Animal Proteins as Important Contributors to a Healthy Human Diet

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Abstract

Adequate protein intake is critical for health and development. Generally, protein of animal origin is of higher quality for humans owing to its amino acid pattern and good digestibility. When administered in mixtures it can enhance the quality of plant proteins, but its availability is often low in low-income communities, especially in young children, the elderly, and pregnant and lactating women, who have increased requirements and in whom high-quality protein also stimulates (bone) growth and maintenance. Although high protein intake was associated with increased type 2 diabetes mellitus risk, milk and seafood are good sources of branched chain amino acids and taurine, which act beneficially on glucose metabolism and blood pressure. However, high consumption of protein-rich animal food is also associated with adverse health effects and higher risk for noncommunicable diseases, partly related to other components of these foods, like saturated fatty acids and potential carcinogens in processed meat but also the atherogenic methionine metabolite homocysteine. In moderation, however, animal proteins are especially important for health maintenance in vulnerable persons.

INTRODUCTION

Protein is an irreplaceable nutrient for all animals, including humans, and is involved in all physiological functions (1–3). An adequate dietary supply of protein, as a source of amino acids and nitrogen, is required to allow the regular turnover of tissue and functional body proteins like enzymes. Particularly relevant are the indispensable (essential) amino acids that cannot be synthesized by the human body, or not in sufficient amounts to meet physiological needs. The latter can be the case at certain life stages, especially during infancy.

The quality of a protein for human nutrition is therefore for the most part determined by its content of indispensable amino acids and its capacity to cover the body's requirements. This is expressed by the amino acid score (AAS) of a given protein, defined as the ratio of its content of indispensable amino acids to the amount required (1).

As the use of protein depends on its bioavailability, this factor is corrected for protein digestibility to give the protein digestibility–corrected AAS (PDCAAS) (2). This latter standard was recently criticized for being based on the overall digestibility of the protein, which is not the same for individual amino acids (2). Moreover, digestibility is determined as fecal digestibility, which is confounded by microbial nitrogen metabolism. This prompted the Food and Agriculture Organization of the United Nations (FAO) to suggest the digestible indispensable AAS (DIAAS) as an improved method of evaluation taking into account the digestibility of individual indispensable amino acids measured in the ileum (2). Another difference from the PDCAAS is that the obtained values are not truncated to 100%, therefore allowing a better comparison, especially of high-quality proteins (2).

However, despite the pivotal role of essential amino acids, nonessential amino acids also have important functions in the body that go beyond being mere components of proteins (3, 4). For instance, some of them serve as precursors for the synthesis of others, like proline and glutamate for arginine. Amino acids whose production depends on the availability of their precursors are considered conditionally indispensable (e.g., tyrosine from phenylalanine). Moreover, an imbalance between dispensable and indispensable amino acids in favor of the latter would result in the use of indispensable amino acids for the synthesis of dispensable amino acids at a substantial metabolic cost. This is prevented by a balanced intake of both essential and nonessential amino acids (3, 4).

THE PARTICULAR QUALITY OF ANIMAL PROTEIN

Generally, proteins of animal origin have an amino acid pattern that is closer to the requirements of the human body, supplying all nine indispensable amino acids in approximately adequate amounts (2) (compare in **Table 1**). Moreover, proteins from animal foods are more easily digestible and hence available to humans. For instance, egg protein has often been used as a reference to rate the biological value of other proteins. In turn, proteins from plant foods are generally less bioavailable owing to antinutritive factors like certain tannins, lectins, and protease inhibitors that require more or less extensive processing of the food to reduce their negative effects (5). Protein accessibility is also reduced by the presence of plant cell walls that are only partly digested in the human gastrointestinal tract, which lacks the enzymes to break down cellulose and related dietary fibers (6, 7). Using the DIAAS for evaluation reveals the superior quality of animal proteins; for instance, whole milk powder has a DIAAS of 122, compared with 64 and 40 for peas and wheat, respectively. Accordingly, the FAO Expert Committee on the evaluation of protein quality in human nutrition recommended that of the three proteins, only the first was eligible for a claim on protein quality (2). Even soy protein, which is generally recognized for its high quality, scores less than animal proteins, with a DIAAS of approximately 90 compared with milk protein concentrate with a DIAAS of 118 in a rat model (8).

Table 1 Amino acid pattern (mg/g protein) of dietary animal and plant proteins compared to the FAO/WHO (1) reference protein

Protein	Amino acid								
	His	Ile	Leu	Lys	SAA	AAA	Thr	Trp	Val
Beef, tenderloin, 1/8 fat, all grades, raw	31.9	45.5	79.6	84.5	39.0	71.4	39.9	6.6	49.6
Pork, tenderloin, lean only, raw	43.2	49.2	85.1	92.7	39.1	80.0	44.9	10.5	52.2
Chicken, breast, meat only, raw	31.0	52.8	75.0	84.9	40.5	73.3	42.2	11.7	49.6
Cod, Atlantic, fillet, raw	29.4	46.1	81.3	91.9	40.3	72.8	43.9	11.2	51.5
Egg, chicken, raw	24.5	53.3	86.3	72.5	51.7	93.7	44.1	13.3	68.2
Milk, cow, whole	27.0	60.0	97.3	78.8	33.9	95.8	44.8	13.9	66.7
Wheat, soft red winter, whole grain	24.9	38.4	74.1	30.6	46.0	81.1	33.2	–	48.3
Wheat flour, white, unenriched	20.3	33.7	64,6	26.8	40.5	54.2	27.2	11.6	40.2
Soybean, mature seeds, raw	30.1	54.0	90.7	74.1	32.9	100.3	48.4	16.2	55.6
WHO/FAO reference infant[a]	21	55	96	69	33	94	44	17	55
WHO/FAO reference child 3+ years, adult[a]	16	30	61	48	23	41	25	6.6	40

Food data from Reference 29.
[a]Food Agric. Organ. (2).
Abbreviations: AAA, aromatic amino acids (i.e., phenylalanine and tyrosine); His, histidine; Ile, isoleucine; Leu, leucine; Lys, lysine; SAA, sulfur amino acids (i.e., methionine and cysteine); Thr, threonine; Trp, tryptophan; Val, valine.

