in changes in glucose, HbA1c, or insulin after 6 mo. Campos-Nonato and colleagues (60) also reported no differences between groups (0.8 compared with 1.34 g/kg) in participants with metabolic syndrome in the context of energy restriction, for either glucose, insulin, or HOMA-IR after 6 mo. Drummen et al. (58), studying 25 participants in weight maintenance after weight loss, also observed no differences between those in 15% compared with 25% energy protein arms at 2 y in glucose, insulin, HbA1c, HOMA-IR, or insulin sensitivity index. Watson and colleagues (59) studied overweight/obese adults for 12 wk of energy restriction followed by 12 wk of weight maintenance, in 22% compared with 32% energy from protein arms. They reported no differences between groups in glucose, insulin, or HbA1c after 24 wk. Similarly, Wright et al. (61), investigating a high-protein diet (1.4 g/kg) with eggs compared with a no-egg standard-protein diet (0.8 g/kg) in weight-maintaining, older, moderately overweight/obese participants, observed no differences after 12 wk between groups with respect to glucose, insulin, or HOMA-IR. However, several of the studies may have been underpowered to detect differences in these largely secondary outcomes. In addition, as mentioned above for kidney function, differential results between studies were likely not solely due to the protein unit, but also to the variably defined outcomes, populations, and methods.

Protein units in studies of circulating lipids

Similarly, many studies have assessed dietary protein in relation to circulating lipids (e.g., total, HDL, or LDL cholesterol or triglycerides), often in the context of metabolic syndrome and/or as secondary outcomes of weight-loss trials. Of the 13 papers we scanned on this topic since 2015, none used grams or g/kg ideal BW, 5 used g/kg actual BW (31, 48, 50, 60, 61), and the remaining 8 studies used percent energy (53, 54, 56–59, 62, 63). Whether experimental or observational, all inconsistently adjusted for various body mass and energy, and results were primarily nonsignificant irrespective of the study design and protein unit. For example, several trials (56, 57, 59, 60), but not all (58, 61), were energy-restricted, with 1 cross-sectional study using g/kg actual BW, adjusted for body fat percentage and skeletal muscle mass but not energy intake (50), and the only prospective cohort study using percent

energy, adjusted for both BMI and WC, as well as energy intake (63).

Protein units in studies of blood pressure

Several of the above studies relating protein intake with metabolic syndrome also investigated blood pressure outcomes, alongside studies with unique protein–blood pressure hypotheses. Of the 15 studies published since 2015 that we reviewed, none used g/kg ideal BW, 1 used grams (64), 9 used percent energy (53, 54, 56, 57, 59, 62, 63, 65, 66), 4 used g/kg actual BW (31, 48, 50, 60), and 1 used frequency (times/week) (67). Again, the studies variably adjusted for body mass and/or total energy, and results were heterogeneous, irrespective of type of study, "replacement" nutrient, unit of intake, or specific outcome [e.g., systolic blood pressure (SBP), diastolic blood pressure (DBP), or hypertension]. Interestingly, Buendia et al. (64) employed a unique approach we did not otherwise observe in the literature, in which protein intake (grams) was adjusted for BW using the residual method in their analyses of protein intake in relation to blood pressure and incident hypertension in the Framingham Heart Study (FHS) Offspring cohort. Finally, as mentioned previously, discrepant associations between studies were likely not solely due to the protein unit, but also to the variably defined outcomes, populations, and methods (e.g., models).

Protein units in studies of body composition

We found 16 recently published trials of protein intake and body composition, with or without energy restriction or exercise components, that compared high with low protein intakes and defined intake groups in terms of either g/kg actual BW (24, 60, 61, 68–73) or percent energy (56–59, 68, 74). However, g/kg ideal BW also appears in this literature (75–77). Several earlier trials (<2015, thus not reviewed here) investigating protein intake in body composition after weight loss in older adults were summarized in a 2016 meta-analysis (78). The meta-analysis concluded that men and women aged ≥50 y are more likely to retain lean mass and lose fat mass when they consume energy-restricted, higher-protein (≥25% energy or ≥1.0 g/kg, presumably actual BW) rather than normal-protein diets (<25% energy or <1.0 g/kg), while losses of body mass were similar in high- and normal-protein groups. Of note, the trials reviewed here and included in Supplemental Table 2 frequently include cardiometabolic risk factors as secondary outcomes, of which results are included in the papers noted in the above sections. Of trials using g/kg actual BW to differentiate intervention groups for weight loss, it is not always clear whether intake recommendations during the intervention period are adjusted for the concurrent actual BW (i.e., incorporating current weight in the context of weight loss, for example) or continue to use the initial BW to define adherence to intervention groups. That is, intake in absolute units (grams) is not universally reported both pre- and postintervention in all studies. In the absence of absolute intake data at both time points, total protein intake may not have changed, and any increase in a g/kg actual BW measure (e.g., using a final or concurrent weight) may simply be a result of weight loss, rather than higher absolute intake. Of the trials, there were generally no significant differences reported between low- and high-protein groups in terms of changes in weight, BMI, or WC. Only 4 of the 16 trials reported greater fat mass losses in high- compared with low-protein groups (24, 57, 71, 76); 3 trials reported higher or maintained lean or fat-free masses in high- v. low-protein groups (24, 61, 76).

The prospective observational literature we examined included 5 unique studies since 2015: 3 were secondary, prospective analyses of trial participants (48, 53, 79) and 2 were population-based cohorts (63, 80). Follow-up ranged from 6 mo (53) to 11 y (63). Of the secondary analyses of trials, Campbell et al. (48) employed g/kg actual BW; van Baak et al. (53) employed percent energy in the Diet, Obesity and Genes (DiOGenes) cohort; and Hernández-Alonso et al. (79) employed both g/kg actual BW and percent energy in the Prevención con Dieta Mediterránea (PREDIMED) cohort. Campbell and colleagues (48) observed lower weight, BMI, and fat mass (absolute) with higher protein intake, but no associations with lean mass or WC, after 36 wk of follow-up. Van Baak et al. (53) observed an inverse relation between protein intake and weight, but not WC or fat mass, across 6 mo of follow-up. Hernández-Alonso et al. (79) observed a higher risk of weight gain, but not weight loss, across 4.8 y of follow-up in participants consuming higher protein when modeled as percent energy and when replacing carbohydrates but not fat, and found no association with weight gain or loss when using g/kg actual BW. There were no significant associations of BMI-adjusted WC for either unit. Baseline BMI was included in all models (79).

Of the population-based prospective cohort studies, Ankarfeldt and colleagues (80), studying the Danish MONItoring trends and determinants of Cardiovascular disease (MONICA) cohort, used both percent energy and biomarker (urinary nitrogen) data in 6 y of follow-up, observing higher year-over-year changes in BMI, fat-free mass, fat mass, and weight with higher intake and biomarker protein. Finally, Shang et al. (63), in the Melbourne Collaborative Cohort Study, observed increases in weight and WC with a higher percent energy of protein intake over 11 y.

Remaining are 6 cross-sectional studies: 3 used g/kg actual BW (31, 50, 81), finding universal benefits of higher protein intake in relation to body composition; 1 used percent energy alone (54), finding lower fat mass with higher protein intake; and 2 used percent energy and grams (51, 82). Popp et al. (82), in the Chinese American Cardiovascular Health Assessment, observed higher BMI and fat mass (both percent and kg) and lower fat-free mass (percent but not kg) with higher protein intake, whether in gram or percent energy units, and differential results by protein unit for weight. Finally, in China Health and Nutrition Survey participants, Liu et al. (51) reported higher body fat and WC with higher protein intake, expressed in both percent energy or grams. Again, as mentioned above, differential results between studies were

likely not solely due to the protein unit, but also to the variably defined outcomes, populations, and models.

In sum, recently published literature on an array of cardiometabolic risk factors use a variety of ways to express units of protein intake. Coupled with inconsistent modeling methods and covariates (i.e., with or without energy intake or a measure of body mass, such as BMI), differential results are the norm, even within a single study, rendering the interpretation of results, not to mention translation to practice, exceedingly challenging.

In the following section, we describe the methods and results of a modeling demonstration assessing cross-sectional analyses of dietary protein, modeled using 7 different units of protein intake, on cardiometabolic outcomes in 4 population-based cohorts.

Methods used in modeling demonstration

The modeling demonstration includes data from 4 cohorts: the National Heart, Lung, and Blood Institute's (NHLBI) FHS Offspring and Third Generation (Gen3) cohorts and the CDC/National Center for Health Statistics (NCHS) NHANES 2003–04 and 2005–06 cycles. These cohorts were selected because *1*) they had publicly available dietary, DXA, and cardiometabolic outcome data on well-characterized populations; *2*) they are samples of diverse ages, races/ethnicities, body compositions, and dietary assessment methods (i.e., FFQ in FHS and dietary recall in NHANES); and *3*) the data would allow for protein intake to be based on different units, BWs (e.g., current compared with ideal), and body composition components (e.g., lean compared with fat-free mass).

The original data collection protocols for FHS were approved by the Institutional Review Board at Boston University Medical Center, and written informed consent was obtained from all participants. In the case of FHS, data were obtained with permission (controlled access) and downloaded from the NHLBI Biologic Specimen and Data Repository Information Coordinating Center (BioLINCC) under study accession numbers HLB00911219a and HLB00060019b (83, 84). NHANES data were downloaded directly from the CDC's NHANES web sites (85, 86). The present study protocol was reviewed by the Tufts University Health Sciences Institutional Review Board.

Study populations

The FHS Offspring and Gen3 cohorts are community-based, longitudinal studies of cardiovascular disease that began in 1971 (87) and 2002 (88), respectively. We used data from Offspring exam 6 (1995–1998; $n = 3532$; mean age, 59 y) and exam 7 (1998–2001; $n = 3539$; mean age, 62 y), and the DXA scans conducted between these exams as part of the Framingham Osteoporosis Study (1996–2001). The Gen3 cohort originally included 4095 children of the Offspring, of which we used exam 2 (2008–2011; $n = 3411$; mean age, 47 y) data for the present analyses, owing to DXA scans being conducted at this exam. Extensive supporting information about the FHS cohorts and the data can be found on the Framingham Heart Study website (http://www.framinghamheartstudy.org/) and BioLINCC (http://biolincc.nhlbi.nih.gov/home/).

Exclusions are provided in **Supplemental Figures 1** and **2** for the Offspring and Gen3 samples, respectively. Briefly, we excluded participants who did not attend the relevant exam(s), those who were not fasting, and those who were missing or had invalid dietary, anthropometric, DXA, laboratory, or covariate data. The final analyzed numbers of participants were 1847 in the Offspring cohort and 2548 in the Gen3 cohort.

