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Animal and plant protein usual intakes are not adversely associated with all-cause, cardiovascular disease–, or cancer-related mortality risk: an NHANES III analysis

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Abstract

We used data from NHANES 1988–1994 to examine associations between animal and plant protein usual intakes and IGF-1 concentration with mortality from all causes, cancer, and cardiovascular disease (CVD). Adult data ($N = 15\,937$) were linked with mortality data ($N = 3843$ events) through 2006. Usual intakes for protein were estimated using the multivariate Markov Chain Monte Carlo method. Hazard ratio (HR) models were fit for mortality types (all-cause, cancer, and CVD) with protein intake measures (per 1 g increase) and IGF-1 concentration ($N = 5753$). There were no associations between animal protein (HR = 0.99; 95% confidence interval (CI): 0.98–1.01; $P = 0.29$) or plant protein (HR = 1.02; 95% CI: 0.95–1.10; $P = 0.55$) intake for all-cause mortality. Similar results were seen for CVD mortality and animal protein (HR = 1.02; 95% CI: 0.99–1.04; $P = 0.14$) and plant protein (HR = 1.01; 95% CI: 0.91–1.13; $P = 0.81$). There was an (inverse) association between cancer mortality and animal protein (HR = 0.95; 95% CI: 0.91–1.00; $P = 0.04$) but no relationship with plant protein (HR = 1.08; 95% CI: 0.93–1.24; $P = 0.30$). We found no association between concentrations of IGF-1 ($N = 5753$) for all-cause mortality (HR = 1.00; 95% CI: 0.99–1.00; $P = 0.81$), CVD mortality (HR = 0.99; 95% CI: 0.99–1.00; $P = 0.53$) or cancer mortality (HR = 1.00; 95% CI: 0.99–1.00; $P = 0.76$). Our results remained unchanged when the sample was separated into younger (<65 years) and older (>65, or between 50 and 65 years) cohorts. Our data do not support the thesis that source-specific protein intake is associated with greater mortality risk; however, animal protein may be mildly protective for cancer mortality. Mortality risk was not associated with circulating IGF-1 in any age group.

Key words: NHANES, aging, health, animal protein, plant protein, disease

Introduction

The recommended dietary allowance (RDA) for protein is set at 0.8 g protein/kg/day in Canada and the US (Medicine 2005). The acceptable macronutrient distribution ranges (AMDR) describe a range of intakes associated with good health (Canada 2019*b*) and ranges from 10% to 35% of energy intake for protein. Oddly, dietary protein intakes that fall well within the AMDR are several times higher than the RDA (Wolfe et al. 2017). There is controversy over how much dietary protein is required to achieve optimal health (Wolfe et al. 2017), particularly in older people (Traylor et al. 2018). Some have reported associations between higher animal protein intakes and increased risk of mortality (Chen et al. 2020) due to cancer (Levine et al. 2014) and cardiovascular disease (CVD) (Chen et al. 2020). Some data suggest that plant protein may confer health benefits (Song et al. 2016; Naghshi et al. 2020; Qi and Shen 2020), other data show associations between increased protein intake and reduced mortality risk (Chan et al. 2019; Naghshi et al. 2020).

Using data from the Third National Health and Nutrition Examination Survey 1988–1994 (NHANES III), a previous study linked higher protein intakes with a 75% increase in overall mortality and an astonishing four-fold increased risk of cancer in adults aged 50–65 years (Levine et al. 2014). Interestingly, these findings were null if the protein source consumed was derived from plants (Levine et al. 2014), which would support guidelines such as Canada's Food Guide, which advocates for greater plant-based protein consumption (Canada 2019*a*).