The addition of animal protein in the form of whey or beef was also more efficient than that of soy to improve the protein quality of wheat in a study in adult humans consuming diets with protein contents of 5%, 15%, or 30% of total energy (9). The combination of 10% of energy from whey protein or beef protein to 5% of wheat protein increased the DIAAS from 53 to 113 and 112, respectively, compared with 84 for the addition of 10% of energy from soy protein. Comparable changes were also seen with the addition of the respective proteins at 25% of energy. Although this study showed that all three protein combinations were able to cover the requirements of indispensable amino acids, a higher intake of soy protein was needed to achieve this goal (9).

The fact that higher amounts of low-quality protein are necessary to meet human amino acid requirements and that foods containing them must be combined with other protein sources, such as foods of animal origin, to improve protein quality also bears the risk of excessive total energy intake, as many protein-rich plant foods, such as soybeans or cereals, also contain high amounts of carbohydrates and/or fat. This is of particular importance for persons with low energy expenditure and/or high protein needs (10). In turn, low amounts of animal protein are sufficient to enhance the quality of plant proteins, as exemplified by Ernst Kofrányi (11), who in 1967 found the highest biological value for a mixture of 37% of nitrogen from egg protein and 63% of nitrogen from potato protein corresponding to approximately one egg and 500 g of potato.

ANIMAL PROTEIN FOR SPECIAL POPULATION GROUPS

Although the amino acid requirements of healthy adults can generally be covered even with protein of lesser quality as long as sufficient amounts are consumed, this might not be the case for some vulnerable population groups, such as infants and young children, for instance, in whom requirements for indispensable amino acids are markedly higher than in older children, adolescents, and adults. Infants up to 6 months need 484 mg indispensable amino acids per gram of protein required. In adolescents and adults, the respective amounts are 286 mg/g protein and 277 mg/g protein (1). The role of protein quality is evidenced by the fact that more nitrogen can be retained by the body from high-quality protein than from low-quality protein (1). Despite their acknowledged weaknesses, for instance, high individual variability, the biological value (BV) and the net protein utilization (NPU) are used as indices to quantify these differences corresponding to the proportion of nitrogen retained from the total amount absorbed (BV) or ingested (NPU). In rats, BV and NPU range above 90 for egg and above 80 for cow's milk compared with BV of about 65 and NPU of 40 to 57 for wheat, rice, and peas (12).

The importance of sufficient protein supply is most apparent in malnourished infants and children. Although in the studies investigating this association it was generally difficult to separate the effects of protein per se from those of energy and various micronutrients, there is evidence for a negative influence of inadequate protein intake on growth and cognitive development (13–15). A beneficial effect of high protein intake has moreover been reported in preterm and low–birth weight children, in whom it promoted mental development and reduced the risk of growth deficits (16).

Few studies looked specifically at the effects of animal protein. However, an older study involving 138 schoolchildren (aged 7.1 to 8.5 years) from Kenya found a significant positive effect between the intake of protein and cognitive performance score that was more pronounced for animal than for total protein, especially in boys ($r = 0.53$ versus $r = 0.28$, respectively, in boys and $r = 0.51$ versus $r = 0.46$, respectively, in girls, all with $p < 0.05$) (17). High-quality protein is also especially important during catch-up growth of malnourished children, who have very high protein requirements and in whom amino acid needs may be altered by infectious states (18).

A special role of milk protein in bone formation is suggested by a study in Danish adolescents in which milk but not meat or other dairy protein was positively correlated with bone mineral content even after correction for total energy, calcium intake, and physical activity. Interestingly, in contrast to meat protein, neither milk nor dairy nor total protein intake was correlated with serum insulin-like growth factor-1 (IGF-1), which is a regulator of bone mineralization and growth (19).

Adequate protein is also important during pregnancy and is associated with improved fetal growth, a lower risk of stillbirth, and higher birth weight of children of undernourished women (20). Although there is little information on the effect of protein from different food sources, milk consumption was associated with improved child growth (21, 22), and the effect was found to be particularly associated with milk protein (23). However, a very high intake of protein does not confer benefits and even seems to increase the risk of infants being small for gestational age (20, 22–24). This underscores the importance of a balanced intake of high-quality protein as it is supplied by animal food sources.

Another group that may benefit from the adequate intake of high-quality protein is older adults. Indeed, several studies suggest that sufficient supply of protein and amino acids is crucial for healthy aging, especially with regard to the maintenance of lean body mass. There is evidence for a beneficial effect of protein intake above the currently recommended level, which is the same as for younger adults (25–27). Moreover, the chronic low-grade inflammatory status that is often observed in elderly individuals also has an influence on protein requirements, especially with regard to certain amino acids, in light of the high content of aromatic amino acids and tryptophan in acute-phase proteins (28). It has also been suggested that the branched-chain amino acid leucine that is abundant in most animal proteins might positively affect muscle protein synthesis in elderly individuals (29, 30). Aging has also been associated with a reduced synthesis of the antioxidant glutathione (GSH) owing to reduced activity of the rate-limiting enzyme glutamate cysteine ligase.

A possible approach to improve GSH production has been to increase the availability of the GSH precursors cysteine and glycine (31, 32).

Increased protein intake (within the range of the dietary reference intake) was associated with a better nutritional status (measured using the geriatric nutritional risk index score) and a higher phase angle in 85 geriatric patients with a high prevalence of insufficient energy and protein intake living in a Viennese long-term care institution (33). In a comparison of the effects of isonitrogenous amounts of beef meat and soy protein on muscle protein synthesis in middle-aged men at rest and after physical exercise, beef induced a significantly higher response (34).

