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## ► To cite this version:

François Mariotti. Animal and Plant Protein Sources and Cardiometabolic Health. *Advances in Nutrition*, 2019, 10 (Supplement\_4), pp.S351-S366. 10.1093/advances/nmy110 . hal-02366513

**HAL Id: hal-02366513**

**<https://hal.science/hal-02366513>**

Submitted on 30 Sep 2022

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## **Animal and Plant Protein Sources and Cardiometabolic Health**

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Word count: 8300

Figures: 3

François Mariotti “no conflicts of interest”.

No source of support for this review.

Author disclosures: FM is editor of a book entitled “Plant-based and vegetarian diets in health and disease prevention”.

Abbreviations used: CVD: cardiovascular disease; EPIC: European Prospective Investigation into Cancer and Nutrition; RCT: randomized controlled trial.

Running title: protein sources and cardiometabolic health

## **Abstract**

The sources or types of protein in the diet have long been overlooked regarding their link to cardiometabolic health. The picture is complicated by the fact that animal and plant proteins are consumed along with other nutrients and substances which make up the “protein package” so plant/animal protein come with clear nutrient clusters. This review aimed at deciphering the relation between plant and animal protein and cardiometabolic health by examining different nutritional levels (such as amino acids, protein type, protein foods, protein patterns and associated overall dietary and nutrient patterns) and varying levels of scientific evidence (basic science, RCT, observational data). Plant protein in western countries is a robust marker of nutrient adequacy of the diet, whereas the contribution of animal protein is highly heterogeneous. Yet recent data from large cohorts have confirmed that total and animal proteins are associated with the risk of CVD and diabetes, even when fully adjusting for lifestyle and dietary/nutritional factors. Here again, there is marked variability depending on the type of animal protein. Protein from processed red meat and total red meat on the one hand, and from legumes, nuts and seeds on the other, are often reported at the extremes of the risk range. RCTs using purified proteins have contributed little to the topic to date, inasmuch as the findings cannot readily be extrapolated to current or near-future diets, but RCTs studying whole protein foods have shown a beneficial effect of pulses. Despite many of the benefits of plant protein reported in observational or interventional studies may stem from the protein package that they convey and the nutrients that they displace, there are also important indications that protein per se may affect cardiometabolic health via the many amino acids that are present at typically contrasting levels in plant vs animal proteins.

Key words: cardiometabolic health, animal protein, plant protein.

## Introduction

As compared to lipid and carbohydrates, protein is the macronutrient that makes the smallest observed contribution to energy intake and the lowest level of recommended intake (1). Protein also has the specific feature that its dietary reference intake has been set on the basis of a strict nutritional requirement. Further, because protein is found in very large amounts in a limited number of foods, very high intakes are infrequent, and this results in the usual intake ranging from 12% to 20% (of energy intake) in economically developed countries (1, 2). While there is a longstanding debate on the levels of fat and carbohydrate that are best for long-term health, the issue of protein is more recent, and indeed there are still limited definitive data to address the question.

As with fat and carbohydrates, the question of the total amount of protein may seem outdated and somewhat misplaced: as I will illustrate in this paper, the type of protein may matter much more than the total amount. As far as cardiometabolic health is concerned, much progress has been achieved in understanding that the total amount of fat is of much less importance than the type of fatty acids (mostly making a distinction between saturated and polyunsaturated fatty acids) and the foods that convey them (distinguishing basically animal foods from vegetal oils) (3-6). Likewise, total carbohydrates now seem a useless descriptor that should be definitely broken down in order to consider different types of carbohydrates and their sources (so making a distinction in particular between low glycemic index foods and those containing added sugars) (7, 8). As for proteins, classic distinctions between sources or types of protein are only made on the basis of digestibility and indispensable amino acid contents, and with respect to the primary criterion of the protein requirement. Future efforts to define protein quality need to focus on studying dietary proteins in terms of health related outcomes, thus encompassing long-term outcomes relative to cardiometabolic health (9). However, this has mostly remained an emerging area of research and, until recently, what mattered in terms

of dietary protein was being above the nutritional allowance and of overall “good quality”, which means an ability to supply sufficient indispensable amino acids to renew body protein (1, 9, 10). Interestingly, by defining protein quality in this way, it becomes dependent on quantity. Therefore, in industrialized countries using varied sources of protein at surfeit levels of intake, this viewpoint cannot take account of the potential influence on health of the type or source of dietary protein. However, the growing importance and urgency of considering dietary protein in terms of sustainability (11-13) means it is now necessary to revise this persistent viewpoint and consider the spectrum of the relationship between the type of protein in the diet (animal vs plant protein, and different protein sources) and long-term health. In this review, I intend to focus on the relation with animal / plant protein sources and cardiometabolic risk.

The topic of protein sources or types and cardiovascular health can be traced back more than a century. That of animal protein and atherosclerosis was the subject of further study during the 1960s. Considerable progress has been made during the past ten years and in particular recently, based on an increasing body of evidence from randomized controlled trials (RCTs) and cohort studies. Our aim in this article is to review the literature on animal/plant protein and cardiometabolic health by consider varying levels of scientific evidence (basic science, RCT, observational data). Furthermore, since animal and plant proteins are consumed along with other nutrients and substances which make up the “protein package”, plant/animal protein come with clear nutrient clusters. Therefore, one specific objective of this review was to explore the relation between the intake of animal and plant protein and cardiovascular risk by considering different nutritional levels for “protein” (such as amino acids, protein type, protein foods, protein patterns and their association with overall dietary patterns and clusters of nutrient intakes).

## **The protein package and dietary protein intake as a marker of diet quality**

Protein intake goes with the “protein package” and results from complex dietary behaviors

There are two reasons for the associations between intakes of proteins of different types and the overall dietary and nutrient patterns seen in individuals. This arises first of all from the nutritional characteristics of the foods containing protein. To take a trivial example, animal protein tends to be accompanied by saturated fatty acids, whereas plant protein tends to come with fiber and phenolic compounds. This is usually referred to as the “whole food package”, or to be more specific the “protein package”; this is important if we are to understand the protein issue with respect to long-term health (14). The more intricate and specific the association between a food protein and other nutrients/substances supplied in the food offer, the stronger the relationship between their intakes in the diet will tend to be (15, 16). The second reason stems from the overarching behavioral or social factors that govern food choices. In western countries, specific protein foods may be chosen over other protein foods which they therefore displace and will be accompanied by different associated foods. For instance, a higher protein intake from fish is usually associated with a lower intake of red meat and high-fat dairy products, a higher intake of vegetables, and a lower intake of sweets as has been reported in the USA (17). These dietary features are also associated with more physical activity along with other markers of a health-conscious set of attitudes (such as less cigarette smoking and a greater use of vitamin supplements). Fish intake is also associated with other features of dietary behavior such as eating three meals a day more frequently, consuming fewer prepared meals and more organic foods, and thus implies a more favorable nutrient profile intake and of course, higher sociodemographic and economic categories, as has been shown in France (16, 18, 19).

