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Naghshi S, Sadeghi O, Willett WC, Esmaillzadeh A. Dietary intake of total, animal, and plant proteins and risk of all cause, cardiovascular, and cancer mortality: Systematic review and dose-response meta-analysis of prospective cohort studies. BMJ. 2020;370.

What We Know, Think We Know, or Are Starting to Know

We know that protein is fundamental to human nutrition, and essential amino acids must be obtained through the diet, while certain conditionally-essential amino acids are required under specific circumstances, for example, injury (1). We also know that there is a substantial energy cost to digestion and absorption of proteins, with up to a quarter of total protein intake being lost as heat [thermogenesis] in the process of digestion (2).

This is because protein utilisation depends on energy in the body at every stage of amino acid transport, conversion, protein synthesis, and protein breakdown (3). Therefore, there are minimum daily requirements for dietary protein are required, and energy from carbohydrate and/or fat to ensure that sufficient dietary proteins remain available to satisfy amino acid demands (3).

We know that the median minimum required amount for daily dietary protein intake is around 0.66g per kilogram of bodyweight: the recommended daily intake of 0.83kg/kg/d is set based on an addition of 2 standard deviations of the variation in requirements from person to person [which is 0.085] (3).

Beyond this, there is extensive debate as to what "optimal" protein intake in the diet is. And there is emerging interest in the potential differential effects of protein source, whether animal proteins or plant proteins, which have been evident in a number of prospective cohort studies (4,5,6). The present study was a systematic review and meta-analysis of prospective cohort studies reporting the relationship between dietary protein and all-cause, cardiovascular, and cancer mortality outcomes.

The Study

The investigators conducted a systematic search of online databases of studies published up to 31st December 2019, including PubMed/Medline, ISI Web of Science, and Scopus. No limitations were placed on language or time of publication prior to the cut-off date.

To be included, studies had to be prospective cohorts in adults examining total protein, animal protein, and/or plant protein, and risk for all-cause mortality, cardiovascular disease [CVD] mortality, and cancer mortality. The primary studies had to have reported effect sizes in hazard ratios, relative risk, or odds ratios, and reported 95% confidence intervals. The exposures of interest were total protein, animal protein, and plant protein.

Both a systematic review* and a meta-analysis were conducted. For the meta-analysis, the effect sizes from the primary included studies were based on the standard practice of comparing the highest vs. lowest levels of intake in that cohort. In addition to this high vs. low analysis, the study also examined both the potential non-linear relationship between protein and mortality, and the potential linear relationship of adding 3% energy from total, animal, or plant proteins.

*Geek Box: Reviews

Reviews are common in research, and have the potential to be valuable to both peers in a given field as well as practitioners, by providing a synthesis of the current evidence and relevant citations. There are generally two types of review which are most common: narrative reviews and systematic reviews. A narrative review is, in effect, a review which discusses the scientific literature, but in narrative form these reviews may be highly prone to investigator bias, cherry-picking of studies, misreporting of findings [i.e., downplaying certain results and overstating other results], and often lack a direction as the specific research question being asked. Narrative reviews may be done well, but they are certainly prone to the aforementioned issues. A systematic review attempts to use objective and transparent methods to qualitatively synthesise evidence in relation to a specific research question. Systematic reviews should use predefined criteria to select, analyse, and synthesise available research. The analysis should include an assessment of the validity of the included studies, i.e., by using a study assessment tool, a popular example of which is the Grading of Recommendations, Assessment, Development, and Evaluation [GRADE] framework, although this has limitations for nutrition. Many of these assessment tools were designed to assess the effectiveness of drug treatments for disease, and do not always translate neatly to food/diet/ nutrients as the exposure of interest. A good systematic review should also rate the overall quality and confidence of the body of evidence, to provide conclusions in relation to the potential health effect of the exposure of interest.

Results: 31 studies were included in the entire paper. We' ll start with reporting the results from the systematic review, which simply stated whether the association was inverse [i.e., lower risk], positive [i.e., higher risk] or no significant association.

> ***Total Protein :*** All-Cause Mortality: 29 articles on the association between intake of total protein and all-cause mortality: 6 reported an inverse association; 1 showed a positive association; 22 reported no significant association.

> ***Animal Protein :*** All-Cause Mortality: 2 reported inverse associations; 13 reported no significant association.

> ***Plant Protein :*** All-Cause Mortality: 7 reported inverse associations; 8 reported no significant association.

> ***CVD Mortality :*** 2 reported inverse associations with total protein; 1 reported inverse associations with animal protein; 6 reported inverse associations with plant proteins.

> ***Cancer Mortality:*** 1 study each reported inverse associations with total protein and plant protein.

For the meta-analysis, 21 studies from the review presented sufficient data for comparison of the highest versus lowest categories of total protein intake.