Evidence for the superiority of animal protein over plant protein in combating sarcopenia and preserving muscle mass also comes from a study in old ovariectomized rats. In this study, only a diet supplemented with animal protein could slow muscle decline and improve muscle structure in sedentary animals, even though the best results were obtained with additional physical resistance training. Although a slightly higher gain of visceral fat mass was observed in the ovariectomized rats on the animal protein diet compared with those fed the plant protein diet, the greatest effect on this parameter resulted from ovariectomy rather than diet or physical exercise (35). In a study on elderly Finnish women, higher total and animal protein intake evaluated through three-day food records was associated with higher lean body mass, whereas no such relationship was observed for plant protein intake (36). Effects of protein intake on bone health are another important aspect to consider, particularly regarding its contribution to healthy aging. In the Framingham Osteoporosis Study, higher total and animal protein intake was associated with lower loss of bone mass (37).

The relevance of protein for the elderly is also suggested by a study based on data from the National Health and Nutrition Examination Study (NHANES) III, in which low protein intake (<10% of energy) was associated with higher all-cause, cancer, and cardiovascular disease (CVD) mortality (38). This study is discussed in more detail below.

ANIMAL PROTEIN IN BODY WEIGHT MANAGEMENT

Protein-rich diets with low contents of carbohydrates have been popular in weight reduction. These diets deviate from the established dietary guidelines with protein contents above 15% of energy and carbohydrate contents well below 50% of energy. Indeed, diets with a higher energy intake from protein can induce body weight reduction, an effect mainly attributed to the satiating properties of protein that lead to an overall reduction of food and hence energy intake (39).

However, high protein intake has also been associated with weight gain over time in epidemiological surveys in the general population (40), as has a high consumption of some animal foods (41, 42). Studies focusing on the special effect of animal protein are limited, and the results for the effect of animal protein on body weight are equivocal. Over a period of 6.5 years, higher intake of animal protein was associated with weight gain in the European Prospective Investigation into Cancer and Nutrition (EPIC) study, whereas no change was observed for plant protein, but this effect was true only for meat protein, not for fish and dairy sources (43). In turn, in an intervention study on the effects of diets with different amounts of protein (20%, 27%, and 35% of energy, of which 80% were of animal origin) in overweight and obese women, the reduction of body weight and body fat mass was greatest in the group with the highest protein intake (44).

A multicenter study in eight European countries with a final sample size of 548 completers looked at the effects of protein content and glycemic index of the diet on weight maintenance after an achieved weight loss. The fat amount was comparable between the test diets (25–30% of energy intake). It was found that weight regain was lower on a higher-protein (23% of energy intake) than a lower-protein (13% of energy intake) diet, and participants on higher-protein diets were more likely to lose additional weight. In this study, however, no separate analyses were made to differentiate between the effects of animal and plant protein (45).

ANIMAL PROTEIN AS A DETERMINANT OF HEALTH AND DISEASE

Epidemiological Evidence

A high intake of animal food, notably red meat and processed meat products, has been associated with several health risks and diseases, such as obesity, CVD, diabetes mellitus, some cancer types, and inflammation (41, 42, 46–53). However, the contribution of animal protein to these effects is not clear (40, 54). In many studies, only total protein is analyzed. Another approach consists in differentiating between protein-rich foods, such as meat, poultry, fish, and dairy, without, however, considering protein itself.

Thus, in the Nurses' Health Study (NHS) and Health Professionals' Follow-Up Study (HPFS), a low-carbohydrate diet pattern emphasizing animal fat and protein sources was associated with higher risk for all-cause, CVD, and cancer mortality [hazard ratio (HR) in the highest versus the lowest decile of low carbohydrate score 1.23, 1.14, and 1.28, respectively] that was significant only for CVD mortality (P for trend = 0.029). In the highest decile, the mean intake of carbohydrates and animal and plant protein was 37.2%, 17.8%, and 4.5% of energy, respectively; in the lowest decile, the respective amounts were 60.5% from carbohydrates, 9.6% from animal protein, and 5.4% from plant protein. However, no separate analysis was conducted for protein, so that the effects of fat intake are contained within the overall low-carbohydrate pattern. Indeed, the intake of animal fat and saturated fatty acids (SFA) was higher for the animal-emphasizing low-carbohydrate pattern (26.3% from animal fat, 12.2% from plant fat, and 26 g of SFA versus 11.6% from animal and 15.5% from plant fat and 16 g of SFA in the high-carbohydrate low-animal group), as was the consumption of red and processed meat (1.3 compared with 0.5 servings/day) (55). It is therefore not possible to draw clear conclusions on health effects of animal protein alone from this study, as the different foods supply a wide array of other nutrients and nonnutritive components that have health impacts of their own that are in many cases stronger than those of protein. This is particularly true for total fat, saturated and unsaturated fatty acids, and cholesterol, as well as for potentially harmful contaminants arising from food processing. These are discussed later in this review.

In turn, a direct comparison of the impact of protein intake from animal and plant sources on ischemic heart disease (IHD) in 40–75-year-old men revealed a higher risk for nonfatal and fatal IHD associated with animal protein and a risk reduction for fatal IHD associated with plant protein (56). However, the associations with animal protein became statistically nonsignificant after full adjustment for potential influence factors, among which were saturated fat, monounsaturated fat, polyunsaturated fat, trans fat, and energy intake (56).

The already-mentioned study based on data from NHANES III found a significantly higher mortality from diabetes associated with total and animal protein intake in men and women aged 50 years and above. In those aged 50 to 65 years, there was also higher all-cause and cancer mortality related to protein intake that persisted after adjustment for fat and carbohydrate intake [HR = 1.74 and 4.98 for all-cause and cancer mortality, respectively, in persons on a high-protein diet (≥20% of energy), and HR = 1.35 and 3.56 for all-cause and cancer mortality, respectively, in persons on a moderate-protein diet (10–19% of energy) compared with those on a low-protein diet (<10% of energy)]. No association was seen for CVD mortality. A limitation of this study, as acknowledged by the authors, is the fact that food intake was estimated from a single 24-h recall on which the classification of protein intake was based. Even though a great majority (93%)

of the participants stated that the recorded food intake corresponded to their typical intake, it is questionable whether this was also true for the following 18 years over which mortality was assessed (38).