Plant and animal protein sources and diet quality: the case of meat eating

It is therefore not surprising that the literature contains numerous reports of associations between protein sources and markers of diet quality. Because of the protein package, animal proteins have long been blamed for their contribution to an inadequate nutrient intake profile,

which may explain the association with cardiovascular risk (14, 20). This has been reported comprehensively with respect to meat intake in different populations and countries. The relationship between meat intake and diet quality has also been considered in studies comparing an omnivorous population qualified as “meat-eaters” versus non-meat eaters who eat fish (pesco-vegetarians) and vegetarians. As discussed by others (21), meat-eaters in the European Prospective Investigation into Cancer and Nutrition (EPIC)-Oxford study (22) had a higher energy intake and a different nutrient intake profile, with a higher qualitative contribution of SFAs and less fiber and PUFAs. However a simple direct comparison of the nutrient profile remains difficult since “meat-eaters” had also a higher intake of vitamin B-12, vitamin D, Zinc and iodine, as found in the EPIC-Oxford study and other studies on vegetarians (22-24). When considering overall profiles of dietary quality, such as those assessed using dietary quality indexes that are mostly based on food groups (such as Healthy Eating Indexes), the quality is much higher for non-meat-eating diets than in the general population (25). However, any direct comparison between vegetarian and non-vegetarian diets is largely confounded by behavioral factors affecting food choices (26, 27). Finer comparisons have been made with populations displaying less extensive ranges of meat consumption and behaviors than the general population, such as the US Adventists (24, 28), or when comparing high-meat eaters, low-meat eaters, poultry-eaters, fish-eaters (and vegetarians) (29, 30). In more general populations that are almost entirely composed of meat-eaters, meat intake has also been associated with poorer diet quality, as assessed from the profile of intake of certain important food groups: higher intakes of total meat were associated with lower intakes of vegetables, fruits and cereals in Europe (20); red meat intake was associated with lower intakes of fruits, whole grains and nuts in Finland (31), and a lower meat:fish ratio was a strong determinant of diet quality and nutrient profiles in Japan (32).

Protein patterns are strong markers of diet quality

The relationship between some specific animal proteins and diet quality indeed extends beyond the case of meat, and a more general link has been reported overall for plant vs animal proteins. Plant and animal protein intakes, both as a whole and in detail, show strong inverse associations with each other and strong associations with patterns of nutrient intake, as was clearly shown recently by Shang et al. (33) when studying the Melbourne Cohort. In more detail, and as shown in Figure 1, animal protein and total protein intakes were strongly associated, and animal protein was associated with lower intakes of plant proteins (including grains), fiber and vitamins E and C, and higher intakes of saturated fats. By contrast, plant protein was associated negatively with all animal proteins except fish, positively with fiber and vitamin E, and negatively with energy and SFAs. Indeed, the associations were much stronger regarding the intake of meat protein rather than total animal protein, and contrasted with fish protein when compared to total animal protein. Phillips and colleagues found in the USA that animal-based protein foods such as meat contributed more to the intake of protein and several “positive” nutrients (notably, zinc, vitamin B-12 and highly-bioavailable iron) as well as several “negative” nutrients (cholesterol and SFAs) than did plant-based protein foods (34). Conversely, the authors found that plant-based protein foods contributed more to the intake of other positive nutrients (notably dietary fiber, vitamin E, magnesium and PUFAs) (34). This confirms that animal-derived nutrients are an important component of the overall pattern of nutrient intake (35). To gain further insight into the nutrient profile associated with animal vs plant protein intake, we analyzed plant and animal protein intakes and the probabilistic overall nutrient adequacy of the diet of French adults (36, 37). We showed that as a general rule, total plant protein and each type of plant protein intake (cereals, legumes, nuts and grains, etc.) were strongly associated with nutrient adequacy (36, 37). The association between plant/animal protein intake and overall nutrient adequacy was explained by a large set of nutrients, including SFAs, sugars, potassium, sodium, folate, vitamin C, manganese, cholesterol and fiber (36). It



is important to note that this list includes many nutrients that are deemed to be of particular importance to cardiometabolic health, and in particular fiber, SFAs, magnesium, potassium and sugars. Some nutrients on this list clearly arise from the “protein package” (such as fiber, magnesium, potassium and SFAs) whereas others (namely sugar) come from a much more complex and indirect association between the nature of protein intake and food choices in the diet. Indeed, it is likely that rather than resulting from a displacement by plant protein foods, a lower sugar intake is due to a smaller contribution of added sugar to the diet of individuals who make healthier choices in general, although we adjusted for potential confounding factors (such as energy, alcohol intake, age and socioeconomic factors). Unlike the robust association with plant protein intake, we found in this population that there was a marked heterogeneity within the animal protein intake with respect to nutrient intakes and adequacy. This was expected because, as mentioned above, meat intake was not associated with the same protein package and dietary behaviors than fish or dairy intakes. In this respect, we found important contrasts between protein from fish and low-fat dairy (positively associated with the nutrient cluster related to global nutrient adequacy) and protein from processed meat, cheese and eggs (negatively associated). These associations also varied according to gender, with a negative association between red meat protein and nutrient adequacy in men only (36). Such a difference in gender with respect to meat intake was also reported by Fogelholm et al. (31) who found a positive association between red meat and vegetables in men only, and by Vergnaud et al. (20) who reported an opposite association between the intakes of meat and “sugar and confectionery” or “added fat”, depending on gender. When analyzing protein intake patterns in the general French population, we recently reported that individuals with a protein pattern with the largest contribution from eating pork had low overall scores for nutrient adequacy whereas the population with a dominant contribution from fish protein had the highest scores (16). In this general population, “pork eaters” and “poultry eaters”, were the groups with the

highest animal:plant protein ratio, whereas fish eaters had the lowest ratio. Likewise, “milk eaters” (i.e. individuals with high intakes of protein from milk) had a high animal:plant protein intake and their diet displayed relatively good nutrient adequacy (16). This further illustrates the definite heterogeneity within animal proteins regarding their associated clusters of nutrients. By contrast, our analysis showed that plant proteins, whatever their source (i.e. grains, legumes, seeds and nuts) and gender were consistently associated with nutrient adequacy.