> *All-Cause Mortality:*

- Total Protein: 6% reduction in risk [HR 0.94, 95% CI 0.89-0.99]
- Animal Protein: No significant association [HR 1.00, 95% CI 0.94-1.05]
- Plant Protein: 8% reduction in risk [HR 0.92, 95% CI 0.87-0.97]

> *CVD Mortality:*

- Total Protein: No significant association [HR 0.98, 95% CI 0.94-1.03]
- Animal Protein: No significant association [HR 1.02, 95% CI 0.94-1.11]
- Plant Protein: 12% reduction in risk [HR 0.88, 95% CI 0.80-0.96]

> *Cancer Mortality:*

- Total Protein: No significant association [HR 0.98, 95% CI 0.92-1.05]
- Animal Protein: No significant association [HR 1.00, 95% CI 0.98-1.02]
- Plant Protein: No significant association [HR 0.99, 95% CI 0.94-1.05]

In the dose-response analyses, there was no significant non-linear or linear association between total protein or animal protein any mortality outcome. For plant protein, there was no significant non-linear or linear association with CVD or cancer mortality.

However, there was a significant association between plant proteins and all-cause mortality in the non-linear dose-response and linear dose-response analysis. In the linear analysis, an additional 3% of energy per day from plant proteins was association with a 5% [HR 0.95, 95% CI 0.93-0.98] reduction in risk for all-cause mortality.

Critical Breakdown

Pros: The separate quantification of total protein, animal and plant protein, and examining both non-linear and linear relationships was a comprehensive analysis. The number of participants and number of deaths among the included studies was very large, and thus there was much greater statistical power to detect associations. Finally, including prospective studies meant that the dietary exposure [protein] was assessed prior to the occurrence of disease.

Cons: A bone of contention with most nutrition meta-analysis is that the actual “high” and “low” categories were not stratified or defined. Many included studies did not control for total energy or adjust for carbohydrate and fat, and thus the effect size of the primary study may not truly reflect an independent effect of protein as the exposure [i.e., are confounded by overall energy intake and macronutrients]. Many included studies used 24hr recalls, which are more prone to bias than food-frequency questionnaires. Finally, the risk of bias of the included studies was based on the ROBINS-E* tool, which is limited in its evaluation scope.

*Geek Box: Risk of Bias in Non-Randomized Studies of Exposures [ROBINS-E]

The Risk of Bias in Non-Randomized Studies of Exposures [ROBINS-E] is a tool for grading risk of bias, one of several tools that may be used in a systematic review or other synthesis of evidence. It is based off seven domains, and within each domain a rating of low, moderate, serious, or critical risk of bias is graded. However, there are a number of potential issues with the ROBINS-E that make it a rather insensitive tool for assessing bias. The first issue is that the ROBINS-E tool can result in studies being rated as 'critical' for bias, even though a study may have critical risk of bias in 7/7 domains, while other studies may have critical risk of bias in only 1/7 domains or 4/7 domains. Only a study with low risk of bias in 7/7 domains will be rated with low risk of bias overall. This is problematic it assumes each domain has equal weighting, when the actual influence of a given domain on the quality assessment of a study differs. It assumes the overall "grade" of each study is equivocal, when they are not. Another issue is that it is based on comparing observational studies to a randomised controlled trial, but this is an imbalanced comparison because there are numerous facets of an RCT that an observational study simply cannot match. It would be far more efficacious to take the best possible design and execution of an observational study as the benchmark for comparison. This is like taking a rally car and comparing it to a formula-1 car and complaining when the rally car doesn't have the characteristics of the F-1 car. It also assumes that RCTs, as the comparative design, are impeccable, which is simply not the case: RCTs have plenty of their own limitations. Overall, it does not appear to be a useful tool for qualitatively assessing bias in observational research.