An increase in diabetes mellitus type II (DM II) risk with high protein intake was also seen in other surveys. In a recent analysis using data from the NHS, NHS II, and HPFS, higher intake of total protein (21.6% of energy compared with 14.8% in the two extreme quintiles) was associated with a higher risk for DM II (HR = 1.39, $p < 0.001$). This association was lost in both NHS studies after adjusting for body mass index (BMI). The risk increase was more pronounced for animal protein intake even after BMI adjustment, whereas plant protein appeared protective (57).

In line with these findings, total and animal protein intake were related to a higher risk for diabetes mellitus of any type in the Dutch EPIC study, resulting in a HR adjusted for age, sex, and other dietary factors of 2.16 and 2.09 for the highest versus the lowest quartile of total (89.8 g/day versus 62.2 g/day) and animal (62.9 g/day versus 35.2 g/day) protein, respectively ($p < 0.001$). These associations persisted after adjustment for diabetes risk factors (HR = 1.67 and 1.58, respectively, $p < 0.001$) but were lost after adjustment for BMI and waist circumference. No significant associations were found for plant protein (58). Substituting carbohydrates for protein was also associated with a lower risk for DM II (RR = 0.77 for 5% of energy from carbohydrate at the expense of protein) in the EPIC Potsdam cohort. No separate analyses were done for animal protein (59).

In the Malmö Diet and Cancer cohort, DM II risk was higher in the highest quintile of total protein intake than in the lowest (20% versus 12.8% of energy in women and 19.5% versus 12.5% in men) (HR = 1.27, $p = 0.01$ for trend) and increased when protein was substituted for carbohydrates or fat (HR = 1.2 and 1.21, respectively). Intake of processed meat, eggs, and, in women only, poultry was also associated with increased DM II risk, whereas no association was observed for red meat, fish, and milk products (60). These findings suggest an increased risk for DM II associated with high intake of protein that might be stronger for animal protein.

Intake of red and processed meat has been particularly associated with a higher risk of colorectal cancer (51, 61). The association between animal protein and colorectal cancers was studied in a meta-analysis including a total of six studies, three prospective cohort studies, and three case-control studies. A significant effect of animal protein intake was identified neither in the meta-analysis nor in any of the included studies (62). Interestingly, in one of the cohort studies based on data from the HPFS, nonmeat animal protein was even associated with a lower risk of colon cancer [RR = 0.68 in the highest quintile (54 g/day) versus the lowest (19 g/day), $p = 0.06$] (63), as was milk protein with colorectal cancer risk in another cohort study in Finnish men [RR = 0.6 in the highest quartile (43.6 g/day) versus the lowest (16.3 g/day), $p = 0.02$] (64). In turn, the HPFS study reported a higher risk (RR = 1.54 in Q5 with 32 g/day versus Q1 with 5 g/day) associated with higher intake of red meat protein (63).

There was also no association of animal protein intake with breast cancer risk in a case-control study from the Montpellier area in France, although a higher risk was found for processed pork meat intake (65). A similar conclusion was also reached in a study on 88,647 women participating in the NHS, where there was no significant difference in breast cancer incidence between the quintiles of animal protein intake (66).

Animal protein intake was positively associated with risk for pancreatic cancer in an Italian study (OR = 1.85 in the highest versus the lowest quintile, $p = 0.039$). However, no adjustments were made for potential carcinogenic factors in protein-rich animal foods, like meat, for instance. The authors suggest these compounds as possible underlying causes in light of the role of red meat as a main source of animal protein in Italy (67).

A higher risk of laryngeal cancer associated with animal protein intake was reported in a study from Switzerland and Italy (68). However, when looking at food groups, effects were identified for red meat and processed meat, eggs, and fish but not for poultry, milk, and cheese, suggesting other agents than animal protein per se as underlying causes (69).

In the Swedish Västerbotten Intervention Program, the incidence of various cancer types did not differ between individuals with different protein contents in their diets. However, a subgroup analysis revealed an inverse relationship between protein intake and colorectal cancer risk in women with a high intake of saturated fat. Moreover, colorectal cancer incidence was higher in men with a high intake of plant protein (70).

Overall, convincing evidence for a link between animal protein per se and cancer is lacking, and it is not listed among the diet-related risk factors for cancer in the report *Food, Nutrition, Physical Activity, and the Prevention of Cancer* of the World Cancer Research Fund and the American Institute of Cancer Research (51).

However, dietary protein, total and in some studies also protein of animal origin, was associated with a lower risk for some diseases and health issues. In a study using data from the Iowa Women's Health Study, the effect of substituting protein for carbohydrates on cancer incidence and mortality, as well as coronary heart disease and all-cause mortality, was studied in postmenopausal women. The substitution of animal protein for carbohydrates was not significantly associated with cancer incidence or any death rate even though the mortality from all causes tended to be lower for higher intake (RR = 0.82 for a median intake of 17.5% of energy versus a median intake of 8.9% of energy, n.s.). In turn, a significant risk reduction was observed with the replacement of carbohydrates with plant protein (71).

In participants of the NHANES III aged >65 years, a higher protein intake was associated with a lower all-cause, cancer, and CVD mortality [HR = 0.72, 0.39, and 0.67 for all-cause, cancer, and CVD mortality, respectively, in subjects on a high-protein diet (≥20% of energy), and HR = 0.79, 0.67, and 0.8 for all-cause, cancer, and CVD mortality, respectively, in subjects on a moderate-protein diet (10–19% of energy) compared with those on a low-protein diet (<10% of energy)]. Only the risk for death from diabetes was higher compared with a low-protein intake in this age group as well (HR = 9.07 and 4.93 for high and moderate versus low protein intake) (38).

