

Proteins in Human Nutrition

Review and recommendations of the Federal Commission for Nutrition (FCN) 2011

Written by a group of experts on behalf of the FCN

Approved by the Federal Commission for Nutrition on February 20, 2011.

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Preface

The present expert report on dietary proteins of the federal commission for nutrition (FCN) is a follow-up of previous reports of the FCN on macronutrients, i.e. on dietary fats (2006) and on carbohydrates (2007). It reviews the current scientific literature and recommendation by authorities on the subject. It aims to extract practical recommendations for the people in Switzerland.

Dietary proteins serve – like carbohydrates and fats – as metabolic fuel. Beside this role, they provide amino acids and thereby nitrogen for protein synthesis. Certain amino acids are essential, and dietary proteins and peptides exert specific physiological effects beyond their role as nutrients.

While health effects of dietary carbohydrates and fats have been studied in numerous investigations, the health effects of dietary proteins – their quantity and quality – has less been considered in the scientific literature in the past. In addition, dietary recommendations on protein intake have usually been directed towards fulfilment of minimal needs; however, specific effects of proteins and of increased intakes have often been ignored. This is particularly noteworthy since dietary protein consumption in industrialized societies has for decades been higher than minimal requirements.

The presents report summarizes basic aspects of dietary proteins and reviews current recommendations on requirements in children, adolescents, adults, pregnant women, and in the elderly. It reviews health effects of dietary proteins in obesity and type 2 diabetes. Here, they are of particular importance since in these conditions, excessive amounts of carbohydrates and fats exert adverse effects.

Further contributions of the report deal with the role of dietary proteins in sport activities, in bone health, in severe illnesses associated with protein catabolism, and in renal and hepatic disease. Dietary proteins are a frequent cause of food allergies with sometimes deleterious consequences for health.

Although the scientific literature often does not provide clear-cut guidance for practical recommendations, i.e. the “ideal” protein intake during various conditions in humans, the report tries to extract from the literature an update of recommendations for various population groups and disease states. It should provide an important tool for all players interested in human nutrition in Switzerland, specifically nutritionists, health authorities, food industry, teachers, dieticians and physicians.

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Basel, February, 2011

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Executive summary

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Dietary proteins and human protein metabolism – basic aspects

Proteins are composed of 21 different amino acids in a definite sequence. Nine of the amino acids are strictly essential and cannot be synthesised within the body. In children, the amino acid histidine is also essential. Six amino acids are conditionally essential, and among the six remaining ones, alanine, glutamic acid and aspartic acid are entirely non-essential. Certain proteins have a high concentration of specific amino acids, for example proline in collagen, together with a very low concentration of the essential amino acids tryptophan and lysine. Animal proteins have usually a higher content of essential amino acids than vegetal proteins, resulting in a higher “biological value”.

At any state of N balance, there is a dynamic flux between whole body synthesis and whole body protein breakdown. During protein-deficient diets or during inadequate energy intake (Very Low Calorie Diet) leading to rapid weight loss, protein breakdown is greater than protein synthesis, so that body protein is lost, mainly by muscle catabolism.

Oxidation of amino acids leads in the liver to the formation of nitrogenous by-products such as urea (non toxic) and ammonia (toxic), both of which are excreted in the urine, where they constitute more than 90% of urinary nitrogenous compounds. The carbon skeleton of amino acids can be used as an energy source and as a substrate for the synthesis of glucose by gluconeogenesis e.g. during fasting and endurance exercise.

Protein synthesis requires energy (ATP) for peptide bonds. About 20% of the remaining heat production (energy expenditure) is accounted for by protein turnover.

Dietary protein consumption in Switzerland

Evaluation of intakes in Switzerland by food supply data yielded an average consumption of about 90 g/d per person, or 1.1 g/kg b.wt./d. About 2/3 of proteins were animal proteins with high biological value (meat and meat products (28%), milk and dairy products (28%), fish (3%), and eggs (3%), and about 1/3 of proteins were of plant origin (25% of total protein as cereals, 3-4% of vegetables).

Actual spontaneous protein consumption in Switzerland by specific groups of subjects is well within the actual recommendations (10-20% of energy). Frail elderly subjects may be at risk of not covering their requirements for protein.

Agricultural production of animal compared to vegetal protein represents a higher ecological burden (required land size, water consumption, production of greenhouse gases etc.). Therefore, the amount of protein consumption not only has an effect on the health of the population as outlined below, but also has a socioeconomic and ecological impact. However, these two latter aspects will not be covered in this report because they are not the focus of this publication.

Dietary protein requirements – general comments

The physiological protein requirements are classically determined by N balance studies (intake expressed as N minus total N output). There may be additional protein needs due to anabolism (growth, pregnancy, "regrowth"). Protein requirements or protein recommendations are generally expressed either in relative terms (g/kg b.wt./d) or relative to energy (% of total energy).

The first protein requirement estimate dates back to before World War II (1.0 g/kg b.wt./d). It was higher than the value of 0.8 g/kg b.wt./d recommended later for almost 4 decades. Today, recent but not yet official recommendations appear to return to the earlier figures (1.0/kg b.wt./d). All these recommendations do not distinguish between sexes and age groups - which is obviously a limitation due to lack of adequate scientific data in these subgroups.

Human protein needs have been calculated on the basis of different criteria by different expert committees. Thereby, various terminologies have been applied. Frequently used terms are: Recommended Dietary Allowances (RDA), Protein Requirement (PR), Reference Nutrition Intake (RNI), Population Reference Intake (PRI), Average Requirement (AR), Dietary Reference Value (DRV), Recommended Daily Nutrient Intake (RDNI), Dietary Reference Intake (DRI), Acceptable Macronutrient Distribution Ranges (AMDR), Optimal Intake (OI), Safe Level of Protein (SLP), Tolerable Upper Intake level (TUI), Lowest Threshold Intake (LTI).

Protein requirements in children and adolescents

Protein requirements in children have been suggested to be approx. 10 g/d at the age of 6 months, increasing at 15-18 years to 58 g in boys and to 47 g in girls, respectively (WHO). However, the actual intake of proteins in children and adolescents is much higher; at 13-15 years - according to a European review - it is about 100 g/d or even more.

Excessive amounts of protein associated with increased energy intakes in infants and small children increase the risk for overweight and obesity.

If at the age of 5-6 years the intake of total protein, and especially that from animal sources, is too high, puberty may start earlier in girls and boys.

Protein requirements in adults

The present official dietary requirements for healthy adult women and men for all age groups are 0.80 g (high quality) protein per kilogram bodyweight. For a normal-weight man (70 kg b.wt.) this corresponds to 56 g/d, and to 46 g/d for a 57 kg woman, respectively.

In pregnancy the minimal protein requirement has been stated to be 1.1 g/kg b.wt./d, and during lactation 1.3 g/kg b.wt./d, respectively.

A concomitant adequate energy intake from non-N-energy sources is a prerequisite for the present recommendation.

Protein requirements in the elderly

The current recommendation for protein intake of healthy elderly people is the same as for younger adults (0.8 g/kg b.wt./d). Despite conflicting evidence and a controversial debate among experts in recent years about these recommendations, the WHO/FAO/UNU expert committee recently confirmed them, irrespective of sex and age.

However, adequate protein intake and the maintenance of nitrogen equilibrium is of particular importance in the elderly because this age group is at increased risk of illness and malnutrition, and very little is known about the protein needs of the frail and unhealthy elderly. The role of protein intake in bone health is dealt with in a separate chapter.

There is general agreement that protein anabolism can be stimulated by moderate amounts of dietary protein. Protein anabolism is also affected by eating patterns and physical activity.

Some suggest that not all elderly subjects can achieve nitrogen balance with 0.8 g/kg b.wt./d protein. However, there are at present no studies convincingly showing that protein requirements of the elderly differ substantially from those of younger adults. Until more evidence is available, it seems reasonable to ensure a protein intake of at least 0.8 g/kg b.wt./d in all elderly people, particularly in those at risk of malnutrition (e.g. frail and multimorbid elderly).

Early recognition of nutritional difficulties is crucial. Adequate protein and energy intake should be ascertained, along with encouragement of physical activity in order to facilitate muscle protein anabolism.

Dietary proteins in sports

The daily intake recommendations for adult athletes suggested by most of the entities are about 1.5 g/kg b.wt./d, with a range of approx. 1.0-2.0 g/kg b.wt./d. Distinction of dietary protein recommendations in strength and endurance athletes is no longer supported by most authorities today.

Research over the past decade indicates a beneficial effect with respect to a positive net muscular protein balance if athletes ingest some protein (about 10 to 20 g/h) before an exercise bout.

The Swiss Food Pyramid for Athletes represents food-based recommendations which ensure a sufficient intake of energy and micronutrients in relation to the daily exercise volume and intensity. It emphasises the need to coordinate protein intake with that of other nutrients.

Dietary proteins in obesity and in diabetes mellitus

Dietary proteins influence body weight by affecting satiety, thermogenesis, energy efficiency, and body composition.

Protein ingestion results in more satiety than aequicaloric amounts of carbohydrates or fat. Their effect on satiety is mainly due to oxidation of amino acids fed in excess; this effect is higher after ingestion of "incomplete" (vegetal) proteins than after animal proteins.

Diet-induced thermogenesis is higher for proteins than for other macronutrients - energy expenditure (thermogenesis) increased by 20-30% after proteins compared to 5-10% after carbohydrates and 0-5% after ingestion of fat. This increase in energy expenditure is caused by protein and urea synthesis and to gluconeogenesis; this effect is larger after animal than after vegetal proteins.

Energy restricted high protein diets in obesity (protein amounts of approx 30% of total energy, or 1.2 g/kg b.wt./d, versus 15-20% of total energy, or 0.8 g/kg b.wt./d) resulted in greater weight loss or in less weight regain after weight loss than diets with lower amounts of protein in studies lasting up to one year. These high protein diets during weight loss maintained fat-free mass (i.e. muscle mass) and increased calcium balance, resulting in the preservation of bone mineral content.

Adequate dietary protein intake in diabetes type 2 is of specific importance since proteins are relatively neutral with regard to postprandial serum glucose and lipid concentrations, and they preserve muscle and bone mass which may be decreased in subjects with poorly controlled diabetes.

An adverse effect of increased dietary proteins such as progression of renal insufficiency has been observed in subjects with kidney diseases - this problem is quite often observed in the elderly, hypertensive and diabetic population.

Dietary proteins and bone health

Dietary proteins may prevent osteoporosis, in addition to dietary calcium and vitamin D. Dietary protein intake was correlated with bone density and mineral content.

Hip fracture incidence was lower during high protein diet (1.3 g/kg b.wt./d) compared to lower protein intake (1.0 g/kg b.wt./d). An intervention study indicated that following orthopaedic management, protein supplementation attenuated post-fracture bone loss, tended to increase muscle strength, reduced medical complications and the duration of rehabilitation.

In the elderly, taking into account the attenuated anabolic response to dietary protein with aging, there is concern that the current dietary protein RDA, as set at 0.8 g/kg b.wt./d, might be too low for the primary and secondary prevention of fragility fractures, and expert groups specialised in bone health stated that approx. 1.2 g protein/kg b.wt./d would be more appropriate.

Protein catabolism and requirements in severe illness

Severe illnesses are characterized by reduced total body protein mass, mainly due to diminished skeletal muscle mass. Protein-energy malnutrition has been associated with increased mortality.

Severe illnesses are characterised by increased protein breakdown and, to a lesser extent, by an increase in whole-body protein synthesis, associated with an increased flux of amino acids from the periphery to the liver.

Nutritional support limits but does not abolish the loss of total body protein mass occurring in acute severe illness. Protein intakes between 1.2 and 1.5 g/kg b.wt./d with neutral energy balance minimise body protein loss. Glutamine and possibly leucine may improve clinical outcomes.

Present recommendations indicate a caloric supply of 20-25 kcal/kg b.wt./d over the first 72-96 hours and an increase in energy intake as the target thereafter. Simultaneously, protein intake should be between 1.2 and 1.5 g/kg b.wt./d.

Tube feeding (enteral nutrition) with "immunonutrients" enriched with arginine, nucleotides and omega-3 fatty acids is indicated in patients with trauma, acute respiratory distress syndrome (ARDS) and mild sepsis. Glutamine (0.2-0.4 g/kg b.wt./d of L-glutamine) should be added to enteral and parenteral (intravenous) nutrition in burned and trauma patients according to current guidelines.

Dietary proteins and atherosclerosis

More than 100 years ago the hypothesis "protein" of the pathogenesis of atherosclerosis and its association with cardiovascular disease was put forward on the basis of animal experiments; however, it has never been verified so far in humans.

Epidemiological studies in the 1960s showed significant associations between dietary animal protein and mortality from cardiovascular disease. However, animal protein intake was also significantly correlated with saturated fatty acid and cholesterol intake. In contrast, in the last decades two prospective cohort studies demonstrated a decreased cardiovascular risk in women during high versus low protein intake when the dietary intake was adjusted for other factors (e.g. saturated fats) and for cardiovascular risk factors.

The Mediterranean diet has been associated with diminished risk of coronary heart disease; this diet has a pattern which is characterised by relatively high amounts of plant-derived food and of fish as alternatives to meat and dairy products.

Protein intake in renal and hepatic disease

The amount and composition of ingested proteins has a direct impact on renal function, especially in kidney disease, and on the risk of kidney stones. Limitation of ingested protein, particularly from animal sources, is therefore crucial in order to slow down the progression of chronic kidney disease and the impairment of renal function. In contrast, patients with chronic renal failure undergoing renal replacement therapy by haemo- or peritoneal dialysis have an increased protein demand. The syndrome of “protein-energy malnutrition” is a relevant factor for morbidity and mortality in this population, requiring early detection and vigorous treatment.

Protein intake in patients with cirrhosis of the liver should not be decreased as suggested earlier but rather increased to counteract the risk of protein malnutrition. Only in patients with advanced hepatic encephalopathy has there been recommended moderate restrictions depending on protein tolerance, with the possible addition of branched chain amino acids (BCAA).

Allergic reactions to food proteins

Four to eight percent of the population have food allergies, and the prevalence rates are increasing in the past years. Most food allergies are against food-derived proteins and they are usually acquired on the basis of a cross-reaction to pollen allergens. These allergens are ubiquitous in the plant kingdom. Therefore pollen-allergic patients might acquire a multitude of different plant food allergies and even react to novel foods, to which they have never been exposed before.

A curative therapy for food allergy does not yet exist. Food-allergic patients have to rely on strict avoidance of the suspected foods. The widespread use of industrially processed foods poses a general problem for food allergic patients. Although the most frequent allergens must be declared openly in the list of ingredients, involuntary contamination with allergy-provoking compounds can occur. The labelling “may contain...” discourages consumption even if the chance of contamination is negligible; on the other hand, the allowance of up to 1 g/kg b.wt./d of allergy-provoking compounds without need for declaration may be too high to protect food allergic individuals if traces of these foods already cause symptoms.

Bioactive proteins and peptides in foods

An increasing amount of data demonstrates specific effects of dietary proteins and peptides beyond their nutritional impact (“bioactivity”). The focus of the investigations has mainly been on vitamin- and mineral-binding proteins, on antimicrobial, immunosuppressing/-modulatory proteins, on proteins with enzyme inhibitory activity as well as on hormones and growth factors derived from food proteins. Most research has been performed on milk proteins.

Biologically active peptides are released during fermentation or digestion of food proteins; these peptides are mainly found in milk, and they exert opiate-like, antihypertensive, mineral-binding, antioxidative, antimicrobial, immuno- and cytomodulating activity.

Intact absorption of these smaller peptides is possible; therefore organs outside the gastrointestinal tract are possible targets for their biological effects.

Bioactive proteins and peptides are normal parts of a balanced diet. However, it is possible to accumulate bioactive peptides in food, for example by using specific microorganisms in fermented dairy products. Although bioactive peptides have been the subject of several investigations *in vitro* and *in vivo* in humans, their health potential is still not clearly established. Therefore, the Commission of European Communities has not (yet) authorised any health claims for bioactive proteins and peptides from food.

Summary of recommendations

Requirements in adults:

Recommended intake of proteins is between 0.8 g and 2.0 g protein/kg b.wt./d; 0.8 g representing the minimum daily needs to maintain short-term nitrogen balance in healthy subjects with moderate activity. The upper tolerable limit (2.0 g) has been set because of uncertainty of the health effects of higher amounts of dietary proteins.

Protein requirements are the same for all age groups of adults and are independent of gender, because there are insufficient scientific data to describe recommendations for subgroups of the population. When expressed as a percentage of energy, protein intake should be 10-20% of energy requirements. Adequate energy intake from non-N-energy sources is a prerequisite for the present recommendation. During pregnancy and lactation, protein requirements are increased (1.1 g /kg b.wt./d during pregnancy, and 1.3 g/kg b.wt./d during lactation, respectively).

Requirements in children and adolescents:

The actual protein reference values (= recommended dietary allowance) for children are 1.8 g/kg b.wt./d in the 1st month, and 1.1 g/kg b.wt./d in the 12th month of age. In the age range of 1 to 4 years it is 0.86 g/kg b.wt./d and 0.91 g/kg b.wt./d up to the age of 10 years. After age 11 until 18 yrs, protein requirements are gender specific, 0.85-0.91 g/kg b.wt./d for male adolescents, and 0.82-0.90 g/kg b.wt./d for females.

WHO also included mean reference values for absolute protein requirements: 10.2 g/d at 6 month of age, increasing to 57.9 g in males and 47.4 g in female adolescents aged 15-18 years.

Current protein intake by Swiss children and adolescents is on the average too high - the actual intake of proteins is about 40 g/d at two years, 60 g/d at three years and 100 g/d and even more at 13-15 years. Excessive protein intake in small children has been associated with adult obesity. During ages of 5-6 years excessive protein intake may lead to early puberty.

Requirements in elderly subjects:

The current recommendation for minimal protein intake of healthy elderly subjects is 0.8 g/kg b.wt./d and, thus, the same as for younger adults. Despite conflicting evidence and a controversial debate among experts in recent years about the adequacy of this amount, a WHO/FAO/UNU committee recently confirmed this recommendation irrespective of gender and age.

It is important to ensure a protein intake of at least 0.8 g/kg b.wt./d in all the elderly, particularly in those at risk for malnutrition (e.g. frail and multimorbid elderly). Protein requirements to improve bone health may be higher than these minimal requirements (see chapter on bone health below).

Requirements in sports:

The daily protein intake for adult athletes recommended by most authorities is about 1.5 g/kg b.wt./d with a range of 1.0 to 2.0 g/kg b.wt./d. Protein ingestion before exercise has been reported to increase protein synthesis. The earlier suggested separate dietary protein recommendations for strength and endurance athletes are no longer supported and this recommendation needs to be adapted to the individual needs of the athlete.

Protein intake in obesity and in diabetes

Consumption of relatively higher amounts of protein in obesity (up to 1.3 g/kg b.wt./d) resulted in greater weight loss or in less weight regain after voluntary weight loss than for lower amounts of protein in studies lasting up to one year. High protein diets maintained fat-free mass (i.e. muscle mass) and increased calcium balance, resulting in preservation of bone mineral content.

The consumption of dietary proteins is frequently insufficient during massive weight loss, e.g. after obesity surgery (i.e. gastric bypass).