NHANES is a series of ongoing surveys designed by the CDC/NCHS to assess the health and nutritional status of US adults and children in a statistically representative fashion (89). These surveys combine both interviews and physical examinations, including dietary assessments and a host of cardiometabolic, functional, and body composition assessments. In the 2003–04 cycle, 10,122 total participants were interviewed, and 9643 were examined. Of those interviewed and examined, 5041 and 4742 were adults ≥20 y, respectively (90). Participants aged ≥8 y were eligible for whole-body DXA scans. In the 2005–06 cycle, 10,348 total participants were interviewed, and 9950 were examined. Of those interviewed and examined, 4979 and 4473 were adults ≥20 y, respectively (90). Participants aged 8–69 y were eligible for whole-body DXA scans.

Exclusions are provided in **Supplemental Figures 3** and **4** for the 2003–04 and 2005–06 cycles, respectively. Briefly, we excluded participants <20 y, those who did not have a morning sample fasting weight, and those who were missing or had invalid dietary, anthropometric, DXA, laboratory, or covariate data, for final analyzed numbers of participants of 1625 in 2003–04 and 1347 in 2005–06.

Dietary assessments

FHS Offspring and Gen3 data.

Dietary intake was assessed at each exam using a semi-quantitative Harvard FFQ (91). The FFQ consisted of a list of ≥126 foods with a standard serving size and a selection of 9 frequency categories ranging from "never or <1 serving/month" to "≥6 servings/day." Participants were asked to report their frequency of consumption of each food item during the last year. The FFQ provides estimates of intake on ≥150 dietary nutritive and nonnutritive components, including total protein, saturated fat, fiber, and alcohol. Because the Offspring DXA scans were conducted between clinical exams 6 and 7, to maximize available data and reduce variability we calculated the mean of intake from FFQs from both exams where available, and otherwise selected the FFQ data from the exam date closest to the DXA scan date.

NHANES data.

Dietary intake was assessed using up to two 24-h recalls. We included adults who completed at least the first recall. In each cycle, the first recall was completed in an in-person interview using measuring guides in the Mobile Examination Center, concurrent with the rest of the examination. A

second recall was completed by telephone 3–10 d later using an automated multi-pass method. The dietary intake data are used to estimate the types and amounts of foods and beverages consumed during the 24-h period prior to the interview (midnight to midnight), and to estimate intakes of energy, protein, and other nutrients. As protein is a habitually consumed nutrient, we calculated the mean intake from both days, where available, for each individual participant (92, 93).

DXA scans and body composition

As mentioned, body composition was assessed by DXA in all 4 samples for estimates of total fat mass, fat-free mass, and lean mass.

FHS Offspring and Gen3 data.

In the Offspring cohort, participants were invited to the Framingham Osteoporosis Study, which included whole-body DXA scans conducted between clinical exams 6 and 7. Scans were performed using a Lunar Dual X-ray absorptiometer (DPX-L; Lunar Radiation Corporation, now GE Healthcare, Inc.) in the "Fast" mode to minimize the participant burden. As the "Fast" mode is not optimal for larger persons, certain scans were underpenetrated. Whole scans or scans except for the legs for the heaviest and/or thickest participants were deleted. In 19 participants who had 2 scans performed on the same day, after repositioning, intraclass correlation coefficients were 0.99 for whole body fat mass (directly measured) and 0.99 for bone-free lean mass (derived from directly measured variables). The methods are described in other works on the Framingham Heart Study (83, 94, 95).

In Gen3, DXA scans were obtained with a GE Lunar Prodigy fan beam densitometer (GE Healthcare, Inc.). For participants too large to fit within the dimensions of the scanning field, a hemi-scan was performed (45.6% of participants had a hemi-scan). For the majority of participants with a hemi-scan, the right side of the participant was scanned and the machine imputed the left side measures to create the whole body. On a subsample of 15 participants measured 3 times after repositioning, coefficients of variation were 1.3% for total fat mass, 1.4% for total bone mineral density, and 0.9% for total lean mass (84).

NHANES data.

Scans were limited to those participants aged ≥8 y in 2003–04 and aged 8–69 y in 2005–06. In addition, excluded from measurements were women who were pregnant or said they were pregnant, and individuals whose body size exceeded 300 lb (136 kg) or 6'5" (1.96 m). The available data sets from NHANES include previously imputed data, with ≤5 data points per participant. In analyses described below, all data points were used and combined using appropriate imputation methods, as recommended.

Characterizing protein intake

Dietary characteristics were adjusted for energy intake using the residual method (96, 97). We expressed energy-adjusted daily protein intake in the following 7 units: grams, percent of total energy (at 4 kcal/g protein), g/kg actual BW, g/kg ideal BW, g/kg lean BW (i.e., bone- and fat-free), g/kg fat-free BW, and BW–adjusted g/kg actual BW (where protein grams were adjusted for BW using the residual method; BW-g/kg actual BW). Fat-free mass (kg) was calculated as total BW less fat mass. Ideal BW (kg) was calculated as the BW for measured height, corresponding to a BMI of 22 kg/m^2 for men and 21 kg/m^2 for women, as per WHO guidelines [Annex 2, Table C, in Joint FAO/WHO/UNU Expert Consultation on Energy and Protein Requirements (4)]. In NHANES, lean and fat-free BW and associated intake units were estimated from the multiply imputed DXA data. Quartile categories of intake were generated for each protein unit (**Supplemental Table 3**).

Cardiometabolic risk factors

We investigated 9 cardiometabolic risk factors to evaluate the confounding (or lack thereof) generated through the use of different units of protein intake when examining associations between protein intake and cardiometabolic health. These included fasting plasma glucose (mg/dL), SBP and DBP (mmHg), total and HDL cholesterol (mg/dL), triglycerides (mg/dL), BMI (kg/m^2), waist circumference (cm), and serum creatinine–based eGFR (mL min^{-1} 1.73 m^{-2}). Lipids were measured in plasma in FHS and serum in NHANES. The methods of standardized data collection and analyses of blood-based measures, blood pressure, and anthropometric indices differed from cohort to cohort. Details of each are provided in the procedural manuals for each cohort (83–86). The eGFR was calculated using the CKD Epidemiology Collaboration equation (98).

Sociodemographic characteristics and other covariates

Sociodemographic variables and other covariates, all self- or proxy-reported by questionnaire or interview, included age, sex, race (in the Offspring cohort: not applicable, as participants were nearly all non-Hispanic White; in Gen3: White, Black, other; in NHANES: non-Hispanic White, non-Hispanic Black, Mexican-American, other Hispanic, other race/ethnicity), smoking status [current smoker (reported cigarette smoking within the last year), non-smoker], educational attainment (high school or less, some college, college degree or higher), marital status (never married, married, divorced/separated/widowed), self-reported treatment for or use of medications for diabetes, hypertension, or dyslipidemia (all yes, no), self-reported history of cardiovascular disease (yes, no), and physical activity (continuous score based on reported hours of activity). Data collection instruments and methodologies are described in detail in the relevant procedural manuals (83–86).

Statistical approach

All analyses were cross-sectional. Demographic, clinical, and dietary characteristics [mean ± SD or weighted mean ± SEM and *n* (%) or *n* (weighted %)] of the participants in each sample are provided. We calculated unweighted,

partial Pearson correlations between BMI, BW, and protein intake and between BMI, BW, and cardiometabolic outcomes, adjusted for age, sex, smoking status, marital status, education, and physical activity. Multivariate adjusted linear regression (PROC GLM in the FHS cohorts and PROC SURVEYREG in the NHANES cohorts, with LSMEANS statements) was used to estimate least square means ± SEs of the cardiometabolic outcomes in each quartile category of protein intake. The median protein intake within each quartile category of protein intake (for each protein unit) was assigned as the category value, and was also used to generate the *P*-trend across quartile categories. Because NHANES DXA data (used for analyses of protein intake expressed as g/kg lean or fat-free BW) consisted of 5 observations per participant, regressions were repeated 5 times. Resulting estimates were analyzed to obtain a single estimate using PROC MIANALYZE. For all NHANES analyses, appropriate sample weights, stratification, and clustering of the complex sampling design were included in the SURVEY PROCs. As mentioned, the weights of the morning fasted sample were used.

Models.

We generated a total of 10 models for each protein unit–cardiometabolic outcome combination. The primary models are as follows: *1*) protein, age, sex, and energy intake (basic model); *2*) model 1 plus current smoking, race/ethnicity, physical activity, marital status, and educational attainment (social behavioral model); *3*) model 2 plus history of cardiovascular disease (CVD) and treatment for diabetes, hypertension, or hyperlipidemia (prevalent disease model); and *4*) model 3 plus saturated fat, fiber, and alcohol intake (dietary model). We did not adjust for total carbohydrates or total fat, so that these energy-constant models remained agnostic about the replacement of protein with carbohydrates or fat. In each of models 1–4, a second model was adjusted for BMI except when BMI was the outcome, as body mass is a known risk factor for cardiometabolic disease and generally included in all diet-disease models. In addition, in models 1 and 4, a third model further adjusted for BW (not BMI), to demonstrate the potential confounding introduced when BW is considered in the context of a ratio measure of intake which includes BW in the denominator. Nutrients, except energy, were expressed in the same units as the protein unit being modeled as the exposure.

All data were analyzed using SAS (v. 9.4, SAS Institute Inc.). Statistical significance was set at a 2-tailed $P < 0.05$. We did not adjust the alpha level for multiple testing, given that *1*) the exposures are constructs representing the same underlying phenomenon, protein intake; and *2*) our objective is not declarative statements about the statistical significance of the associations, but rather their directionality.

Results of modeling demonstration

Demographic and clinical characteristics of the 4 samples (weighted characteristics in the NHANES samples) are provided in **Table 1**, and dietary characteristics are provided in **Table 2**. The samples were broadly similar, although the Offspring cohort were ∼20 y older (mean, 60.3 y; SD, 9.5 y) than the other 3 cohorts, and several characteristics (e.g., percent women, percent treated for CVD) correspond with the cohort's older mean age. NHANES participants, by design, were considerably more racially/ethnically diverse than FHS. FHS participants had higher educational achievement than NHANES participants, and a lower proportion were current smokers. Mean BMIs were similar across the cohorts, from 26.3 kg/m^2 in the Offspring cohort to 28.3 kg/m^2 in the latter NHANES. Obesity prevalence was lowest in the Offspring cohort, at 14.2%, and highest in the latter NHANES, at 33.7%. The underweight prevalences were <2% in each sample.

Mean intake and intake by BMI categories

Mean daily protein intake was comparable across the 4 samples, irrespective of protein unit: 77.7–85.3 g, 17.3–18.8% energy, 1.05–1.10 g/kg actual BW, and 1.32–1.39 g/kg ideal BW (Table 1). When mean intake was stratified by BMI (**Supplemental Table 4**), participants with obesity reported ∼3 g and 1–2% energy higher intake, and ∼0.4 g/kg actual BW lower intake, while intake based on ideal BW was, as expected, very similar across BMI categories. When viewed by the recommended intake cut points of 0.8 g/kg BW and stratified by BMI (**Supplemental Table 5**), a smaller proportion of those with obesity than those with normal BMI met the US RDA in each sample (e.g., in NHANES 2005–06, 91.1% with normal BMI compared with 62.2% with obese BMI). When based on the WHO recommendation (g/kg ideal BW) (3, 4), proportions of those meeting the recommendation were generally higher and more comparable across all BMI categories.