A proposed mechanism for the cancer-promoting effects of protein is through IGF-1 (Levine et al. 2014; Rahmani et al. 2022). Previous studies have shown that protein intake is linearly associated with circulating IGF-1 levels (Giovannucci et al. 2003; Levine et al. 2014; Travis et al. 2016) and that lower protein intake may contribute to a lower risk of IGF-1-related cancer-related and all-cause mortality (Giovannucci et al. 2003; Levine et al. 2014; Travis et al. 2016). However, meta-analyses have proposed a U-shaped relationship between IGF-1 and mortality, with increased mortality in subjects with

lower or higher IGF-1 levels (van Bunderen et al. 2010; Burgers et al. 2011; Svensson et al. 2012).

Given the heterogeneous findings from cohort trials of the association of protein intake and mortality and the large (to our knowledge, the largest reported) increase in cancer risk associated with protein intake (Levine et al. 2014), the goal of the present study was to use data from NHANES III to examine associations between usual intake of animal and plant protein (which was no use din Levine et al. 2014) with all causes, cancer, and CVD mortality risk in adults of various age groups. We hypothesized that usual intakes of protein from animal sources would be associated with increased risk for mortality (Chan et al. 2019; Chen et al. 2020), but this relationship may depend on age (Levine et al. 2014). We also hypothesized that circulating IGF-1 would not be associated with mortality and that increased plant-sourced protein would not be associated with mortality and would be protective in the case of CVD- or cancer-related mortality (Song et al. 2016; Naghshi et al. 2020; Qi and Shen 2020).

Methods

The National Center for Health Statistics conducts the US NHANES survey, which has been thoroughly described in peer-reviewed publications (Fulgoni 2008; Pasiakos et al. 2015; Pikosky et al. 2022). For the current analysis, data were obtained from NHANES III, which comprised data from 1988 to 1994 and included protein intake data on all adults ≥ 19 years old. The analysis contained 15 937 (N = 7483 men and N = 8454 women) participants following exclusions for unreliable (<750 kcal/day) intake data, follow-up ineligible data, and pregnant or lactating females (refer to Fig. 1 for details).

SAS 9.4 was used for all analyses, and NHANES III survey parameters, including examination weights, strata and primary sampling units, were utilized. Data from men and women were linked with mortality data through 2006. Usual intakes were estimated for calories, total protein, animal protein, plant protein, total fats, and carbohydrates, and were modelled simultaneously using the multivariate Markov Chain Monte Carlo (MCMC) method (https://epi.grants.cancer.gov/hei/multivariate-mcmc-method.html) (Zhang et al. 2011). Briefly, the MCMC method is used when the diet is measured with error and when estimating an association of dietary intake with a relative risk, hazard ratio (HR), or other chosen health outcome. Simply using each individual's actual reported dietary intake without any adjustments leads to a biased estimate of any associations (Zhang et al. 2011). The MCMC method allows for usual intake estimates to be jointly modelled, thus reducing measurement error. To help address measurement error, for these analyses, we co-modelled energy, total protein, animal protein, plant protein, total fats, and carbohydrates with the addition of age (in four groups 19–30, 31–50, 51–70, and 71+ years), day of dietary recall (1 or 2), weekday/weekend dietary, and race/ethnicity in the MCMC models and adjusting for NHANES sample design using exam weights.

Detailed procedures for estimating, from diet recall, dietary protein intakes have been detailed extensively elsewhere (Fulgoni 2008; Pasiakos et al. 2015; Pikosky et al. 2022). Briefly, estimates of the nutrient content of reported foods are determined by linking food composition data provided by the USDA Nutrient Database for Standard Reference (SR). The ingredients of disaggregated survey food recipes (coded using the SR food codes) were linked to the appropriate food composition databases using the SR-Link file of the FNDDS (versions 4.1 and 5.0 link SR releases 22 and 24, respectively). Protein gram amounts by type associated with an intake in the NHANES individual foods file were obtained via the SR Links and SR nutrients files. Every SR code with protein was assigned via the SR code description to a source: animal, dairy, plant, or mixed protein. Mixed protein was used to denote that the source for the SR code was from more than one of animal, dairy, or plant protein. For each food code, the SR weights and links were used to determine the percentage of

Fig. 1. Flowchart of exclusions and total sample in the analyses. Values are presented with sexes combined, ≥19 years.