A relatively high amount of protein (up to approx 1.3 g/kg b.wt./d) in the diet may be of particular importance in obese diabetic or hyperlipidemic subjects. Dietary protein has no significant negative effects on blood glucose control, on serum lipids or on other cardiovascular risk factors. However, dietary protein intake should not be excessive, and there are insufficient long-term data (beyond 2 yrs) of such eating habits. Increased protein intakes are contraindicated in elderly or obese subjects with renal impairment.

Protein intake and bone health

Several studies point to a positive effect of relatively high protein intake (up to 1.5 g/kg b.wt./d) on bone mineral density or content and on hip fracture risk. However, excessive amounts of dietary protein (i.e. more than 2.0 g/kg b.wt./d) associated with low calcium intake (i.e. less than 600 mg/d) may have adverse effects on bone health.

Protein intake in catabolic illness

Present recommendations indicate that protein intake should be between 1.2 and 1.5 g/kg b.wt./d, combined with an energy intake of 20-25 kcal/kg b.wt./d over the first 72-96 hours after an acute catabolic event (trauma, severe illness) in order to minimise body protein loss in severe catabolic illness. In addition, glutamine and possibly leucine may specifically improve clinical outcomes. Thereafter, energy intake should be increased to target levels.

Enteral "immunonutrition" via tube feeding and enriched with arginine, nucleotides and omega-3 fatty acids is indicated in patients with trauma, ARDS and mild sepsis. Glutamine (0.2-0.4 g/kg b.wt./d of L-glutamine) should be added to enteral and to parenteral (intravenous) nutrition in burned and trauma patients (ESPEN guidelines 2006 and 2009).

Protein intake in renal and in hepatic diseases

Limitation of ingested protein, particularly from animal sources, to approx. 0.8 g/kg b.wt./d is important to slow down the progression of chronic kidney disease with impaired excretory function. In contrast, patients with chronic renal failure undergoing renal replacement therapy by haemo – or peritoneal dialysis have an increased dietary protein demand. Protein intake in patients with cirrhosis of the liver should be increased to 1.0-1.2 g/kg b.wt./d in order to prevent protein malnutrition. In patients with advanced hepatic

encephalopathy, moderate protein restriction depending on protein tolerance (0.5-1.2 g/kg/d) has been recommended, with the possible addition of branched chain amino acids (BCAA).

Key practical recommendations for the consumer

- Dietary proteins should be included in each meal of the day; consume at least 3 meals per day.
- Do not skip breakfast. A protein-containing breakfast prevents whole body protein catabolism during the morning.
- Some groups in the population are at an increased risk of not eating enough dietary proteins:
 - a. Elderly people - because of poor appetite, co morbidities, psychosocial circumstances.
 - b. People with impending or established osteoporosis,
 - c. Subjects eating hypo caloric or imbalanced diets. Remember that your dietary protein needs are maintained if your caloric intake is decreased; during a hypo caloric diet, the percentage of dietary proteins should be increased.
 - d. Sick individuals - they have increased protein and energy needs but often consume too little proteins due to anorexia.
- Dietary proteins should be consumed in higher amounts than to cover minimal needs in athletes; the same is true if optimal bone health (prevention of osteoporosis) is desired.
- Dietary proteins play a specific favourable role in obesity and diabetes due to their high satiating effect and their “neutral” behaviour regarding glucose and lipid metabolism.
- Subjects with renal impairment should limit the intake of dietary proteins to the minimum needed to prevent protein catabolism.
- Read the food labels where pertinent information on protein content is available.

Chapters of the report

1. Dietary proteins in humans: Basic aspects and consumption in Switzerland

Yves Guigoz, Epalinges

1.1. Summary/ Zusammenfassung/ Résumé

This introductory review gives an overview on protein metabolism, and discusses protein quality, sources and requirements as well as the results from recent studies on Swiss spontaneous protein consumption.

To assess protein quality in protein mixes and foods, the "protein digestibility-corrected amino acid score" (PDCAAS) is presented as a valuable tool in addition to the biological value (BV).

Considering protein intake recommendations, the lower limit recommended has been defined according to the minimal amount needed to maintain short-term nitrogen balance in healthy people with moderate activity.

Evaluation of intakes in Switzerland from food consumption data is about 90 g/d per person. 2/3 of proteins consumed in Switzerland are animal proteins with high biological value (meat and meat products (28%), milk and dairy products (28%), fish (3%), and eggs (3%)) and about 1/3 of proteins are of plant origin (25% of cereals, 3-4% of vegetables).

Actual spontaneous protein consumption in Switzerland by specific groups of subjects is well within the actual recommendations (10-20% of energy) with only the frail elderly being at risk of not covering their requirements for protein.

Zusammenfassung: Nahrungsproteine: Grundlagen und aktueller Verzehr in der Schweiz

Diese Einführung gibt einen Überblick über den Stoffwechsel von Proteinen, erläutert die Proteinqualität und Proteinquellen und diskutiert den Proteinbedarf. Weiter werden Daten zum Proteinverbrauch in der Schweiz aus jüngsten Untersuchungen präsentiert.

Zur Beurteilung der Proteinqualität verschiedener Proteine und von Lebensmitteln stellt der sogenannte *Protein Digestibility-Corrected Amino Acid Score* (PDCAAS) neben der biologischen Wertigkeit (BW) ein wertvolles Instrument dar.

Die Empfehlungen zur Proteinzufuhr basieren auf dem Minimalbedarf, bei welchem die Stickstoffbilanz über kurze Zeit bei gesunden Menschen mit mässiger Aktivität noch im Gleichgewicht gehalten wird.

Die Evaluation des Verzehrs von Proteinen in der Schweiz anhand von Lebensmittelverbrauchsdaten ergab etwa 90 g Proteine/Tag pro Person. Ungefähr 2/3 der Proteine waren tierischer Herkunft mit hoher biologischer Wertigkeit. Davon stammten 28% aus Fleisch und Fleischprodukten, 28% aus Milch und Milchprodukten, 3% aus Fisch und 3% aus Eiern. Ungefähr 1/3 der Proteine waren pflanzlicher Herkunft, entsprechend 25% aus Getreide und 3-4% aus Gemüse.

In der Schweiz liegt der geschätzte Verzehr an Nahrungsproteinen von einzelnen Bevölkerungsgruppen im Bereich der aktuellen Empfehlungen (10-20% der täglichen Energieaufnahme). Eine Ausnahme bilden gebrechliche und ältere Menschen; diese sind für eine Unterversorgung mit Proteinen gefährdet.

Résumé : Protéines alimentaires chez l'homme : principes de base et consommation actuelle en Suisse

Ce résumé introductif donne un aperçu du métabolisme protéique, présente les sources de protéines et leur qualité variable, ainsi que les besoins en la matière, et aborde la consommation spontanée de protéines en Suisse, sur la base d'études récentes.

Le PDCAAS (protéine digestibility-corrected amino acid score) s'avère un outil valable, en plus de la valeur biologique, pour évaluer la qualité protéique des mélanges de protéines et des aliments.

L'apport en protéines recommandé est défini selon la limite inférieure correspondant au besoin quotidien minimum de protéines, pour un bilan azoté équilibré à court terme chez une personne en bonne santé ayant une activité modérée.

En Suisse, chaque personne absorbe en moyenne 90 grammes de protéines par jour, comme le montre l'évaluation des données disponibles sur la consommation alimentaire en Suisse. Il s'agit pour 2/3 de protéines animales possédant une valeur biologique élevée (viande et produits à base de viande: 28%; lait et produits laitiers: 28%; poisson: 3%; œufs: 3%). Près de 1/3 des protéines sont d'origine végétale (céréales: 25%; légumes: 3-4%). La consommation spontanée de protéines des divers groupes de population en Suisse correspond pleinement aux recommandations en vigueur (10-20% des besoins d'énergie). La seule exception concerne les personnes âgées fragiles, dont les besoins en protéines risquent de ne pas être couverts.

1.2. Dietary proteins

Proteins are fundamental structural and functional elements within every cell and undergo extensive metabolic interaction (for review see (1), (2)). Dietary protein provides the amino acids required for synthesis of various body proteins such as skeletal muscle and other structural proteins, transport proteins, hormones and enzymes. They can provide energy but usually not as a major source.

Definitions according to the amino acid composition

Proteins are polymers of amino acids, which determine their specific properties through the type of amino acids they contain, and the sequence in which they are linked together.

There are 20 different proteinogenic amino acids which are classified in indispensable and dispensable amino acids. *Indispensable* amino acids are leucine, isoleucine, valine, lysine, threonine, tryptophan, methionine, phenylalanine, and histidine, which our body is unable to synthesize for building proteins, and must be obtained totally from the diet in order to maintain N balance. *Dispensable* amino acids are alanine, aspartic acid, asparagine, glutamic acid, glycine, and proline, which our body can synthesize in adequate amounts from metabolic intermediates by transamination. Some dispensable amino acids cannot be synthesized in sufficient amounts under specific conditions, e.g. growth or stressful conditions, and are therefore *conditionally indispensable* amino acids. Representatives are arginine, cystine, glutamine, serine, and tyrosine.

Nitrogen content of dietary proteins

The protein content in foodstuff is estimated by multiplying the measured total nitrogen content by a nitrogen-to-protein conversion factor set usually as 6.25. This factor has been proposed for all protein sources by the ESPGHAN (3), and retained in the last revision of food standard for infant formula by Codex (4) and EFSA (5). In food chemistry, however, specific nitrogen-to-protein conversion factors are used (6, 7). These range from 5.71 for soybean (seed, flour and soy products) to 6.38 for milk and dairy products, with the 6.25 factor for mixed protein food sources.

In Switzerland, according to the federal ordinance on labelling and advertising of foodstuffs (8), the protein content is calculated using the 6.25 factor of total N content (Kjeldahl method), which is also used in the Swiss Food Composition Database (9). In future versions of this database more food specific conversion factors are intended to be used.

Features of dietary proteins and use in food technology

Protein interactions and reactions are very important in food systems, especially in infant food and affect the quality and structure of food, as well as its processing. Chemical modification of proteins by processes can affect their bioavailability, such as the Maillard reaction (or non-enzymatic browning reaction) which occurs between the free amino group of a protein (mostly lysine) and a free carbonyl group of reducing sugars (glucose, fructose, lactose, maltose) to form lysinoalanine, followed by early Maillard products (Amadori products) and advanced Maillard reaction products (Advanced Glycation End products, AGE). Intense heating of proteins enhances formation of these products. They affect protein digestibility (10), protein solubility, and product flavour (for review see (11)). Potential bio-available lysine is in this case assessed by the quantisation of lysine that has not undergone any form of structural change: "reactive lysine" (12). Recently, adverse effects of these products in humans by increasing markers of risk of diabetes type 2 and cardiovascular disease have been described (13).

Some proteins are used as ingredients in many food products. Examples are whey products (14) and others:

- a) Dairy in ice cream, yogurt, dairy spreads, cheese, and products, beverages as dairy solids, and for emulsification, viscosity, whip ability.
- b) Meats in processed meat, sausages, fish for fat/water binding, gelling.
- c) Dry seasoning mixes in potato chips, Mexican dishes, gravy for dispersibility, flavour, low sweetness bulking.
- d) Bakery in muffins, crusts for pies, breads, buns for colour, flavour development, heat setting, shelf-life.
- e) Confections in candy bars for whip ability, low sweetness bulking, and egg replacement stability.
- f) Snack foods in cookies, bars for thermal expansion, nutrition.
- g) Beverages in cocoa mix, coffee creamer, sports drinks, health drinks for solubility, viscosity, nutrition, dairy flavour, protein quality.
- h) "Nutraceuticals" and sports foods in energy bars and drinks, nutrient supplements for special needs for bioactivity and protein quality.

1.3. Definitions of protein quality

Nutritional quality of a specific protein corresponds to the capacity of this protein source to cover requirements for nitrogen and amino acids of humans, as well as total amino nitrogen necessary for the

synthesis of dispensable amino acids. It depends mainly on the essential amino acid composition and digestibility, and can differ widely. Protein quality can be assessed by following indices (2, 15, 16):

- Biological Value (BV), i.e. the proportion of absorbed protein retained in the body.
- Net Protein Utilization (NPU), i.e. the proportion of dietary protein that is retained in the body (i.e. it takes account of the digestibility of the protein).
- Protein Efficiency Ratio (PER), which is the gain in weight of growing animals per gram of protein eaten (standard method for protein quality assessment of infant formula).
- Relative Protein Value (RPV), i.e. the ability of a test protein, fed at various levels of intake, to increase nitrogen balance, compared with a standard protein.
- Chemical Score, based on chemical analysis of the protein, is the amount of the limiting amino acid compared with the amount of the same amino acid in a reference protein (egg protein).
- Amino acid score: This uses a reference pattern of amino acid requirements as the standard

Protein digestibility-corrected amino acid score

To overcome the difficulty in assessing protein quality in protein mixes and foods, the use of the **protein digestibility-corrected amino acid score** (PDCAAS) has been proposed. This is a method based on a comparison of the amino acid content of food with human requirements (amino acid scoring system). It is considered to be the most suitable approach for routine assessment (15, 17). The PDCAAS corresponds actually to the digestibility multiplied with the amino acid score according to following formula:

PDCAAS % = True protein digestibility coefficient x amino acid score

PDCAAS % = (mg of the first limiting amino acid in 1 g of test protein) / (mg of the same amino acid in 1 g reference protein) x true digestibility of the test protein (expressed in %).

PDCAAS above 100 are considered as 100. This method takes into account:

- a) the first limiting essential amino acid in a protein or a mixture of proteins to meet the amino acid requirements, and
- b) the digestibility of that protein or food.

It is very useful for routine assessment of quality, keeping in mind the limitation of the true digestibility assessed by rat assay, the impact of anti-nutritional factors, modified lysine by processing/storage and biological efficiency of supplemental amino acids.

The PDCAAS of a mixture of proteins from meat, milk and wheat in the ratio 1:1:1 is 100%, indicating that none of the essential amino acids is limiting, so optimum use can be made of all essential amino acids in the synthesis of body proteins. In the diets of lacto-ovo vegetarians and vegans, lysine is the limiting amino acid. Assuming that milk proteins and wheat proteins are present in a 1:1 ratio, then the PDCAAS for a lacto-ovo vegetarian diet is estimated at 84%. In calculating the PDCAAS for a vegan diet, assuming a ratio of wheat proteins to soya proteins of 1:1, it is estimated that this diet has a PDCAAS of 77% (18)

The PDCAAS value could be used to adjust dietary protein intake to meet requirements:

Recommended intake = safe level of protein/PDCAAS value of diet

This suggests that protein requirements of lacto-ovo vegetarians and vegans are higher than that of people on a mixed diet: Lacto-ovo vegetarians 1.2 times higher (1/0.84) and 1.3 times (1/0.77) higher for vegans (18).

Actual amino acids scoring patterns are given in **Tab. 1** and true digestibility in **Tab. 2** for calculation of the PDCAAS (see examples in **Tab. 3**). Protein quality assessment using the limiting indispensable amino acid will, however, be subject to possible changes as the requirements for essential amino acids are under revision. The actual protein quality gives indication on the roles of the first limiting amino acid for growth or nitrogen balance, but does not take into account the complex roles for protein and amino acids in regulation of bone health, gastrointestinal function and bacterial flora, glucose homeostasis, cell signalling, and satiety (see other reviews of this report and (19)).

Tab. 1: Amino Acids Scoring Pattern for Calculation of PDCAAS (Amino acid requirements/protein requirements)

	FNB/IOM-USA 2002/2005		WHO/FAO 2007	
	Children ≥1 Year of Age and All Other Older Age Group	Preschool Children (1-2 years)	Older Children and Adolescents (4-18 years)	Adults (>18 years)
Indispensable amino acid	mg/g protein		mg/g protein	
Histidine	18	18	16	15
Isoleucine	25	31	30	30
Leucine	55	63	60	59
Lysine	51	52	48	45
Methionine + cysteine	25	26	23	22
Phenylalanine + tyrosine	47	46	41	38
Threonine	27	27	25	23
Tryptophan	7	7.4	6.5	6
Valin	32	42	40	39

Tab. 2: True digestibility of proteins (sum of digestible protein/total protein)^a

Protein source	True digestibility (%)
Meat, fish	94
Milk, cheese	95
Egg	97
Wheat, cereal	77
Wheat, whole	86
Wheat flour, white	96
Oatmeal	86
Oats, cereal	72
Chickpea	80
Maize	85
Maize + beans ^b	78
Maize + beans + milk ^b	84
Wheat + chickpea + milk powder ^b	85
Western (American) mixed diet	96

^afrom WHO technical report series ; no. 935, 2007 (2)

Tab. 3: Protein digestibility – corrected amino acid score (PDCAAS^{a,b})

Protein source	PDCAAS (%)
Meat, fish	100
Milk, cheese	100 (121)
Egg	100 (118)
Beef	92
Soy	91
Wheat, cereal	42
Wheat, whole	67
Chickpea	91
Wheat + chickpea + milk powder ^c	84

^afrom WHO technical report series ; no. 935, 2007 (2)

^bSchaafsma J. Journal of Nutrition. 2000;130:1865S-1867S (70)

1.4. Metabolism of dietary and whole body proteins

Dietary proteins are broken down in the upper intestinal tract into small peptides and amino acids, and subsequently absorbed. Free amino acids form the amino acid pool and are used for protein synthesis. In the body there is a continuous process of protein synthesis and degradation, *protein turnover*. In adults under normal conditions, synthesis and degradation rates are similar, allowing equilibrated nitrogen balance. During growth, synthesis exceeds protein degradation, allowing protein deposition and positive nitrogen balance. For a daily protein intake of 75 g, under balance conditions, there is 300 g of protein synthesis from a small amino acid pool (70 g), 300 g of protein degradation feeding the amino acid pool, and about 75 g of protein loss (for review see (20)). The turnover of specific proteins varies from minutes to months, depending on the protein function: transport/communication (plasma protein, hormones, and cell membrane and receptors) structural (bone, skin, epithelia) enzymatic (digestive, clotting, and metabolic pathways) and protective (acute phase protein, immunoglobulins, cytokines). The turnover of muscle proteins represents about 20% of the total protein turnover, liver about 10%, skin and digestive tract proteins about 15% each. Nutritionally we consider the global turnover, which is for an adult of 70 kg under stable conditions of about 4 g protein/kg b.wt./d. This, however, depends:

- of the age: for a newborn, it is much more rapid, about 10-15 g/kg b.wt./d, allowing a protein deposition of 1-1.5 g protein/kg b.wt./d or a daily body weight gain of 20-30 g/d (12% protein)
- of the nutritional status: protein turnover diminishes under fasting, resulting in negative protein balance (proteolysis being higher than synthesis).
- of the pathological state: Inflammation, trauma or sepsis induce a 3-4-fold increase in protein turnover, resulting in negative protein balance and reduced muscle mass.

Protein synthesis and degradation are energy requiring, which under reduced energy intake may result in negative nitrogen balance. Energy costs of protein synthesis correspond to about 1 kcal/g protein *in vivo* in man. Proteolysis is the main source of amino acids ~75% against ~25% for nutritional supply. Both, synthesis and degradation are very well regulated, however, the nutritional and hormonal conditions regulating degradation are not as well understood as those regulating synthesis.