Issues when interpreting current and future diet quality in terms of plant vs animal protein patterns.

The complex links between animal:plant protein intake and the overall characteristics of the diet were also found when examining the design of dietary interventional trials. The Dietary Approach to Stop Hypertension (DASH) diet, Omniheart diet and others such as the “Beef in an Optimal Lean diet” (BOLD) could be compared on the basis of their different levels of plant:animal proteins but neither the composition of these diets, nor their nutrient contents, were indeed simply related to this ratio (38, 39). The diets were indeed designed to increase the levels of certain nutrients (such as potassium, magnesium and fiber) and were based on an overall healthy dietary pattern which included fruits and vegetables and low-fat versions of dairy and meat products (40). The levels of plant versus animal proteins per se were secondary to the design of these diets and the results of these studies cannot be compared using this parameter alone (39).

It remains unclear whether the association between plant protein intake and overall nutrient adequacy can be ascribed mainly to the intrinsic characteristics of the foods that are currently available to compose our diet (i.e. to the “protein package” of the usual protein food groups), or if this might be largely confounded by the healthy behaviors of individuals who purposely adopt a diet containing more plants (i.e. linked to overarching factors of diet quality)

(41). Dietary diversity is an overarching factor in diet quality which we found was associated with both overall nutrient adequacy and plant protein intake in French adults (37). When analyzing this relationship in more detail, we found that the association between plant protein intake and nutrient adequacy was independent of overall dietary diversity (37). This result further strengthens the view that plant protein intake is a robust marker of the nutritional adequacy of the diet (36). Satija et al. (42) studied the relation between health and overall score of adherence to a plant-based diet or to a healthy plant-based diet (as measured using a plant-based diet index that positively counts plant food groups that are not fruit juices, sweetened beverages, refined grains, potatoes and sweets/dessert. The authors found that the negative association between a plant-based diet and a risk of diabetes in three USA cohorts was stronger when the healthy plant-based diet was considered). Similar findings were reported recently regarding the cardiovascular disease (CVD) mortality risk in the general US adult population (43). It is important to note that food groups that contribute negatively to a healthy plant-based diet are not rich in protein. When looking more closely at the data on the relationship with protein intake, it can be seen that contrary to other indexes, the healthy plant-based diet index is positively associated with total protein intake (42, 43). This association appears to be the result of a higher intake of plant protein and a moderately lower intake of animal protein. Therefore, plant/animal protein intake appears to be tightly associated with diet quality, even across different types of plant-based or animal-based diets in the general population.

However, what is difficult to infer from the present literature is the extent to which the association between plant protein intake and the intake patterns for nutrients that are important to cardiometabolic health may depend on the characteristics of the plant protein foods that are traditionally available, and whether this may change in the near future when new products come onto the market. This indeed reflects the classic nutrients-foods-patterns complexity (44). As pointed out in the analysis by Satija and colleagues, not all plant foods are healthy but the

plant protein foods currently available are mostly good contributors to healthy diets. This is typically the case of legumes, nuts and seeds, for instance. By contrast, it remains unclear whether the development of new plant protein products to be used as substitutes for meat products by the general population will cause a healthy revision of the plant protein food benchmark; there could be a risk that it will lead to a more heterogeneous relationship between plant protein intake and a healthy nutrient intake, and hence long-term health.

Finally, this intricate association between intakes of various protein sources, the overall dietary pattern and the profile of nutrient intake means that we should be very cautious when analyzing and interpreting observational data. There are indeed two types of confusion that should be borne in mind and do not have the same implications. The first concerns the association between the intake of plant/animal proteins, dietary patterns and overall health-related behaviors. This is indeed a classic pitfall in nutritional epidemiology. Carefully designed models for very large samples are good solutions to overcome this issue using residualization (45), but because the associations with protein intakes are strong, there will always be an important risk of residual confusion. The second type of confusion arises from the fact that when referring to “protein” in the diet we do indeed mean protein-rich products, i.e. protein plus the “protein package”, along with nutrients and other substances that are more or less closely associated with the protein per se. This second issue has implications when interpreting the results of observational studies. It means that it may not be possible to extrapolate the association found for “protein” in a given study to either another dietary context (another population not sharing the same dietary background). It also means that it may not be possible to extrapolate in the future if the population changes the nature of the foods conveying their protein, after a change in the food offer. Beyond observational data, this issue also has implications regarding the setting and interpretation of RCTs, as I shall discuss further below.

## **Plant and animal proteins in observational studies**

Until recently, only a few studies had specifically examined the association between dietary plant protein (versus animal protein) and the cardiovascular or diabetic risk, and they produced a mixed picture. When modelling the data from the Iowa Women's Health Study, Kelemen et al. (46) reported that plant protein could have potentially favorable effects on coronary heart disease mortality when compared with both animal protein and total carbohydrate. Indeed, an inverse association was observed when plant protein replaced either carbohydrate or animal protein whereas no association was observed when animal protein replaced carbohydrates. When analyzed in the context of low-carbohydrate diets, plant (vs animal) -based diets were associated with lower rates of mortality from CVD in US cohorts (47), but there was little evidence for such a contrast in European cohorts (48). This difference could thus be ascribed to countries with different background diets and metabolic statuses of individuals, and different types of dietary substitutions between dietary proteins and carbohydrates (49). Because the models were adjusted to take account of numerous potential confounding factors (such as physical activity, BMI, energy intake, alcohol intake, smoking) but to take little or no account of the dietary factors associated with animal vs plant protein intakes, the associations with plant and animal protein could rather have been ascribed to some association with plant-based or animal-based diets, and the amount of animal/plant protein and fat that they conveyed. In other words, plant and animal proteins could in this case be taken as markers of underlying dietary patterns.