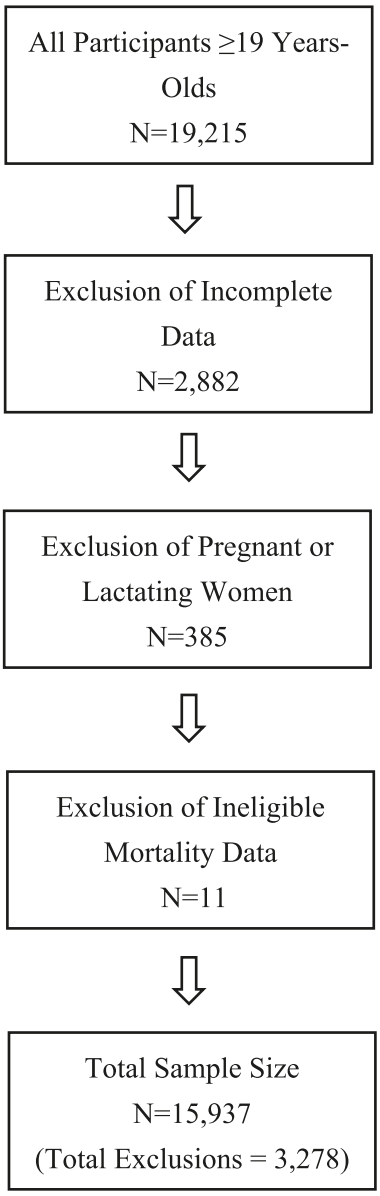


Table 1. Mean usual intakes of protein type by percentiles and IGF-I in US adults ≥ 19 years old.[a]

Variable	*N*	All		Percentiles of protein intake						*P*
		Mean	SE	33.3	SE	50	SE	66.7	SE	
Total protein (g)	15 937	82	1	69	1	72	2	97	1	<0.001
Animal protein (g)	15 937	57	1	46	1	50	2	68	1	<0.001
Plant protein (g)	15 937	25	1	21	1	22.6	1	29	1	<0.001
Carbohydrates (g)	15 937	266	1	223	1	239	6	307	1	<0.001
Total fat (g)	15 937	84	1	68	1	74.6	2	99	1	<0.001
Energy (kcal)	15 937	2186	4	1782	2	1915	63	2580	40	<0.001
IGF-1 (ng/mL)	5753	270	3	219	3	259	3	305	4	<0.001

[a]Sexes combined data from NHANES III; SE—standard error. Usual intakes were determined using the Markov Chain Monte Carlo method.

protein of each type (animal, dairy, plant, mixed) that made up the protein in the food code. These percentages were then applied to the total protein for the food code of each food consumed by each subject. Several food categories (such as mixed dishes, burritos and tacos, soups, cakes and pies, and eggs and omelets) were common for more than one source of protein.

HR models using SAS PROC SUREVYPHREG were fit for mortality types (all-cause, cancer, CVD) with protein intakes (total, animal, and plant) or IGF-1. The BOOTSTRAP option was used to estimate variances using 1000 replications. Survival models were created using follow-up months from the examination date reported in NHANES III, where loss to follow-up was censored. Pre-specified covariates added to the models were age (continuous), biological sex (categorical in combined analyses), physical activity level (categorical: sedentary, moderate, and vigorous based on response to physical activity questionnaire), current smoking status (categorical: yes/no), and dietary energy (continuous). Mortality status and date of death were recorded in the NHANES-linked National Death Index (NDI) public-access files through 31 December 2006, to replicate analyses of Levine et al. (2014). We merged the baseline data from NHANES III with follow-up data from the NDI. For participants with mortality recorded, follow-up time was defined as months until death date, while for other participants, follow-up was defined as months until 31 December 2006. Hazard models were run only with subjects with a complete set of covariates: (≥19 years old) males ($n = 7483$) and females ($n = 8454$) and median follow-up time was 174 months, and 2084 and 1759 deaths occurred in males and females, respectively.