In a recent intervention study in 91 overweight or obese women, a diet with 35% of energy from protein, of which 80% was of animal origin, resulted in weight reduction over 6 months (≥10% in approximately 65% of the participants) and had positive effects on body fat mass, plasma lipids, and insulin resistance that were more pronounced than for a protein intake of 20% of total energy. However, this study had no control group with high intake of plant protein, and effects were compared only with baseline values (44).

A possible protective effect of animal protein has been reported for death from stroke in a Japanese sample, but this association disappeared after adjustment for animal fat and cholesterol intake (72). However, animal protein in the participants of this study came mostly from milk, eggs, and fish, whereas consumption of meat and meat products was rather low (red meat beef and pork, chicken, and pork meat products were consumed daily by only 8.7%, 1.8%, and 3.5% of the participants, respectively) (73). Nevertheless, a recent study in Swedish women also showed a lower risk of stroke and cerebral infarction for higher total and animal protein intake that persisted after adjustment for other stroke risk factors on stroke risk [RR = 0.71 in the highest versus the lowest quintile of animal protein intake (≥58 g/day versus <38.3 g/day), $p = 0.01$] (74). A protective effect of moderate total and animal protein was also concluded in a meta-analysis including seven prospective studies (75). In turn, no significant association between animal protein intake and stroke risk was found in middle-aged men in the HPFS (76).

In accordance with a lower stroke risk, protein intake was also inversely correlated to blood pressure in some studies, thus acting protectively against hypertension (77, 78). Although this effect was in most cases stronger for plant protein, the Japanese Circulatory Risk in Communities Study reported a stronger inverse association with blood pressure for animal than for plant protein in both men and women, and this relationship was also found for both major animal protein sources, fish and meat, separately (79).

In contrast to the higher diabetes risk associated with high protein intake, which was reported in some surveys (38, 57–60), a beneficial influence on glucose metabolism and glucose tolerance was described for dairy and especially whey proteins. This has been attributed to the insulinotropic effect of these proteins that seems to be mediated directly by their amino acid composition as well as their modulation of the gastrointestinal incretin hormones glucagon-like peptide-1 and gastric inhibitory peptide, which trigger insulin release. Besides, these mediators also have a satiating effect (80, 81).

Results of Interventional Animal and Human Studies

A very early discovery was the hypercholesterolemic effect of casein in rabbits compared with soy protein, which was independent from the fat or cholesterol content of the diet. However, findings in humans were ambiguous, with some studies reporting no difference between casein and soy protein (82–85). Differences in the regulatory effects of individual amino acids on hepatic apolipoprotein B100 synthesis have been shown and might be the underlying cause of the differing effects of casein and soy on cholesterolemia inasmuch as some of the amino acids inhibiting apo B100 synthesis (Met, Cys, and Trp) were found to be limiting in casein (86, 87). Different effects of soy and meat proteins on gene expression in the liver were also described in rats, but although only soy protein reduced food intake and thereby the weight gain of the animals, both proteins showed positive effects on blood lipid metabolism, although via different mechanisms (88, 89).

A link between animal protein intake and cancerogenesis was deduced from several studies in rats in which a high intake of casein promoted the tumor-inducing effect of aflatoxin B1 on the animals' livers, whereas a low-casein or a wheat gluten diet inhibited tumor growth. However, in these studies, the animals fed the protein-deficient diets developed severe and lethal hepatic lesions caused by the acute toxicity of aflatoxin B1 (90–92). This suggests negative effects of protein deficiency rather than a protective influence of low protein intake.

In similar experiments involving rhesus monkeys and rats, a protective effect of a high-casein diet was found. In this case, using a lower dose of aflatoxin B1, the animals fed 20% of casein did not develop tumors, as opposed to the protein-deficient animals (93, 94). High protein intake in the form of 20% casein also mitigated the effects of diethylnitrosamine on hepatic cancer development and reduced ensuing mortality in mice (95).

Regardless of the effects, findings from such animal models must be interpreted carefully. The animal protein–rich diet was based on 20% (on a per weight basis) of casein as the only protein source. Such a predominance of casein is not typical for human nutrition even if high amounts of animal food are consumed. In the United States, a country with high dairy consumption, the average contribution of milk products to total protein intake is 20%, corresponding to approximately 22 g/day of protein and approximately 17 g/day of casein in 40–49-year-old men, the group with the highest protein intake (96). This has generally to be considered in relation to studies on isolated proteins.

Nevertheless, a study in mice showed greater tumor development growth in animals that were implanted with murine melanoma or murine breast cancer cells and fed a high-protein diet (18% of energy) compared with those fed a low-protein diet (4–7% of energy). However, in this same study, only a marginal nonsignificant difference was observed between animal (casein) and plant (soy) protein (38).

Possible Mechanisms Behind Health Effects of Animal Protein

Several causal factors have been suggested to underlie health effects of animal proteins. Some of these have already been discussed above, such as the differential effects of proteins from different sources on the expression of several genes involved in amino acid, lipid, and energy metabolism. For example, both soy and meat protein were associated with downregulation of hepatic lipid synthesis in rats (88).

Amino acids are also regulators of the mammalian target of rapamycin (mTOR) pathway, which is central for energy and nutrient metabolism as well as protein synthesis. In this function, it acts as a sensor of amino acid availability, explaining its sensitivity to protein quantity and quality. mTOR has been implicated in tumor development and growth, and its inhibition, with tumor suppression. Furthermore, mTOR is involved in glucose metabolism by causing insulin resistance and has been suggested as a mechanism underlying the diabetogenic effect of high protein intake (97). A state of insulin resistance and hyperglycemia that has been associated with high protein intakes could also be attributed to the interference of amino acids with glucose uptake in muscle and other peripheral cells (97).