More recently, positive findings regarding plant vs animal proteins and cardiovascular risk have been reported by major cohort studies. After combining the large Harvard cohorts, Song et al. (50) reported significant associations between plant vs animal protein intake and CVD mortality. In models adjusting for lifestyle risk factors and for numerous dietary and fatty acid intakes, the authors found that a 10% increase in energy intake from animal protein would

translate into an 8% increase in CVD mortality. Conversely, a 3% increase in energy from plant protein would reduce this risk by 12%. When compared to the energy contribution of animal protein (mean: 14%, 5<sup>th</sup>-95<sup>th</sup> percentile: 9%-22%) and plant protein (4%, 2%-6%) in the population, the impact of equally rebalancing plant and animal protein could therefore be important. These associations were confined to participants with at least one unhealthy lifestyle factor (based on smoking, heavy alcohol intake, overweight or obesity and physical inactivity) and were not evident among those without any of these risk factors. In a Mediterranean population at high cardiovascular risk, animal protein (and not plant protein) was also associated with a higher risk of CVD events and mortality (51). In the large cohort of the Adventist Health Study 2, we found an association between CVD mortality and animal protein intake, but not total plant protein intake. An 18g increment of animal protein was associated with a 7% increase in CVD mortality, which is a higher estimate of the risk than that previously found. In the Adventist cohorts, the mean and range of animal protein was lower than in the general population, and the population had a better overall dietary and nutrient pattern, with fewer differences in lifestyle confounding factors across the different types of diet. Therefore, the results could mean that even at low/moderate levels of intake in a population with a good overall health status, an intake of animal protein is associated with a higher CVD risk.

An association between animal/plant protein and type-2 diabetes has also been confirmed by recent studies. In a very large cohort of type 2 diabetics in eight European countries (EPIC-InterAct), it was found that animal protein was associated with a higher risk of diabetes, after adjusting for dietary and nutrient intakes. The relationship tended to be attenuated but persisted after adjustment for BMI and waist circumference, suggesting that the effect of animal protein intake was not entirely mediated by its association with body weight and composition (52, 53), a classical association in the literature (52, 53). Similar results were found in Chinese women, with evidence for mediation by insulin resistance, independently of

BMI (54). Genetic susceptibility to diabetes did not change the associations (55). Based on the Melbourne Cohort (21,523 participants), Shang et al. (56) also reported a higher risk of incident diabetes in individuals with a higher animal protein intake. These positive associations appeared to be stronger in individuals with normal baseline plasma glucose, BMI or blood pressure, and were more marked in men. An association with plant protein intake and diabetes was only found in women. In another recent analysis of USA cohort studies that included a large number of diabetics, the relationship was confirmed for animal protein, but it was also found that plant protein was inversely associated with risk, irrespective of gender (57). A recent meta-analysis (58) confirmed that the data overall clearly demonstrate an association between total and animal protein intake and diabetes risk, but do not reveal a significant association with plant protein, as reported previously (56). Interestingly, it has been argued that the relation with animal protein may be non-linear, with important effects only being observed under high intakes, and may only be mediated in part by BMI (59). It was recently found that total and animal protein intake, and the animal:plant protein ratio was strongly and positively associated with insulin resistance in a cross-sectional analysis of middle aged and older people from the Adventist Health Study 2 (60). These results suggest that the effect of animal protein on insulin sensitivity can also occur at the low levels of intake seen in this population.

Overall, these results on diabetes risk and CVD mortality are generally in line with the findings regarding intermediary endpoints for cardiometabolic health. A relation between the type of protein intake and incident metabolic syndrome was recently documented in the Melbourne Cohort study (33). The authors reported a higher risk with higher total and animal protein intakes and lower plant protein intakes. A higher energy intake from animal protein was associated with increases in systolic blood pressure, waist circumference and body weight over 11 years, whereas a higher plant protein intake was associated with reductions in waist circumference and weight (33).

Considerable study over many years has focused on the relation between animal/plant protein intake and blood pressure (61). A number of transversal or longitudinal studies reported negative associations between plant protein and blood pressure (62-66). However, total protein intake was shown to be inversely associated with blood pressure in many studies, and some reported inverse associations between animal protein intake and blood pressure or the risk of hypertension (67-69). Some of the heterogeneity affecting these findings could be linked to the background dietary context and the type of animal protein consumed, which would explain the contrasting findings in Asian vs Western populations. Taken together, however, the difference between the effects of plant or animal protein on blood pressure does not appear to be so marked (70-72), unlike the harder endpoints discussed above.

#### **Beyond total plant and total animal protein: the type of animal/plant protein matters**

It has become clear in the last decade that plant protein and animal protein are overly coarse descriptors of protein intake. When reviewing the epidemiological studies that have attempted to analyze in detail the association between more specific protein sources and some health endpoints, we can see dramatic differences between specific protein sources within the “animal protein” type. In a recent meta-analysis of diabetes risk, the intake of proteins from red meat and processed meat was associated with a higher risk, whereas the intake from dairy products was associated with a lower risk (58). Studies with fully adjusted models of substitution have been helpful in further analyzing this heterogeneity. When modeling the effect of replacing one portion of animal protein food with one portion of average plant protein food (comprised of whole grains, legumes, peanuts, peanut butter and other nuts), Malik et al. (57) found that substitution for processed meat was associated with a greater benefit with respect to diabetes risk (a 21% reduction in risk) while the association with dairy protein was not significant (Figure 2). Red meat was intermediate in this gradation. Interestingly, high-glycemic index



foods such as refined grains and potatoes were also associated with a higher risk when compared to plant protein foods (Fig. 2). In the Melbourne Cohort, Shang et al. (33) ascribed the positive effect of animal protein on incident metabolic syndrome and its components to red meat and chicken, and the negative effect of plant protein to legumes and nuts. Red meat was the animal protein which was found to be significantly associated with diabetes risk in a Chinese cohort (54). In the context of low carbohydrate diets, red and processed meats have been implicated in the positive association between intakes of animal protein and fat and a higher risk of diabetes (73). Since these studies adjusted for BMI, there could be additional effects of meat on diabetes risk than those mediated by changes to BMI (74-76). An elucidation of the underlying mechanisms supports the causal role of processed and total red meat consumption in increasing risk of type 2 diabetes (77). These possible mechanism include role of saturated fatty acids, sodium, advanced glycation end products, nitrates/nitrites for processing, heme iron, trimethylamine N-oxide, branched amino acids, and endocrine disruptor chemicals (77).