Correlations of BMI and BW with intake and cardiometabolic outcomes

Partial Pearson correlations between BMI, BW, and intake were generally weak but significant ($-0.08 \leq$ partial $r \leq 0.14$) between BMI or BW and grams, percent energy, and g/kg ideal BW (**Supplemental Table 6**). Correlations were inverse, larger, and more statistically significant between BMI or BW and units with actual BW or BW compartments in the denominator. BMI and BW were modestly correlated with SBP, DBP, glucose, HDL cholesterol, and triglycerides, and strongly correlated with WC (**Supplemental Table 7**). Neither BMI nor BW were correlated with eGFR [except partial $r = -0.08$ ($P = 0.006$) with BW in NHANES 2005–06] and total cholesterol [except partial $r = 0.12$ ($P \leq 0.001$) with BMI and partial $r = 0.07$ ($P = 0.003$) with BW in Offspring cohort].

Associations with cardiometabolic outcomes

Adjusted least square means of 9 cardiometabolic outcomes and protein units, across all models, are shown in **Supplemental Tables 8–16**. Each table is presented in the following order of protein units: grams, percent energy, g/kg actual BW, BW-adjusted g/kg actual BW, g/kg ideal BW, g/kg fat-free BW, and g/kg lean BW.

TABLE 1 Demographic and clinical characteristics of FHS and NHANES participants[1]

Demographic and clinical characteristics	FHS Offspring[2]	FHS Gen3[2]	NHANES 2003–04[3]	NHANES 2005–06[3]
n	1847	2548	1625	1347
Age, y	60.3 ± 9.45	46.6 ± 8.67	46.2 ± 0.61	43.7 ± 0.55
Sex, female	1154 (62.5)	1408 (55.3)	50 (0.8)	49 (1.3)
Current smoker	212 (11.5)	230 (9.0)	27 (1.4)	31 (2.1)
Marital status				
Never married	101 (5.5)	351 (13.8)	24 (2.3)	26 (1.4)
Married	1396 (75.6)	1885 (74.0)	60 (2.5)	58 (1.5)
Divorced, separated, widowed	350 (19.0)	312 (12.2)	17 (1.5)	15 (0.8)
Education				
High school or less	640 (34.7)	357 (14.0)	44 (1.8)	41 (2.5)
Some college	608 (32.9)	744 (29.2)	33 (1.8)	33 (1.6)
Bachelor's degree or higher	334 (18.1)	917 (36.0)	24 (2.0)	26 (3.1)
Graduate or professional degree	265 (14.4)	530 (20.8)	—	—
Race/ethnicity				
Non-Hispanic White	1847 (100)	2540 (99.7)	74 (3.7)	71 (2.6)
Non-Hispanic Black	—	1 (0.04)	10 (1.88)	11 (1.54)
Mexican-American	—	—	8 (2.16)	9 (1.20)
Other Hispanic	—	—	3 (1.03)	4 (1.17)
Other race/ethnicity	—	7 (0.3)	4 (0.7)	6 (1.0)
Height, m	1.66 ± 0.09	1.70 ± 0.09	1.70 ± 0.003	1.70 ± 0.003
Weight, kg	72.9 ± 12.6	80.3 ± 18.4	81.1 ± 0.45	81.7 ± 0.61
BMI, kg/m^2	26.3 ± 3.63	27.8 ± 5.44	28.0 ± 0.14	28.3 ± 0.21
BMI category, kg/m^2				
<18.5	12 (0.7)	23 (0.9)	1 (0.3)	2 (0.5)
18.5–24.9	666 (36.1)	842 (33.1)	32 (1.5)	32 (1.7)
25–29.9	908 (49.2)	943 (37.0)	35 (1.8)	32 (1.5)
30–34.9	227 (12.3)	482 (18.9)	20 (1.0)	19 (1.5)
35–39.9	29 (1.6)	171 (6.7)	7 (0.6)	9 (1.0)
≥40	5 (0.3)	87 (3.4)	4 (0.6)	5 (0.7)
Fat mass,[4] kg	26.4 ± 8.23	27.1 ± 11.2	28.2 ± 0.31	28.0 ± 0.32
Fat mass,[4] %	36.2 ± 9.24	33.1 ± 9.00	34.4 ± 0.25	33.7 ± 0.25
Lean mass,[4] kg	43.4 ± 10.3	48.9 ± 11.3	51.1 ± 0.29	51.9 ± 0.40
Lean mass,[4] %	59.6 ± 9.28	61.6 ± 9.16	63.3 ± 0.23	64.1 ± 0.24
Fat-free weight,[4] kg	46.5 ± 10.9	53.2 ± 12.1	52.9 ± 0.30	53.7 ± 0.41
Ideal weight,[5] kg	59.3 ± 7.65	62.0 ± 7.91	62.4 ± 0.25	62.4 ± 0.27
Waist circumference, cm	94.3 ± 10.9	96.1 ± 14.4	97.1 ± 0.37	96.6 ± 0.61
Cholesterol,[6] mg/dL	204 ± 35.9	186 ± 33.5	199 ± 1.43	199 ± 1.91
HDL cholesterol,[6] mg/dL	55.7 ± 16.9	59.8 ± 17.6	53.8 ± 0.65	55.4 ± 0.40
Triglycerides,[6] mg/dL	124 ± 63.5	107 ± 57.7	147 ± 5.59	141 ± 4.31
Plasma glucose, mg/dL	99.1 ± 19.6	95.5 ± 15.7	99.9 ± 1.04	102 ± 1.07
SBP, mmHg	126 ± 18.9	116 ± 13.9	121 ± 0.62	120 ± 0.63
DBP, mmHg	73.8 ± 9.32	74.0 ± 9.30	70.8 ± 0.46	70.5 ± 0.46
eGFR, mL min^{-1} 1.73 m^{-2}	84.0 ± 19.8	96.8 ± 13.9	94.2 ± 1.12	94.0 ± 0.87
Treatment for diabetes	64 (3.5)	64 (2.5)	1 (0.2)	2 (0.3)
Treatment for hypertension	500 (27.1)	423 (16.6)	23 (1.8)	21 (1.5)
Treatment for dyslipidemia	301 (16.3)	418 (16.4)	18 (1.4)	14 (1.5)
Treatment for CVD	623 (33.7)	70 (2.8)	8 (1.0)	7 (1.1)

[1] Abbreviations: CVD, cardiovascular disease; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; FHS, Framingham Heart Study; Gen3, FHS Third Generation cohort; SBP, systolic blood pressure.
[2] In FHS, data are presented as mean ± SD for continuous characteristics and *n* (%) for categorical characteristics.
[3] In NHANES, data are presented as weighted mean ± SEM for continuous characteristics and % (standard error of percent [SEP]) for categorical characteristics, using methods accounting for complex survey design.
[4] Lean and fat-free body weight compartments were derived from DXA measures. In NHANES, these were additionally derived from multiply imputed DXA estimates.
[5] Ideal body weight as weight for actual height corresponding to a BMI of 22 kg/m^2 in men and 21 kg/m^2 in women.
[6] Lipids were measured in plasma in FHS and in serum in NHANES.

Models of fasting plasma glucose as the outcome readily illustrate the problem of confounding by BW when using a protein unit that includes BW in the denominator (models 1, 1 + BMI, and 1 + BW are shown in **Figure 1**A–G; Supplemental Table 8). Within each cohort, protein as grams (Figure 1A) or percent energy (Figure 1B) showed consistent direct associations with glucose, irrespective of adjustments for either BMI or BW. Similarly, g/kg ideal BW (Figure 1C) was also consistent with and without adjustments for BMI or BW, and also appeared generally consistent directionally with gram and percent energy units. However, units with actual BW or BW compartments in the denominator

TABLE 2 Daily dietary intakes of FHS and NHANES participants[1]