While the primary focus of this work was on repeating analyses of results from a previous publication (Levine et al. 2014) with an updated methodology, the present study conducted additional novel analyses examining protein intake and mortality risk. Namely, we assessed the association of total protein, plant protein, and animal protein in both 5 and 10 g increments with all-cause, CVD, and cancer mortality risk rather than just on a per-gram basis as in the original analysis. Additionally, we also assessed the impact of simultaneously, including both animal and plant protein in HR models. Covariates in the models for these additional analyses were the same as those in the original analyses.

Results

The population characteristics of the participants in this round of NHANES have been described previously (Ford 1998; Seeman et al. 2008).

Subjects' usual intakes and IGF-1 levels

Table 1 shows the mean usual intakes of macronutrients, including animal and plant protein and percentiles of intake. The mean and distribution of IGF-1 values are also presented.

Usual animal protein and plant protein intake and all-cause, CVD, and cancer mortality risk

Table 2 shows the HR for usual animal and plant protein intakes and all-cause, CVD, or cancer mortality risk. Animal protein was not associated with increased risk; however, age, sedentary lifestyle, and smoking were associated with increased all-cause mortality risk (see Supplemental data). Usual intake of animal protein was shown to have a modest but significant inverse association with cancer mortality (HR = 0.95; LCL = 0.91, UCL = 1.00; $P = 0.04$). Usual intake of plant protein was not associated with all-cause, CVD, or cancer mortality risk. However, age, sedentary lifestyle, and smoking were associated with an increased mortality hazard (see Supplemental data). The results of our analysis were unaffected if mortality, either all-cause, CVD-, or cancer-related, was examined in adults who were 19–65 years old or older than 65 (data not shown).

In those 19+ years old, there were no associations of total protein and plant protein with all-cause, CVD, and cancer mortality risk when assessed on a 5 or 10 g increment basis (data not shown). For animal protein, there was no association with all-cause and CVD mortality when assessed on a 5 or 10 g increment basis. However, there was a significant inverse association with cancer mortality for both the 5 g (HR = 0.77; LCL = 0.61, UCL = 0.97; $P = 0.03$) and 10 g increment basis (HR = 0.60; LCL = 0.38, UCL = 0.95; $P = 0.03$). When animal protein and plant protein were included in hazard models simultaneously, the results remained the same. Thus, no association of either protein with all-cause or CVD mortality risk was observed. Further, results showed no association of plant protein with cancer mortality risk and an inverse association with animal protein with cancer mortality risk.

Table 2. Hazard ratios for usual animal and plant protein intake and all-cause, CVD, and cancer mortality.

	HR	LCL	UCL	*P*
All-cause mortality				
	Animal protein			
Animal protein intake (g)	0.92	0.78	1.08	0.286
	Plant protein			
Plant protein intake (g)	1.24	0.61	2.55	0.682
CVD mortality				
	Animal protein			
Animal protein intake (g)	1.19	0.95	1.51	0.132
	Plant protein			
Plant protein intake (g)	1.13	0.38	3.43	0.813
Cancer mortality				
	Animal protein			
Animal protein intake (g)	0.60	0.37	0.94	0.029
	Plant protein			
Plant protein intake (g)	2.08	0.49	8.88	0.314

Note: $N = 15\,937$; all-cause mortality $N = 4280$; cardiovascular disease (CVD) mortality $N = 1742$; and cancer mortality $N = 862$; adults $\geq$ 19 years old. HR—hazard ratio (per 10 g increase). LCL—lower 95% confidence limit, and UCL—upper 95% confidence limit.

Table 3. Hazard ratio and mean IGF-1 concentrations and risk of all-cause, CVD, and cancer mortality.

	HR	LCL	UCL	*P*
All-cause mortality				
IGF-1 (ng/mL)	0.95	0.91	1.17	0.81
CVD mortality				
IGF-1 (ng/mL)	0.92	0.93	1.21	0.53
Cancer mortality				
IGF-1 (ng/mL)	0.91	0.88	1.08	0.76

Note: $N = 5753$; all-cause mortality $N = 1199$; cardiovascular disease (CVD) mortality $N = 523$; and cancer mortality $N = 289$; adults $\geq$ 19 years old. HR—hazard ratio (per 10 g increase). LCL—lower 95% confidence limit; UCL—upper 95% confidence limit.