As far as cardiovascular mortality is concerned, and using the same approach of protein source substitution in the Harvard cohorts, it was found that replacing processed meat by plant protein was the most beneficial (39% reduction in risk), while replacing unprocessed meat (17% reduction in risk) or dairy (11% reduction) procured the most modest benefits (50). These findings have clarified earlier reports regarding the association between the risk of coronary heart disease and the consumption of protein food groups in the Nurses' Health Study in 2010. In this report, Bernstein et al. (78) found that in a model controlling statistically for energy intake, one serving/day of nuts was associated with a 30% lower risk when compared to one serving/day of red meat. Similarly, when compared to one serving/day of red meat, a lower risk was associated with one serving/day of low-fat dairy (13% lower risk), poultry (19%) or fish (24%). This was also in line with the recent report from the *Prevención con Dieta Mediterránea*

(PREDIMED) study, where portions of red and processed meats involved the highest risk of incidence of metabolic syndrome, when compared to legumes, poultry and rabbit, fish or eggs (79). Because intakes of protein from various food groups are indeed much multicollinear, our first aim was to analyze the underlying structure of the protein pattern in order to identify the most salient component. Therefore, we analyzed the pattern of protein intake in the Adventist Health Study 2 and found five protein factors (independent of each other), which we then used in models to analyze cardiovascular mortality. We found significant and strong associations with two of these factors. Comparing the highest vs lowest quintiles of factor scores, we found a 61% higher risk for the "Meat" protein factor and a 40% lower risk for the "Nuts & Seeds" protein factor. These estimates were little influenced by other characteristics of the diet, such as vegetarian dietary patterns or nutrients related to CVD health. These associations were particularly strong among young adults aged 25–44 (with a 100% higher risk for the "Meat" protein factor and a 60% lower risk for the "Nuts & Seeds" protein factor); Figure 3.

Finally, although there is a very important body of evidence for their effects on diabetes and CVD risk, it should be kept in mind that red meat, and especially processed meat, are strongly associated with health-oriented behaviors that still make it difficult to disentangle the causal effects of red meat from observational studies (80).

### **Lessons from clinical trials**

Clinical trials offer another line of evidence regarding the specific effects of some plant and animal proteins. The literature proposes a large body of studies that have examined the effects of specifically manipulating plant proteins on intermediary endpoints related to cardiometabolic health. The studies can be divided into two different types. The first contains studies that manipulate protein in quite a specific manner by using purified proteins that can be included in experimental foods with a closely controlled composition. The second type

399 concerns studies that test the utilization of protein foods as included in individual diets, usually  
400 controlling for the macronutrient content of the diets. In a clinical trial, if a “plant protein” is  
401 tested using foods that are rich in that protein, the trial will refer to the effect of consuming  
402 certain plant protein-rich foods along with the protein package of these foods and not  
403 specifically the plant protein. It would be erroneous to assign the results to either a particular  
404 protein or to the protein food group in general, i.e. irrespective of variations in the nutrient  
405 contents of these protein foods (81). However, the findings of such studies have important  
406 implications for public nutrition, because they can be extrapolated to how individuals actually  
407 consume dietary proteins or would eat more of a dietary protein if willing to do so, using the  
408 protein foods that are available. By contrast, if a trial uses a protein ingredient that is purified  
409 (e.g. protein isolate) and used to prepare experimental foods, the results will apply more to  
410 “protein” but are less extrapolable to real life. Furthermore, it should be kept in mind that  
411 proteins are never pure and they usually convey >10% nutrients and other substances intimately  
412 associated with them (notably in the case of plant protein) (82). Even if they are intimately  
413 associated, there may be variations in the amounts and nature of the substances involved, thus  
414 making the “protein” quite heterogeneous. This has long been discussed regarding the amounts  
415 of isoflavones in soy protein, the opinion being that isoflavones may convey a large part of the  
416 benefit associated with “soy protein” (83-85). Another point (which might seem a detail and is  
417 often overlooked) is that protein ingredients may be isonitrogenous but differ regarding the  
418 amount of amino acids they contain, as we discussed several years ago (86). In any case, the  
419 more the trial design aims to decipher a specific effect of the protein isolated, the more the  
420 results will apply to protein per se, but the less they will readily apply to public nutrition, unless  
421 the food industry can rapidly deliver new food products containing purified protein that  
422 individuals are prepared to consume. In that case, it is questionable whether such trials are  
423 relevant to the actual situation where individuals would incorporate these new protein foods in

their diet, because these new protein foods would be expected to replace the other protein foods that they are currently consuming, in a way that has not been analyzed by the design of the RCT.

I do not intend to review all the literature concerning RCT on specific protein and protein sources and cardiometabolic health, but I would like to discuss certain aspects of this literature.

Briefly, as far as purified protein ingredients are concerned, much as been published on soy protein, and the reader is directed to some interesting reviews and opinions (85, 87, 88). I would like to use this example to consider the amounts (“doses”) of soy protein that could be expected to convey the effects reported in the literature. The effects of soy protein on blood lipids and blood pressure have mostly been observed with high intakes of protein, generally around ~35g/day (83, 89), while the effects of soy protein at intakes below 25g/day are either weak or found not statistically significant; this was discussed recently in a meta-analysis concerning the effects on blood pressure (84). In order to judge the practical implications of such levels of intake, it is worth mentioning that the high soy protein intake of Adventist vegans in the USA averages only 13g (24). Other plant proteins, which have been less studied, may have effects on cholesterol or blood pressure, as was suggested for lupin protein (90-92), but the data are scarce. It may therefore be difficult to determine whether a combination of purified plant proteins in large quantities (~50g/day) could result in reproducing some of the findings regarding soy protein at high doses reported in RCT.