Dietary protein supply under Western diet corresponds to 1-1.5 g/kg b.wt./d or 70-105 g protein/d for men of 70 kg. On top of the dietary supply, amino acids secreted in the gastrointestinal tract are about 50 g, giving a total daily amino acid supply of about 150 g (20, 21). Splanchnic extraction amounts to 60-80% of the

absorbed amino acids, except 20% for the branched chain amino acids. Splanchnic extraction is affected by aging and the nutritional status.

Protein metabolism is under hormonal and nutritional regulation: anabolic hormones (insulin, IGF-1 and catecholamine) favour protein gain and catabolic hormones (glucocorticoids, thyroid hormones and cytokines) promote protein loss. Nutritional regulation indicates that ingested amino acids stimulate protein synthesis, and a sufficient energy supply is necessary to maintain nitrogen balance in equilibrium. A high protein meal through its supply in amino acids and the post-prandial insulin level promote a positive nitrogen balance.

Protein quality influences the rate of muscle protein synthesis, and the presence of proteins rich in branched chain amino acids (e.g. leucine) seems to be more effective (22); for example, whey hydrolysate (inducing large increase in plasma leucine concentration) stimulated muscle protein synthesis after resistance exercise in men more than soy protein or casein (for review see: (23, 24).

Whole body and muscle protein turnover decline with age in men and women, indicating that there is a progressive decline in the body's remodelling processes with aging; but aerobic exercise can enhance muscle protein synthesis irrespective of age (25). In addition, muscle protein synthesis is influenced by the type, amount and timing of protein supply (26, 27). Further aspects of protein metabolism are given with more in depth discussion in accompanying papers of this report.

1.5. Protein sources

Dietary proteins are mainly derived from animal (meat, fish, dairy products and eggs) or plant sources (cereals, vegetables). The protein content of foods (as % of total weight) varies considerably from 2.7% for bread, 18% for meat, to 25% for cheese and dry vegetables. Plant proteins are generally of lower biological value, due to their lower digestibility and primary limiting amino acids, mainly lysine and sulfur containing amino acids (methionine, cysteine), as well as tryptophan for maize. Good quality supply of essential amino acids can be, however, obtained by complementing cereal proteins (poor in lysine, but rich in sulfur containing amino acids) with leguminous proteins (poor in sulfur amino acids, but rich in lysine), quality can be further improved with dairy products. Examples of protein sources high in leucine and branched chain amino acids are given in **Tab. 4**.

Tab. 4: Leucine and branched-chain amino acids (BCAA) content of proteins

	Leucine (g/100g protein)	BCAA (g/100g protein)
Whey protein isolate	14	26
Milk protein	10	21
Egg protein	8.5	20
Muscle protein	8	18
Soy protein isolate	8	18
Wheat protein	7	15

(from : Millward DJ et al. Am J Clin Nutr 2008;87:1576S-1581S (19)

Other characteristics of protein sources in the total diet should also be taken into account (28): e.g. egg whites are high in protein and low in cholesterol, but low in zinc; soy products are rich in flavonoids and phytoestrogens, but they can also contain phytates that diminish zinc and iron absorption. Processed meat and full-cream milk are rich in saturated fats; however fish, low-fat milk products as well as lean meat, beans

are protein sources low in saturated fat. Animal protein sources provide key nutrients, such as meat providing vitamins A, B6, B12 and folate, and iron, zinc. Milk products are rich in calcium, vitamin B2, B12, and D (if added), and trace elements (Zn, iodine) (29). These nutrients are not widely available from plant sources.

Proteins and peptides are frequent causes of food allergies; the management of food allergies is avoidance of the relevant food allergens. (for review see (30) and accompanying chapter by Ballmer-Weber B).

For the introduction of food proteins in infant nutrition and avoidance of food allergies, see (30, 31), and accompanying chapter by Baerlocher K.

Nutrition claims and conditions applying to protein sources:

According to the “Verordnung des EDI über die Kennzeichnung und Anpreisung von Lebensmitteln (LKV)”¹ and the European regulation (32) the following claims can be done:

SOURCE OF PROTEIN: “A claim that a food is a source of protein, and any claim likely to have the same meaning for the consumer, may only be made where at least 12% of the energy value of the food is provided by protein.”

HIGH PROTEIN: “A claim that a food is high in protein, and any claim likely to have the same meaning for the consumer, may only be made where at least 20% of the energy value of the food is provided by protein.”

For review on nutrient profiling for foods bearing nutrition and health claims see (33, 34).

1.6. Protein requirements – general remarks

The **dietary requirement** is the amount of protein or its constituent amino acids, or both, that must be supplied in the diet in order to satisfy the metabolic demand and achieve nitrogen equilibrium” (Definition in: WHO technical report series ; no. 935, 2007 (2)).

How protein requirements and safe levels of intake are determined

The nitrogen balance has been the most frequently used method to define the minimum required levels of proteins and indispensable amino acids. The subjects are fed varying amounts of protein or amino acids, and the N balance is measured, until the amount of dietary protein intake achieves a neutral or slightly positive balance. These measurements require an adequate energy intake. A meta-analysis by Rand WM *et al.* (35) provides new recommendations for dietary reference values, i.e., an estimated average requirement (EAR median) and a recommended dietary allowance (RDA, safe level of intake, 97.5th percentile) for healthy adults of 0.65 and 0.83 g good-quality protein/kg b.wt./d, respectively. An extension of this analysis concluded that EAR and RDA were 0.66 g and 0.80 g protein/kg b.wt./d, respectively, and these are the most recent recommendations from the WHO (2), the dietary reference intake (DRI) for the U.S. (36), the Dutch dietary reference intakes (18) and the D-A-CH recommendations (37, 38). This amount of protein achieves protein balance in almost all individuals (97.5%), and refers to average daily intake over time (5-10 days). These recommendations correspond to 8-10% of total energy for good quality dietary proteins and to 9-12% of total energy for usually consumed medium quality proteins.

¹http://www.admin.ch/ch/d/sr/c817_022_21.html

Tolerable upper level of intake

The report of the international Dietary Energy Consultative Group (39) and studies on low-fat diet with a high content of protein (40, 41) show that levels of protein consumption up to about 25 percent of total energy intake have no adverse effects on health in normal subjects. However, there is little data available concerning higher levels of protein consumption (20-25% of total energy intake and above). Exercising a certain degree of caution, the Health Council's Committee on Dietary Reference Intakes of the Netherlands, has set the tolerable upper intake level for children from birth to five months of age at 10% of total energy intake, for babies aged from 6 to 11 months at 15% of total energy intake, for the 1 to 3-year age group at 20% of total energy intake, and for children aged 4 and above at 25% of total energy intake (18), or for a men of 72 kg on 2900 kcal (12.2 MJ) corresponds to 2.9 protein/kg b.wt./d. In Switzerland (37, 38) an upper level of protein intake has been set at 2 g of protein/kg b.wt./d, or 120 g protein/d for women (60 kg) and 140 g protein/d for men (70 kg), corresponding to ~20% of total energy intake (equalling 2300 kcal (9.5 MJ) in women and 2900 kcal (12 MJ) in men as recommended by D-A-CH 2000/2002; **Tab. 5**). The Institute of Medicine (36) found insufficient data to establish a tolerable upper level of intake for total protein and for any single amino acids. But in complement to lower limits in the Acceptable Macronutrient Distribution Ranges (AMDRs) for fat and carbohydrates, an upper limit of 35% of energy from protein has been set to ensure a safe diet for adults.

Tab. 5: Recommended intakes of protein ^a (D-A-CH)

Age	Protein recommended intake			
	g/kg b.wt./day		g/day	
	Male	Female	Male	Female
Infants				
< 1 month				12
1 to < 2 month	2.0			10
2 to < 4 month	1.5			10
4 to < 6 month	1.3			10
6 to < 12 month	1.1			10
Children and young adolescents				
1 to < 4 year	1		14	13
4 to < 7 year	0		18	17
7 to < 10 year	0.9		24	24
10 to < 13 year	0.9		34	35
13 to < 15 year	0.9		46	45
Adolescents and Adults				
15 to < 19 year	0.9	0.8	60	46
19 to < 25 year	0.8		59	48
25 to < 51 year	0.8		59	47
51 to < 65 year	0.8		58	46
65 year and over	0.8		54	44
Pregnancy ≥ 4 th month				58
Lactation				63

^aD-A-CH. Société allemande de nutrition, Société autrichienne de nutrition société Suisse de recherche sur la nutrition, association Suisse pour l'alimentation. Valeurs de référence pour les apports nutritionnels. Association Suisse pour l'alimentation, Berne. 2000/2002 (37, 38).

Acceptable Macronutrient Distribution Ranges (AMDRs)

Recommendations on macronutrients are also stated as “Acceptable Macronutrient Distribution Ranges” (AMDRs). AMDR is not just one acceptable value but a broad range to minimize health risks, e.g. the risk of coronary heart disease, diabetes, and obesity.

An AMDR is defined as “a range of intakes for a particular energy source that is associated with reduced risk of chronic disease while providing adequate intake of essential nutrients”. They have been estimated to be between 10 to 35% of total energy for protein, 20 and 35% of energy for total fat, and 45 to 65% of energy for carbohydrate (36). AMDRs allow individuals to make choices based on their preferences and stress the importance of the total diet approach (Position of the American Dietetic Association: Total Diet approach to communicating food and nutrition information. (28)). This AMDR corresponds to the RDA and the upper limit for urea synthesis, and expressed per body weight, corresponds to 0.8-3.5 g protein/kg b.wt./d. Actual D-A-CH recommendations are, however, at 10-20% of energy for protein intakes (0.8-2.0 g protein/kg b.wt./d) (37, 38).

Optimal Intake

Optimal intake of protein should be between 0.8 g protein/kg b.wt./d, i.e. the “minimum daily needs for protein to maintain short-term nitrogen balance in healthy people with moderate activity” (36), and the set upper level of 2.0 g protein/kg b.wt./d (37, 38). Recent data on evaluation of protein requirements using the method of stable isotope-based indicator amino acid oxidation (IAAO) suggest that the requirements were underestimated by the nitrogen balance studies, and that the protein requirements values should be of an EAR of 0.93 g/kg b.wt./d with an RDA of 1.2 g/kg b.wt./d (for review see (42)).

The RDA of 1.2 g protein/kg b.wt./d corresponds to 84 g protein/d for a 70 kg person consuming a diet providing 2900 kcal/d, or 12% of energy intake from protein. This is within the recommended range for protein intake of 10-35% of total energy intake (AMDR) and does not exceed the value of 2.0 g protein/kg b.wt./d, the upper limit of protein currently consumed safely by well-nourished populations (D-A-CH 2000/2002). While the current RDA for a 70 kg person (D-A-CH 2000/2002) of 58 g protein/d corresponds to 8% of energy intake for a diet providing 2900 kcal/d this value of 8% is much lower than usual 12-15% of energy from protein in Western diets. Furthermore, diets with these levels of protein suggest protective effects on osteoporosis, metabolic syndrome, type 2 diabetes, heart disease and sarcopenia (for reviews see Protein Summit 2007: Exploring the Impact of High-Quality Protein on Optimal Health: (43-47); and accompanying papers for health aspects). A protein intake of 30 g per meal optimally stimulates skeletal muscle protein synthesis independent of age (for review see (48)). To conclude, this suggests that optimal protein intake may be above the actual RDA (minimum intake for N-balance), and that the protein intake at each meal is of importance to maintain muscle mass, but at the level of actual usual spontaneous protein intakes (see below).

1.7. Protein intakes in Switzerland

Methodological issues in estimation of protein intake

Large individual variations already reported by Widdowson and McCance in early 50's (49), suggest that dietary requirements/recommendations should be given in ranges (even if they are large), due to the individual variation, and that reported protein intake data have to be considered as an estimation of protein intake *of specific groups*, and must therefore be evaluated with caution.

Comparison of protein intakes between different dietary assessment methods (16-day weighed record, food frequency, 7-day checklist, 7-day open diary, 24-hour recalls) yielded relatively similar results. Miss- and under-reporting seems to be more person-specific than method-specific (50, 51); this suggests that validated food frequency questionnaire 24-hour recalls are useful tools in estimating protein intake.

Another method of estimating food and nutrient consumption is the use of food consumption data (52). Food supply data, however, differ from dietary survey data: Food supply data measure food and nutrient availability as national totals whereas dietary survey data provide data on food and nutrient intakes reported by individuals. But both use food composition data to translate food intakes into nutrient intakes.

Calculations of nutrient intake are done on the basis of food composition databases, and comparisons of national Food composition data bases have shown that major improvements are needed in standardization and documentation at the food and nutrient levels. Following the SENECA and the EPIC studies an European Nutrient Database (ENDB) has been set-up to reduce or minimize systematic and random errors in nutrient intake estimations (53), as well as assignment of mixed foods are also of importance (54). For Switzerland, the new Swiss Food Composition Database, was first published in 2002, now maintained by the ETH Zürich with the support of Network of Excellence EuroFIR (9). Further developments, however, are needed as acceptable macronutrient distribution ranges (AMDR) require the ability to quantify the percentages of calories provided by each macronutrient (55).

Evaluation of food supply in Switzerland for the years 2001/2002

Data on food supply in Switzerland during the period 2001 to 2002 were calculated on the basis of food balance data (52). This work follows the same basic principles given by the UN FAO, ESS: Supply Utilization Accounts and Food Balance Sheets (56) for the calculation of the use of the foodstuffs intended for human consumption starting from the food balances and the nutrient content of US Food supply data (57). In short, the assessment considers the availability (production, imports, and stocks) compared to the various uses (exports, human consumption, animal feeds, stocks, losses.). The other data being known, the use of the foodstuffs for human consumption is then deduced from this assessment. If one considers only the share used for human consumption, the equation can be simplified to:

Food supply per capita [kg/capita/year] = (indigenous production ± variation stocks + imports-exports)/actual population.

These estimates of per capita consumption for each food are multiplied by the amount of food energy of each available nutrient and dietary component in the raw edible portion of the food. Results for each nutrient from all foods are added and then converted to amount available for consumption on a per capita and per day basis (using the above mentioned country specific food composition databases). Uncertainty on the estimate of consumption from supply depends on the estimate of the losses during storage, uncertainties on

the population, and on the imported and exported quantities. Further, this methodology does not allow conclusions on specific group of the population (52).

Evaluation of approximate intake of proteins based on food supply in Switzerland

Based on the above described method for food supply, estimation of protein intakes in Switzerland was calculated based on the Swiss Food Composition Database (Jacob S 2005). Intake estimates were corrected for losses during food preparation, mainly peel, stones, hard shells for fruit and vegetables, bone, tendons, shells for meat, fish and shell fish, and crust for hard cheese.

Protein supply (intake not corrected) was about 95 g/d and corrected intake 88 g/d. The main protein sources were meat and meat products (28%), milk and dairy products (28%), and cereals (25%), representing about 80% of the estimated total intake of 88 g/d. Fish (3%), eggs (3%) and non-alcoholic beverages (3%) represent about 10% of the total protein intake, while vegetable represents 3-4% (29).

For comparison, the same methodology of food supply is available for the U.S. population (U.S. Food Supply – Food Supply Database (57)). Average protein intake for the same period (2001-2002), according to the US Food Supply database was on average 109 g per capita per day, with the following food sources: meat, poultry & fish 39.7%, dairy products 19%, grain & breakfast cereals 22.4%, legumes, nuts & soy 6.2%, vegetables & vegetable juices 5.1%, eggs 4.1%.

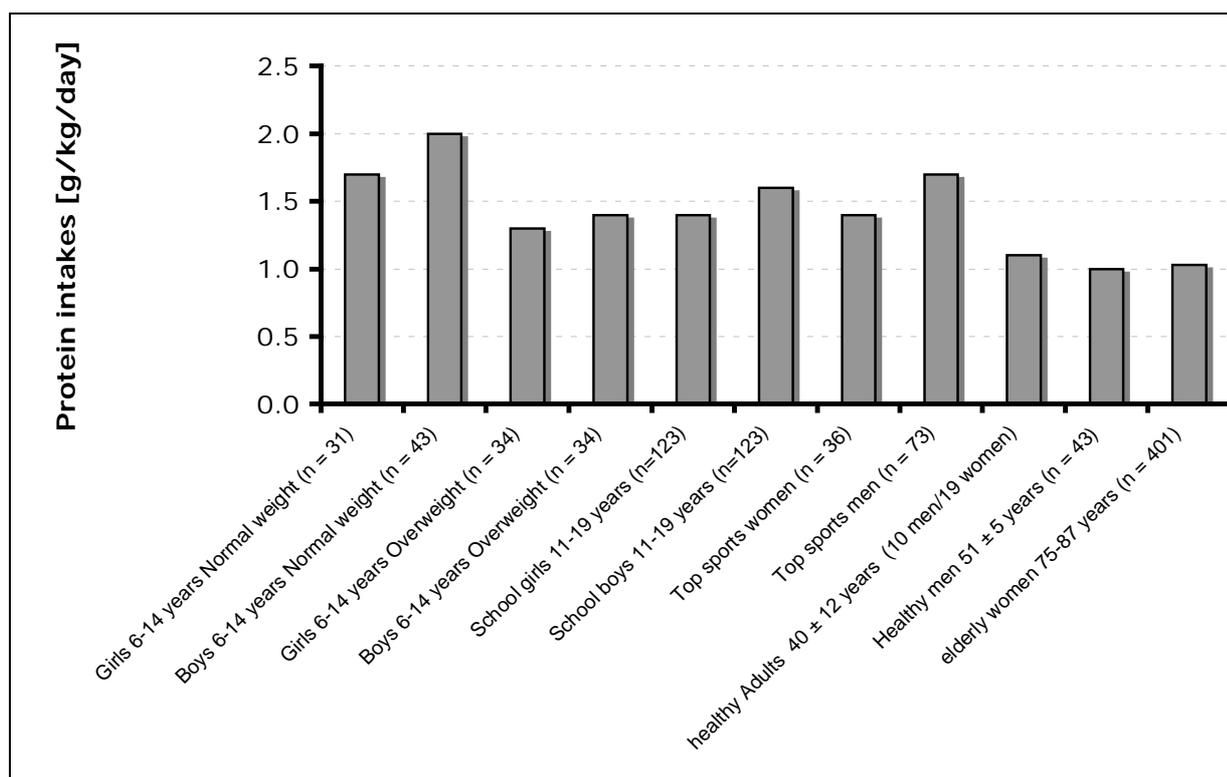
Therefore the pattern of Swiss protein sources is quite similar to the US diet, with animal proteins such as meat and meat products, milk and dairy products representing 60% of the protein intake, and plant protein intake (cereals/vegetable/legumes/fruits/nuts) just over 30%. These protein intakes of 88 g/d and 13% of energy compare well to the D-A-CH recommended intakes (58 g/d for men/10% of energy); they seem to be adequate on a population basis and of good quality.