Dietary characteristic	FHS Offspring[2]	FHS Gen3[2]	NHANES 2003–04[3]	NHANES 2005–06[3]
n	1847	2548	1625	1347
Energy, kcal	1828 ± 548	1993 ± 640	2222 ± 29.1	2199 ± 37.1
Protein				
g	77.7 ± 12.9	84.2 ± 15.4	81.6 ± 0.77	85.3 ± 0.74
% energy	18.6 ± 6.54	18.8 ± 7.11	17.3 ± 0.21	18.4 ± 0.43
g/kg actual BW	1.10 ± 0.27	1.10 ± 0.32	1.05 ± 0.01	1.10 ± 0.01
BW-adj g/kg actual BW	1.10 ± 0.39	1.10 ± 0.44	1.10 ± 0.02	1.11 ± 0.02
g/kg lean[4] BW	1.89 ± 0.54	1.82 ± 0.54	1.67 ± 0.02	1.73 ± 0.02
g/kg fat-free[4] BW	1.76 ± 0.51	1.67 ± 0.49	1.62 ± 0.02	1.67 ± 0.02
g/kg ideal[5] BW	1.33 ± 0.29	1.39 ± 0.32	1.32 ± 0.01	1.39 ± 0.01
Carbohydrate				
g	236 ± 37.6	241 ± 39.4	255 ± 2.94	257 ± 2.42
% energy	56.6 ± 19.9	53.9 ± 20.3	54.4 ± 1.03	55.3 ± 1.02
g/kg actual BW	3.35 ± 0.86	3.18 ± 0.94	3.32 ± 0.04	3.33 ± 0.05
BW-adj g/kg actual BW	3.34 ± 1.31	3.16 ± 1.36	3.46 ± 0.03	3.34 ± 0.07
g/kg lean[4] BW	5.74 ± 1.61	5.21 ± 1.50	5.30 ± 0.07	5.26 ± 0.07
g/kg fat-free[4] BW	5.36 ± 1.52	4.78 ± 1.36	5.11 ± 0.07	5.08 ± 0.07
g/kg ideal[5] BW	4.05 ± 0.84	3.96 ± 0.84	4.16 ± 0.06	4.20 ± 0.04
Fat				
g	60.3 ± 13.0	71.7 ± 14.0	80.2 ± 1.25	82.4 ± 0.65
% energy	32.7 ± 12.8	36.1 ± 14.4	38.4 ± 0.73	39.8 ± 0.80
g/kg actual BW	0.85 ± 0.23	0.94 ± 0.28	1.04 ± 0.01	1.06 ± 0.01
BW-adj g/kg actual BW	0.85 ± 0.36	0.94 ± 0.42	1.09 ± 0.02	1.07 ± 0.02
g/kg lean[4] BW	1.46 ± 0.45	1.54 ± 0.46	1.66 ± 0.03	1.68 ± 0.01
g/kg fat-free[4] BW	1.36 ± 0.42	1.42 ± 0.42	1.61 ± 0.03	1.62 ± 0.01
g/kg ideal[5] BW	1.03 ± 0.26	1.18 ± 0.28	1.31 ± 0.02	1.34 ± 0.01
Saturated fat				
g	21.0 ± 5.85	23.2 ± 5.75	26.3 ± 0.41	27.4 ± 0.25
% energy	11.4 ± 4.77	11.7 ± 4.92	12.6 ± 0.25	13.2 ± 0.28
g/kg actual BW	0.30 ± 0.10	0.30 ± 0.10	0.34 ± 0.00	0.35 ± 0.00
BW-adj g/kg actual BW	0.30 ± 0.14	0.30 ± 0.14	0.36 ± 0.01	0.36 ± 0.01
g/kg lean[4] BW	0.51 ± 0.18	0.50 ± 0.16	0.54 ± 0.01	0.56 ± 0.01
g/kg fat-free[4] BW	0.47 ± 0.17	0.46 ± 0.15	0.53 ± 0.01	0.54 ± 0.01
g/kg ideal[5] BW	0.36 ± 0.11	0.38 ± 0.10	0.43 ± 0.01	0.45 ± 0.00
Fiber				
g	18.9 ± 5.44	22.3 ± 7.23	15.4 ± 0.37	15.6 ± 0.32
% energy	4.53 ± 1.80	4.97 ± 2.18	3.25 ± 0.07	3.36 ± 0.10
g/kg actual BW	0.27 ± 0.10	0.30 ± 0.13	0.20 ± 0.01	0.20 ± 0.01
BW-adj g/kg actual BW	0.27 ± 0.12	0.29 ± 0.15	0.21 ± 0.01	0.20 ± 0.01
g/kg lean[4] BW	0.46 ± 0.17	0.49 ± 0.21	0.32 ± 0.01	0.32 ± 0.01
g/kg fat-free[4] BW	0.43 ± 0.16	0.45 ± 0.19	0.31 ± 0.01	0.31 ± 0.01
g/kg ideal[5] BW	0.33 ± 0.10	0.37 ± 0.14	0.25 ± 0.01	0.26 ± 0.01
Alcohol				
g	9.77 ± 13.5	11.7 ± 15.0	9.42 ± 1.35	12.7 ± 1.07
% energy	4.08 ± 5.35	4.58 ± 5.24	3.57 ± 0.32	4.78 ± 0.37
g/kg actual BW	0.13 ± 0.18	0.15 ± 0.19	0.12 ± 0.02	0.17 ± 0.01
BW-adj g/kg actual BW	0.14 ± 0.19	0.15 ± 0.20	0.13 ± 0.02	0.17 ± 0.01
g/kg lean[4] BW	0.22 ± 0.30	0.24 ± 0.30	0.19 ± 0.03	0.26 ± 0.02
g/kg fat-free[4] BW	0.21 ± 0.28	0.22 ± 0.28	0.18 ± 0.02	0.25 ± 0.02
g/kg ideal[5] BW	0.16 ± 0.22	0.19 ± 0.23	0.15 ± 0.02	0.21 ± 0.02

[1] Abbreviations: BW, body weight; BW-adj, intake adjusted for body weight using the residual method; FHS, Framingham Heart Study; Gen3, FHS Third Generation cohort.
[2] In FHS, data are presented as mean ± SD.
[3] In NHANES, data are presented as weighted mean ± SEM, using methods accounting for complex survey design.
[4] Lean and fat-free BW compartments were derived from DXA measures. In NHANES, these were derived from multiply imputed DXA estimates.
[5] Ideal BW as weight for actual height corresponding to a BMI of 22 kg/m^2 in men and 21 kg/m^2 in women.

(Figure 1D–G) appear confounded by BMI and BW. For example, for g/kg actual BW (Figure 1D), the solid lines, indicating the trends unadjusted for BMI or BW, suggest favorable associations of higher protein intake, in contrast with gram, percent energy, and g/kg ideal BW curves. However, these trends disappeared or inverted after adjusting for BMI or BW (dotted and dashed lines, respectively). Even where protein intake was residually adjusted for BW to remove the correlation between intake and BW, the association of protein intake in units of BW-adjusted g/kg actual BW with glucose remained confounded by BMI or BW, as shown in Figure 1E. Fat-free and lean compartments

(Figure 1F and G, respectively) showed similar curves, both tending to attenuate or modestly reverse the unadjusted association with glucose after adjusting for BMI or BW.

Of the lipid outcomes we examined, only associations with total cholesterol were generally consistently nonsignificant, irrespective of protein unit and model (Supplemental Table 9). As mentioned above, neither BMI nor BW were correlated with cholesterol (Supplemental Table 7). Results of protein intake and HDL cholesterol (Supplemental Table 10) and triglycerides (Supplemental Table 11) showed patterns similar to that of glucose. That is, any associations of protein, and in particular those modeled using g/kg actual BW, were substantially attenuated with adjustments for BW or BMI. However, for NHANES 2003–04, higher protein intake, irrespective of unit, model, or adjustments for BMI or BW, appeared to be largely consistently associated with higher HDL cholesterol.

With respect to blood pressure, associations of protein intake with SBP (Supplemental Table 12) were generally nonsignificant in the fully adjusted model (model 4) with or without body size adjustment, except for FHS Gen3, in which percent energy, g/kg actual BW, and BW-adjusted g/kg actual BW were inversely associated with SBP. Results for DBP were similar to those for SBP (Supplemental Table 13).

Highly significant inverse associations of protein intake with BMI were observed when protein intake was modeled as g/kg actual BW or BW-adjusted g/kg actual BW prior to adjusting for BW, which were fully attenuated after adjustment for BW (Supplemental Table 14). In contrast, when modeled as g/kg ideal BW, results indicated that higher protein intake was directly associated with BMI, even after adjusting for actual BW. For protein modeled in g/kg lean BW or g/kg fat-free BW, prior to the actual BW adjustment, intake was inversely associated with BMI, but was reversed to being directly associated with BMI after BW adjustment. Results for intake in grams or percent energy suggested direct associations with or without an adjustment for BW, but were inconsistent across models and cohorts.

Neither grams nor percent energy were associated with WC after adjusting for body size, and only the Framingham cohorts indicated direct associations prior to such adjustment (Supplemental Table 15). When protein was modeled as g/kg actual BW or BW-adjusted g/kg actual BW, protein was generally inversely associated with WC even after adjusting for BMI or BW. For g/kg ideal BW, unique associations emerged depending on the body size unit of adjustment. For example, in model 4 without body size, associations were nonsignificant with WC, except for in Framingham Gen3, which showed a direct relation. In model 4 + BMI, protein was significantly, inversely associated with WC. In model 4 + BW, protein was directly associated with WC. Such reversals were also evident in the g/kg lean or fat-free BW analyses.

Protein intake was not associated with eGFR when intake was modeled in grams or g/kg ideal BW (Supplemental Table 16). When intake was modeled in percent energy, a suggestive direct association emerged for Framingham Offspring only. For g/kg actual BW, BW-adjusted g/kg actual BW, g/kg lean BW, and g/kg fat-free BW, suggestive direct associations were observed primarily after adjusting for body size, and only in the Offspring cohort and NHANES 2003–04 cycle.

Discussion

In the present investigation, we found ample evidence of confusion in recent literature about the WHO andIOM protein recommendations, and differential conclusions about the associations of protein intake on human health, which may in part be due to units. Results of our modeling demonstration in 4 large samples substantiated that confusion, frequently showing differential results by unit, as well as between cohorts. Nevertheless, some important consistencies did emerge: when protein was modeled as grams, percent energy, or g/kg ideal BW, with and without adjustments for BMI or BW, results of our demonstration followed similar trends for each outcome. When protein was modeled as g/kg actual BW, the reciprocal of BW introduced an artificial bias (confounding), which appeared corrected after adjusting for BW or BMI, rendering results similar to grams, percent energy, and g/kg ideal BW.

We additionally observed some potentially intriguing and potentially explanatory associations between protein intake in a ratio with fat-free or lean mass and several outcomes, such as eGFR. Whereas there appeared to be few associations across most units of intake and eGFR, g/kg lean BW and g/kg fat-free BW showed some direct, significant associations. Because the role of protein intake in kidney function continues to be hotly debated, these results may point to areas for future consideration.

Beyond directly pointing out the confusion inherent in the literature, the present paper also demonstrates the need for population-based assessments of meeting intake recommendations to specify which recommendations are being used. It could be argued that g/kg actual BW grossly overestimates protein inadequacy in populations that are overweight or obese; similarly, it could be argued that those based on g/kg ideal BW are underestimating inadequacy. This in turn points to another important avenue of research: the need to identify whether excess adiposity demands additional protein and, if so, at what rate per kilogram of excess fat mass. It is not, to our knowledge, biologically plausible for fat mass to have the same protein requirements as lean mass; thus, a "pro-rating" of protein intake above requirements of ideal BW may be useful in future recommendations.

Model interpretation

The present modeling demonstration is predicated on the idea that body mass must be accounted for in diet models of outcomes associated with body mass, whether those models explicitly include body mass in observational studies or intrinsically include body mass as considerations during recruitment, sampling, or analysis in experimental studies. Body mass, being related to both diet and most cardiometabolic outcomes, is often considered a mediator