It is more likely that plant protein might benefit cardiometabolic risk factors when consumed as a protein food. This would not be surprising because some components in the plant protein package could favorably replace others that are associated with animal protein (e.g. fatty acid types), but also because some of them have been shown to be effective in modulating cardiometabolic risk factors, such as fiber. In RCT, the positive effects of plant

protein when using plant foods or unpurified protein ingredients have been ascribed to the effects of protein and fiber that are found at high levels in the unrefined ingredients. For instance, this is the case for lupin (93, 94), although there have been some conflicting results (95). Indeed, as shown in the literature when comparing the results with soy products against those obtained with soy protein ingredients, the evidence generally appears much stronger when studying whole plant foods or raw ingredients than purified proteins (96-98). Overall, a recent analysis reported that the evidence available from randomized controlled trials suggests that 1 to 2 servings of plant protein foods instead of animal protein foods decreases low-density lipoprotein cholesterol, non-high-density lipoprotein cholesterol and apolipoprotein B by ~4% in adults with and without hyperlipidemia. (99). Because of inconsistencies or inaccuracies in the estimates the overall certainty of the evidence is moderate and suggests that more research will be required to refine these estimates (99). The effect of pulses on cardiovascular risk factors in RCT is reviewed elsewhere in the same volume of this journal [add Sievenpeper's review reference]. In my view, further studies are needed to assess the effects of plant protein on various cardiometabolic risk factors, such as low-grade inflammation, endothelial vascular function and insulin sensitivity, because there have been some null results (e.g., 100) and the field has not been studied sufficiently. These studies would be better served by the use of different types of plant protein and different types of substitution.

When considering even more general changes to food consumption involving foods rich in plant proteins, a consensus has now been reached that legumes improve cardiometabolic risk factors (as compared to various diets controlled for energy nutrients). For a full review on legumes and beans and cardiometabolic risk, the reader can refer to the review by Salas Salvado in the same volume of this journal [add Salas Salvado's review reference here]. By contrast, it is difficult to draw any conclusions from RCTs that have tested the effects of meat consumption because the dietary treatments and results have varied. O'Connor et al.'s recent meta-analysis

(101) reported no effect of total red meat intake >0.5 servings/day on blood lipids and blood pressure. It had already been found that substituting red meat for poultry or fish did not affect blood lipids (102) and substituting protein in general for some types of carbohydrates in a healthy diet could benefit CVD risk factors (103, 104). However, this information from RCTs contrasts with observational findings that associated red meat intake and CVD risk factors (105) or CVD events and mortality (106-114).

Approaches that rely on simultaneous changes to several foods in a diet, such as a more food-based and dietary pattern method, are useful to clarify dietary recommendations aimed to achieve a reduction in cardiometabolic risk (115). If trying to summarize the findings of observational and interventional studies, what stands out is that a healthier protein pattern would consist in reducing the consumption of processed and red meats and preferring more protein sourced from legumes, nuts and seeds. This general conclusion would also fall in line with the common features of generally prudent diets such as the Mediterranean diet (116-118). More data are required to investigate the specific effects of other animal based protein products (such as chicken and dairy) and other plant-based products (such as grains), and the factors which modulate their effects on cardiometabolic health.

#### **Could amino acids form part of the relationship?**

As I discussed at the beginning of this paper, one might consider that a large part of the relationship between plant and animal protein intakes and cardiometabolic risk could be ascribed to the large cluster of nutrients and other substances (e.g. phytochemicals) that they convey, either directly or indirectly (39, 119). Indeed, in observational studies, it remains difficult to separate the effects of a specific protein from that of the closely associated “protein package”, and also from other characteristics of the overall diet, as I have argued. It also seems clear that the literature is more conclusive when RCTs involve whole protein plant foods rather

than purified proteins. Nonetheless, it should also be considered that the type of protein per se (i.e. the relative amounts of amino acids that it supplies) may affect cardiometabolic risk. Support for this proposal can be found in human and animal studies that manipulated amino acid intakes, and also from an analysis of observational studies. Many studies have reported a significant association with cardiometabolic outcomes, even when fully adjusted models were used, i.e. including dietary/nutrient intakes and despite classic confounding factors related to behaviors and socioeconomic status. For example, this was the case regarding a recent report on plant/animal protein intake and the risk of diabetes, which used a model that, as well as numerous genetic and lifestyle factors, also adjusted for total energy intake, percentages of energy from trans-fats, saturated fats, monounsaturated fats and polyunsaturated fats, dietary cholesterol, dietary fiber, and the glycemic index (57). Song et al. (50) also recently found significant relations between CVD mortality and plant vs animal protein intakes when adjusting for dietary intakes (e.g. whole grains, fruits and vegetables, glycemic index) and the intakes of different fatty acids. Likewise, in the Adventist Health Study-2 cohort, we found that the strong association between protein intake factors was not modified when account was taken of potential confounders such as the vegetarian diet category and the intake of a series of nutrients considered as being relevant to cardiovascular risk (e.g. PUFAs, SFAs, sodium and vitamins A, C, E, B-6, folate and B-12) (120).

As well as their utilization for protein synthesis or oxidation, amino acids enter specific metabolic pathways that lead to the synthesis of metabolites which play key roles in physiology and pathophysiology. For this review, I would like to mention a few amino acids that are present at varying levels in plant and animal proteins and have been studied widely for their probable impacts on physiology: arginine, cysteine and branched chain amino acids.

Arginine provides the substrate for the synthesis of nitric oxide, the key mediator of vascular homeostasis (121-123). Indeed, an impairment of NO production and/or bioactivity

has been largely reported as a central feature associated with cardiometabolic risk, including that of coronary artery disease, stroke and diabetes (124). As shown by a meta-analysis, arginine supplementation improves endothelial function when its levels are low at baseline (125). The beneficial effects of arginine supplementation on endothelial function can be achieved with a low intake (such as that seen by modifying the amount or type of protein in the diet) as has been demonstrated by its ability to blunt postprandial endothelial function after a high-fat meal (126, 127). The kinetics of arginine bioavailability may also play an important role inasmuch as arginine that is made available slowly – as is the case for dietary, protein-bound arginine – is directed more towards nitric oxide synthesis than arginine that is rapidly available (for example, in a dietary supplement) (128). The potential of arginine-rich proteins to prevent alterations to postprandial endothelial function has been documented (129, 130) and arginine may mediate some of the beneficial effects of arginine-rich proteins in the diet (90, 130). Arginine may also have other benefits on cardiometabolic health (131-135). The benefits of high arginine intake have also been studied for many years in association with lysine intakes, stemming from seminal works on plant and animal proteins and atherosclerosis (136, 137). More recently, in a closely controlled trial, Vega-Lopez et al. (138) found that a low (vs high) lysine:arginine ratio lowered fasting and postprandial C-reactive protein and lowered postprandial plasma levels of triglycerides in moderately hypercholesterolemic subjects.