Tab. 6: Protein intakes in Switzerland

	Protein intake		D-A-CH recommended intake	Reference
	g/day	% of energy	g/day	
Approximate intake from food supply	95	13	58	Jacob S 2005 (29)
Approximate intake from food supply corrected for waste	88	13	58	
Boys, 6-14 years; normal weight	62±10	12	34	Aeberli I et al. 2007 (58)
Boys, 6-14 years; overweight	67±9	13	34	
Girls, 6-14 years; normal weight	53±6	12	35	
Girls, 6-14 years; overweight	61±9	14	35	
Children, 6-14 years ; normal weight	58±15	13	34	Aeberli I et al. 2009 (59)
Children, 6-14 years ; overweight	63±9	15	34	
Children, 6-14 years ; obese	68±14	15	34	
Girls, 11-16 years	65±14	14	45	Decarli B et al. 2005 (60)
Boys, 11-16 years	76±18	14	46	
Sportswomen	83	16		Colombani P and Mannhart C 2005 (61)
Sportsmen	131	14		
Healthy men	82±22	?	58	Morin P et al. (63)
Elderly Women	65±20	17	44	Dumartheray EW et al. 2006 (64)

A representative survey (similar to the National Health and Nutrition Examination Survey (NHANES) in the U.S. or National Diet and Nutrition Survey (NDNS) in U.K.) is not available for Switzerland; but specific group studies have been published (**Tab. 6, Fig. 1** and below), however, seldom representative of the whole group, with one exception, the SENECA study.

Fig. 1: Protein intakes [g/kg body weight/day] by specific groups of subjects in Switzerland



Protein intake of 6 to 14 year-old Swiss children

In two studies with children aged 6-14 years, Aeberli I et al. (58,59) reported the macronutrient intakes for normal weight, overweight (above the 85th percentile and below the 95th percentile of percentage of body fat), and obese (above the 95th percentile of percentage of body fat) children (total number of children: n = 142 in 2007; n = 79 children in 2009). Nutritional intake was assessed using two 24-hour-recalls and a one-day dietary record.

Intakes for both study was quite similar, and mean protein intake was 12-13% of energy in normal weight children, and 14-15% of energy in overweight/obese children, representing 1.8 g/kg b.wt./d in normal weight children, 1.3 g/kg b.wt./d in overweight and obese children, which is well above the D-A-CH recommended intakes 0.9 g/kg b.wt./d. Within food sources, the only significant difference was the increased meat intake (28 g/d for boys and 22 g/d for girls) in overweight and obese children, while all other food sources were somewhat (but not significantly) higher in overweight and obese children compared to normal weight children.

Protein intake of a Swiss teenagers aged 11 to 16 years (Canton of Vaud)

Within a study on physical activity and sporting practices of school boys aged 11 to 19 years, a food consumption survey was carried out in a sub-group of 246 teenagers 11 to 16 years old (50% girls and boys), using a questionnaire composed of questions of general order concerning meals and snacks, of food frequency consumption, and a 3-day diary (one day of the weekend and two in week), completed by an interviewing dietician (60).

The reported protein intakes were 65 ± 14 g/d for girls and 76 ± 18 g/d for boys, respectively, with 10 girls (8%) and 3 boys (2.5%) below the D-A-CH recommended intake of 45 g/d.

Protein intakes expressed as g per kg of body weight were 1.4 g/kg b.wt./d in girls and 1.6 g/kg b.wt./d in boys ($p < 0.001$). The percentage of subjects with consumptions lower than the recommendations remained marginal among boys (6.4%) but was 13.6% in girls

Both in girls and boys, two thirds of the proteins were of animal origin, ensuring good nutritional quality.

Protein intake in Swiss top-class sports-women and sports-men

In a study on 36 top sports-women and 73 top sports-men, food intake was measured by weighed records (61).

A sufficient protein supply was generally reached in these subjects, with an average protein intake of 1.4 g/kg b.wt./d in Swiss sports-women and 1.7 g/kg b.wt./d in Swiss sports-men, respectively. These intakes were in the range of the recommendations of 1.2-1.7 g/kg b.wt./d (American College of Sports Medicine (62)). Nevertheless, 39% of Swiss sports-women as well as 10% of the sports-men exhibited lower intakes than the minimum recommended (1.2 g/kg b.wt. for active persons). A low protein intake was observed in sports-women and men who also had low total energy intakes.

Protein intakes in healthy adults

For validation of a self-administered food frequency questionnaire, protein intakes were evaluated in healthy subjects aged 40±12 years (10 men and 19 women) and healthy men aged 51±5 years (n = 43). Protein intakes for the younger adults were 72±25 g protein/d, corresponding to 1.1 g/kg b.wt./d. The healthy men intakes of protein was 81.5±22 g/d, corresponding to 1.0 g/kg b.wt./d. These mean intakes are well above the recommended intakes (0.8 g protein/kg b.wt./d), however, variation in reported individual intakes is rather large (63).

1.8. Protein intakes of community-living Swiss elderly women aged 75-87 years

In a subgroup of 401 elderly women, who participated in the Swiss Evaluation of the Methods of Measurement of Osteoporotic Fracture Risk (SEMOF) prospective study, nutrient intakes were assessed by food frequency questionnaires (FFQ), followed by a review of each FFQ by a dietician with the respondent (64).

Related to body weight, the elderly women consumed in the average 1.03 g/kg b.wt./d, however, 26.2% had an intake below 0.8 g/kg b.wt./d, and even 8.5% were below the minimal requirement of 0.6 g/kg b.wt./d. On the high intake side, 29.9% of the elderly women had intakes above 1.2 g/kg b.wt./d, and 12% were even above 1.5 g/kg b.wt./d. Protein intake was 65±20 g/d which was somewhat higher than the 52±14 g/d reported in Yverdon, SENECA study (65), and low intakes were correlated to low energy intakes.

1.9. Conclusions

Current protein consumption in Switzerland is 12-17% of total energy, which is well within the actual recommendations (10-20% of energy), covering the newest suggested requirements (**Tab. 6**). Optimal levels of recommended protein intakes by US guidelines (MyPyramid food pattern, (66)) are, however, even slightly higher, i.e. 17-21% of total energy.

Only frail elderly might be at risk of not covering their requirements for protein.

2/3 of proteins consumed in Switzerland are animal proteins with high biological value (meat and meat products (28%), milk and dairy products (28%), fish (3%), and eggs (3%)) and about 1/3 of proteins are of plant origin (25% of cereals, 3-4% of vegetables). This ratio animal/plant protein is quite similar to the ones observed in France, Germany and the Netherlands, but much higher than that observed in health conscious UK adults and in Greece (67), leaving room for increased amounts of plant proteins in our diet, as suggested by the food guide pyramid of the Swiss Society for Nutrition. providing higher fibre intakes and lower amounts of saturated fat (e.g. see (68)) and to reduce the risk of chronic disease (see e.g. (69)).

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2. Protein turnover, ureagenesis and gluconeogenesis

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2.1. Summary/ Zusammenfassung/ Résumé

The major processes discussed below are protein turnover (degradation and synthesis), degradation into urea or conversion into glucose (gluconeogenesis, **Fig. 1**).

Daily protein turnover is a dynamic process characterised by a double flux of amino acids: the amino acids released by endogenous (body) protein breakdown can be reutilised and reconverted to protein synthesis, with very little losses. Daily rates of protein turnover in humans (300 to 400 g per day) are largely in excess of the level of protein intake (50 to 80 g per day). A fast growing rate, as in premature babies or in children recovering from malnutrition, leads to a high protein turnover rate and a high protein and energy requirement. Protein metabolism (synthesis and breakdown) is an energy requiring process, dependent upon endogenous ATP supply. The contribution made by whole body protein turnover to the resting metabolic rate is important: it represents about 20% in adults and more in growing children. Metabolism of proteins cannot be disconnected from that of energy since energy balance influences net protein utilisation, and since protein intake has an important effect on postprandial thermogenesis – more important than that of fats or carbohydrates.

The metabolic need for amino acids is essentially to maintain stores of endogenous tissue protein within an appropriate range, allowing protein homeostasis to be maintained. Thanks to a dynamic free amino acid pool, this demand for amino acids can be continuously supplied. The size of the free amino acid pool remains limited and is regulated within narrow limits.

The supply of amino acids to cover physiological needs can be derived from 3 sources:

1. Exogenous proteins that release amino acids after digestion and absorption
2. Tissue protein breakdown during protein turnover
3. *De novo* synthesis, including amino acids (as well as ammonia) derived from the process of urea salvage, following hydrolysis and bacterial metabolism in the hind gut.

When protein intake surpasses the physiological needs of amino acids, the excess amino acids are disposed of by 3 major processes:

1. Increased oxidation, with terminal end products such as CO₂ and ammonia
2. Enhanced ureagenesis i.e. synthesis of urea linked to protein oxidation eliminates the nitrogen radical
3. Gluconeogenesis, i.e. *de novo* synthesis of glucose.

Most of the amino groups of the excess amino acids are converted into urea through the urea cycle, whereas their carbon skeletons are transformed into other intermediates, mostly glucose. This is one of the mechanisms, essential for life, developed by the body to maintain blood glucose within a narrow range, (i.e. glucose homeostasis). It includes the process of gluconeogenesis i.e. *de novo* synthesis of glucose from non-glycogenic precursors, in particular certain specific amino acids (for example alanine), as well as glycerol (derived from fat breakdown) and lactate (derived from muscles).

The gluconeogenic pathway progressively takes over when the supply of glucose from exogenous or endogenous sources (glycogenolysis) becomes insufficient. This process becomes vital e.g. during starvation.

Zusammenfassung: Protein-Turnover, Ureagenese und Gluconeogenese

Die wichtigsten der hier besprochenen Prozesse sind: Protein-Turnover (Abbau und Synthese), Abbau von Proteinen in Harnstoff (Ureagenese) oder Umwandlung in Glucose (Gluconeogenese; Abb. 1). Der tägliche Proteinturnover ist ein dynamischer Prozess, der aus einem Fluss von Aminosäuren in zwei Richtungen besteht: Aminosäuren werden einerseits aus endogenen Proteinen freigesetzt, andererseits werden sie mit sehr wenig Verlusten wieder in Körperproteine eingebaut (Proteinsynthese). Der Proteinturnover des Menschen beträgt ungefähr 300 bis 400 g pro Tag und ist, im Vergleich zur Proteinzufuhr durch die Nahrung von ungefähr 50 bis 80 g pro Tag, wesentlich höher. Rasches Wachstum, wie z.B. bei Frühgeborenen oder Kindern, die sich von einer Unterernährung erholen, führt zu einem erhöhten Proteinturnover mit einem erhöhten Protein- und Energiebedarf. Der Proteinturnover ist ein Energie konsumierender Prozess und daher abhängig von zur Verfügung stehendem endogenem ATP. Der Energiebedarf des Proteinturnovers beträgt bei Erwachsenen ungefähr 20% des Energieumsatzes und noch mehr bei wachsenden Kindern. Der Proteinstoffwechsel kann nicht vom Energiestoffwechsel getrennt werden, da die Energiebilanz die Netto-Proteinverwendung beeinflusst; und da die Proteinaufnahme einen wichtigen Einfluss auf die postprandiale Thermogenese hat – die thermogene Wirkung der Proteine ist stärker als diejenige von Fetten oder Kohlenhydraten.

Der Bedarf an Aminosäuren leitet sich im Wesentlichen davon ab, welche Aminosäuren in welcher Menge benötigt werden, um die Protein-Homöostase sicherzustellen (die Menge an Körperproteinen aufrecht zu erhalten). Dank einem dynamischen freien Aminosäure-Pool können bei Bedarf Aminosäuren kontinuierlich nachgeliefert werden. Dieser freie Aminosäuren-Pool ist begrenzt in der Grösse und wird eng reguliert. Der physiologische Bedarf an Aminosäuren kann aus drei Quellen gedeckt werden:

1. Nahrungsproteine, aus denen während der Verdauung Aminosäuren freigesetzt werden, die anschliessend absorbiert werden
2. Abbau von Gewebeproteinen während des Proteinturnovers
3. *De novo* Synthese, einschliesslich der Aminosäuren wie auch dem Ammoniak, die beim Harnstoffabbau durch Hydrolyse und bakterielle Zersetzung im Dickdarm entstehen.

Wenn die Proteinaufnahme den physiologischen Bedarf übertrifft, werden überschüssige Aminosäuren durch drei Prozesse abgebaut:

1. Oxidation zu terminalen Endprodukten CO₂, Harnstoff und Ammoniak
2. Oxidation zu Harnstoff (Ureagenese)
3. Gluconeogenese, d.h. *de novo* Synthese von Glucose.

Die meisten überschüssigen Aminogruppen werden im Harnstoffzyklus zu Harnstoff umgewandelt, während ihr Kohlenstoffskelett in andere Produkte, vor allem in Glucose, umgewandelt wird. Dieser Prozess der Gluconeogenese ist einer der lebenswichtigen Mechanismen, die es erlauben, den Blutzucker in einem kleinen Bereich aufrecht zu halten (Glucose-Homöostase). Die Gluconeogenese umfasst einerseits die *de novo* Synthese von Glucose aus nicht glycogenen Vorstufen, insbesondere gewissen ausgewählten Aminosäuren, wie z.B. Alanin und andererseits auch aus Glycerin (aus dem Abbau von Fetten) und Laktat

(aus den Muskeln). Die Gluconeogenese übernimmt schrittweise die Deckung des Bedarfs an Glucose, wenn der Nachschub durch die exogene oder endogene Glucoseversorgung (Glycogenolyse) nicht mehr ausreicht. Dieser Prozess wird z.B. während des Fastens überlebenswichtig.

Résumé: Turnover protéique, uréogénèse et néoglucogénèse

Les principaux processus discutés ici concernent le turnover protéique (dégradation et synthèse), la dégradation en urée (uréogénèse) et la conversion des acides aminés en glucose (néoglucogénèse ; **fig. 1**).

Le turnover protéique journalier constitue un processus dynamique, caractérisé par un double flux d'acides aminés: les acides aminés libérés par la dégradation des protéines endogènes peuvent être réutilisés et reconvertis en protéines, avec un bon rendement. Le taux de turnover protéique chez l'homme (300 à 400 g/jour) excède largement l'apport de protéines (50 à 80 g/jour). Une croissance rapide – comme chez les enfants prématurés ou les enfants récupérant d'une malnutrition – engendre un taux de turnover protéique élevé ainsi que des besoins protéino-énergétiques accrus.

Le métabolisme protéique (synthèse et dégradation) est un processus qui nécessite de l'énergie et donc qui dépend d'un apport endogène en ATP. L'impact du turnover protéique sur le métabolisme de repos est important: il représente environ 20% chez l'adulte, voire davantage chez les enfants en croissance. Le métabolisme des protéines ne peut être déconnecté du métabolisme énergétique pour deux raisons: La balance énergétique influence l'utilisation protéique nette; et le niveau d'apport protéique a un effet important sur la thermogénèse postprandiale – plus encore que les lipides et les glucides.

Les acides aminés exogènes sont essentiels pour maintenir les stocks de protéines endogènes dans une fourchette appropriée, ce qui permet de maintenir l'homéostasie protéique. Grâce à la présence d'un pool d'acides aminés libres en équilibre dynamique, la demande en acides aminés peut être satisfaite de manière continue. La dimension du pool d'acides aminés libres est limitée et régulée dans une fourchette étroite.

L'apport d'acides aminés qui couvre les besoins physiologiques provient de trois sources :

1. Les protéines exogènes, qui libèrent les acides aminés après la digestion et l'absorption ;
2. La dégradation protéique au niveau tissulaire au cours du turnover protéique ;
3. La synthèse *de novo* qui inclut les acides aminés (ainsi que l'ammoniaque) dérivés des processus de recyclage de l'urée au niveau du côlon, à l'issue de l'hydrolyse et du métabolisme bactérien.

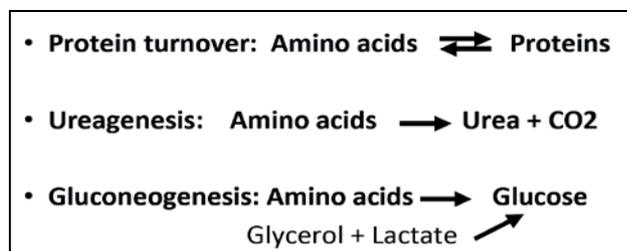
Lorsque l'apport protéique dépasse largement les besoins physiologiques en acides aminés, le surplus d'acides aminés est éliminé au cours de trois grands processus :

1. Augmentation de l'oxydation, avec comme produits terminaux le CO₂, l'urée et l'ammoniaque ;
2. Stimulation de l'uréogénèse – à savoir la synthèse de l'urée liée à l'oxydation des protéines – afin d'éliminer le radical azoté ;
3. Activation de la néoglucogénèse (synthèse *de novo* de glucose).

La plupart des radicaux aminés sont convertis en urée via le cycle de l'urée, alors que leurs squelettes carbonés sont transformés en d'autres intermédiaires, en particulier le glucose. Il s'agit d'un des mécanismes essentiels à la vie, susceptible de maintenir le glucose sanguin (glycémie) dans d'étroites limites (homéostasie du glucose). Le processus de néoglucogénèse, c'est-à-dire la synthèse *de novo* de glucose à partir de précurseurs non glucidiques, à savoir les acides aminés glucoformateurs (p. ex., alanine), le glycérol (issu de l'utilisation des graisses) ainsi que le lactate (dérivé essentiellement des

muscles). La voie gluconéogénique est progressivement stimulée lorsque l'apport de glucose exogène (alimentation) et endogène (glucogénolyse) devient insuffisant par rapport aux besoins métaboliques. Ce processus s'avère vital en cas de jeûne total ou partiel.

Fig. 1: Schematic biochemistry of protein metabolism



2.2. Protein turnover

General Aspects

The maintenance of body protein stores (i.e. lean body mass) at an appropriate level forms the basis of protein homeostasis. The proteins of the body are not metabolically inert, but are continuously being broken down and replaced by new molecules. This forms the basis of the concept of protein turnover, indicating that body proteins are in a dynamic equilibrium, which largely contributes to protein homeostasis (1). Note that the concept of Nitrogen balance, which is the net difference between total N (protein) intake and total N excretion is a different, more static, concept since it disregards the magnitude of overall protein synthesis and protein breakdown (2).

Protein synthesis, protein degradation, and amino acid oxidation are tightly regulated to preserve lean body mass in healthy individuals. Protein turnover can be considered as the sum of protein synthesis + protein degradation. A greater rate of whole body synthesis than breakdown indicates an anabolic state that results in lean tissues deposition, whereas more breakdown than synthesis indicates a catabolic state that degrades lean tissues.

Not only growing or reproducing cells require new protein molecules to be constantly synthesised and, as a result, need a supply of exogenous amino acids from food. In fact, in children and in the adult, body proteins are continually synthesised and degraded in all tissue. Individual endogenous proteins turn over at various different rates. Their half-lives (time interval required for the amount of protein to be degraded to half of its initial value) can vary from a few hours (some hepatic proteins) to one year or so (collagen protein in connective tissue). However, the half-life of a specific protein in different organs is generally similar. An efficient protein turnover is essential since it allows some regulatory proteins to be rapidly synthesized respectively degraded so that the cell can rapidly respond to constantly changing conditions, a typical manifestation of protein homeostasis. The proteins which have very short half-lives are specifically targeted for protein degradation, since their turnover is high; as a result they quickly respond to an acute change in protein intake.

The magnitude of daily protein turnover is in adults 3-4 fold greater (5-6 fold greater in growing children) than the intake of protein, respectively the oxidation of protein. For example, it has been demonstrated that the rate of protein synthesis (10-12 g/kg b.wt./d. in rapidly growing infants greatly exceeds that necessary for net protein gain e.g. 2 g/kg b.wt./d (3). This indicates an efficient recycling of amino acids in the free amino acid

pool, the size of which is tightly regulated (2). In other words, the amino acids released by protein breakdown can be reutilized for protein synthesis rather efficiently for N recycling but this has an energetic cost (see further).