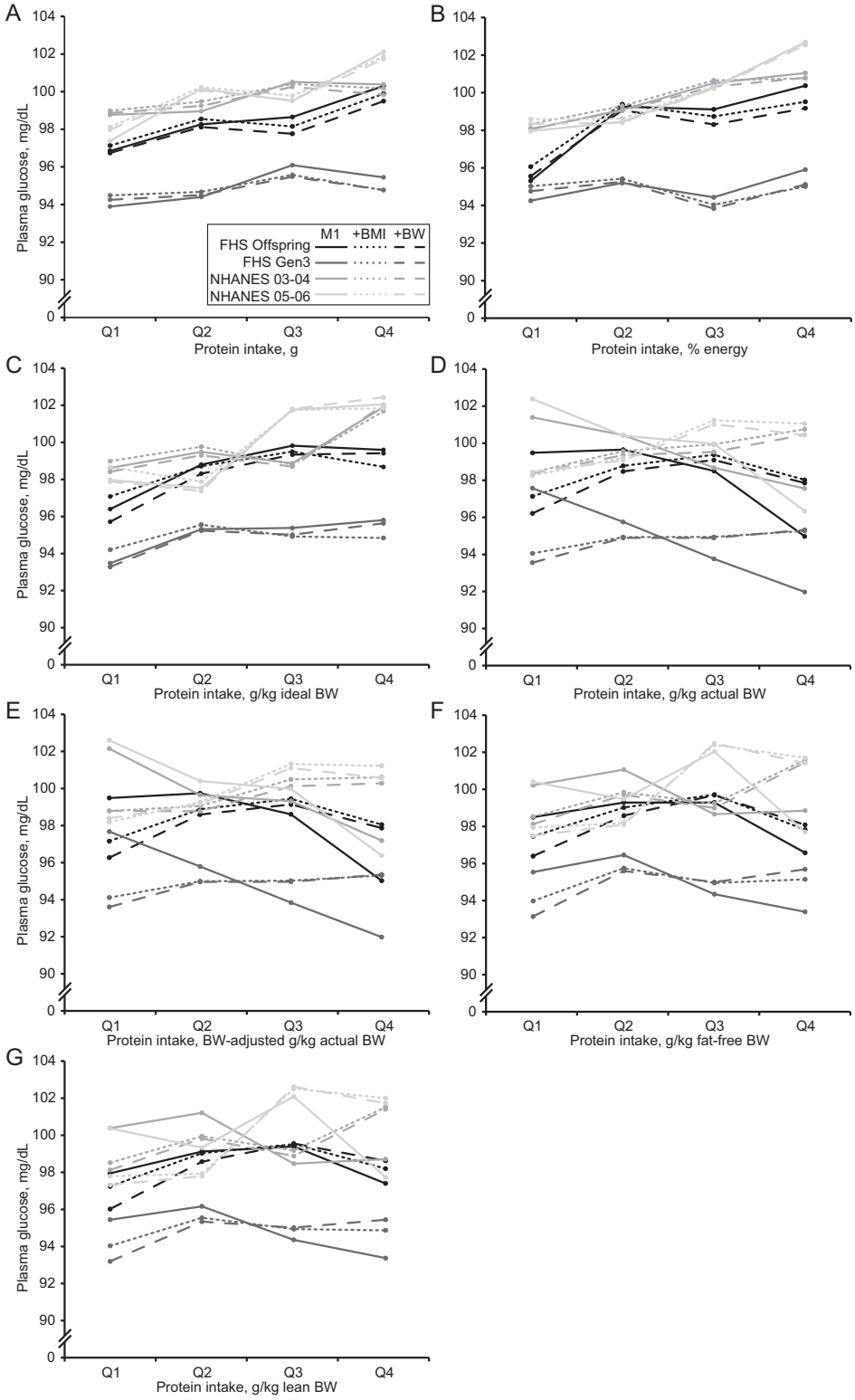

FIGURE 1 Associations between protein intake, expressed in quartile categories of various units, and fasting plasma glucose (mg/dL) in 4 cohorts: FHS Offspring, FHS Gen3, NHANES 2003–04, and NHANES 2005–06. Points represent least square adjusted means of plasma glucose in each quartile category of intake, adjusted for age, sex, and energy intake (solid line), and additionally adjusted for BMI (dotted line) or BW (dashed line). Daily intake units are grams (A), percent energy (B), g/kg ideal BW (C), g/kg actual BW (D), BW-adjusted g/kg actual BW (E), g/kg fat-free BW (F), and g/kg lean BW (G). Participant numbers in quartile categories of intake (Q1 through Q4) are as follows: FHS Offspring: 461, 462, 462, and 462; FHS Gen3: 637, 637, 637, and 637; NHANES 2003–04: 406, 406, 407, and 406; and NHANES 2005–06: 336, 337, 337, and 337. Abbreviations: BW, body weight; FHS, Framingham Heart Study; Gen3, FHS Third Generation cohort; M1, model 1.

of diet–disease associations, not a confounder, owing to its presence on the causal path.

In the protein gram model, the question being answered is: what is the relation between protein intake and an outcome, when energy is held constant? This, and all models, are isoenergetic; in theory, higher protein, in grams, displaces other macronutrient contributions to energy (e.g., fat and carbohydrate, not included in the model). With BW (or BMI) in the model, we additionally ask about that association in the context of constant weight (i.e., weight maintenance). The interpretation of the percent energy model is similar to that of the gram model, where energy, but not other macronutrients, are held constant. It remains agnostic to the "replacement" macronutrient. Results are, unsurprisingly, similar to those of the gram model. Of note, total energy should ideally be included in models of diet–disease associations, given the biological relevance and potential confounding of energy intake in most health/disease outcomes. For protein as percent energy, authors should carefully consider the effect and interpretation of energy and macronutrient contributions in statistical modeling, as often all energy sources are included simultaneously, rendering any such model nonsensical.

In the protein g/kg actual BW model, the question being answered is: what is the relation between protein per kilogram of BW with an outcome, when energy is held constant? Although the model is also isoenergetic, whether protein increases, BW decreases, or both, per unit increase in the ratio, is ambiguous. For example, say a hypothetical 50 kg individual consumes 0.8 g/kg actual BW, or 40 g protein. A tenth of a unit increase in the ratio (e.g., from 0.8 to 0.9 g/kg) could mean that the individual increased their protein intake by 5 g (i.e., to 45 g) with their BW unchanged, or it could mean that the individual lost ∼5.6 kg (i.e., to 44.4 kg) with the protein intake unchanged. Almost certainly, a ∼10% loss in BW will have more of an effect on cardiometabolic outcomes than a 5 g increase in protein. From a statistical standpoint, another way to think about a model using the ratio of g/kg is as a model that includes an interaction term (g × 1/kg), but fails to include the first-order terms. After adjusting for BW in the model, the denominator of the ratio is effectively cancelled out. Yet another way to consider this is that evaluating g/kg actual BW as an independent variable and any measure of body mass (e.g., fat mass, BMI, weight) as the dependent variable automatically puts mass on both sides of the equation. As shown in the present results, the g/kg actual BW model adjusted for BW is equivalent to the gram model unadjusted for BW, as would be expected. However, the model is incomplete because body mass has not been accounted for (just "cancelled out") and, as noted, a measure of body mass needs to be included in examinations of diet–disease associations. Adjusting this model for BMI does not solve the problem of BW confounding, but rather cancels the association of BW and introduces the reciprocal of height, squared.

The g/kg ideal BW model is important, given the WHO recommendation unit, and intriguing in terms of its interpretation both before and after BW is introduced in the model. The simple energy-adjusted model asks, essentially, "if a person is at an ideal weight, and maintains energy intake, what is the effect of higher protein per kilogram on a given cardiometabolic outcome (thus displacing other energy-contributing macronutrients)?" Adding actual BW, or more ideally, BW in excess of ideal, as a variable in this model, could thus be thought of as controlling for (a proxy for) excess BW.

Protein as g/kg lean or fat-free BW are slightly more challenging to interpret. Similar to g/kg actual BW, both units are also correlated with BW. In the modeling demonstration, results generally align with those for g/kg ideal BW. Adjusting for total BW reflects again the idea of excess adiposity being accounted for in the adjusted model.

Integrating the demonstration and the literature

Buendia et al. (64) employed an apparently rarely used approach of adjusting protein intake (grams) for BW using the residual method (as opposed to the more frequent energy intake adjustment) in their analyses of protein intake in relation to blood pressure and incident hypertension at exams 3 and 5 of the Framingham Offspring cohort. This, the authors argued, removed the correlation between protein intake and BW, as it is the objective of residual adjustment methods. Interestingly, this approach was advocated for in a recent response letter by Greenberg (99) to a study that expressed protein in g/kg BW (49) [actual, not ideal BW, although that was unclear until the authors' own response (100)] in relation to incident diabetes. In the letter, Greenberg (99) argued that the results of Sluik et al. (49), which become nonsignificant after adjusting for BMI, were confounded by BW, in a perfect illustration of the problem we are addressing in the present endeavor. He suggested that protein intake be adjusted for both energy and BW prior to generating a ratio measure and further analysis. In the present analysis, we included a measure of protein intake, both energy and BW adjusted, in a ratio with BW. Despite such adjustments, the associations remained confounded by BW and/or BMI, not because protein intake was correlated with either BW or energy (both $r \leq 0.1$ in all cohorts), but because of the ratio itself.

Including 4 different sample populations in the demonstration allowed us to observe the natural variability that exists from sample to sample. Despite using the same analytic approach (indeed a single analyst) in each cohort, variability in associations between protein and cardiometabolic outcomes was evident. Yet, we were able to observe a consistent confounding by BW in ratio units. Modeling 7 different protein intake units allowed us to assess those commonly used in the literature. Considering the multiple ways in which protein intake is expressed additionally strengthened this modeling exercise, because it demonstrated the consistency across models based on the units that include or do not include aspects of BW.

Importantly, the objective of this paper was neither to conclusively reach a consensus about protein's effects on

human health nor to generate estimates about the adequacy of protein intake in the American population, but rather to illustrate the challenges contributing to reaching a consensus on protein's effects on human health when various units of protein intake are used across the scientific literature, in the context of the population prevalence of overweight/obesity. This is not withstanding differences that may be attributable to protein quality and source (e.g., animal- or plant-based), aspects of which were frequently investigated in the studies we reviewed.

Recommendations and suggested best practices for future research

Units of g/d and percent energy, ideally in quantile categories, are appropriate, and generally give similar results. In observational studies, the residual adjustment of grams for energy intake is recommended to correct for misreporting, and should continue to be used; however, if available, other ways of adjusting self-reported intake, such as doubly-labeled water for total energy expenditure or using biomarker data, may be preferable (101). The unit of g/kg actual BW is ideally avoided in epidemiologic studies because of confounding by BW. However, given that the RDA is expressed in this unit, analyses will undoubtedly continue to be performed using this unit in observational studies. We showed that adjusting protein grams for BW using the residual method, prior to creating the g/kg actual BW unit, does not sufficiently address confounding by body mass, despite removing the numerator correlation with BW; thus, we would not recommend that approach. If using g/kg actual BW, then at a minimum, analyses should be stratified by BMI (in $\geq$3 categories, ideally more), as some author groups have done (102). In complementary analyses, or perhaps preferably, authors should use the unit of g/kg ideal BW. If using g/kg ideal BW, any BW in excess of the ideal value may be included in the model as a proxy of excess adiposity, to account for this important predictor of disease. Indeed, the use of g/kg ideal BW, whether in trials or with additional adjustments in observational studies for weight beyond ideal BW, would clarify protein needs in the context of overweight/obesity; that is, it would allow for investigators to assess the associations on cardiometabolic health of not only protein intake per unit of ideal BW, but separately of BW in excess of the ideal, as a proxy for the protein "needs" of excess fat mass. In fact, we believe this should be a part of ongoing reevaluations of the protein DRIs.