Sulfur amino acids, and particularly cysteine, have also been studied for their potential effects on cardiometabolic risk, because of their links with homocysteine (a probable risk factor for CVD) and glutathione (a pivotal molecule in redox homeostasis). The relationship between cysteine intake, glutathione metabolism and cardiometabolic health has been widely studied and reviewed elsewhere (139-141). In brief, dietary cysteine supplementation has been reported to reduce diet-induced oxidative stress and insulin resistance in rats (142). This protective effect was notably accompanied by an alteration of glutathione redox status, which has been reported



as being an early marker of atherosclerosis in healthy humans (143). In humans, variations in sulfur amino acid intakes affect fasting plasma free cysteine concentrations and the redox status, as shown by the extracellular cysteine/cysteine redox potential (144). Supplementation with a cysteine donor lowers plasma homocysteine, improves glutathione levels and redox status, and lowers blood pressure (145). Cysteine intake has also been inversely associated with the incidence of stroke (146).

BCAAs have been widely studied for their relation with insulin resistance since it was reported that high BCAAs plasma levels formed part of a metabolic signature correlated with insulin resistance in obese individuals. BCAAs concentrations are elevated in obese subjects with insulin resistance and/or metabolic syndrome (147). They are associated with plasma acylcarnitines and cardiovascular risk factors (147-152) and they are predictive of diabetes and CVD (153-155). BCAAs intake has been associated with the incidence of insulin resistance and diabetes, although this intake could simply be a marker of total and animal protein intake (156, 157). BCAAs supplementation has also been reported to modulate insulin sensitivity, notably in the context of diet-induced obesity (158-160) or in individuals with low baseline levels of intake (161). These findings remain controversial, because opposite results were reported with leucine alone in mice (162, 163) and high plasma BCAAs concentrations resulted from a complex change in their metabolism (150, 164). Importantly, dietary BCAAs largely interplays with fatty acid oxidation, and we recently showed that this could explain why the type of protein in a high-fat meal modulates the extent of postprandial mitochondrial overload and incomplete substrate oxidation (165). However, it remains difficult to ascertain whether dietary and plasma BCAAs contribute to the onset of the dysregulations contributing to cardiometabolic risk, or if high plasma BCAAs concentration is a marker of dysregulated metabolism (166, 167). Recent Mendelian randomization analyses suggested a causal role of

BCAAs metabolism, which is impaired by insulin resistance, in the etiology of type 2 diabetes (166, 168, 169).

Based on experimental studies in animal or humans, or associations found in epidemiological studies, other amino acids have been studied for their links with cardiovascular risk factors and identified as candidates for mediating the effects of protein intakes. For instance, oral glycine was shown to be particularly efficient in potentiating the action of insulin (170, 171). Intakes of glutamic acid have been associated with lower systolic blood pressure, independently of other amino acids (172). Plasma glycine and glutamine are inversely associated with the incidence of type-2 diabetes (153). Other candidate amino acids include histidine, phenylalanine and tyrosine (135, 173).

It would be interesting to study both the separate and combined effects of amino acid intakes, to identify the most influential amino acids when taken together (with additive or synergetic effects) and try to determine how they might account for the effects of plant proteins. Because amino acid intakes are not independent of each other, and are also associated with other characteristics of the diet, the analysis of observational data needs to take account of this complexity and ways must be found to reduce it to the most salient components. In this regard, Teimoori et al. (174) recently analyzed patterns of amino acid intake using models with multiple dietary/nutritional confounders. They found that an amino acid factor with high loadings from BCAAs, aromatic amino acids, serine and threonine was strongly associated with the incidence of hypertension in a model adjusting for fatty acid types, calcium, sodium, magnesium, potassium and fiber. In the study by Jennings et al. (173), the association between the intake of several amino acids and blood pressure was found to depend on whether they originated from plant or animal protein sources, which is indicative of the complexity of this issue. Further analysis of this complex situation is necessary in the context of large cohorts.

Although it is much too soon to draw any definite conclusions, the amino acids identified in the literature that I have reviewed here as possibly being detrimental to cardiometabolic health are found at higher levels in animal protein and their intake are more contributed by animal protein intake. These are mostly BCAAs and aromatic amino acids, – indispensable amino acids (175). Conversely, the amino acids identified as being potentially beneficial are mainly found at higher levels in plant proteins. These are mostly arginine, cysteine, glutamine/glutamate, and glycine, or – non-indispensable amino acids (173). These findings warrant further study to understand patterns of amino acid intake and cardiometabolic health. This research may indeed change our vision of amino acids as being classified as “indispensable” vs “non-indispensable”, and drive attempts to shift our understanding of protein quality for human health.

## **Conclusion**

Plant protein in western countries is a robust marker of nutrient adequacy of the diet, whereas the contribution of animal protein largely varies according to animal source. Plant and animal proteins are indeed consumed with other nutrients and substances that makes the ‘protein package’ and this may explain their relation to cardiometabolic health in the current dietary patterns. Yet recent data from large cohorts have confirmed that total and animal proteins are associated with the risk of CVD and diabetes, even in models that are largely adjusted for lifestyle and dietary/nutritional factors. Here again, there is marked variability depending on the type of animal protein. Protein from processed red meat and total red meat on the one hand, and from legumes, nuts and seeds on the other, are often reported at the extremes of the risk range. RCTs using purified proteins have contributed little to the topic to date, inasmuch as the findings cannot readily be extrapolated to current or near-future diets, but RCTs studying whole protein foods have shown a beneficial effect of pulses. Despite many of the benefits of plant protein reported in observational or interventional studies may stem from the protein package

622 that they convey and the nutrients that they displace, there are also important indications that  
623 protein *per se* may affect cardiometabolic health via the many amino acids that are present at  
624 typically contrasting levels in plant vs animal proteins.

## Acknowledgements.

I would like to thank Prof. Jean-François Huneau for interesting discussions on this topic.