The rate of protein turnover can be assessed non-invasively by using non-radioactive stable isotopes (4). It is fortunate that, in nature, nitrogen possesses a heavy non-radioactive isotope in nature i.e. N¹⁵, present in low abundance (0.36%). The amino acid classically used for human studies has been N¹⁵-Glycine (non-essential amino acid) as a single acute dose (or continuous infusion) since it is ubiquitous. The isotopic abundance of the 2 terminal end products of protein oxidation (N¹⁵-urea & N¹⁵-ammonia) is measured in urine by isotopic ratio mass spectrometry. Thanks to a rather straightforward model developed by Waterlow (1), the protein (N) flux, the rate of overall protein synthesis and protein breakdown are calculated. This requires simultaneous measurement of total protein (N) intake and total protein utilisation (i.e. total N excretion). Note that the difference between the former and the latter represents the Nitrogen (Protein) balance, which is mathematically equivalent to the difference between total Protein syntheses minus total protein breakdown. An identical N (protein) balance can be obtained by different rates of whole body synthesis and breakdown. For example, catabolic disorders result in increased protein breakdown. Protein feeding during this condition results in a relative increase in protein synthesis, thereby minimizing N losses. Conversely, short-term protein restriction (of a few week duration) leads to an adaptation of protein metabolism in the direction of a decrease protein turnover (synthesis and degradation) as well as amino acid oxidation. As a result, the net protein mobilisation (as measured by N balance) is lower than the total reduction in protein degradation.

Kinetic models developed to assess whole body protein turnover have been presented a decade ago (2, 5).

What factors influence protein turnover?

Endogenous factors include age and aging, growth, body composition (fat-free mass, adipose tissue) and various diseases (catabolic, hyper-metabolic). For example, the rate of protein turnover is known to decrease with age in humans, particularly in muscles. The changes in body composition (in particular a fall in fat-free mass and muscle mass) with aging may explain the fall in protein turnover observed (6). However whether there is a decrease, depends upon how the results are expressed, in absolute value or per unit fat-free mass. Let's take the example of obesity in children. Whole-body nitrogen flux, protein synthesis, and protein breakdown were measured postprandial over 9 h from ¹⁵N abundance in urinary ammonia by using a single oral dose of ¹⁵N glycine (7). Absolute rates of protein synthesis and breakdown were significantly greater in obese children than in control children (208±24 compared with 137±14 g/d and 149±20 compared with 89±13 g/d, respectively). Obesity in prepubertal children was associated with an absolute increase in whole-body protein turnover, which contributed to explain the greater energy expenditure in obese children than in control children, when expressed in absolute values. However when expressed per kg body weight, all values were lower in the obese children.

Exogenous factors include the nutritional status in terms of protein and energy (8), anabolic substances, prolonged exercise, recovery from malnutrition or "regrowth" in adults. During endurance exercise there is an increase in the utilization of protein as a metabolic fuel (i.e. increased urea excretion) and the net rate of whole body protein is depressed (9). Note that daily rates of protein turnover are rather insensitive to protein intake over a wide range of intakes (10).

2.3. Energy-protein interaction of whole body protein synthesis and breakdown

A comprehensive review showing the complexity of protein-energy interaction has been published (11, 12). Energy and protein (amino acid) metabolism interacts at various levels both molecular and cellular: a step change in energy intake will result in a change in net protein utilization, the magnitude of which will depend upon the magnitude of dislocation and the host condition (nutritional status). For example a drop in total energy intake (keeping protein intake constant), as encountered during a weight loss diet will give rise to an increase in protein needs consecutive to an increase body protein loss due to enhanced protein oxidation (13). If a reduced energy intake results in an acute negative nitrogen balance, carbohydrates, and fat are nitrogen sparing to about the same extent. However, their mechanisms of action (hormones, substrate competition) are different.

There are a number of energy-dependent processes associated with protein and amino acid metabolism: a. Protein synthesis (and breakdown) in particular activation of amino acids, the formation of peptide bonds during the elongation of the peptides (see below) b. r-RNA and t-RNA turnovers, c. amino acid transport, d. ion pumps and channels, e. signal transduction and protein translocation, f. the glucose-alanine cycle and g. urea synthesis (see further).

In summary, the high rate of protein synthesis costs energy primarily because it requires ATP for the synthesis of peptide bonds connecting amino acids together, but other concomitant biochemical processes also cost energy. In addition, the energetic cost of protein degradation, generally neglected, is not zero, although the exact cost related to this process remains unknown.

An estimate of this cost has been made either from a biochemical unidirectional approach, using stoichiometric calculations. Alternatively it can be assessed from in vivo human studies, by relating the resting energy expenditure of the subjects to its rate of whole body protein synthesis measured simultaneously. Both approaches yield different values; the former (static, in vitro) yielding lower values than the latter (dynamic, in vivo).

Estimate based on stoichiometric calculations indicate that 4 ATP equivalent are required (per bond) in order to bind amino acid together for the biosynthesis of polypeptides and consequently to produce specific protein. If this estimate includes the active transport of amino acid into cells + mRNA synthesis a value of about 1 kcal/g of protein synthesised is found. This accounts for 25% of the metabolizable energy value of exogenous protein (4 kcal/g). Assuming a whole body protein synthesis of 250 to 350 g/d in an average adult, this indicates that 250 to 350 kcal/d of heat are produced in the intermediary metabolism because of this relatively inefficient process.

In vivo, estimates based on the relationship between whole body protein syntheses and resting energy expenditure, suggest that, in adults, the contribution of protein turnover is roughly 20% of the resting energy expenditure in adults i.e. about 300 kcal/d (14,15). This proportion can increase to 30% in fast growing premature babies (3).

2.4. What is the fate of exogenous (food derived) amino acids?

Amino acids (AA's) derived from the hydrolysis of food protein, reach a highly active intracellular amino acid pool, a metabolic pool limited in size and not expandable, from which they can follow 3 major pathways:

1. AA's can be used for the synthesis of new endogenous proteins and other biological substances
2. AA's can be irretrievably oxidized by the body, yielding urea (+ ammonia) and carbon dioxide (CO₂) as terminal end-products (see process of ureogenesis) and
3. AA's can be converted into other compounds (see process of gluconeogenesis).

A schematic diagram showing these key metabolic processes which occur in the liver i.e. elimination of Nitrogen from the body and endogenous production of glucose when it's availability is low, is shown in **Fig. 2**. Catabolism of AA's by the liver is mainly regulated, on a short-term basis, by substrate availability from exogenous supply (i.e. food) or endogenous mobilization in the post absorptive state, i.e. from protein breakdown.

In summary, protein and amino acids ingested in excess of those needed for biosynthesis cannot be stored, in contrast to fatty acids and glucose, nor are they excreted as such (aminoaciduria is negligible in healthy subjects) without prior transformation. This is explained by limited size of the intracellular free amino acid pool, which cannot be much expanded. In other words, in adulthood, protein cannot be stored in body tissue by just increasing the amount of exogenous protein...and staying in bed. Surplus of amino acids are used as metabolic fuel and are oxidized, unlike glucose and fatty acids substrates, which are stored in the liver (+ muscles) and adipose tissue respectively. In contrast, enhancing physical exercise in chronic conditions, particularly of the strength type, can lead to an increase in skeletal muscle protein storage.

2.5. The production of urea by the liver: ureagenesis

All animals, from flatworms to mammals, have the genetic capacity to synthesize urea and therefore have a functional urea cycle (16). The complete cycle is present in the liver of terrestrial vertebrates, and in man it represents the sole mechanism for ammonia disposal (17). Embryological development of the urea cycle in mammalian foetal liver therefore permits use of amino acids as new sources of energy to meet oxidative demands for continuing growth.

Urea is water soluble a substance produced by the liver, and removed from the blood by the kidneys. The role of urea is not only as carrier of waste nitrogen. Being practically neutral, urea is a safe vehicle for the body to both transport and excrete excess nitrogen. Being highly soluble in water and easily distributed within the body by simple diffusion (no active transport across membranes required), the urea synthesized in the liver is distributed and diluted in a very large body pool considered almost equivalent to total body water (about 45 litres of intracellular + extracellular water in a 70 kg man).

The liver produces urea in the so-called urea cycle as a "waste" end product of the metabolism of protein. Urea is found dissolved in blood (from 2.5 to 7.5 mmol/l) and is excreted by the kidney as a component of urine, primarily as a function of dietary protein intake, the higher the protein intake, and the higher the urea production and vice versa. The handling of urea by the kidneys is a vital part of human metabolism. Urea serves an important role in the metabolism of nitrogen-containing compounds. It is the main nitrogen-containing substance in the urine in man (about 85% of total N), the remaining being ammonia and creatinine, the latter being derived from endogenous, non enzymatic creatine precursor in muscles.

A large excess of protein (2-3 g/kg b.wt/d i.e. more than 2 to 3x the basic recommendations) modestly increases blood urea nitrogen but largely increases total urinary urea excretion (and urea in sweat along with sodium chloride and water).

The biosynthesis of urea requires ATP: less than 20% of the energy derived from the metabolism of amino acids is required for ureagenesis (17). This partly explains (in addition to the cost of whole body protein turnover) the high thermogenic effect of protein ingestion.

Finally, note the role of the micro flora (micro biota) in the lower hind gut (colon) which should not be neglected. It is called the “urea salvage” pathway (18). There is a no negligible degree of urea recycling by bacterial hydrolysis in the colon, which allows some N to be recovered in the amino acid pool, subsequently retransformed into amino acids in the liver. The potential of this *de novo* synthesis of amino acid from urea recycling is quantitatively small but still remains an accepted phenomenon (19).

2.6. The endogenous *de novo* production of glucose: gluconeogenesis

Gluconeogenesis serves as an alternative source of glucose when endogenous supplies are limited (in size and rate of availability) or when exogenous supply of carbohydrates is too low or absent. It is also influenced by the nature and level of dietary intakes: a fasting diet and a low carbohydrate diet (less than 100 g/d) stimulate this process whereas a high carbohydrate diet (hyper energetic or not) blunts gluconeogenesis.

Note that from a biochemical point of view, the gluconeogenesis pathway is not simply a reversal of the glycolytic pathway: the irreversible steps of glycolysis are bypassed. Thanks to the tight enzymatic control in the glycolysis and gluconeogenesis pathways, these 2 processes are regulated to prevent them from being simultaneously activated, since this would result in a waste of energy (ATP) known as “futile cycle”. The physiological conditions stimulating the gluconeogenesis pathway, simultaneously inhibit the glycolytic pathway, and vice versa, indicating that these pathways are controlled in a reciprocal fashion (20).

Although glycolysis occurs universally, gluconeogenesis is confined to the liver and kidney. The key hormone regulating the metabolic transformation of amino acids into glucose in the liver and kidney i.e. gluconeogenesis, is glucagon. Another hormone (cortisol) also plays an important role.

The role of glucagon (which has an opposing metabolic effect to insulin) largely concerns glucose metabolism:

- It stimulates gluconeogenesis i.e. *de novo* glucose synthesis, simultaneously inhibiting glycolysis i.e. degradation of glucose in the intermediary metabolism.
- It enhances glycogen breakdown, whereas glycogen synthesis is inhibited and, as a result,
- It allows releasing free glucose into to the circulation to maintain glycaemia.
- It has also an effect on ureagenesis: hyperglucagonemia in normal man induces mild nitrogen losses by stimulation of hepatic ureagenesis.

The process of gluconeogenesis ultimately leads to a mobilization of muscle tissue to produce glucose. Fortunately, other precursor substrates are involved in this gluconeogenesis process namely glycerol, which comes from the breakdown of fat (in situation of accelerated lipolysis), and also a very important intermediate substrate viz. lactate which is produced at low rate in resting post absorptive conditions and at a high rate during intense exercise.

Fig. 2 shows the importance of the liver as a central metabolic carrefour, where the above metabolic processes (ureagenesis and gluconeogenesis) take place.

The energy cost of gluconeogenesis still remains a matter of debate. A recent study investigating in healthy humans the extent to which a high-protein (carbohydrate-free) diet increases gluconeogenesis and whether this can explain the increase in energy expenditure (21). The increase in resting energy expenditure was found to be significantly related to the increase in gluconeogenesis. Forty two % of the increase in energy expenditure after the high protein diet was explained by an increase in gluconeogenesis. The energy cost of gluconeogenesis was estimated to be 33% of the energy value of the produced glucose.

Fig. 2:

Ureagenesis, gluconeogenesis and amino acid oxidation in the liver. Oxidation of amino acids is incomplete and leads mainly to urea, in addition to CO₂ and H₂O. Urea is water soluble with residual energy (2.5 kcal/g) and is excreted in urine.

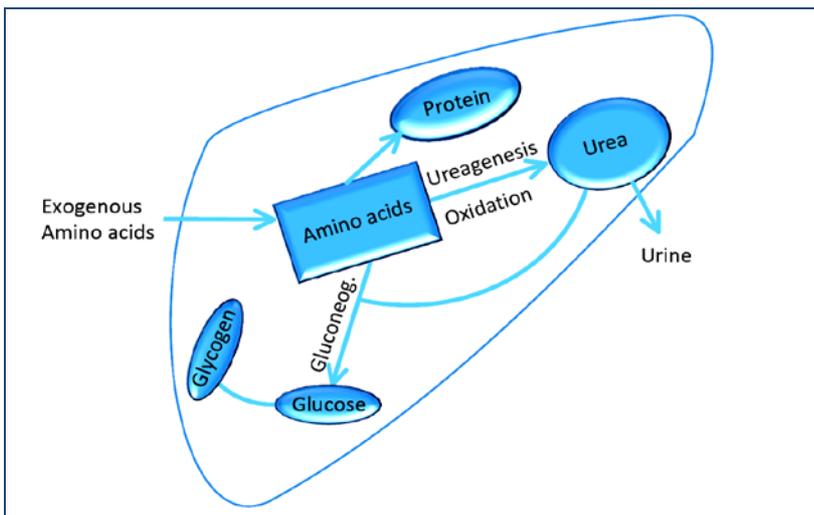
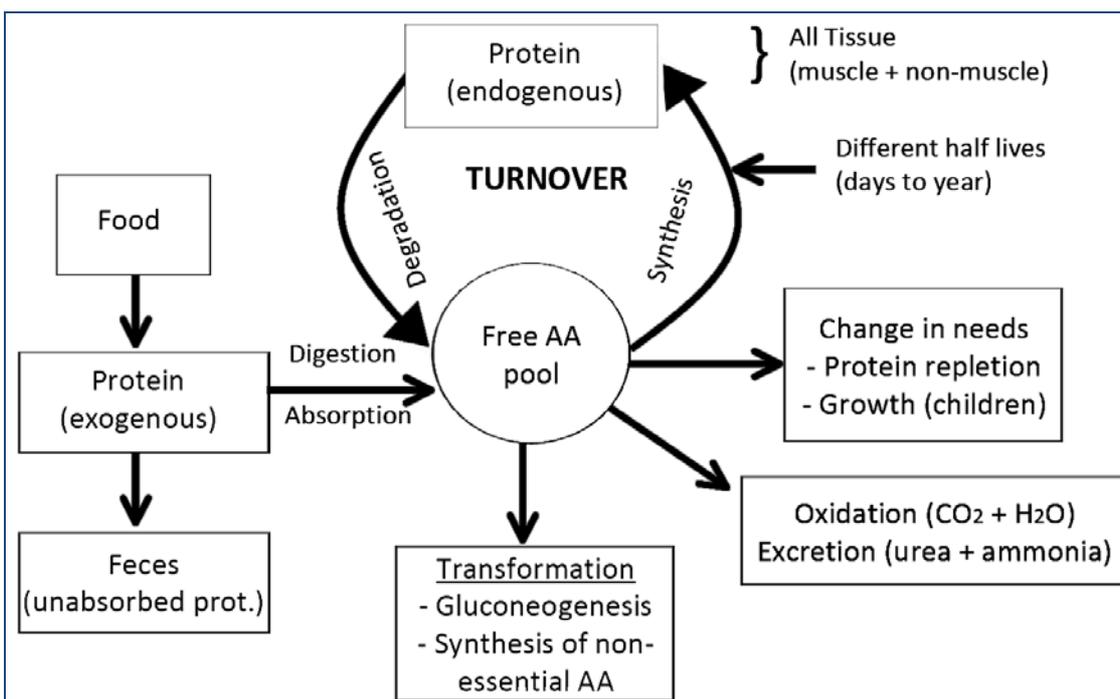


Fig. 3:

Protein turnover results from synthesis and degradation of proteins. The rate of protein oxidation which primarily depends on protein intake, the process of protein transformation and the excretion of end products are shown. AA = amino acids.



2.7. Conclusion

A simplified schematic diagram of overall protein metabolism, which includes the 3 processes discussed above is shown in **Fig. 3**. The key component of the metabolism of protein is without any doubt protein turnover, which allows moderating total protein needs and minimizing protein losses. Adequate day-to-day exogenous protein/amino acid supply will “feed” the small free amino acid pool and allows replacing amino acids which have not been reutilized via the recycling process.

Protein turnover is not a futile cycle. Life depends upon the exportation of nitrogenous compounds (urea and ammonia), both being excreted in urine. The fact that amino acids from muscles can be mobilized to produce glucose in situations where glucose is scarce, maintains plasma glucose levels, although at the expense of muscle proteolysis.

Finally one final consideration to highlight is the capacity of the organism to adapt to different intakes of proteins, resulting in rapid changes in N excretion protein turnover as seen in chronically malnourished individuals (8).

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3. Protein requirements in children and adolescents

Kurt Baerlocher, St. Gallen

3.1. Summary/ Zusammenfassung/ Résumé

The actual protein reference values (= recommended dietary allowance) for children in Switzerland are documented in the „German/ Austrian/Swiss (DACH) References Values for Dietary Intakes“ (2000). They are based upon the report of experts of FAO/WHO in 1985 and the investigations by Dewey in 1996. These reference values are as follows: In the first year 2.0 g/kg b.wt./d (1st month) to 1.11 g/kg b.wt./d (12th month), 1 g/kg b.wt./d from 1-4 years and afterwards 0.9 g/kg b.wt./d up to 15 years of age. As a consequence of new data on energy requirements and body composition, an expert group of FAO/WHO revised the protein requirements in 2002 (published in 2007).

The protein requirements in children consist of the requirements for maintenance (as in adults) plus those for growth (protein deposition). These data are calculated from nitrogen balance studies and by the so called factorial method (statistical and mathematical calculations). The new data result in protein requirements that are slightly lower, especially in infants and small children, than so far recommended. They are 1.77 g/kg b.wt./d (1st month) to 1.14 g/kg b.wt./d (12th month), 0.86 g/kg b.wt./d for 1-4 year old children and 0.91-0.92 g/kg b.wt./d up to 10 years of age.

From 11 years onwards the values are gender specific, 0.91-0.85 g/kg b.wt./d for male (m) adolescents (up to 18 years) and 0.90-0.82 g/kg b.wt./d for female (f) adolescents of the same age. The WHO also included in the recommendations a mean reference value for weight and values for protein requirements in g/d: 10.2 g/d at 6 months of age increasing to 57.9 g (m) and 47.4 (f) at 15-18 years of age. However, the actual intake of proteins in children and adolescents is – according to a European review – much higher, about 40 g/d at two years of age, 60 g/d at three years of age and 100 g/d and even more at 13-15 years of age.