IGF-1 concentrations and mortality risk

Table 3 shows the risk of all-cause, cancer, and CVD mortality and IGF-1 concentrations. We observed no association between blood concentration of IGF-1 and mortality risk, whether all-cause, cancer-, or CVD-related.

Hazard ratio risk analysis by usual total protein intake tertiles

HR analysis in all adults (Fig. 2) revealed no significant associations between usual protein intakes by tertile and all-cause mortality risk, CVD-related risk, or cancer mortality risk.

Impact of age on hazard ratio risk analysis

According to tertiles of protein intake, we also analyzed all-cause, CVD-, and cancer-related deaths, and separated our sample into 19–65 years ($N = 13\,296$; mortality $N = 2905$) and 66 years and older ($N = 3903$; mortality $N = 1375$). Our results were unaffected and showed no HR differences between the younger and older cohorts for animal protein or plant protein for all-cause (Fig. 3), CVD-, and cancer-related mortality (results not shown). We also analyzed our data in an age group of 50–65 years men and women to compare our data to those from a previous study that reported marked differences in mortality risks for all-cause and cancer-related mortality with protein intakes (Levine et al. 2014). We observed no significant association between total protein intake by tertile and all-cause mortality risk in this age group or in those >65 years (Fig. 3).

Fig. 2. Usual tertile (69–97 g; see Table 1) of total dietary protein intake and hazard ratio (HR) for all-cause, cardiovascular disease- (CVD), and cancer-related mortality. Values are presented as HR with lower and upper 95% confidence intervals (CI). *P* values are indicated above each ratio. $N = 15\,937$; mortality $N = 4280$; adults $\geq$ 19 years.

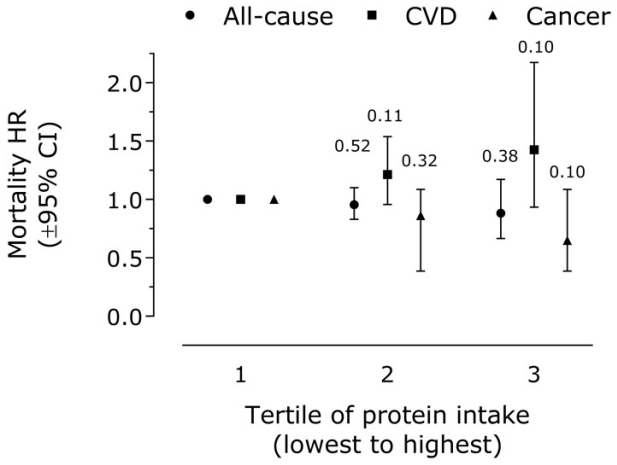
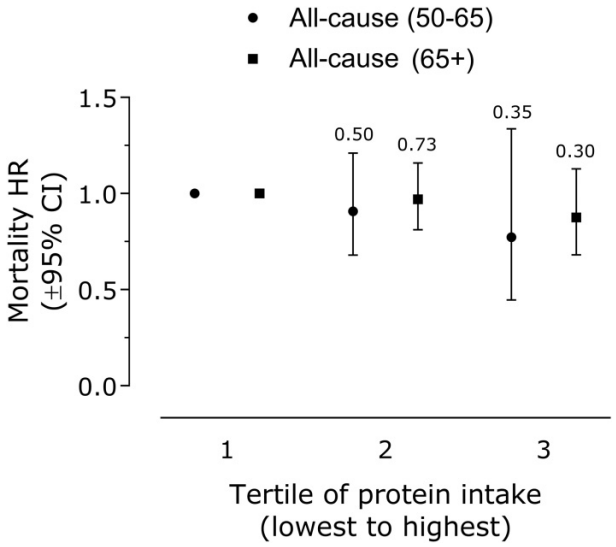


Fig. 3. Usual tertile (69–97 g; see Table 1) of total dietary protein intake and hazard ratio (HR) for all-cause mortality in >65 years old and 50–65 years old. Values are presented as HR with lower and upper 95% confidence intervals (CI). *P* values are indicated above each ratio. Adults 50–65: $N = 3185$, mortality $N = 857$; adults > 65 years; and $N = 3530$; mortality $N = 2526$.