Notably, a short-time intervention in male Danish schoolchildren (aged 8 years) comparing the effects of the daily intake of 53 g protein in the form of skimmed milk or lean meat during one week resulted in a significant increase in fasting serum insulin levels and a significantly higher insulin resistance in the milk but not the meat group. In both groups, the serum concentration of insulinogenic amino acids (i.e., branched-chain amino acids and arginine) increased, and although this effect was slightly more marked in the milk group, there was no significant difference between both groups, suggesting additional factors contributing to the effect of animal proteins on glucose metabolism and insulin action (98).

Despite a lack of convincing evidence for an association between animal protein and cancer as stated above, a stimulatory influence of protein on IGF-1 has been found in several studies in animals as well as humans (38, 99, 100), although an association was not always found (101). IGF-1 has been related to increased risk of breast, colorectal, and prostate cancer, among others, although its involvement is not entirely clear (102).

However, IGF-1 is crucial for growth and bone mass development in children, and its reduction is considered the major factor in stunting caused by protein-energy malnutrition (103). Serum IGF concentrations were positively correlated to body height in healthy Danish preschool children (aged 2.5 years) and also to the intake of animal protein and milk, but not of vegetable protein and meat. The intake of animal protein and milk was also positively related to height (104).

Moreover, IGF-1 levels decrease with aging, and this is associated with the loss of lean body, muscle, and bone mass in the elderly (100, 105). IGF-1 enhances bone mineralization through its effect on the kidneys, where it stimulates the synthesis of 1–25-$(OH)_2$ vitamin D and the reabsorption of phosphate, thereby increasing the availability of both minerals for bone synthesis. Adequate protein intake is essential for this function not only as it promotes IGF-1 release but also in light of the stimulating effect of dibasic amino acids like arginine and lysine on intestinal calcium absorption (106, 107) (see **Figure 1**).

Another amino acid, methionine, has been suggested to underlie the potential atherogenic effects of high intake of animal protein acting as a precursor for homocysteine (HCys). High levels of HCys in the blood, which must be discussed in relation to the status of vitamin B_{12}, folate, and vitamin B_6 (see **Figure 2**), have been considered a marker for increased CVD risk and

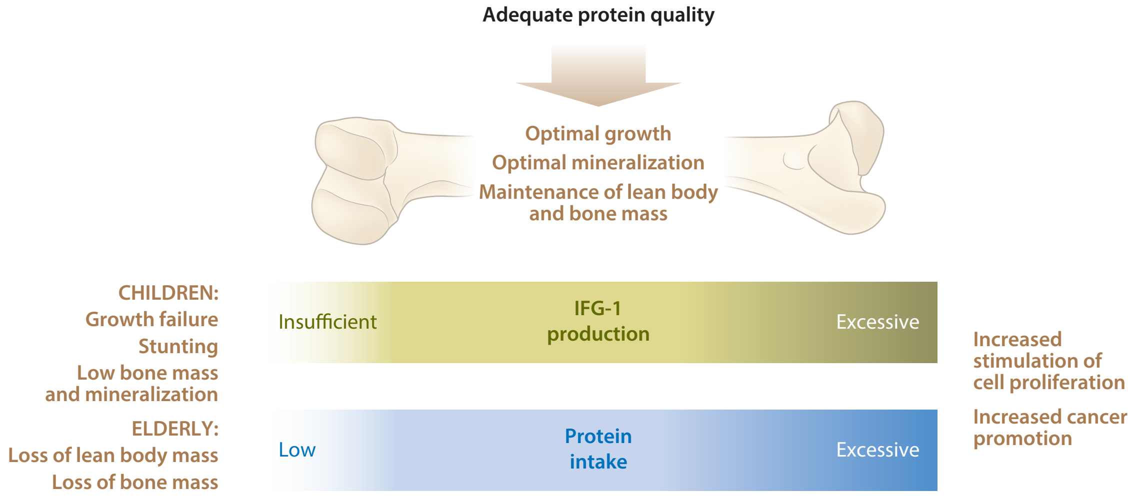

Figure 1

The influence of protein intake on insulin-like growth factor (IGF)-1 and health. Protein in sufficient quantity and quality is required for the synthesis of IGF-1. IGF-1 is essential for cell proliferation, growth, and bone formation in children as well as the maintenance of lean body mass and bone mass and density in the elderly. However, excessive protein intake, especially of high quality, can lead to increased levels of IGF-1 in the body that promote cell proliferation and thereby may increase the risk of cancerogenesis.

are associated with endothelial dysfunction, vascular damage, and atherosclerosis. However, these effects were observed after high methionine intake independently from the HCys concentration, casting doubt on the role of this latter by itself (108). Methionine exerts a particularly high toxicity when fed in excess to rats, but in humans the ingested dose would have to be extremely high to see comparable effects (109, 110). Nevertheless, transitory elevation of methionine increases HCys concentration and causes reduction in endothelial function (111). Methionine contents are higher in casein and other animal proteins compared with soy, and this has been used to explain the inverse effects of this latter on CVD risk. However, chronic high protein and methionine intake was associated with lower HCys blood levels in healthy subjects (112). Moreover, methionine supplementation prevented the mild hypercholesterolemia induced by a soy protein but not a casein protein diet richer in methionine in rabbits (113). Altogether, there is no evidence for negative effects of the higher concentrations of methionine in animal proteins consumed in usual amounts.

In turn, animal protein, especially from fish and shellfish, is a source of another sulfur-containing amino acid, taurine, that, although at least in adults is not indispensable and not a proteinogenic amino acid, is abundant in the human body, especially in the muscle tissue, and exerts a variety of functions. It is not found in plant foods, and it has been suggested that human synthesis is rather limited. Its role in the conjugation of bile acids to form taurocholic acid links it to cholesterol excretion, but it is also involved in cell osmoregulation and membrane stability and possesses antioxidant as well as immune-modulating properties (114, 115). Beneficial effects of taurine on blood pressure and cardiovascular health have been suggested, and the fact that fish and shellfish are particularly good sources of taurine might be related to the protective impact of these foods on cardiovascular health (116).