Not only the quantity but also the quality of proteins is important. Animal proteins have a more precious composition of amino acids (AA) than vegetal proteins, because they contain all the indispensable AA. The digestibility is also better. The limiting AA of vegetal proteins are lysine, tryptophan and the sulfur-containing AA. The requirement for the indispensable amino acids is age dependent.

Health aspects of proteins are nowadays very important, especially in childhood. If infants and small children consume excessive amounts of protein they are at risk of becoming overweight and of obesity. If at the age of 5-6 years the intake of total protein, and especially that from an animal source, is too high puberty may start earlier in girls and boys. Such observations may lead to new questions, which indeed are a challenge for more studies on the importance of proteins in nutrition.

Zusammenfassung: Proteinbedarf von Kindern und Jugendlichen

Die aktuellen Referenzwerte für den Proteinbedarf von Kindern und Jugendlichen in der Schweiz sind in den Referenzwerten der Deutschen, Österreichischen, Schweizerischen Gesellschaften für Ernährung (DACH-Referenzwerte) für die Nährstoffzufuhr aus dem Jahre 2000 enthalten. Sie basieren auf dem Expertenbericht der FAO/WHO aus dem Jahre 1985 und den Untersuchungen von Dewey im Jahre 1996. Diese Werte betragen im ersten Lebensjahr 2.0 g/kg KG/Tag (1. Monat) bis 1.11 g/kg KG/Tag (12. Monat), 1 g/kg KG/Tag von 1-4 Jahren und anschliessend 0.9 g/kg KG/Tag bis zu 15 Jahren. 2002 überprüfte eine Expertengruppe

der FAO/WHO den Proteinbedarf aufgrund neuerer Untersuchungen im Bereich des Energiebedarfs und der Körperzusammensetzung. Im Kindesalter setzt sich der Proteinbedarf aus dem Erhaltungsbedarf (wie bei Erwachsenen) und zusätzlich aus dem Wachstumsbedarf zusammen. Die Berechnung beruht auf Stickstoffbilanzen und der faktoriellen Methode (statistische und mathematische Berechnungen). Die neuen Daten zeigen einen etwas tieferen Proteinbedarf, v.a. im Säuglings- und Kleinkindesalter, nämlich 1.77 g/kg KG/Tag (1. Monat) bis 1.14 g/kg KG/Tag (12. Monat), 0.86 g/kg KG/Tag bis zu 4 Jahren und anschliessend 0.91-0.92 g/kg KG/Tag bis zu 10 Jahren. Ab 11 Jahren gelten abhängig vom Geschlecht verschiedene Werte von 0.85-0.91 g/kg KG/Tag für männliche (m) Jugendliche bis 18 Jahre und von 0.82-0.90 g/kg KG/Tag für weibliche (w) Jugendliche. Die Werte sind also, ausser für die 7-10-jährigen, etwas niedriger als bisher empfohlen. Neu hat die WHO auch mittlere Referenzwerte für das Gewicht in den entsprechenden Alterskategorien miteinbezogen, wie auch die Angaben des Protein-Bedarfs in g/Tag. Dieser steigt von 10.2 g/Tag bei 6 Monate alten Kindern auf bis zu 57.9 g/Tag (m) und 47.4 g/Tag (w) bei den 15-18-jährigen.

Gemäss einer Europäischen Zusammenstellung sind die aktuellen Daten der Proteinzufuhr allerdings wesentlich höher und betragen z.T. schon 40 g/Tag bei 2-jährigen, 60 g/Tag bei 3-jährigen Kindern und 100 g/Tag und mehr bei 13-15-jährigen.

Neben der Quantität ist auch die Qualität der Proteine entscheidend. Tierische Proteine sind bezüglich der Aminosäuren (AS)-Zusammensetzung wertvoller als pflanzliche, da sie mehr essentielle AS enthalten und auch die Verdaulichkeit besser ist. Limitierend sind bei den pflanzlichen Proteinen meist die AS Lysin, Tryptophan und die schwefelhaltigen AS. Der Bedarf an essentiellen AS ist ebenfalls altersabhängig.

Von grosser Bedeutung sind heute – gerade im Kindesalter – die gesundheitlichen Aspekte der Proteine. Eine zu hohe Proteinzufuhr bei Säuglingen und kleinen Kindern gilt als Risikofaktor für Übergewicht und Adipositas. Eine erhöhte totale Proteinzufuhr im Alter von 5-6 Jahren, v.a. von tierischem Protein, nicht aber von pflanzlichem, kann zu einem früheren Beginn der Pubertät bei Knaben und Mädchen führen. Solche neuen Erkenntnisse bringen auch neue Fragen, die uns in Zukunft weiterhin hinsichtlich der Bedeutung der Proteine in der Nahrung herausfordern werden.

Résumé : Les besoins en protéines des enfants et adolescents

L'apport nutritionnel conseillé (ANC) en protéines des enfants et adolescents de Suisse correspond aujourd'hui aux valeurs de référence DACH, formulées en 2000 par les sociétés de nutrition allemande, autrichienne et suisse. Ces valeurs se fondent sur le rapport d'experts FAO/OMS de 1985 et sur les travaux de Dewey remontant à 1996. Elles oscillent, la première année, entre 2,0 g/kg/jour (1er mois) et 1,11 g/kg/jour (12e mois), passant à 1 g/kg/jour de la 1re à la 4e année, puis à 0,9 g/kg/jour jusqu'à quinze ans. En 2002, un groupe d'experts FAO/OMS a révisé les besoins en protéines, à la lumière des découvertes récentes sur les besoins énergétiques et sur la composition de l'organisme (résultats publiés en 2007).

Les besoins en protéines durant l'enfance se composent d'une ration d'entretien (comme pour les adultes) et d'une ration de croissance (dépôt de protéines). Ils se calculent à partir d'études du bilan azoté, à l'aide de la méthode « factorielle » (calculs statistiques et mathématiques). Les nouvelles données indiquent des besoins en protéines légèrement inférieurs aux recommandations en vigueur, notamment pour les

nourrissons et les petits enfants. Ils se situent d'abord entre 1,77 g/kg/jour (1er mois) et 1,14 g/kg/jour (12e mois), puis à 0,86 g/kg/jour jusqu'à quatre ans, et à 0,91-0,92 g/kg/jour jusqu'à dix ans.

Dès l'âge de onze ans, les valeurs varient selon le sexe, avec 0,91-0,85 g/kg/jour pour les garçons (jusqu'à 18 ans) et 0,90-0,82 g/kg/jour pour les filles du même âge. L'OMS a également formulé ses recommandations en fonction du poids de référence. Les besoins passent ainsi de 10,2 g/jour à six mois à 57,9 g (garçons) et 47,4 g (filles) entre 15 et 18 ans. L'apport protéique actuel est toutefois bien supérieur chez les enfants et les adolescents – selon une enquête menée au niveau européen. Il avoisine déjà 40 g/jour à deux ans, 60 g/jour à trois ans et 100 g/jour ou davantage entre 13 et 15 ans.

Outre la quantité, la qualité des protéines s'avère importante. Les protéines animales, qui contiennent tous les acides aminés (AA) essentiels, ont une plus grande valeur que les protéines végétales et sont également plus faciles à digérer. Les protéines végétales sont carencées en lysine, en tryptophane et en acides aminés soufrés. Les besoins en AA essentiels varient eux aussi en fonction de l'âge.

De même, les aspects sanitaires des protéines requièrent une grande attention aujourd'hui, pendant l'enfance notamment. Une surconsommation de protéines chez les nourrissons et les petits enfants comporte un risque de surpoids et d'obésité. Si l'apport total en protéines, en particulier celles d'origine animale, est trop élevé à cinq ou six ans, le début de la puberté risque d'être accéléré chez les deux sexes. De telles observations suscitent de nouvelles questions et justifient d'autres études consacrées à l'importance des protéines dans l'alimentation.

3.2. Introduction

The present recommendations for protein requirements of children and adolescents in Switzerland are based on the DACH-reference values for the supply of nutrients [1]. They are divided in different age-groups: infants (first 6 months and 6-12 months), children (1-<4, 4-<7, 7-<10, 10-<13 and 13-<15 years) and adolescents (15-<19 years). These reference values from the year 2000 follow the report of the WHO from 1985 on energy and protein requirements [2] and the investigations of Dewey, reported in 1996, on the requirements of proteins in infants and children [3]. Since then, further basic details of protein requirements of children and adolescents have been elaborated, especially in the new report from WHO in 2007, which summarizes the data of an FAO/WHO/UNU expert conference in 2002 [4] or those from the Institute of Medicine, Food and Nutrition Board, US National Academy of Sciences [5]. These data have been adopted in the Paediatric Nutrition Handbook 2004 [6] and also in the AHA Scientific Statements for children and adolescents [7]. In 2003, the regional office of WHO for Europe published guidelines on „Feeding and nutrition of infants and young children“ with an emphasis on the situation in the former Soviet Union. In this report the protein requirements are also discussed [8].

Since most of the work and information on protein requirements in paediatrics concern children below the age of 2 years, the International Life Sciences Institute (ILSI) organized a workshop on „Nutrition in children and adolescents in Europe: what is the scientific basis“ and summarized the reference values for nutrients in the different European countries [9].

In the following presentation on the protein requirements in children and adolescents we rely on the above cited information. A special issue is protein requirements of premature babies. These are mostly nourished according to special guidelines [10] under medical supervision in clinics. Their needs can therefore not be part of nutritional recommendations described in this report which is directed to healthy children and adolescents.

3.3. Protein requirements and their measurement

Protein requirements are therefore defined as the minimal intake of a qualitatively high graded protein that will provide the maintenance of an appropriate body composition, allowing growth at the normal rate for age, assuming a good energy balance and normal physical activity [11]. This physiological protein requirement is expressed as „estimated average requirement“ (EAR). The recommended dietary allowance (RDA) or „reference value“ is the safe level of protein intake which will assure the needs of nearly all individuals of an age group. The RDA is assessed from the EAR plus two times the standard deviation (+2SD) of the EAR of each age group [11].

During the first 6 months of life human milk is the optimal source of nutrients for the normal full term infant. The intake of protein of human milk from a healthy, well nourished mother is therefore the optimal quantity of protein. The average milk intake in this age group is 780 ml/d and the average protein content 11.7 g/l. The mean adequate intake is therefore 9.1 g/d or 1.52 g/kg b.wt./d in this age group [11]. However, one has to consider that human milk contains a quite high fraction of „Non-Protein-Nitrogen“ (NPN; - 25%) (e.g. urea, amino sugars, peptides, amino acids, creatinin etc), what is not anymore the case in later nutrition (cows milk contains only 5% NPN). In older infants, children and adolescents other techniques are used for the measurement of protein requirements like nitrogen-balances at different levels of protein intake, the so called factorial method (calculation of a mean value for the needs of maintenance and growth), non radioactive investigations with stable isotopes or biochemical analyses [6].

3.4. Quantitative aspects of protein requirements

The values for protein requirements in childhood, reported in 1985, by the factorial method [2] have been revised by Dewey in 1996 [3]. He concluded that the 1985 values for breast fed infants (0-6months) should be 10-25% lower. In the WHO Report of 2002/2007 [4], new experimental data were included and the values of the requirement for maintenance and growth (protein deposition) are specified as follows:

Protein requirements for maintenance

The basic needs of proteins in infants and children were calculated by estimation of nitrogen loss at low or even zero protein intakes. These studies indicated a mean value of 62 ± 12 mg nitrogen/kg b.wt./d. Further 10 balance studies in children of 6 months to 12 years of age gave an average basal nitrogen loss of 57.4 mg/kg b.wt./d, which is almost similar. From these data, considering an efficiency of protein utilization of 70%, a mean maintenance value of 110 mg nitrogen/kg b.wt./d could be determined (range: 66-149 mg nitrogen, corresponding to 0.42-0.93 g protein/kg b.wt./d). In children who consumed animal proteins (milk, eggs), the maintenance value was slightly lower (93 mg nitrogen = 0.58 g protein/kg b.wt./d) [4]. Therefore, this value had been chosen for infants below 6 months, which are breastfed or formula fed. For children above 6 months and adolescents a mean maintenance value of 110 mg nitrogen/kg b.wt./d was adopted, which corresponds to 0.69 g protein/kg b.wt./d. These values are lower than in the 1985 report. Dewey et al.

calculated a value of 90 mg nitrogen/kg b.wt./d (= 0.56 g protein/kg b.wt./d) [3]. The new maintenance value of 110 mg nitrogen is very close to the one which has been calculated for adults (105 mg nitrogen/kg b.wt./d = 0.66 g protein/kg b.wt./d). Therefore the same value of 0.66 g protein is used in children over 2 years and in adults.

Protein requirements for growth (protein deposition)

New methods for the assessment of protein deposition in children from 6 months to 18 years of age and new data of the amino acid composition of body proteins added to an improvement of the factorial method [4].

At one month of age the average protein deposition is 0.548 ± 0.113 g/kg b.wt./d and will be reduced until the 12th month to 0.168 ± 0.019 g/kg b.wt./d.

Tab. 1 summarizes the new values (4), which are calculated from the studies of Butte et al. [12] and Ellis et al. [13] about the body composition of children.

Tab. 1: Protein requirements for growth (protein deposition) in children and adolescents

Age Years	Protein deposition (g/kg b.wt. per day)		
	m	f	SD
0-0.5	0.266		0.035
1	0.168		0.031
1.5	0.108		0.029
2	0.073	0.076	0.026
3	0.034	0.044	0.022
4-5	0.011	0.024	0.018
6-10	0.049	0.047	0.016
11-15	0.041	0.031	0.017
16-18	0.023	0.009	0.008

Comparing these new protein data for growth with the previous ones for infants of Fomon and Dewey, the new values are lower until 3 months of age but slightly higher after 3 months.

Therefore, for children of 1-6 months of age, the results of average protein requirements (EAR) (including those for maintenance and growth) is 1.41 g protein (1 month) to 0.98 g/kg b.wt./d (6 months) resulting in a safe level of protein intake (RDA) of 1.77 g (1 month) to 1.14 g/kg b.wt./d (6 months) [4]. The values of the 1985 WHO report were definitely higher (2.25 to 1.3 g) and also the DACH values are slightly higher. The proportion of protein deposition (growth) is about 60% of the total protein requirement in the first month and is reducing thereafter to about 16% by two years of age.

The recommendations for safe levels of protein requirements in infants, children and adolescents are depicted in **Tab. 2**.

They are separated for sex and compared with the previous values of 1985 [2], the DACH values of 2000 [1] and the newer US Academy of Sciences values of 2005 [5]. The data are expressed in g/kg b.wt./d or as g per day. The differently chosen age groups by the expert committees make a true comparison difficult.

Tab. 2: Average protein requirements and protein reference values of children and adolescents

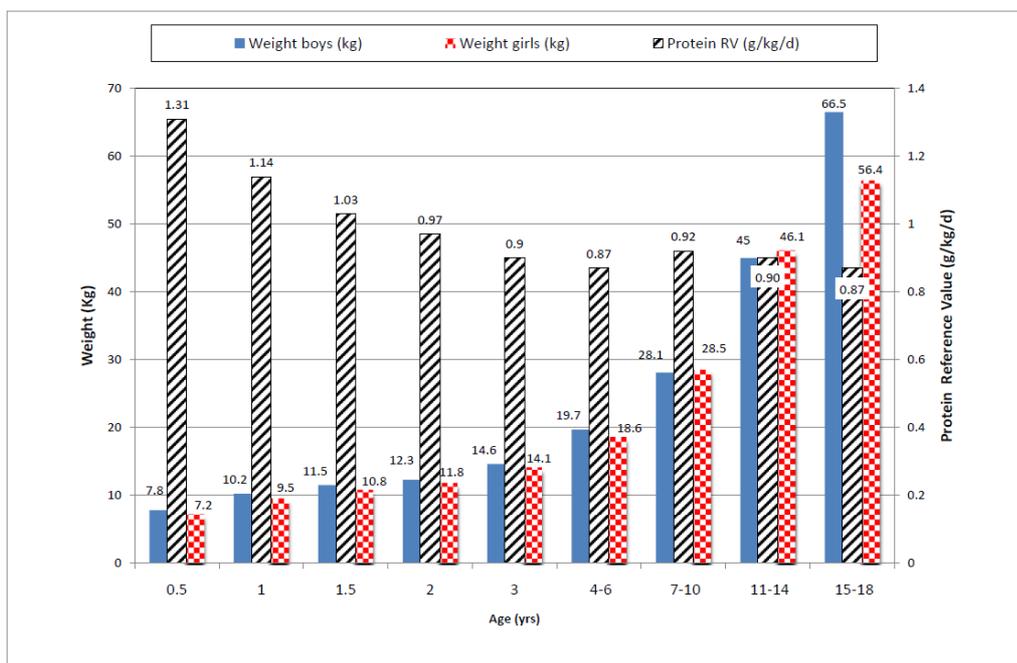
Age Month or Year	Maintenance value	Growth-value		Average Requirement		Ref. value	FAO/WHO 1985 [2]		DACH [1]		US Nat. Acad. 2005 [5]	
		m	f	m	f		m	f	m	f		m
g/kg per day; m=male, f= female												
	m & f	m	f	m	f	m	f	m	f	g / day		
1 Mo*	0.58	0.83		1.41		1.77		2.25		2.0	12	
2	0.58	0.65		1.23		1.5		1.82		1.5	10	
3	0.58	0.55		1.13		1.36		1.47		1.3	10	
4	0.58	0.49		1.07		1.24		1.34		1.3	10	
6	0.58	0.40		0.98		1.31		1.30		1.3	10	
7-12	0.66	0.29		0.95		1.14		1.57		1.1	10	9
1.5 yr	0.66	0.19		0.85		1.03		1.26				
2	0.66	0.13		0.79		0.97		1.17	1 (1-<4 y)	14	13	11 (1-3 y)
3	0.66	0.07		0.73		0.90		1.13				
4	0.66	0.03		0.69		0.86		1.09				
5	0.66	0.06		0.69		0.85		1.06				
6	0.66	0.04		0.72		0.89		1.02	0.9 (4-<7 y)	18	17	15 (4-8 y)
7	0.66	0.08		0.74		0.91		1.01				
8	0.66	0.09		0.75		0.92		1.01				
9	0.66	0.09		0.75		0.92		1.01	0.9 (7-<10 y)	24		
10	0.66	0.09		0.75		0.91		0.99				
11	0.66	0.09	0.07	0.75	0.73	0.91	0.90	0.99	1.0			27 (9-13 y) 28
12	0.66	0.08	0.06	0.74	0.72	0.90	0.89	0.98	0.98	0.9 (10-<13 y)	34	35
13	0.66	0.07	0.05	0.73	0.71	0.90	0.88	1.0	0.98			
14	0.66	0.06	0.04	0.72	0.70	0.89	0.87	0.97	0.94	0.9 (13-<15 y)	46	45
15	0.66	0.06	0.03	0.72	0.69	0.88	0.85	0.96	0.90			
16	0.66	0.05	0.02	0.71	0.68	0.87	0.84	0.92	0.87	0.9(15-<19 y) 0.8	60	46
17	0.66	0.04	0.01	0.70	0.67	0.86	0.83	0.90	0.83			44 (14-18 y) 38
18	0.66	0.03	0.00	0.69	0.66	0.85	0.82	0.86	0.80			

Comparison of the new protein reference values with the previous values of FAO/WHO 1985 [2], DACH 2000 [1] and US National Academy of Sciences 2005 [5]

*1-6 months: values of breastfed infants.