We also observed no significant association between risk for CVD mortality in 50–65 years old and usual intake of animal protein (HR = 1.00; 95% CI: 0.97–1.04; $P = 0.76$) or plant protein (HR = 1.17; 95% CI: 0.88–1.54; $P = 0.25$) in this age group. Interestingly, there was a modest but significant protective relationship between cancer mortality and usual animal protein intake (HR = 0.87; 95% CI: 0.76–0.98; $P = 0.034$) but not for usual intake of plant protein (HR = 1.05; 95% CI: 0.85–1.31; $P = 0.64$). We observed no association with cancer mortality and IGF-1 concentration (HR = 1.00; 95% CI: 0.99–1.00; $P = 0.87$) in those 50–65 years old.

Discussion

Our analysis revealed no significant adverse associations between dietary protein from either plant or animal origin and all-cause or CVD-related mortality. We also did not observe any association between total protein intake, systemic IGF-1 concentrations, and cancer-related mortality. We observed a small but significant protective effect of animal protein and cancer mortality. The current findings contradict some previously published findings that have linked animal protein intake to increased mortality risk (Levine et al. 2014; Chan et al. 2019; Chen et al. 2020). We also did not find beneficial mortality outcomes with increasing plant protein consumption, contrasting with previous reports (Song et al. 2016; Budhathoki et al. 2019; Yamaoka et al. 2020). Nonetheless, our results are not at odds with other analyses that reported no association between protein intake and mortality or small, statistically significant, positive associations between protein intake and mortality (Budhathoki et al. 2019; Chan et al. 2019; Naghshi et al. 2020) or a recent study that found positive links between higher mid-life protein consumption and the odds of healthy aging (Ardisson Korat et al. 2024).

Studies of dietary protein intake on circulating GH and IGF-1 in humans have shown that dietary protein intake is associated with greater levels of IGF-1 (Kazemi et al. 2020). Interestingly, however, conditions of GH deficiency or GH resistance in humans are not associated with major increases in longevity (Bartke and Darcy 2017). Associations between increased levels of IGF-1 and modestly increased risk of prostate (Travis et al. 2016), breast (Key et al. 2010), and colorectal (Rinaldi et al. 2010) cancers have been reported; nonetheless, the observation is inverse for ovarian cancer (Li et al. 2016). Also, the association between IGF-1 and mortality has been suggested to be parabolic (Burgers et al. 2011; Rahmani et al. 2022), particularly for CVD mortality (Laughlin et al. 2004). Thus, it appears less than clear that lower levels of IGF-1 should be used as a hallmark of reduced chronic disease risk as systemic hormone levels are not prognostic for cancer (Morris et al. 2006), and the relationship to other age-related disease risks is nonlinear (Laughlin et al. 2004; Burgers et al. 2011; Westwood et al. 2014; Rahmani et al. 2022). These reports (Laughlin et al. 2004; Burgers et al. 2011; Westwood et al. 2014; Rahmani et al. 2022) yield a heterogeneous set of health recommendations related to IGF-1. For example, only ischemic heart disease and not all-cause mortality was associated with lower levels of IGF-1 (Laughlin et al. 2004). Alternatively, others have determined an association between IGF-1 and all-cause mortality but no association between IGF-1 and cancer- or CVD-related mortality (Burgers et al. 2011). Another study cited no association between higher IGF-1 and mortality (Rahmani et al. 2022). Our analysis showed no association between IGF-1 and disease-specific or all-cause mortality. We also observed no difference in HR and IGF-1 levels fitting alternative (nonlinear) curve-fitting models. Notably, the parabolic (or spline-derived) associations between IGF-1 and mortality did not actually test whether dietary protein and IGF-1 were related (Laughlin et al. 2004; Burgers et al. 2011; Westwood et al. 2014).