In trials that specify intervention arms based on g/kg actual BW, especially in cases of weight-loss objectives among participants who are obese or overweight, authors should specify whether recommendations/intake changed in an ongoing fashion based on the fluctuating actual weight (in the context of weight loss) or whether the initial weight value continued to be used to prescribe intake. To our knowledge, $\geq$1 weight-loss study has been specific in this regard (68). In addition, pre-post assessments of intake should include estimates of absolute intake (grams) as well as g/kg actual BW, based on the actual BW at the concurrent time point. This would allow readers to assess whether absolute protein intake (grams) actually increased, or whether intake changed simply because of weight loss (g/kg actual BW). Thus, authors should definitively and clearly state whether they use actual or ideal BW in generating trial arms, if the BW-based measure (as opposed to percent energy or AMDR) is being used. To take a hypothetical parallel-arm intervention trial, with Group 1 intake targeted at 1.0 g/kg actual BW at baseline and Group 2 intake targeted at 1.5 g/kg actual BW, a participant weighing 100 kg would initially be allotted 100 g/d in Group 1 and 150 g/d in Group 2. At the 6-mo trial point, by which point a Group 1 participant lost 20 kg, continuing to use the baseline weight would result in intake exceeding 1.0 g/kg actual BW (i.e., 1.25 g/kg actual BW). This would call into question whether a given participant in Group 1 is still adhering to the Group 1 intake, or lands closer to the Group 2 intake. In trials of protein intake in normal-weight participants, g/kg actual BW and g/kg ideal BW are very similar; thus, there is less of a need for close attention to what time point "actual BW" refers to.

In all studies of macronutrient composition, whether observational or experimental, particular care needs to be taken in selecting appropriate covariates. In any energy-constant approach, increasing or decreasing any single macronutrient will, by definition, alter $\geq$1 of the other 2 primary macronutrients (as well as alcohol, unless held constant). In observational studies of macronutrients as percent energy, if energy is held constant, only 2 of the 3 primary macronutrients can maximally also be in the model, such that if energy from protein and carbohydrates are in the model, any increase or decrease in protein intake reflects a comparable decrease or increase in energy from fats. Such "substitution" modeling needs to be explicit, as it would be in a trial. Other approaches to compositional energy modeling have also been proposed, such as those suggested by Leite (103, 104).

Finally, more research is needed on protein needs and requirements in the context of obesity driven by excess fat mass. We would further urge the US Dietary Guidelines committees to review the current RDA, which has remained unchanged since the beginning of the obesity "epidemic," in light of the prevalence of obesity in the United States, as well as the confusion around the BW denominator described herein.

Limitations

Our review of the literature was neither systematic nor exhaustive; rather, we aimed to locate and describe studies published since 2015 on a given cardiometabolic outcome that applied different units to arrive at different results. Although we have not sought to establish additional evidence on protein–disease associations, given the nonsystematic nature of our review, we may have missed important studies that would have contributed to our understanding of protein in human health.

There are known limitations to using DXA in quantifying lean and fat-free mass compartments (105, 106), which may

have affected our estimation of intake using these measures in the unit denominator, and thus any cardiometabolic associations shown in this paper. In addition, DXA scans in the various populations studied here were limited to nonpregnant women, and were variably limited by body size [e.g., <300 lbs (136 kg) due to machine restrictions] or age. Thus, any inferences derived from the present analyses may not be directly applicable to obese or elderly populations.

Dietary, and notably protein, intake self reports, whether by FFQ or recall, are prone to misreporting. There are multiple approaches to correcting misreported intakes, including energy adjustment by the residual method, as employed here (101). In addition, in NHANES, we used the mean of 2 d of recall, where available, rather than more sophisticated methods of estimating usual intake. Given that this was a modeling demonstration, that protein intake is a habitually rather than episodically consumed nutrient, and that we were not attempting to quantify population distributions of intake or reach conclusions about the quantity of protein intake in relation to outcomes, we did not employ the usual intake methodology. Therefore, the NHANES estimates presented here do not fully account for intra-individual variation, and may over- or underestimate habitual intake. In addition, we did not use protein biomarker data in the present analyses, as our work is based on the most common methods used in experimental and observational literature, which rely mainly on predefined intervention quantities and/or self-reported intake. That said, future work including biomarker data may illuminate additional associations between protein intake and human health, and further our understanding of the role of excess adiposity in protein requirements.

Finally, there remain a number of additional ways we could have modeled protein intake. For example, we might not have residually adjusted protein for energy, using crude estimates from the FFQs or 24-h recalls instead. For fat mass– or lean mass–based units, we might have adjusted these measures for height (e.g., a "fat mass index," which is similar to BMI in that kg fat mass is divided by height, squared). Given the exponentially greater challenge of interpreting additional units beyond the 7 herein, and the truly endless permutations possible, we limited ourselves to those which we presented.

Conclusions

Our understanding of the effects of protein intake on human health should not depend on the unit used to express protein intake in human nutrition studies; our review of the literature and modeling demonstration indicate it currently does. Authors should acknowledge differences between, and be specific about their use of, WHO compared with US recommended intake units. BW is a confounder of actual BW-based units; therefore, observational studies assessing the relation between dietary protein and disease outcomes should ideally use grams or percent energy, while adjusting for BW. In populations where overweight and obesity affect a majority, recommended intake based on actual BW should be reevaluated.

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References

1. Institute of Medicine. Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein, and amino acids (macronutrients) [Internet]. The National Academies Press; 2005. Available from: http://www.nap.edu/openbook.php?record_id=10490.
2. Wolfe RR, Cifelli AM, Kostas G, Kim I-Y. Optimizing protein intake in adults: Interpretation and application of the recommended dietary allowance compared with the acceptable macronutrient distribution range. Adv Nutr 2017;8:266–75.
3. Joint Expert Consultation on Protein and Amino Acid Requirements in Human Nutrition, Weltgesundheitsorganisation, FAO, United Nations University, editors. Protein and amino acid requirements in human nutrition: Report of a joint WHO/FAO/UNU expert consultation [Geneva, 9 - 16 April 2002]. Geneva, Switzerland: WHO; 2007.
4. Joint FAO/WHO/UNU Expert Consultation on Energy and Protein Requirements, editor. Energy and protein requirements: Report of a joint FAO/WHO/UNU expert consultation. Albany, NY: WHO Publications Center USA; 1985.
5. European Food Safety Authority Panel on Dietetic Products, Nutrition and Allergies (NDA). Scientific opinion on dietary reference values for protein: Dietary reference values for protein. EFSA J 2012;10:2557–623.
6. Nordic Council of Ministers. Nordic nutrition recommendations 2012. Nord Nutr Recomm 2014;5:1–3.
7. Richter M, Baerlocher K, Bauer JM, Elmadfa I, Heseker H, Leschik-Bonnet E, Stangl G, Volkert D, Stehle P, on behalf of the German Nutrition Society (DGE). Revised reference values for the intake of protein. Ann Nutr Metab 2019;74:242–50.
8. Agence Française de Sécurité Sanitaire des Aliments. Apport en protéines: Consommation, qualité, besoins et recommandations (Protein intake: Dietary intake, quality, requirements and recommendations Report No. 19.) [Internet]. Maisons-Alfort, France: Agence Nationale de Sécurité Sanitaire de L'alimentation, de L'environnement et du Travail; 2007. Available from: https://www.anses.fr/fr/system/files/NUT-Sy-Proteines.pdf.
9. National Research Council. Recommended dietary allowances [Internet]. Washington, DC: National Academies Press; 1941. Available from: http://www.nap.edu/catalog/13286.
10. Institute of Medicine. The role of protein and amino acids in sustaining and enhancing performance [Internet]. Washington, DC: National Academies Press; 1999. Available from: http://www.nap.edu/catalog/9620.
11. Murphy CH, Oikawa SY, Phillips SM. Dietary protein to maintain muscle mass in aging: A case for per-meal protein recommendations. J Frailty Aging 2016;5:49–58.
12. Traylor DA, Gorissen SHM, Phillips SM. Perspective: Protein requirements and optimal intakes in aging: Are we ready to recommend more than the recommended daily allowance? Adv Nutr 2018;9(3):171–82.
13. Thomas DT, Erdman KA, Burke LM. Position of the Academy of Nutrition and Dietetics, Dietitians of Canada, and the American College of Sports Medicine: Nutrition and athletic performance. J Acad Nutr Diet 2016;116:501–28.
14. Mazzulla M, Sawan SA, Williamson E, Hannaian SJ, Volterman KA, West DWD, Moore DR. Protein intake to maximize whole-body anabolism during postexercise recovery in resistance-trained men