Intestinal fermentation of undigested protein has been identified as a potential risk factor associated with high protein intake. Protein intake in very high amounts or of low digestibility results in the accumulation of undigested protein in the colon that is metabolized by the gut

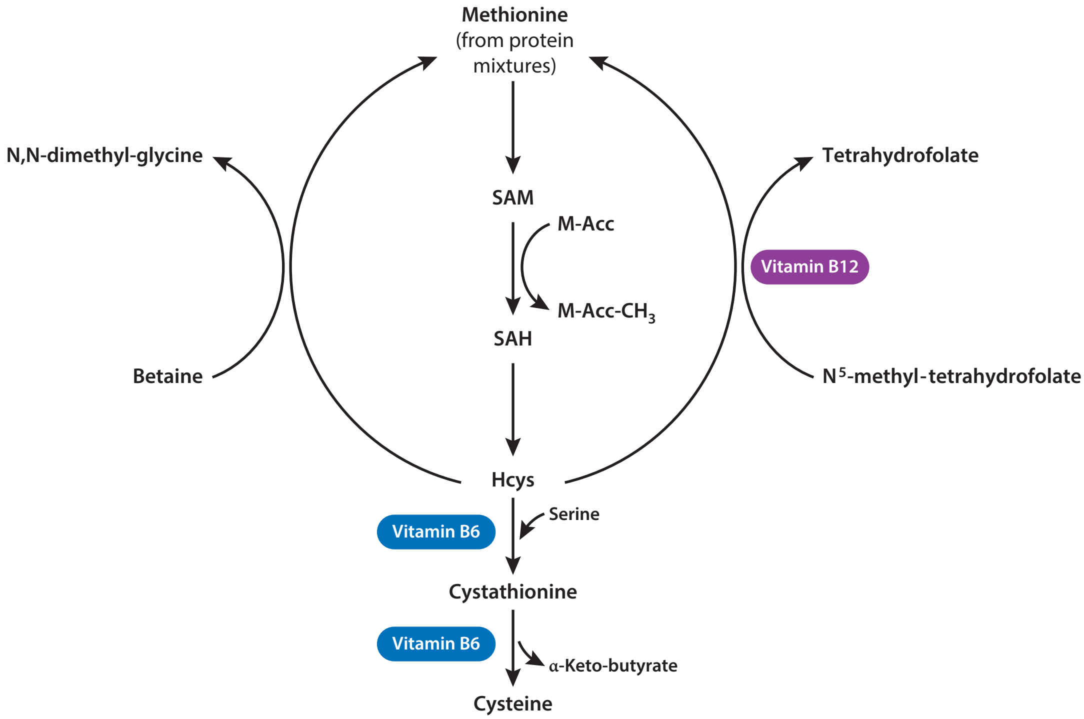

Figure 2

The methionine cycle. Methionine is transformed to S-adenosyl-methionine (SAM) in an ATP-consuming reaction. SAM transfers a methyl group to methyl-accepting molecules (M-Acc), thereby being transformed to S-adenosylhomocysteine (SAH). SAH is metabolized to homocysteine (Hcys) that can be reconverted to methionine by methylation. Two alternative pathways exist, the first using tetrahydrofolate as a methyl-donator and requiring vitamin B12 as a cofactor, the second using betaine. Alternatively, Hcys can also be degraded to cysteine in the transsulfuration pathway. This latter requires vitamin B6.

microorganisms. The substances produced in this process encompass ammonia (NH_3), phenolic compounds like p-cresol and phenol, and hydrogen sulfide (H_2S), which have shown detrimental effects on the gut epithelial cells. H_2S was shown to induce DNA damage and interfere with the oxidation of butyrate, a major energy substrate for colon cells. Fermentation of sulfur amino acids that are contained in higher amounts in meat leads to the formation of H_2S, and this might contribute to the association of high meat intake with higher incidence of colorectal cancer as well as inflammatory bowel diseases (117). Although this nonexhaustive overview shows several possible ways in which protein might be involved in health effects that have been attributed to animal foods, these latter contain several other components with effects on health that can be positive or negative.

ADDITIONAL FACTORS POTENTIALLY INVOLVED IN NEGATIVE EFFECTS OF PROTEIN-RICH ANIMAL FOODS

Among animal foods, red and processed meat are most often associated with negative health effects, particularly with regard to cancer (51, 61, 118). Different factors have been suggested as causal agents (119, 120).

Mutagenic substances, most of which are formed during processing and/or heating of meat, are among the most studied. N-nitroso compounds (NOCs) are formed by the reaction of nitric oxide and other nitrogen oxides with amines under low-pH conditions (121). Processed meat is a particular source of nitrosating agents, as these latter are used in the curing process that inhibits microbial growth and oxidative processes and prevents spoilage while at the same time preserving the red color of the meat through binding of myoglobin by nitrite. NOCs have been shown to form adducts with DNA, RNA, and proteins, thereby causing mutations and damage (122). Moreover, NOCs are metabolized by CYP450 enzymes in the liver, a process in which they are activated to the actual cancerogenic substances and that results in the formation of toxic by-products like formaldehyde and reactive carbocations (121). Although processed meat is a source of preformed NOCs, they are formed endogenously after consumption of unprocessed red meat owing to the catalyzing impact of haem iron (123).

The dry heating of meat can result in the generation of another class of carcinogenic substances, the polycyclic aromatic hydrocarbons (PAHs) that are formed during incomplete combustion. Accordingly, their concentrations in food increase with the degree of frying and grilling. Thus, the highest concentrations were measured in very-well-done grilled or barbecued steaks and, to a lesser degree, hamburgers, whereas the pan-fried or broiled meat showed much lower levels. However, meat and other animal products are not the only source of PAHs, as these substances arise from environmental pollution. Depending on the individual substance, cereal products, but also vegetables, pulses, and nuts contribute notably to intake, mostly owing to the greater amounts in which they are consumed. The highest concentrations per weight are found in dried tea, coffee, and cocoa powder, but as they are consumed in small weight quantities, their contribution is generally low (124, 125).