The expert group of FAO/WHO/UNU recommends for the first time also reference values for the different age groups as well as reference values for the mean weight [14] (**Fig. 1**).

Fig. 1: Protein reference values of Infants, children and adolescents and reference values of the average weight in the different age groups



This should make the practical use of the data easier. The new data of protein requirements for children over 7 years of age and adolescents are only slightly different from those of DACH in the year 2000 [1]. The values of protein requirements are slightly lower in infants and small children than previously thought.

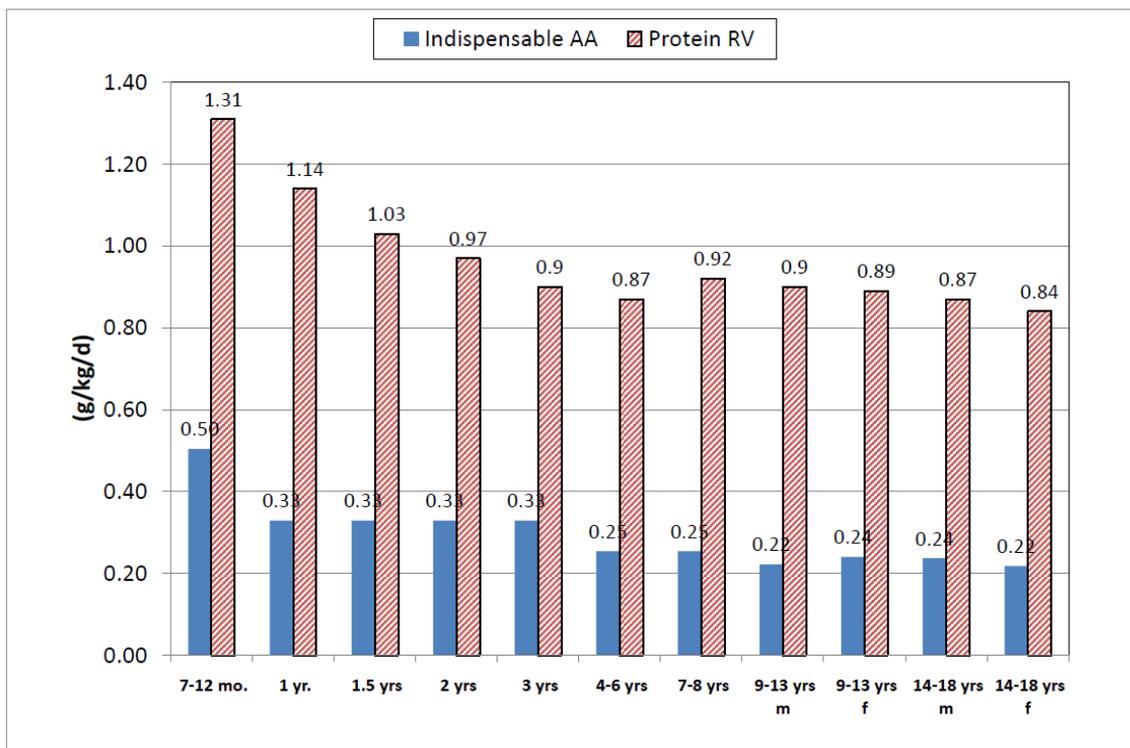
3.5. Qualitative aspects of protein requirements

Protein quality

The recommended values for protein intake assume the intake of high graded proteins, e.g. proteins which are well digestible and which contain the indispensable amino acids in the necessary amount (**Fig. 2**).

This is valid for animal proteins like milk, egg, meat and fish. Plant proteins (legumes, grains, seeds and vegetables) are less digestible (70-80%) and provide often not sufficient amounts of indispensable amino acids, like lysine or sulfur containing amino acids.

Fig. 2: Requirements of indispensable amino acids and protein reference values (RV) in the different age groups



To describe the potential of specific dietary proteins to provide essential amino acids an amino acid score has been introduced: PDCAAS (protein-digestibility corrected amino acid score): the ratio of mg amino acids per 1 g of food protein to mg amino acids in the reference pattern, corrected with the true digestibility. Values below 1 or 100 signify an insufficient provision of the amino acid [1,6]. **Tab. 3** shows values for the digestibility of some dietary proteins and presents the corresponding amino acid score, how it had been calculated for preschool- and school children in USA [6].

Tab. 3: Mean values for digestibility and amino acid score of various protein sources in a mixed diet [6].

Protein Source	Digestibility %		Amino acid Score	
	true	relative	Preschool child	school-aged child
Whole egg	97	100	109 (trp)	133 (trp)
Cow milk, cheese	95	100	127 (trp)	155 (S)
Meat, fish	94	100	100 (trp)	122 (trp)
Maize	85	90	48 (lys)	64 (lys)
Rice polished	88	101	62 (lys)	82 (lys)
Wheat whole	86	90	48 (lys)	64 (lys)
Wheat refined	96	101	38 (lys)	50 (lys)
Beans	78	82	100 (S)	114 (S)
Soy protein isolate	86	90	108 (S)	123 (S)
Mixed US-diet	96	101	109 (trp)	113 (trp)

True digestibility %: Nitrogen intake minus (faecal nitrogen on test protein – faecal nitrogen on zero-protein diet) x 100 divided by nitrogen intake.

Relative digestibility: digestibility of the protein relative to that of a reference protein (milk or egg).

Amino acid score: see text.

Abbreviations: trp = tryptophan, lys = lysine, S = cysteine + methionine.

Value shown is for the most limiting amino acid; values of more than 100 indicate that the protein source contains relatively more of that amino acid as the reference protein.

The limiting amino acids are indicated in brackets. Especially five amino acids can influence the quality of proteins: Lysine, threonine, tryptophan and the sulfur containing amino acids methionine and cysteine.

Amino acid requirements for infants, children and adolescents

Indispensable (essential) amino acids have to be obtained from the diet. These are: histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine. Those amino acids that the infant or child is unable to produce in sufficient amounts and hence have to be provided by the diet partly or fully – during some periods – are named „conditionally indispensable“ amino acids. These are: arginine, cysteine, glutamine, glycine, proline and tyrosine.

The amino acid requirement for infants of 0 months of age are based on the average intake of human milk (780 ml/d) and the mean content of each indispensable amino acid in human milk [11].

The requirements of the indispensable amino acids for children are age dependent and presented in **Tab. 4**. If an indispensable amino acid in the diet is less than the individual's requirement, it can limit the utilization of other amino acids and hence the protein synthesis.

Tab. 4: Requirements of indispensable amino acids for infants, children and adolescents

Amino acid	Age														
	0-6 Mo	7-12 Mo		1-3 years		4-8 years		9-13 years m		9-13 years f		14-18 years m		14-18 years f	
Definition*	c	a	b	a	b	a	b	a	b	a	b	a	b	a	b
Histidine	36	22	32	16	21	13	16	13	17	12	15	12	15	12	14
Isoleucine	88	30	43	22	28	18	22	18	22	17	21	17	21	16	19
Leucine	156	65	93	48	63	40	49	40	49	38	47	38	47	35	44
Lysine	107	62	89	45	58	37	46	37	46	35	43	35	43	32	40
Methionine + cysteine	59	30	43	22	28	18	22	18	22	17	21	17	21	16	19
Phenylalanine + tyrosine	135	58	84	41	54	33	41	33	41	31	38	31	38	28	35
Threonine	73	34	49	24	32	19	24	19	24	18	22	18	22	17	21
Tryptophan	28	9	13	6	8	5	6	5	6	5	6	5	6	4	5
Valine	87	39	58	28	37	23	28	23	28	22	27	22	27	20	21

*a. Average requirement (mg/kg b.wt./d); b. Reference value (mg/kg b.wt./d);
c. Adequate intake (mg/kg b.wt./d)

Data from Dietary Reference Intakes 2002/2005
m = male f = female
Institute of Medicine, Food and Nutrition Board [5]

3.6. Comparison of protein requirements in the surrounding countries

Prentice et al. compared the situation across Europe with regard to dietary recommendations and reference values for children aged 2-18 years and the methodological approaches used in each country [9]. Information was obtained from 29 countries out of 39. There are considerable disparities in the perceived nutritional requirements of European children and adolescents. The reference values for protein are differently expressed either as g/kg body weight and day or as g/day and often without data of representative weight references for each age group. In most of the West-European countries and North America the used methods are the same and comparable. The values are based on the factorial method (WHO Expert Consultation Report 1985 [2]) and on the assumption that children have a similar protein maintenance requirement as adults when referred relative to body weight (0.66 g/kg b.wt./d). The requirement for growth is calculated from the body composition and the growth velocity. Nevertheless, the values diversify clearly. This may be due to remarkable differences in the corrections for day to day, differences in growth data, in the efficiency of the dietary proteins for the synthesis of specific proteins and in the relative quality of the dietary protein versus the reference protein. As example, in the DACH References [1] the protein requirement for children is given as 8-10% of the estimated energy requirement whereas other countries recommend 10-15%.

In any case, the actual dietary protein intake is clearly higher than recommended in Europe.

The intake is about 40 g per day at the age of 2 years (corresponding 3.5 g/kg b.wt./d), 60 g per day at three years and even exceeding 100 g per day at 13-15 years, what is about three times the recommended value [9]. Only a few countries differentiate between males and females in the protein reference intakes expressed on a body weight basis.

In the „Consensus Guidelines“ 2007 it is noted that dietary reference values and recommendations differ enormously in Europe and that harmonisation could or should be a desirable challenge for the future [15].

3.7. Catch up growth

Catch up growth in children applies in the most part to malnourished infants and small children with protein-energy-malnutrition in the developing countries. In the FAO/WHO report 1985, considerations, calculations and data on the nutritional needs in such children are presented [2] and completed in the new report of 2007 [4]. In children with a weight deficit, catch up growth will vary according to the nature of the deficit. In many cases clinical nutrition will be necessary to improve the condition. Either the supply of energy or/and of protein has to be adapted, often in a higher dose than the recommended values for healthy children.

3.8. Protein intake and health aspects

An inadequate intake of protein may have potential adverse effects. An insufficient intake of protein in form of the „Protein-Energy-Malnutrition“ is still a great problem in children living in developing countries. In contrast, the protein intake in developed countries is definitely above the recommended reference values. Does this have consequences?

In infancy, a higher intake of protein can overstress the not yet mature kidneys. And the intake of undiluted cows milk (high protein and calcium) induced a severe malnutrition at the time infant formulas were not yet introduced [16]. In adolescents, long term consumption of high amounts of protein may lead to the same disorders than in adults like kidney stones due to increased calcium and oxalate excretion or negative effects on bone health as consequences of an altered dietary acid-base balance and increased renal calcium elimination [4].

The main focus on adverse nutritional effects in children concerns nowadays a high protein intake in infancy and small childhood with the potential of an increased risk for overweight and obesity. The initial point for this finding were epidemiological observations that breastfeeding (low protein content of human milk) has a preventive effect on later overweight and obesity, and in fact this effect was also relative to the duration of breastfeeding [17]. This led to the „Early-Protein-Hypothesis“, which so far has been further supported by many experimental data [17]. Infants who are fed with infant formulas receive 10-18% more protein than breastfed infants and increase their weight above the sixth week of life compared to the breastfed infants. This correlates with increased plasma values for IGF-1, insulin and the insulin stimulating amino acids [18] and hence explains the increase in weight and adipogenic activity [17].

Within the „Donald-Longterm-Nutrition-Study“ of the Research Institute of Childhood Nutrition in Dortmund data have been collected which show that an increased amount of total and especially animal protein at the age of 5-6 years led to an earlier pubertal growth spurt, to an earlier maximal growth velocity and also an earlier menarche and pubertal vocal change. Plant proteins involved a later appearance of the same parameters [19].

From such studies it is nowadays clear, that nutrition can influence and program endocrine and metabolic processes in a sensitive period of development, processes which can influence health in later life. In this domain there will be even more new and important information in the next future.

3.9. Conclusions

The actual daily protein intake in infants, children and adolescents is higher than officially recommended. Epidemiological studies comparing breastfed and formula fed infants initiated the discussion about the optimal intake of proteins in children. This led to a revision of the data on protein requirements. The new

protein reference values for infants and small children are clearly lower than the ones presented by FAO/WHO in 1985, but only slightly lower than those of DACH in 2000. The values für schoolchildren and adolescents are only a little lower than those of 1985 and about the same as the reference values of DACH.

The long term effects of a high protein intake are of great importance. Not only the quantity of intake but also the quality of proteins is crucial, and many questions are yet unresolved. This applies also to the requirements of proteins and amino acids in childhood, which are still calculated by complex statistical analyses, despite new methods for the measurement of the body composition, which have already improved the new data presented in this paper.

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4. Protein requirements of adults

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4.1. Summary/ Zusammenfassung/ Résumé

Body proteins are subject to a continuous degradation and synthesis (i.e. protein turnover) and accordingly a steady i.e. daily supply of high quality dietary protein has to be assured. For health maintenance, protein catabolism has to be minimised and protein synthesis optimised whenever possible. The present recommendations for protein intake are based mostly on N-balance studies with some supportive evidence from data generated by other methods. The present dietary requirements for healthy adult women and men for all age groups is 0.8 g (high quality) protein per kilogram bodyweight (0.8 g/kg b.wt.). For a normal man (70 kg b.wt.) this intake recommendation corresponds to a recommended daily intake of 56 g/d and 46 g/d for normal women (57 kg b.wt.). A concomitant adequate energy intake from non-N-energy sources is a prerequisite for the present recommendation. The intake recommendation and the requirements are identical for women and men and independent of age.

Zusammenfassung: Proteinbedarf von Erwachsenen

Körperproteine durchlaufen einen kontinuierlichen Prozess des Ab- (Degradation) und Aufbaus (Synthese) (= Proteinturnover). Folglich müssen ständig, d.h. täglich Proteine von hoher Qualität mit der Nahrung zugeführt werden. Für die Aufrechterhaltung einer optimalen Gesundheit sollte wenn immer möglich der Proteinabbau minimiert und die Proteinsynthese optimiert werden. Die aktuellen Empfehlungen für die Proteinzufuhr basieren mehrheitlich auf Stickstoffbilanzberechnungen. Daten, die mit anderen Methoden erhoben wurden, unterstützen diese Ergebnisse. Der aktuelle Bedarf von gesunden erwachsenen Frauen und Männern beträgt für alle Alterskategorien 0.8 g/kg KG/Tag Protein von hoher Qualität. Für einen normalgewichtigen Mann (70 kg KG) entspricht dies einer empfohlenen Zufuhr von 56 g/Tag und 46 g/Tag für eine normalgewichtige Frau (57 kg KG). Voraussetzung für die vorliegende Empfehlung ist eine angemessene Energiezufuhr aus nicht-stickstoffhaltigen Energiequellen. Die Bedarfsempfehlungen unterscheiden sich nicht für Männer und Frauen und sind zudem altersunabhängig.

Résumé : Les besoins en protéines des adultes

Les protéines corporelles subissent un cycle permanent de dégradation et de synthèse (turnover protéique). D'où la nécessité d'un apport alimentaire quotidien en protéines de qualité élevée. Pour rester en excellente santé, il importe de réduire autant que possible le catabolisme protéique et d'optimiser la synthèse protéique. Les présentes recommandations se fondent le plus souvent sur des études du bilan azoté, étayées par des données obtenues à l'aide d'autres méthodes. Les besoins actuels d'une femme ou d'un homme en bonne santé s'élèvent, toutes classes d'âge confondues, à 0,8 g/kg/jour de protéines de qualité élevée, soit usuellement un apport nutritionnel conseillé de 56 g/jour pour un homme (70 kg) et de 46 g/jour pour une femme (57 kg). La présente recommandation suppose également un apport énergétique adéquat de matières non azotées. Les recommandations et les besoins sont identiques pour les deux sexes et indépendants de l'âge.

4.2. Introduction

Proteins are polymers of amino acids (AA). Proteins and amino acids have multiple functions at the level of the cells but also at the organ and whole body level (protein biosynthesis, N-source, energy source, carbon source, precursor for hormone synthesis and others) [1]. The average nitrogen content of proteins is about 16%. For reasons of simplicity protein metabolism corresponds to amino acid or N metabolism, respectively [1-2]. A normal person has about 10-12 kg of protein in the body, a little more than 40% in the muscle [3]. In this chapter the protein requirements for healthy adults will be summarized and discussed.

For health maintenance and assurance of proper function of all body functions an adequate intake of the energy substrates (fat, carbohydrate and protein) as well as the essential micronutrients has to be assured. Proteins have to be regarded not only as an energy source, but more so as a source of the nine essential amino acids, which are absolutely essential for the synthesis of body proteins and thus normal body function. Basically all amino acids could be used as a source of energy, however, not all can be used for gluconeogenesis [1]. Protein synthesis can only occur in the presence of all essential amino acids.

For health maintenance protein catabolism has to be minimized and protein synthesis optimized whenever possible. Body protein underlies a continuous turnover, i.e. continuous degradation and synthesis. The free amino acids originate thus either from the diet, *de novo* synthesis or from degradation of tissue protein. The free amino acids from tissue degradation can either be reutilized for the synthesis of new tissue protein, or they can be oxidized or a minor fraction is shuttled away for utilization in non-protein pathways. The major fraction of the free amino acids comes from the diet. These are the simplified pathways of amino acid metabolism and turnover (see also **Fig. 1**), i.e. the continued degradation and synthesis of body protein, which is under normal conditions in a healthy person equal to ≥ 250 g per day. The constant degradation underlines the importance of continued (i.e. daily) ingestion of adequate amounts of high quality protein. Obviously the protein turnover per unit body mass is smaller in the elderly and decreases with aging.

The protein turnover from up to 250 g/d has to be viewed in relation to the range of the normal protein intake between 50-100 g/d and thus is indirectly a reflection of the ideal protein intake respectively the protein requirements (see below). Protein digestion and -absorption is (independently from the protein source and also independently from the diet composition) generally comparatively high (i.e. $>90\%$) and can thus be met in healthy adults in the setting of an adequate energy intake (i.e. an energy intake according to the needs) in Europe and Switzerland without any special efforts consuming a normal mixed diet [4]. Protein catabolism (e.g. during fasting or also in disease conditions) leads always to a loss of body proteins and should be avoided (or at least minimized) under all circumstances.

The present recommendations for protein intake are based mostly on N-balance studies with some supportive evidence from data generated by other methods [5-8]. Despite some controversial discussions regarding methodological issues the present recommendations are based on different studies and from very different population groups which have been gathered during a considerably long time period and can thus be regarded as rather robust and also reliable [2, 5-6, 9-10]. Every method – including the N-balance method – has its advantages and disadvantages according to the research question and also studied population [5-6, 11-13], which is the major reason for divergent and inconsistent recommendations of the total protein and also single amino acid requirements [13]. Despite rather intense research efforts there is still no generally accepted, validated and also meaningful alternative for the N-balance method [5]. In general the N-balance

overestimates (N- or protein-) intake and underestimates (N- or protein-) excretion thus leading to a *false positive* N-balance [9]. The present DACH recommendations, as used in Switzerland [14] are mainly based on the US recommendation [2].