with high habitual intakes is severalfold greater than the current recommended dietary allowance. J Nutr 2019;150(3):505–11.
15. Haring B, Selvin E, Liang M, Coresh J, Grams ME, Petruski-Ivleva N, Steffen LM, Rebholz CM. Dietary protein sources and risk for incident chronic kidney disease: Results from the Atherosclerosis Risk in Communities (ARIC) study. J Ren Nutr 2017;27:233–42.
16. Lee SW, Kim Y-S, Kim YH, Chung W, Park SK, Choi KH, Ahn C, Oh K-H. Dietary protein intake, protein energy wasting, and the progression of chronic kidney disease: Analysis from the KNOW-CKD Study. Nutrients 2019;11:121–33.
17. Hurt RT, McClave SA, Martindale RG, Ochoa Gautier JB, Coss-Bu JA, Dickerson RN, Heyland DK, Hoffer LJ, Moore FA, Morris CR, et al. Summary points and consensus recommendations from the international protein summit. Nutr Clin Pract 2017;32:142S–51S.
18. Tajima N, Noda M, Origasa H, Noto H, Yabe D, Fujita Y, Goto A, Fujimoto K, Sakamoto M, Haneda M. Evidence-based practice guideline for the treatment for diabetes in Japan 2013. Diabetol Int 2015;6:151–87.
19. Hales CM, Fryar CD, Carroll MD, Freedman DS, Ogden CL. Trends in obesity and severe obesity prevalence in US youth and adults by sex and age, 2007-2008 to 2015-2016. JAMA 2018;319(16):1723–5.
20. Ford ES, Dietz WH. Trends in energy intake among adults in the United States: Findings from NHANES. Am J Clin Nutr 2013;97:848–53.
21. Katz DL, Doughty KN, Geagan K, Jenkins DA, Gardner CD. Perspective: The public health case for modernizing the definition of protein quality. Adv Nutr 2019;10(5):755–64.
22. Gardner CD, Hartle JC, Garrett RD, Offringa LC, Wasserman AS. Maximizing the intersection of human health and the health of the environment with regard to the amount and type of protein produced and consumed in the United States. Nutr Rev 2019;77:197–215.
23. Wolfe RR, Rutherfurd SM, Kim I-Y, Moughan PJ. Protein quality as determined by the Digestible Indispensable Amino Acid Score: Evaluation of factors underlying the calculation. Nutr Rev 2016;74:584–99.
24. Mitchell CJ, Milan AM, Mitchell SM, Zeng N, Ramzan F, Sharma P, Knowles SO, Roy NC, Sjödin A, Wagner K-H, et al. The effects of dietary protein intake on appendicular lean mass and muscle function in elderly men: A 10-wk randomized controlled trial. Am J Clin Nutr 2017;106:1375–83.
25. Esmeijer K, Geleijnse JM, de Fijter JW, Kromhout D, Hoogeveen EK. Dietary protein intake and kidney function decline after myocardial infarction: The Alpha Omega Cohort. Nephrol Dial Transplant 2020;35(1):106–15.
26. Møller G, Rikardt Andersen J, Ritz C, P Silvestre M, Navas-Carretero S, Jalo E, Christensen P, Simpson E, Taylor M, Martinez JA, et al. Higher protein intake is not associated with decreased kidney function in pre-diabetic older adults following a one-year intervention-A preview sub-study. Nutrients 2018;10(1):54–65.
27. Malhotra R, Lipworth L, Cavanaugh KL, Young BA, Tucker KL, Carithers TC, Taylor HA, Correa A, Kabagambe EK, Ikizler TA. Protein intake and long-term change in glomerular filtration rate in the Jackson Heart Study. J Ren Nutr 2018;28:245–50.
28. Lew Q-LJ, Jafar TH, Koh HWL, Jin A, Chow KY, Yuan J-M, Koh W-P. Red meat intake and risk of ESRD. J Am Soc Nephrol 2017;28:304–12.
29. Oosterwijk MM, Soedamah-Muthu SS, Geleijnse JM, Bakker SJL, Navis G, Binnenmars SH, Gant CM, Laverman GD. High dietary intake of vegetable protein is associated with lower prevalence of renal function impairment: Results of the Dutch DIALECT-1 cohort. Kidney Int Rep 2019;4:710–9.
30. So R, Song S, Lee JE, Yoon H-J. The association between renal hyperfiltration and the sources of habitual protein intake and dietary acid load in a general population with preserved renal function: The KoGES Study. PLOS One 2016;11:e0166495.
31. Berryman CE, Agarwal S, Lieberman HR, Fulgoni VL, Pasiakos SM. Diets higher in animal and plant protein are associated with lower adiposity and do not impair kidney function in US adults. Am J Clin Nutr 2016;104:743–9.
32. Herber-Gast G-CM, Biesbroek S, Verschuren WM, Stehouwer CD, Gansevoort RT, Bakker SJ, Spijkerman AM. Association of dietary protein and dairy intakes and change in renal function: Results from the population-based longitudinal Doetinchem cohort study. Am J Clin Nutr 2016;104:1712–9.
33. Rebholz CM, Coresh J, Grams ME, Steffen LM, Anderson CAM, Appel LJ, Crews DC. Dietary acid load and incident chronic kidney disease: Results from the ARIC study. Am J Nephrol 2015;42:427–35.
34. Jhee JH, Kee YK, Park S, Kim H, Park JT, Han SH, Kang S-W, Yoo T-H. High-protein diet with renal hyperfiltration is associated with rapid decline rate of renal function: A community-based prospective cohort study. Nephrol Dial Transplant 2020;35(1):98–106. https://academic.oup.com/ndt/advance-article/doi/10.1093/ndt/gfz115/5511599
35. Kaji A, Hashimoto Y, Kobayashi Y, Wada S, Kuwahata M, Yamazaki M, Fukui M. Protein intake is not associated with progression of diabetic kidney disease in patients without macroalbuminuria. Diabetes Metab Res Rev 2019;35:e3150.
36. Teo BW, Toh QC, Xu H, Yang AYT, Lin T, Li J, Lee EJC. Dietary protein intake in a multi-ethnic Asian population of healthy participants and chronic kidney disease patients. Ann Acad Med Singapore 2015;44:145–9.
37. Cirillo M, Zingone F, Lombardi C, Cavallo P, Zanchetti A, Bilancio G. Population-based dose-response curve of glomerular filtration rate to dietary protein intake. Nephrol Dial Transplant 2015;30:1156–62.
38. Ogna A, Forni Ogna V, Bochud M, Guessous I, Paccaud F, Burnier M, Wuerzner G. Association between obesity and glomerular hyperfiltration: The confounding effect of smoking and sodium and protein intakes. Eur J Nutr 2016;55:1089–97.
39. Shang X, Scott D, Hodge AM, English DR, Giles GG, Ebeling PR, Sanders KM. Dietary protein intake and risk of type 2 diabetes: Results from the Melbourne Collaborative Cohort Study and a meta-analysis of prospective studies. Am J Clin Nutr 2016;104:1352–65.
40. Tian S, Xu Q, Jiang R, Han T, Sun C, Na L. Dietary protein consumption and the risk of type 2 diabetes: A systematic review and meta-analysis of cohort studies. Nutrients 2017;9:982–99.
41. Ye J, Yu Q, Mai W, Liang P, Liu X, Wang Y. Dietary protein intake and subsequent risk of type 2 diabetes: A dose-response meta-analysis of prospective cohort studies. Acta Diabetol 2019;56:851–70.
42. Fan M, Li Y, Wang C, Mao Z, Zhou W, Zhang L, Yang X, Cui S, Li L. Dietary protein consumption and the risk of type 2 diabetes: A dose-response meta-analysis of prospective studies. Nutrients 2019;11:2783–99.
43. Ke Q, Chen C, He F, Ye Y, Bai X, Cai L, Xia M. Association between dietary protein intake and type 2 diabetes varies by dietary pattern. Diabetol Metab Syndr 2018;10:48–58.
44. Li J, Sun C, Liu S, Li Y. Dietary protein intake and type 2 diabetes among women and men in Northeast China. Sci Rep 2016;6:37604–13.
45. Mirmiran P, Bahadoran Z, Esfandyari S, Azizi F. Dietary protein and amino acid profiles in relation to risk of dysglycemia: Findings from a prospective population-based study. Nutrients 2017;9:971–80.
46. Virtanen HEK, Koskinen TT, Voutilainen S, Mursu J, Tuomainen T-P, Kokko P, Virtanen JK. Intake of different dietary proteins and risk of type 2 diabetes in men: The Kuopio Ischaemic Heart Disease Risk Factor Study. Br J Nutr 2017;117:882–93.
47. Azemati B, Rajaram S, Jaceldo-Siegl K, Sabate J, Shavlik D, Fraser GE, Haddad EH. Animal-protein intake is associated with insulin resistance in Adventist Health Study 2 (AHS-2) calibration substudy participants: A cross-sectional analysis. Curr Dev Nutr 2017;1:e000299.
48. Campbell WW, Kim JE, Amankwaah AF, Gordon SL, Weinheimer-Haus EM. Higher total protein intake and change in total protein intake affect body composition but not metabolic syndrome indexes in middle-aged overweight and obese adults who perform resistance and aerobic exercise for 36 weeks. J Nutr 2015;145:2076–83.

49. Sluik D, Brouwer-Brolsma EM, Berendsen AAM, Mikkilä V, Poppitt SD, Silvestre MP, Tremblay A, Pérusse L, Bouchard C, Raben A, et al. Protein intake and the incidence of pre-diabetes and diabetes in 4 population-based studies: The PREVIEW project. Am J Clin Nutr 2019;109:1310–8.
50. Nabuco HCG, Tomeleri CM, Sugihara Junior P, dos Reis Fernandes R, Cavalcante EF, Antunes M, Burini RC, Venturini D, Barbosa DS, Silva AM, et al. Lower protein and higher carbohydrate intake are related with altering metabolic syndrome components in elderly women: A cross-sectional study. Exp Gerontol 2018;103: 132–7.
51. Liu X, Gan W, Gao C, Qi X, Liao X, Lin J, Zhao Y. The independent associations of protein consumption with body fat and glycaemic control in adult Chinese. Eur J Nutr 2019;58:1981–90.
52. Mansourian M, Yazdani A, Faghihimani E, Aminorraya A, Amini M, Jafari-Koshki T. Factors associated with progression to pre-diabetes: A recurrent events analysis. Eat Weight Disord-ST 2018;25:135–41 .
53. van Baak M, Larsen T, Jebb S, Martinez A, Saris W, Handjieva-Darlenska T, Kafatos A, Pfeiffer A, Kunešová M, Astrup A. Dietary intake of protein from different sources and weight regain, changes in body composition and cardiometabolic risk factors after weight loss: The DIOGenes study. Nutrients 2017;9:1326–39.
54. Wang J, Hong Z, Wu L, Ding B, Bi Y, Gu Z, Li W. Dietary intake and cardiometabolic biomarkers in relation to insulin resistance and hypertension in a middle-aged and elderly population in Beijing, China. Appl Physiol Nutr Metab 2017;42(8):869–75.
55. Nanri A, Mizoue T, Kurotani K, Goto A, Oba S, Noda M, Sawada N, Tsugane S, Japan Public Health Center-Based Prospective Study Group. Low-carbohydrate diet and type 2 diabetes risk in Japanese men and women: The Japan Public Health Center-Based Prospective Study. PLOS One 2015;10:e0118377.
56. Marco-Benedí V, Pérez-Calahorra S, Bea AM, Lamiquiz-Moneo I, Baila-Rueda L, Cenarro A, Civeira F, Mateo-Gallego R. High-protein energy-restricted diets induce greater improvement in glucose homeostasis but not in adipokines comparing to standard-protein diets in early-onset diabetic adults with overweight or obesity. Clin Nutr 2020;39(5):1354–63.
57. Mateo-Gallego R, Marco-Benedí V, Perez-Calahorra S, Bea AM, Baila-Rueda L, Lamiquiz-Moneo I, de Castro-Orós I, Cenarro A, Civeira F. Energy-restricted, high-protein diets more effectively impact cardiometabolic profile in overweight and obese women than lower-protein diets. Clin Nutr 2017;36:371–9.
58. Drummen M, Dorenbos E, Vreugdenhil ACE, Raben A, Fogelholm M, Westerterp-Plantenga MS, Adam TC. Long-term effects of increased protein intake after weight loss on intrahepatic lipid content and implications for insulin sensitivity: A PREVIEW study. Am J Physiol-Endocrinol Metab 2018;315:E885–91.
59. Watson N, Dyer K, Buckley J, Brinkworth G, Coates A, Parfitt G, Howe P, Noakes M, Murphy K. Effects of low-fat diets differing in protein and carbohydrate content on cardiometabolic risk factors during weight loss and weight maintenance in obese adults with type 2 diabetes. Nutrients 2016;8:289–304.
60. Campos-Nonato I, Hernandez L, Barquera S. Effect of a high-protein diet versus standard-protein diet on weight loss and biomarkers of metabolic syndrome: A randomized clinical trial. Obes Facts 2017;10:238–51.
61. Wright CS, Zhou J, Sayer RD, Kim JE, Campbell WW. Effects of a high-protein diet including whole eggs on muscle composition and indices of cardiometabolic health and systemic inflammation in older adults with overweight or obesity: A randomized controlled trial. Nutrients 2018;10:946–63.
62. Mente A, Dehghan M, Rangarajan S, McQueen M, Dagenais G, Wielgosz A, Lear S, Li W, Chen H, Yi S, et al. Association of dietary nutrients with blood lipids and blood pressure in 18 countries: A cross-sectional analysis from the PURE study. Lancet Diabetes Endocrinol 2017;5:774–87.
63. Shang X, Scott D, Hodge A, English DR, Giles GG, Ebeling PR, Sanders KM. Dietary protein from different food sources, incident metabolic syndrome and changes in its components: An 11-year longitudinal study in healthy community-dwelling adults. Clin Nutr 2017;36: 1540–8.
64. Buendia JR, Bradlee ML, Singer MR, Moore LL. Diets higher in protein predict lower high blood pressure risk in Framingham Offspring Study adults. Am J Hypertens 2015;28:372–9.
65. Mattos CB, Viana LV, Paula TP, Sarmento RA, Almeida JC, Gross JL, Azevedo MJ. Increased protein intake is associated with uncontrolled blood pressure by 24-hour ambulatory blood pressure monitoring in patients with type 2 diabetes. J Am Coll Nutr 2015;34:232–9.
66. Kuwabara M, Niwa K, Ohno M, Hisatome I. High rate of calories from protein is associated with higher prevalence of hypertension. J Hum Hypertens 2019;33:340–4.
67. Mehrabani S, Asemi M, Najafian J, Sajjadi F, Maghroun M, Mohammadifard N. Association of animal and plant proteins intake with hypertension in Iranian adult population: Isfahan healthy heart program. Adv Biomed Res 2017;6:112–9.
68. Porter Starr KN, Pieper CF, Orenduff MC, McDonald SR, McClure LB, Zhou R, Payne ME, Bales CW. Improved function with enhanced protein intake per meal: A pilot study of weight reduction in frail, obese older adults. J Gerontol A Biol Sci Med Sci 2016;71:1369–75.
69. Bales CW, Porter Starr KN, Orenduff MC, McDonald SR, Molnar K, Jarman AK, Onyenwoke A, Mulder H, Payne ME, Pieper CF. Influence of protein intake, race, and age on responses to a weight-reduction intervention in obese women. Curr Dev Nutr 2017;1:e000703.
70. Smith GI, Commean PK, Reeds DN, Klein S, Mittendorfer B. Effect of protein supplementation during diet-induced weight loss on muscle mass and strength: A randomized controlled study. Obesity (Silver Spring) 2018;26:854–61.
71. Bhasin S, Apovian CM, Travison TG, Pencina K, Moore LL, Huang G, Campbell WW, Li Z, Howland AS, Chen R, et al. Effect of protein intake on lean body mass in functionally limited older men: A randomized clinical trial. JAMA Intern Med 2018;178: 530–41.
72. Antonio J, Ellerbroek A, Evans C, Silver T, Peacock CA. High protein consumption in trained women: Bad to the bone? J Int Soc Sports Nutr 2018;15:6–11.
73. Rossato L, Nahas P, de Branco F, Martins F, Souza A, Carneiro M, Orsatti F, de Oliveira E. Higher protein intake does not improve lean mass gain when compared with RDA recommendation in postmenopausal women following resistance exercise protocol: A randomized clinical trial. Nutrients 2017;9:1007–22.
74. Witjaksono F, Jutamulia J, Annisa NG, Prasetya SI, Nurwidya F. Comparison of low calorie high protein and low calorie standard protein diet on waist circumference of adults with visceral obesity and weight cycling. BMC Res Notes 2018;11:674–9.
75. Zhang L, Pagoto S, Olendzki B, Persuitte G, Churchill L, Oleski J, Ma Y. A nonrestrictive, weight loss diet focused on fiber and lean protein increase. Nutrition 2018;54:12–8.
76. Schiavo L, Scalera G, Pilone V, De Sena G, Quagliariello V, Iannelli A, Barbarisi A. A comparative study examining the impact of a protein-enriched vs normal protein postoperative diet on body composition and resting metabolic rate in obese patients after sleeve gastrectomy. Obes Surg 2017;27:881–8.
77. Muscariello E, Nasti G, Siervo M, Di Maro M, Lapi D, D'Addio G, Colantuoni A. Dietary protein intake in sarcopenic obese older women. Clin Interv Aging 2016;11:133–40.
78. Kim JE, O'Connor LE, Sands LP, Slebodnik MB, Campbell WW. Effects of dietary protein intake on body composition changes after weight loss in older adults: A systematic review and meta-analysis. Nutr Rev 2016;74:210–24.
79. Hernández-Alonso P, Salas-Salvadó J, Ruiz-Canela M, Corella D, Estruch R, Fitó M, Arós F, Gómez-Gracia E, Fiol M, Lapetra J, et al. High dietary protein intake is associated with an increased body weight and total death risk. Clin Nutr 2016;35:496–506.
80. Ankarfeldt MZ, Gottliebsen K, Ängquist L, Astrup A, Heitmann BL, Sørensen TIA. Dietary protein and urinary nitrogen in relation to 6-year changes in fat mass and fat-free mass. Int J Obes 2015;39:162–8.