Associations with dietary nutrients and IGF-1 have also been observed with intakes of, among other nutrients: protein, carbohydrate, dietary fibre, retinol, beta-carotene, and wholegrain starch (Watling et al. 2021; Rahmani et al. 2022). Nonetheless, an "optimal" IGF-1 level has been proposed as between 120 and 160 ng/mL (Rahmani et al. 2022), which is an interesting concept. However, if the previously mentioned nutrients are associated with greater IGF-1 (and thus if the model is correct, risk), then the foods one would need to limit are difficult to determine. Nonetheless, an attempt to ascertain this was put forward by Rahmani et al. (2022), stating, "High consumption of dairy products including milk, cheese and yogurt, and margarine was associated with increased IGF-1 levels… while high consumption of butter, eggs, and egg products was associated with decreased levels of IGF-1". From the same analysis (Rahmani et al. 2022), there was no association between meat, processed meat, poultry, fish and shellfish, and vegetables with IGF-1. Our data are at odds with those reported by Rahmani et al. (2022), who also used NHANES III but only reported data on 2605 participants from

30–65 years; a reason for this choice was not immediately clear.

Our results showing a lack of relationship between dietary protein and IGF-1 and cancer contrasts with analyses for some (Key et al. 2010; Rinaldi et al. 2010) studies but not all (Chan et al. 2019; Naghshi et al. 2020; Ardisson Korat et al. 2024). Our results are, however, in stark contrast to the conclusions from Levine et al. (2014), who reported that in 50–65-year-old persons, those in their highest protein intake group had a 74% increase in their relative risk of all-cause mortality (HR = 1.74; 95% CI: 1.02–2.97) and were more than four times more likely to die of cancer (HR = 4.33; 95% CI: 1.96–9.56) when compared to those in their lower protein intake group. Even those in these authors' (Levine et al. 2014) self-defined moderate protein group showed a three times greater risk of cancer mortality (HR: 3.06; 95% CI: 1.49–6.25) than their low protein group. The protein groups defined by Levine et al. (2014) were not evenly sized quantiles of protein intake but were defined by the authors as high (20% or more of energy intake from protein; $n = 1146$, ~94 g/day), moderate (10%–19% of energy from protein; $n = 4798$, ~70 g/day), or low (<10% of energy from protein; $n = 437$, ~42 g/day). By comparison, our usual intake of tertiles comprised (from lowest to highest) equal energy intakes from protein. Partitioning our data into similar age intervals (50–65 years ($N = 3185$; mortality $N = 857$)) as those used by Levine et al. (2014), we observed no relationships between usual dietary protein intake and mortality (Fig. 3), and the same was true for the animal- and plant-derived proteins and CVD- and cancer-related mortality. In the same age group (50–65 years), we also saw no association between IGF-1 concentrations and mortality. Comparing the adjustments made to their model (Levine et al. 2014), we used the same adjustments with some differences; however, no adjustment variables that we tested changed our results or conclusions. Given that we used the same dataset, we are unable to fully account for the marked discrepancies between our data and those of Levine et al. (2014). We propose that many of the previously reported relationships (Levine et al. 2014) were spuriously high due to uneven numbers of participants in their respective groups and small numbers of cases of all-cause and disease-specific mortality artificially inflating risks when comparing arbitrarily defined protein intake groups. Additionally, Levine et al. (2014) did not use usual intakes but actual intakes; thus, their results may have been complicated due to intake measurement errors.

The 24-hour recall (24HR) method is used in NHANES for assessing dietary intake; however, the impracticality of conducting multiple 24HR (interviewer-administered, high volumes of food information that must be coded) has led to the development of models to estimate usual intake (Tooze et al. 2010). A limitation of the use of the 24HR (actual) intake method is the potential for violating an assumption it is unbiased for the consumption of the nutrient on that day (Tooze et al. 2010; Freedman et al. 2014).