Heat is also involved in the generation of heterocyclic amines (HCAs), for which genotoxic and mutagenic effects have been shown. They are formed in a Maillard reaction of amino acids with creatinine and sugars (123). Of the various HCA compounds so far detected, eight have been classified as cancerogenic for humans by the International Agency for Research on Cancer, mostly based on evidence from animal studies (126). Although these substances act as cancerogens on their own, the formation of some of them is influenced by the proteins in the food inasmuch as it involves reactions with amines or amino acids.

The fact that especially red meat intake shows a relationship to colorectal cancer, whereas the production of mutagens like HCAs is not necessarily higher in this type of meat as compared with others (127), supports the role of haem iron that has been suggested. Indeed, haem not only is more abundant in red meat varieties but also has shown prooxidative effects on polyunsaturated fatty acids (PUFAs). PUFA oxidation by free haem iron leads to the formation of malondialdehyde and 4-hydroxynonenal, which are cyto- and genotoxic, providing a possible explanation for the promoting effects of high-fat diets on colorectal cancer. Besides the prooxidative properties of haem iron itself, metabolites resulting from its detoxification by haem oxidase might contribute to oxidative damage (128). Moreover, haem catalyzes the formation of NOCs, thereby increasing the endogenous production of these mutagens (120).

Although the implication of these and some other factors in cancerogenesis is based on solid evidence, the situation in vivo is complicated by the multiple interactions of various nutrients and food components, many of which are protective against cancer. Animal foods are rich sources of many nutrients contributing to overall health.

PROTEIN-RICH ANIMAL FOODS AS A SOURCE OF OTHER ESSENTIAL NUTRIENTS

Marine fish and seafoods in particular are unique in their richness in n-3 PUFAs (especially eicosapentaenoic acid), vitamin D, and iodine, nutrients that are otherwise difficult to obtain in sufficient amounts. n-3 PUFAs play a beneficial role in cardiovascular health as precursors of less inflammatory and vasoconstricting eicosanoids than their n-6 counterparts, like arachidonic acid (129). Recently, several functions were uncovered for vitamin D that are not related to its role in calcium and phosphate homeostasis and bone metabolism. Vitamin D exerts immune-modulating effects that are anti-inflammatory and antiautoreactive. It has also been shown to promote cardiovascular health and protect from certain cancers. The latter function might be related to its control of cell proliferation (130–132).

Meat, in particular the red varieties, is an important source of iron and zinc. Despite the potential involvement of iron in colorectal cancer etiology, its adequate supply is essential for health and well-being. Iron-deficiency anaemia is among the most relevant nutritional deficiency disorders worldwide, affecting industrialized countries as well, particularly among vulnerable population groups like menstruating and pregnant women, children, and the elderly. Haem iron from meat is characterized by its higher bioavailability. However, the addition of meat to the diet also improves the absorption of non-haem iron from plant foods despite high contents of phytate that interfere with trace mineral uptake (119, 133, 134). Protein quantity and quality of the diet have an effect on the bioavailability of zinc. In a diet study in humans, the addition of fish or chicken to beans increased Zn absorption by 50–70% (135).

Meat is also a good source of B vitamins, such as thiamine, vitamin B_6, and vitamin B_{12}, that play a particular role as cofactors of enzymes involved in energy and nutrient metabolism and cell proliferation. Thus, the methionine cycle is highly dependent on vitamin B_{12}, vitamin B_6, and folate, and deficiencies of these vitamins are associated with hyperhomocysteinemia and damage to the blood vessels, as well as with disturbed cell replication. This is all the more relevant as meat is also a source of substances with potential negative effects on cardiovascular health, such as saturated fatty acids, cholesterol, or the aforementioned methionine. In the case of the latter, the status of B vitamins was shown to be a no less or even more important factor in vascular health than methionine (108).

Calcium and riboflavin are two nutrients of importance that are supplied by milk products. Calcium especially has been found to be critical in many population groups in low- as well as high-income countries. Its relevance for bone health is well known, but its deficiency has also been connected to a higher colorectal cancer risk. Milk promotes calcium absorption through the effect of casein phosphopeptides and possibly also through lactose, although the role of this latter is still controversial (136). A higher risk for suboptimal status of certain nutrients has been reported for vegetarians and particularly vegans, especially for vitamin B_{12}, iron, zinc, and n-3 fatty acids but also for protein (137–140).

CONCLUSION

Protein is an essential nutrient with many functions in the body that are not limited to the supply of amino acids for the body's own protein synthesis. Indeed, amino acids have a multitude of effects on metabolic pathways. However, although findings gained in cell culture and animal trials offer potential explanations for observed effects of protein intake, it is difficult to apply them to the much more complex situation in humans, in whom a multitude of nutrients and other food components act together. It also underscores the importance of a varied diet that supplies sufficient essential nutrients while at the same time limiting the risk of excessive intakes of nutrients and harmful substances. As with other nutrients, imbalances of amino acids can induce adverse effects on health. This also concerns interactions with other nutrients. Proteins of high quality are characterized by their ability to supply amino acids in optimal amounts and relations. This is generally more the case of animal than plant proteins. It implies that lower amounts of high-quality protein are required to cover the body's needs so that potential negative metabolic effects of excessive amino acid intakes can be prevented.

However, the overconsumption of animal foods that characterizes unhealthy dietary patterns like the Western diet of many industrialized countries and an increasing number of low- and middle-income countries is associated with negative effects. In moderate amounts, animal proteins are important parts of a healthy diet that complement plant foods, which in their turn can help prevent potential negative effects of animal foods via their antioxidant, secondary plant compound, and dietary fiber content.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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