81. Beasley JM, Deierlein AL, Morland KB, Granieri EC, Spark A. Is meeting the recommended dietary allowance (RDA) for protein related to body composition among older adults?: Results from the Cardiovascular Health of Seniors and Built Environment Study. J Nutr Health Aging 2016;20:790–6.
82. Popp CJ, Beasley JM, Yi SS, Hu L, Wylie-Rosett J. A cross-sectional analysis of dietary protein intake and body composition among Chinese Americans. J Nutr Sci 2019;8:e4.
83. NIH National Heart, Lung, and Blood Institute, Biologic Specimen and Data Repository Information Coordinating Center. Framingham Heart Study (FHS) Offspring (OS) and OMNI 1 cohorts. Accession Number HLB00060019b [Internet]. 2009. Available from: https://biolincc.nhlbi.nih.gov/studies/framoffspring/.
84. NIH National Heart, Lung, and Blood Institute, Biologic Specimen and Data Repository Information Coordinating Center. Framingham Heart Study (FHS) Third Generation (Gen III), OMNI 2, and New Offspring (NOS) cohorts. Accession Number HLB00911219a [Internet]. 2012. Available from: https://biolincc.nhlbi.nih.gov/studies/gen3/.
85. Centers for Disease Control and Prevention (CDC), National Center for Health Statistics (NCHS). National Health and Nutrition Examination Survey [Internet]. Hyattsville, MD: US Department of Health and Human Services, Centers for Disease Control and Prevention; 2003. Available from: https://wwwn.cdc.gov/nchs/nhanes/ContinuousNhanes/Default.aspx?BeginYear=2003.
86. Centers for Disease Control and Prevention (CDC), National Center for Health Statistics (NCHS). National Health and Nutrition Examination Survey [Internet]. Hyattsville, MD: US Department of Health and Human Services, Centers for Disease Control and Prevention; 2005. Available from: https://wwwn.cdc.gov/nchs/nhanes/ContinuousNhanes/Default.aspx?BeginYear=2005.
87. Feinleib M, Kannel WB, Garrison RJ, McNamara PM, Castelli WP. The Framingham Offspring Study. Design and preliminary data. Prev Med 1975;4:518–25.
88. Splansky GL, Corey D, Yang Q, Atwood LD, Cupples LA, Benjamin EJ, D'Agostino RB, Fox CS, Larson MG, Murabito JM, et al. The Third Generation cohort of the National Heart, Lung, and Blood Institute's Framingham Heart Study: Design, recruitment, and initial examination. Am J Epidemiol 2007;165:1328–35.
89. Centers for Disease Control and Prevention (CDC), National Center for Health Statistics (NCHS). NHANES: National Health and Nutrition Examination Survey homepage [Internet]. 2018. Available from: https://www.cdc.gov/nchs/nhanes/index.htm.
90. Centers for Disease Control and Prevention (CDC), National Center for Health Statistics (NCHS). NHANES: Response rates and population totals [Internet]. Available from: https://wwwn.cdc.gov/nchs/nhanes/ResponseRates.aspx.
91. Rimm EB, Giovannucci EL, Stampfer MJ, Colditz GA, Litin LB, Willett WC. Reproducibility and validity of an expanded self-administered semiquantitative food frequency questionnaire among male health professionals. Am J Epidemiol 1992;135:1114–26.
92. Centers for Disease Control and Prevention (CDC), National Center for Health Statistics (NCHS). NHANES 2003-2004: Dietary interview - Total nutrient intakes, first day data documentation, codebook, and frequencies [Internet]. Available from: https://wwwn.cdc.gov/Nchs/Nhanes/2003-2004/DR1TOT_C.htm.
93. Centers for Disease Control and Prevention (CDC), National Center for Health Statistics (NCHS). NHANES 2005-2006: Dietary interview - Total nutrient intakes, first day data documentation, codebook, and frequencies [Internet]. Available from: https://wwwn.cdc.gov/Nchs/Nhanes/2005-2006/DR1TOT_D.htm.
94. Visser M, Kiel DP, Langlois J, Hannan MT, Felson DT, Wilson PW, Harris TB. Muscle mass and fat mass in relation to bone mineral density in very old men and women: The Framingham Heart Study. Appl Radiat Isot 1998;49:745–7.
95. Visser M, Harris TB, Langlois J, Hannan MT, Roubenoff R, Felson DT, Wilson PW, Kiel DP. Body fat and skeletal muscle mass in relation to physical disability in very old men and women of the Framingham Heart Study. J Gerontol A Biol Sci Med Sci 1998;53:M214–221.
96. Willett W. Nutritional epidemiology. New York, NY: Oxford University Press; 1998.
97. Willett WC, Stampfer MJ. Total energy intake: Implications for epidemiologic analyses. Am J Epidemiol 1986;124:17–27.
98. Levey AS, Stevens LA, Schmid CH, Zhang Y, Castro AF, Feldman HI, Kusek JW, Eggers P, Van Lente F, Greene T, et al. A new equation to estimate glomerular filtration rate. Ann Intern Med 2009;150:604–12.
99. Greenberg JA. Adjusting nutrition intake exposure variables for body weight. Am J Clin Nutr 2019;110:1511.
100. Brouwer-Brolsma EM, Raben A, Feskens EJM. Reply to J Greenberg and D Ibsen et al. Am J Clin Nutr 2019;110:1512–3.
101. Korth AL, Bhutani S, Neuhouser ML, Beresford SA, Snetselaar L, Tinker LF, Schoeller DA. Comparison of methods used to correct self-reported protein intake for systematic variation in reported energy intake using quantitative biomarkers of dietary intake. J Nutr 2020;150(5):1330–6.
102. Pasiakos SM, Lieberman HR, Fulgoni VL. Higher-protein diets are associated with higher HDL cholesterol and lower BMI and waist circumference in US adults. J Nutr 2015;145:605–14.
103. Leite MLC. Applying compositional data methodology to nutritional epidemiology. Stat Methods Med Res 2016;25:3057–65.
104. Leite MLC, Prinelli F. A compositional data perspective on studying the associations between macronutrient balances and diseases. Eur J Clin Nutr 2017;71:1365–9.
105. McLean RR, Kiel DP, Berry SD, Broe KE, Zhang X, Cupples LA, Hannan MT. Lower lean mass measured by dual-energy X-ray absorptiometry (DXA) is not associated with increased risk of hip fracture in women: The Framingham Osteoporosis Study. Calcif Tissue Int 2018;103:16–23.
106. Cawthon PM. Assessment of lean mass and physical performance in sarcopenia. J Clin Densitom 2015;18:467–71.