Inverse associations between protein intake from plant-based sources and mortality risk due to a reduced CVD risk have been reported (Song et al. 2016; Chen et al. 2020). There are numerous hypotheses as to why increasing plant consumption may be inversely associated with CVD risk related to cholesterol-lowering effects, plant bioactive compounds, amino acid (methionine) restriction, improved insulin sensitivity, and interactions via the gut microbiome (Richter et al. 2015; Norman and Klaus 2020). Based on a lower risk for mortality seen with increased plant-based protein consumption (Song et al. 2016; Budhathoki et al. 2019; Naghshi et al. 2020; Qi and Shen 2020), recommendations to replace animal-derived with plant-derived protein have been made (Fernandez et al. 2020); however, our analysis shows no apparent benefit of this recommendation. Our data are not at odds with the conclusion that consuming plant foods does not benefit health (Aune 2019; Kahleova et al. 2019). We propose that plant-derived protein per se may not underpin the lower mortality risk with increasing consumption of plant-derived foods.

Our study has inherent limitations that require acknowledgement. We did not include comparator nutrients in our analysis, nor did we complete any substitution analysis, which has been criticized as a shortcoming of the types of analyses that we have conducted (Tobias 2022). We acknowledge that the IGF-1 results were done on only a sub-sample of the population, so the numbers of mortality events were lower than for the entire sample than the total sample; however, there were still sufficient events to make meaningful estimates of risk. The shorter follow-up time (12 years) could also have been extended to include data up to 2012 (18 years). We acknowledge controversy over the accuracy of the methods used to assess food intake using the methods employed in NHANES (Ahluwalia et al. 2016). While an in-depth discussion is not warranted here, we acknowledge that it has long been recognized that self-reported dietary intakes show consistent under-reporting of energy intake and, to some extent, macronutrients (Ahluwalia et al. 2016). We employed sophisticated procedures to determine usual intakes (MCMC method), simultaneously adjusting all intake variables and HR estimates were generated using a regression calibration approach with a bootstrap procedure with 1000 replications (see Methods for greater details). We acknowledge that using biomarkers may improve intake estimates, and urinary urea nitrogen could be used to assess (in a crude sense) protein intakes (Freedman et al. 2014).

In conclusion, using NHANES-linked mortality data through 2006, we report no significant associations between dietary protein intake and mortality risk, whether all-cause, CVD-, or cancer-related. We noted a small reduction in cancer mortality risk with increasing animal protein intake. We found no association between IGF-1 and mortality; these relationships were unmodified regardless of age.

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Data described in the manuscript, code book, and analytic code will be made publicly and freely available without restriction at the CDC's National Center for Health Statistics at wwwn.cdc.gov/nchs/nhanes.

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Formal analysis: SMP, VLF
Funding acquisition: YP, VLF
Investigation: YP, SMP, VLF
Methodology: YP, SMP, VLF
Project administration: YP
Validation: VLF
Visualization: VLF
Writing – original draft: YP, SMP
Writing – review & editing: YP, SMP, VLF

Competing interests
YP, as President of Nutritional Strategies, provides food, nutrition, and regulatory affairs consulting services for food and beverage companies and food-related associations. VLF, as Senior Vice-President of Nutrition Impact, provides food and nutrition consulting services for food and beverage companies. VLF also conducts analyses of NHANES data for members of the food industry. SMP reports grants or research contracts from the US National Dairy Council, Canadian Institutes for Health Research, Dairy Farmers of Canada, Nestle Health Sciences, Cargill, National Science and Engineering Research Council, Friesland Campina, and the US NIH during the conduct of the study; personal fees from Nestle Health Sciences, Nutricia, Optimal Nutrition and non-financial support from Enhanced Recovery, outside the submitted work. SMP has patents licensed to Exerkine but reports no financial gains from patents or related work.

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Supplementary material
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