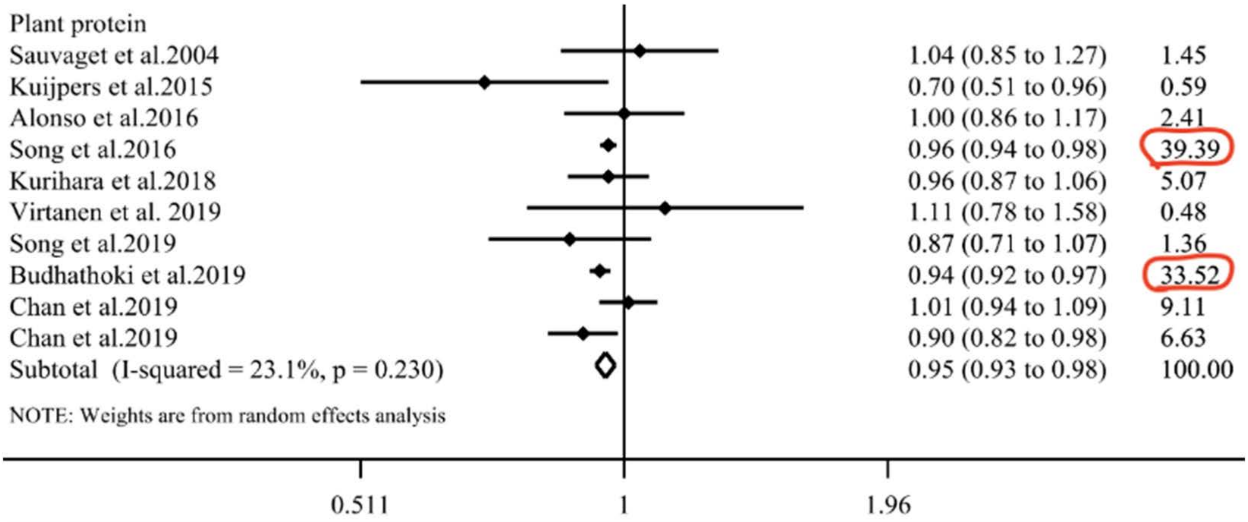
Key Characteristic

There was no stratification of studies based on similar levels of "high" or "low" intakes, which can be a major limitation for nutrition meta-analysis. For example, if "high" animal protein in one study is 20% energy, and it is 14% in another, it could be that 14% is the "high" quantile in another study. It is not possible to assume that these levels are equivocal in their health effects, particularly given that the source of the protein is important, i.e., higher animal protein may reflect higher red meat intake.

Let's think about this by reference to the two studies which contributed substantial statistical weight to the findings in the study. One study [5] was a Japanese cohort: highest vs. lowest animal protein intake was 11.7% vs. 4.1%, respectively, and the majority of animal protein from fish. The other study [6] was a US cohort: highest vs. lowest animal protein intake was 20% vs. 8.9%, respectively, and the majority of animal protein from meats. Mixing studies like this together is what noted epidemiologist Sander Greenland has called "distortive lumping". If meta-analysis in nutrition matched studies based on these important variables, perhaps findings more reflective of a given exposure, and more consistent findings, would be reported.

Interesting Finding

The dose-response analysis, particularly the linear dose-response analysis examining the effects of adding 3% energy from different sources, suggests that modest increases of plant proteins as a percentage of energy may improve health outcomes. Let's take a closer look at the forest plot from this dose-response analysis of all-cause mortality, you'll see that the statistical weight of two studies are circled in red: together these studies constitute 75% of the statistical weight contributing to the overall effect size [which was a 5% reduction in risk from adding 3% of energy from plant proteins].



Forest plot *of linear dose-response analysis for plant proteins and all-cause mortality from supplementary data.*

So, what were the levels of plant proteins in these studies? In the paper by Song et al., the lowest quintile was 2.6% and the highest was 6.6%; in the paper by Budhathoki et al., the lowest quintile was 4.9% and the highest was 8.6%. Bear in mind that each of these cohorts were omnivorous, i.e., the plant protein was a proportion of total protein intake, in addition to animal proteins. Based on these levels of intake, it is possible that modest increases in the quantity of plant protein in the diet may have benefit. The caveat is that the majority of the statistical weight in this dose-response analysis was derived from 2 studies, and the overall effect size was modest.

Relevance

It is important to bear in mind that in this meta-analysis the findings were largely "null": no significant association in the majority of included studies, and in the majority of the outcomes assessed. Overall, the systematic review indicates that the majority of the literature, whether reporting total protein, animal or plant protein, largely yields statistically insignificant findings. This qualitative synthesis was largely confirmed in the quantitative synthesis through meta-analysis.

However, there are still more pieces of the evidential puzzle that remain to be fully elucidated, in particular the mechanisms through which animal and plant proteins modulate risk, either through other constituents of the food matrix [i.e., fat, fibre, bioactive food components], or wider diet pattern [i.e., high meat vs. high plant]. For example, studies have found protein from processed red meat to be strongly associated with mortality, but no association found for protein from fish or poultry [6]. Conversely, studies have found the inverse relationship with plant proteins associate with higher soy food consumption [5]. Thus, the effects of food sources, and the balance of other foods and nutrients in the diet, may all be relevant factors influencing any associations with dietary protein and health outcomes.

In addition, practically all cohorts have omnivorous populations, and vegetarian cohorts still consume animal proteins from any combination of fish, eggs, or dairy. The epidemiology of vegan diets is in its infancy, and so there remain gaps in our knowledge in relation to factors, like protein digestibility, which are highly relevant to dietary protein effects [7]. In addition, similar to the relevance of the ratio of polyunsaturated to saturated fats for cardiovascular disease, it could be that particular ratios of animal to plant protein may be elucidated.

Overall, it is difficult to come to any cogent conclusions from prospective cohort studies alone. This systematic review and meta-analysis indicates largely null effects, with the potential for modest benefits from increasing plant proteins as a proportion of total energy.

Application to Practice

It is important to build any analysis with a nutrient as the exposure of interest back up into food-based recommendations. While this study did not analyse specific food sources of proteins, the benefit to adding modest amounts of energy from plant protein sources gives us an inference that legumes, soy foods, nuts, seeds, and other sources of plant proteins could be added to the diet, and likely confer a range of benefits beyond merely protein.

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