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## Legends for Figures

**Figure 1.** Partial correlation analysis between intakes of protein according to sources and intakes of nutrients in the Melbourne Cohort study. Pearson's correlation analysis was used to calculate age- and gender- adjusted partial correlation coefficients. Blue and red colors indicate positive and negative correlations, respectively, and color intensity and the size of the circle are proportional to the correlation coefficient. Adapted with permission from Shang et al. (33).

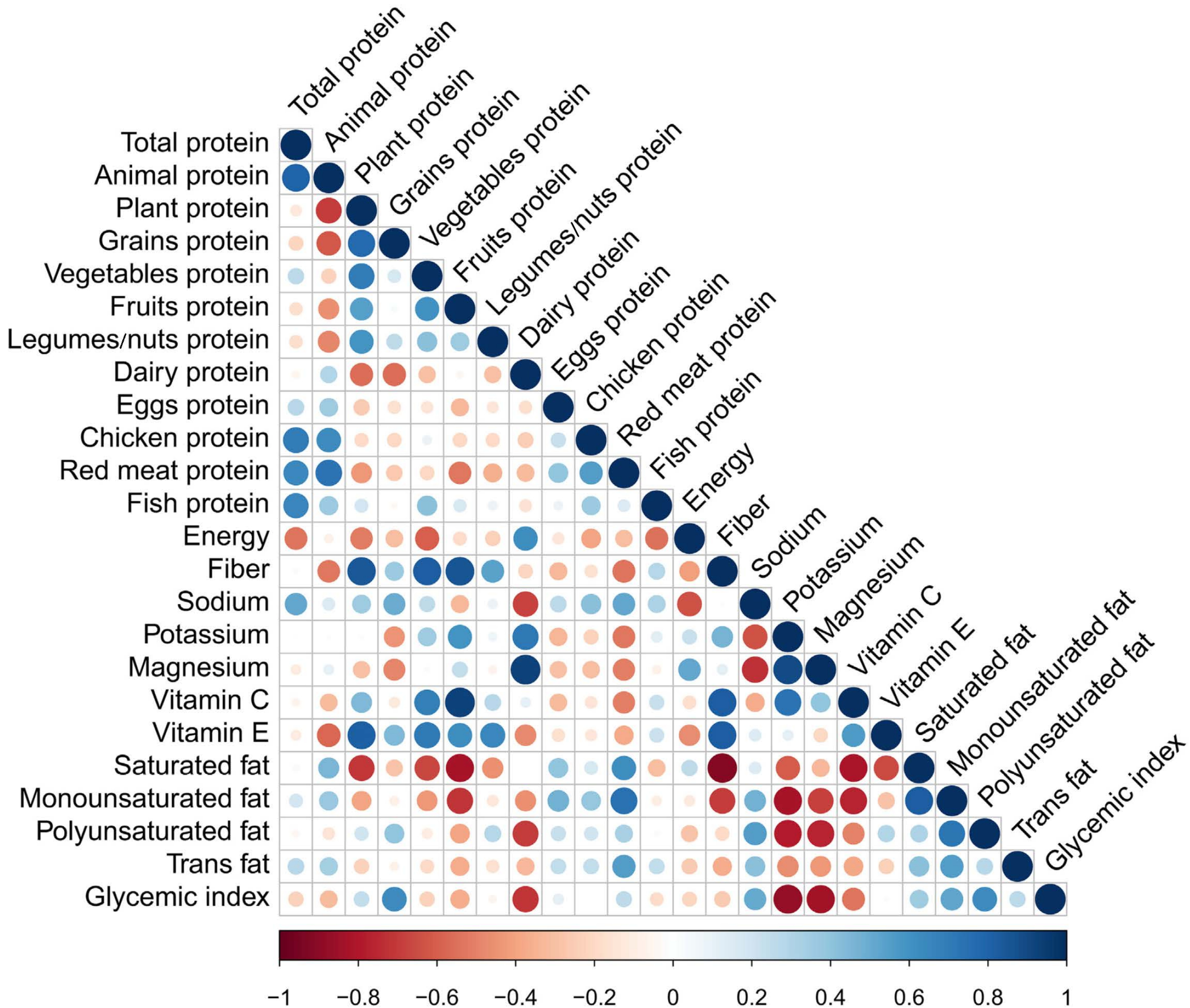
**Figure 2.** Pooled hazard ratios (HRs) and 95% confidence intervals (CIs) for type 2 diabetes associated with replacement of 1 serving of individual animal protein foods (dairy foods, poultry, eggs, red meat, and processed meat), refined grains, and potatoes with 1 serving of vegetable protein foods (composite variable comprised of whole grains, legumes, peanuts, peanut butter, and other nuts) in the Nurses' Health Study (1984–2008), Nurses' Health Study II (1991–2009), and the Health Professionals Follow-up Study (1986–2008). The models adjusted for age, family history of diabetes, smoking, alcohol intake, physical activity, race/ethnicity, total energy intake, postmenopausal hormone use (Nurses' Health Study, Nurses' Health Study II), oral contraceptive use (Nurses' Health Study II), intakes of sugar-sweetened beverages, fruit, and vegetables, and body mass index. Results were mutually



adjusted for other food sources of animal protein, refined grains, and potatoes. Reproduced with permission from Malik et al. (57).

**Figure 3.** Multivariate-adjusted hazard ratio (HR) of CVD mortality by quintile of the "Meat" protein factor (Panel A) and "Nuts & Seeds" protein factor (Panel B) in 81,337 participants in the Adventist Health Study-2, presented at two age categories. The model was adjusted for age, sex, race, BMI, individual socioeconomic factors, health-related lifestyle, energy intake and the type of diet in the vegetarian spectrum. Significant age interactions were found for the "Meat" and "Nuts & Seeds" protein factors. HRs were estimated at the mean age of each age category. Adapted from Tharrey et al. (120).







Type of Food Replaced

HR (95% CI)

Dairy

0.98 (0.96, 1.00)

Refined grains

0.94 (0.91, 0.96)

Poultry

0.91 (0.86, 0.96)

Eggs

0.89 (0.83, 0.95)

Red meat

0.89 (0.86, 0.93)

Fish

0.87 (0.81, 0.94)

Potatoes

0.84 (0.78, 0.91)

Processed meat

0.79 (0.74, 0.84)

0.70

1.00

1.05

Hazard Ratio