4.3. Protein requirements

The present dietary recommendations for requirements of healthy adult women and men for all age groups is 0.80 g (high quality) protein per kilogram body weight (0.8 g/kg b.wt./d) [2, 5].

Tab. 1: Recommended protein intake for healthy adults (i.e. 70 kg man / 57 kg women)

	Men	Women
19-50 years	0.80 g/kg b.wt./d (56 g/d)	0.80 g/kg b.wt./d (46 g/d)
51-70 years	0.80 g/kg b.wt./d (56 g/d)	0.80 g/kg b.wt./d (46 g/d)
Pregnancy*		1.1 g/kg b.wt./d or additionally 25 g/d
Lactation*		1.3 g/kg b.wt./d or additionally 25 g/d

*independent of age; b.wt./d: body weight per day

This intake recommendation corresponds to an estimated average requirement of 0.66 g protein per kilogram b.wt. per day. According to the present recommendations protein should account between 10% and maximally 35% of the total energy requirements. For a normal weight man (70 kg b.wt.) this intake recommendation corresponds to a recommended daily intake of 56 g/d and 46 g/d for women (57 kg b.wt.)

An adequate energy intake (to cover the needs) from non-N-energy sources is a prerequisite for the present recommendation [15]. The intake recommendation and the requirements are identical for women and men and independent from age [5, 16].

During pregnancy and lactation different adaptations in protein metabolism as well as metabolism of the different amino acids occurs [17-18]. Despite the different adaptive changes daily protein intake has to be increased as summarized in **Tab. 2 and 4**.

Protein requirements are controversial [19-22]. During the whole adult life span, and especially in the elderly, one should pay less attention to total protein intake but more to protein quality, i.e. the amount of the essential amino acids in the diet [23].

The protein requirements and intake recommendations during pregnancy and lactation and in the elderly will be discussed in separate chapters of this report.

From the metabolic point of view not only the amount of protein but more so the composition regarding the (essential) amino acids is of crucial importance. Accordingly to define protein requirements one has to pay attention not only to the *quantity* of the ingested proteins but more so to the *quality* of the proteins. The quality of a protein is mainly determined by the amino acid pattern, which is in agreement with the specific body needs of essential amino acids for the endogenous protein synthesis. The quantitative aspect of protein ingestion, i.e. the amount which needs to be consumed to cover the recommendations, is determined by different characteristics of the food, however, mainly by the “protein-density” and the concomitant energy intake [6]. The energy requirement is further determined by different factors such as body weight, muscle

mass, age, physical activity and others. All these single factors have to be included for the determination and evaluation of the adequacy of protein intake [2].

Food protein consists of essential and non-essential amino acids (in total 20 amino acids, see **Tab. 2**). For an adequate synthesis of body proteins a healthy adults needs only the nine essential amino acids: histidine, leucine, isoleucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine [1-2]. In the setting of an inadequate intake of protein (inadequate regarding the ingested total amount but especially the amount and ratio of essential amino acids) body protein synthesis is impaired.

Tab. 2: Overview of the essential and the non-essential or conditionally essential amino acids

Essential amino acids	Non-essential or conditionally essential amino acids
Histidine	Arginine
Leucine	Cysteine
Isoleucine	Glutamine
Lysine	Glycine
Methionine	Proline
Phenylalanine	Tyrosine
Threonine	
Tryptophan	
Valine	

These important issues are often ignored and forgotten in the formulation of recommendations of protein intake and especially also in clinical practice. Often it is better to eat a smaller amount of essential amino acids together with an adequate amount of energy instead of a larger amount of an (incomplete) protein mixture. Several studies reported that the intake of a mixture of essential amino acids leads to an equivalent or even better muscle protein synthesis than the ingestion of a larger amount of a mixture of essential and non-essential amino acids together [24].

4.4. Relationship between protein requirements and energy intake

The energy balance is one of the major determinants of N-balance. A sufficient intake of energy from carbohydrates or fat is a prerequisite for the maintenance of an adequate (normal) N-balance. This is of special importance during periods of growth, in the elderly and in situations of inadequate energy intake (classical malnutrition or inadequate energy intake during disease conditions or voluntarily during periods of fasting for weight reduction). All steps of protein metabolism, i.e. from protein utilization, protein synthesis till proteolysis, need considerable amounts of energy [1]. Accordingly any positive or negative change in energy intake respectively energy fluxes affects N-utilization [10, 25-26]. The close interrelationship between energy and protein metabolism is already known for many years [25, 27] and is one reason for the methodological difficulties and discrepancies using the N-balance (and also other methods) to define protein requirements. The discrepancy between studies can often be explained by differences in energy metabolism and energy balance.

The protein sparing effects of the major energy substrates, i.e. carbohydrates and fat, are more or less identical and the small differences found in certain studies is of no importance in daily life and clinical practice [5, 28]. The protein sparing effects of carbohydrates are stronger in the setting of low (i.e. below the recommendations) protein intakes and/or concomitant low energy intakes [28-29], which can be explained by

the pronounced insulin mediated anti-proteolysis. One part of the controversies around the protein requirements respectively the ideal amount of protein intake can be explained by insufficient energy intake or short term alterations in energy intake and thus balance, resulting in an overestimation of protein requirements. The protein sparing effects of energy are of special importance in the elderly, who often consume insufficient amounts of both, high quality proteins and energy [30].

In overweight and obese individuals not only fat mass but also lean body mass is increased, thus leading to increased protein requirements per kilogram body weight. In these subjects calculation of protein requirements should be based on kilogram lean body mass. **Protein turnover** per kilogram lean body mass is identical in normal weight and overweight individuals.

In daily practice, when lean body mass is not known, it is usually the actual weight which is used for the per kg calculation of protein requirements in overweight subjects. However, this leads to an overestimation of protein requirements in massively obese subjects. Therefore, in obesity, a lower than the actual body weight (e.g. normal weight plus 20%) can be used for the calculation of protein requirements.

Often individuals pursue a complete fasting diet without protein intake and no intake of other energy substrates to reduce their body weight. Due to the above outlined characteristics of protein metabolism already a short bout of fasting with lacking or insufficient protein (and energy) intake leads to a negative protein balance with a loss of lean body mass. The latter condition is often associated with insufficient physical activity (insufficient aerobic as well as resistance training activity) further aggravating the loss of lean body mass. The physiological consequence of the loss of lean body mass in the latter setting is still underestimated. The loss of lean body mass can eventually be counteracted by intermittent fasting: In a two-week study in young normal weight adults (aged 18-35 years) intermittent fasting induced a neutral metabolic situation with no increase in proteolysis and maintenance of lean body mass [31].

4.5. Protein quality

Recommendations for the estimated adequate intake as well as the recommended intake of the different amino acids have been defined [5] (see **Tab. 3 & 4**). In this brief review the requirements of single amino acids should not be discussed in detail since in daily life not single amino acids are consumed but complex mixtures of amino acids alone respectively with other substrates. Nevertheless the concept of the “complete protein source” should be recalled, which is a protein source containing all essential amino acids.

The nutritional value and indirectly also the classification of a protein sources is based on the pattern of the amino acid composition as compared to a reference protein source (e.g. egg protein). The initially defined protein requirements are based on the assumption that the ingested protein is of high quality, i.e. that it contains all essential amino acids in ideal amounts for optimal endogenous protein synthesis (see **Tab. 3 and 4**). The protein quality is determined by the occurrence and completeness of the essential amino acids which are the major determinant of the efficiency of the protein (i.e. amino acid) utilization for the endogenous protein synthesis.

Tab. 3: Intake recommendations for the single essential amino acids in healthy adults (>19 years, independent of gender). Essential amino acids should account for approximately 24% of the total intake.

Amino Acid	Intake recommendation (mg/kg b.wt./d)
Histidine	14
Leucine	42
Isoleucine	19
Lysine	38
Methionine & cysteine	19
Phenylalanine & tyrosine	33
Threonine	20
Tryptophan	5
Valine	4

Tab. 4: Intake recommendations for the single essential amino acids in healthy women during pregnancy and lactation. The intake of the essential amino acids should account for approximately 26% during pregnancy and 24% during lactation.

Amino Acid	Pregnancy (mg/kg b.wt./d)	Lactation (mg/kg b.wt./d)
Histidine	18	19
Leucine	56	62
Isoleucine	25	30
Lysine	51	52
Methionine & Cysteine	25	26
Phenylalanine & Tyrosine	44	51
Threonine	26	30
Tryptophan	7	9
Valine	31	35

Different indices to measure protein utilization have been defined [5-6, 32]. For daily practice the biological value (BV) of a protein source is the most useful index. The consumption of food sources with a high BV should thus be preferred (e.g. egg, fish, meat, milk and milk products). In daily practice we should thus pay less attention to the absolute amount of protein ingested but more so to the amino acid pattern i.e. the absolute amounts of essential amino acids. It is well known that the consumption of a smaller amount of a complete protein leads to an increased muscle protein synthesis as compared to the consumption of a higher amount of an incomplete protein source [24]. In daily practice the consumption of a complete protein is not always possible but can be achieved with simple and cost efficient strategies. The concomitant consumption of a glass of milk (or another milk product such as curd) or also a whey concentrate increases the “biological value” of a meal in a cheap and physiologically efficient way [32-33].

Contrary to the general opinion the need for essential amino acids can also be covered by a plant-based diet. A vegetarian needs to ingest basically the same amounts of protein (i.e. same protein requirements) as along as the energy requirements are covered and as long as he chooses a food pattern in agreement with the concept of protein complementarity [34-35]. Most plant protein sources are incomplete proteins. Thus in the setting of a plant-based diet the choice of the food becomes critical to achieve protein complementarity. Lysine and isoleucine are the limiting amino acids in cereals. Beans and other pulses are rich in lysine and isoleucine. Thus cereals need to be consumed together with pulses: the consumption of food sources with a

complementary amino acid pattern leads to a renewed complete amino acid pattern i.e. containing all the essential amino acids. Accordingly the protein requirements (to cover the needs of all essential amino acids) of vegetarians can vary as a function of the composition of the protein sources. This is also reflected in studies reporting a lower muscle mass in vegetarians (as compared to age and gender matched controls) [36], which might be reflection of the ingestion of an incomplete amino acid pattern for muscle protein synthesis leading in the longer term to a lower muscle mass. However, most vegetarians know the rules of amino acid complementarity and combine the different food sources in their diet e.g. cereals together with pulses. Ideally protein complementarity should occur on a daily basis or even in each meal to assure the ideal amino acid availability for optimal protein synthesis. The only plant food with a complete amino acid pattern is soy protein [37]. Vegans as compared to lacto-ovo-vegetarians might have an increased risk for an unbalanced or even insufficient intake of essential amino acids. In our society the monitoring of an adequate intake of complete protein sources seems to be often more important than the monitoring of the overall protein intake. Further a large fraction of the population is dieting for reasons of weight control ignoring the protein sparing effects of small amounts of energy.

4.6. Controversy about the current recommendations

During the last few years it has been postulated that the present recommendations for protein intake are too low [12-13]. These authors postulate based on studies using the indicator amino acid oxidation method that the present US protein intake recommendation (from which the DACH recommendations are derived) are 40-50% too low and that the average daily protein need should be 0.93 g/kg b.wt./d. These authors recommend a safe protein intake of 1.2 g/kg b.wt./d [13]. The indicator amino acid method is a very sophisticated and complex method subject to many exogenous and endogenous confounders and can not be compared to the N-balance methodology. The indicator amino acid method is based on the increasing supply of an "indicator amino acid" (i.e. usually the amino acids for which the requirement should be determined) and the concomitant measurement of the oxidation rate of the indicator amino acid. In the setting of an insufficient supply (intake) of the indicator amino acid the indicator amino acid is oxidized and not used for protein synthesis. Only a supply of the indicator amino acid at the level of its requirement leads to a stabilization of the oxidation rate at a more or less constant level which corresponds to the level of the true requirement of the indicator amino acid. Contrary to the N-balance method the indicator amino acid method is a rather artificial setting since it uses only one amino acid at a time. Using the indicator amino acid method the measured and calculated requirements of the single amino acids is higher and thus also the calculated total protein requirements. Based on a critical review of the available literature about the present protein requirements no final conclusion around these controversies can be made. Nevertheless it seems wise to stick to the present recommendations.

The metabolic behaviour of single amino acids (the key issues on which the indicator amino acid method is based) are very different as compared to the metabolic behaviour of a mixture of amino acids (which is assessed by the N-balance method and which reflects actually the more physiological situation of food intake). Thus the data from the indicator amino acid method have to be interpreted with caution only. Different population groups consume often insufficient amounts of protein (as compared to the present recommendations) and insufficient amounts of energy, putting them at increased risk of protein-energy malnutrition with the well known consequences [38]. Accordingly it seems to be more meaningful to implement strategies to improve in a sustainable manner the adequacy of the intake of proteins and of other

substrates according to the present recommendations in the majority of subjects at risk (such as the elderly) within the entire population [38].

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5. Protein requirements in the elderly

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5.1. Summary/ Zusammenfassung/ Résumé

Adequate protein intake and the maintenance of nitrogen equilibrium are of particular importance in the elderly because this age group is at increased risk of illness and malnutrition. Age-related changes in body composition and protein metabolism may contribute to altered dietary protein requirements for older adults.

The current recommendation for protein intake of healthy elderly subjects is 0.8 g/kg b.wt./d and, thus, the same as for younger adults. Despite conflicting evidence and a controversial debate among experts in recent years about the adequacy of this amount, the WHO/FAO/UNU expert committee recently confirmed this recommendation irrespective of sex and age. Very little is known about the protein needs of frail and unhealthy elderly.

Nitrogen balance studies in the elderly revealed conflicting results and some suggest that not all elderly can achieve a nitrogen balance with 0.8 g/kg b.wt./d, particularly if energy supply is not adequate. On the other hand, there are, at present, no studies convincingly showing that protein requirements of elderly people differ substantially from those of younger adults. Beyond the amount of protein needed for nitrogen balance, the optimal protein intake for preservation of muscle mass and health and prevention of sarcopenia is of paramount interest. At present, there is insufficient longer-term research with defined health outcomes to derive recommendations in this regard. Also with respect to protein metabolism in the elderly, still many uncertainties exist at present. There is general agreement that protein anabolism can be stimulated by moderate amounts of dietary protein, and is affected by eating patterns, insulin action and physical activity. It is difficult to draw firm conclusions, because most of the data come from short-term studies under experimental conditions and are contradictory. Until more evidence is available, it seems reasonable to ensure a protein intake of at least 0.8 g/kg b.wt./d in all elderly persons, particularly in those at risk of malnutrition (e.g. frail and multimorbid elderly). Early recognition of nutritional difficulties and subsequent elimination is crucial. In addition to ascertaining adequate protein and energy intake, physical activity should be encouraged in order to increase energy expenditure and food intake and to facilitate muscle protein anabolism.

Long-term studies with clinical endpoints are needed to further clarify the optimal amount, kind and timing of protein intake for younger and older, healthy, frail and ill elderly subjects to prevent deficiency and optimally preserve lean body mass, body functions and health.

Zusammenfassung: Proteinbedarf von älteren Menschen

Die ausreichende Proteinzufuhr und die Aufrechterhaltung des Stickstoffgleichgewichts sind von zentraler Bedeutung bei älteren Menschen, da in dieser Alterskategorie ein erhöhtes Risiko für Erkrankungen und für eine Proteinmangelernährung besteht. Altersbedingte Veränderungen der Körperzusammensetzung und des Proteinmetabolismus tragen möglicherweise zum veränderten Proteinbedarf von älteren Menschen bei.

Die aktuelle Empfehlung für die Proteinzufuhr älterer Menschen beträgt 0.8 g/kg KG/Tag und entspricht somit der Empfehlung für jüngere Erwachsene. Trotz widersprüchlicher Evidenz und kontroversen Diskussionen unter Experten in den vergangenen Jahren, ob die empfohlenen Mengen ausreichen,

bestätigte kürzlich ein WHO/FAO/UNU Expertenkomitee die Empfehlungen unabhängig von Alter und Geschlecht. Über den Proteinbedarf von gebrechlichen und kranken älteren Menschen ist sehr wenig bekannt.

Stickstoffbilanzberechnungen bei älteren Menschen ergaben widersprüchliche Ergebnisse und einige wiesen darauf hin, dass mit 0.8 g/kg KG/Tag nicht alle älteren Menschen das Stickstoffgleichgewicht aufrechterhalten können – insbesondere wenn die Energiezufuhr nicht ausreichend ist. Zurzeit liegen noch keine Studien vor, die zeigen, dass der Proteinbedarf von älteren Menschen wesentlich von jenem jüngerer Menschen abweicht. Neben der benötigten Proteinmenge für das Stickstoffgleichgewicht, ist die optimale Proteinzufuhr auch für die Erhaltung der Muskelmasse, Gesundheit und der Prävention von Sarkopenie von zentraler Bedeutung. Im Moment liegen allerdings noch zu wenige Langzeitstudien mit definierten Endpunkten vor, um dazu Empfehlungen abgeben zu können. Zahlreiche Unsicherheiten sind auch im Hinblick auf den Proteinmetabolismus bei älteren Menschen vorhanden. Einigkeit herrscht darin, dass der Proteinanabolismus durch moderate Nahrungsproteinmengen stimuliert werden kann und durch das Ernährungsmuster, die Insulinantwort und die körperliche Aktivität beeinflusst wird. Es ist jedoch schwierig eindeutige Schlussfolgerungen zu ziehen, da die meisten Daten aus Kurzzeitstudien unter experimentellen Bedingungen stammen und widersprüchlich sind. Solange nicht mehr wissenschaftliche Evidenz vorliegt, sollte die Proteinzufuhr von älteren Menschen mindestens 0.8 g/kg KG/Tag betragen, insbesondere bei jenen mit einem erhöhten Risiko für Fehlernährung, d.h. bei gebrechlichen und multimorbiden älteren Menschen. Die frühe Erkennung von Ernährungsschwierigkeiten und deren Behebung sind zentral. Zusätzlich zur Sicherstellung der ausreichenden Protein- und Energiezufuhr, sollte zu körperlicher Aktivität ermuntert werden, um den Energieverbrauch und damit die Nahrungszufuhr zu erhöhen sowie den Muskelproteinanabolismus zu erleichtern.

Langzeitstudien mit klinischen Endpunkten sind notwendig um Klarheit zu erhalten über die optimale Menge, Art und Zeitpunkt der Proteinzufuhr für jüngere und ältere, gesunde, gebrechliche und kranke ältere Menschen, damit eine Unterversorgung vermieden und fettfreie Körpermasse, Körperfunktionen und Gesundheit erhalten werden kann.

Résumé : Les besoins en protéines des personnes âgées

Un apport adéquat en protéines et le maintien de l'équilibre azoté sont particulièrement importants chez les personnes âgées, catégorie de la population exposée à un risque accru de maladie et de malnutrition. Les changements, liés à l'âge, de la composition du corps humain et du métabolisme protéique peuvent contribuer à modifier les besoins protéiques durant la vieillesse.

L'apport en protéines recommandé aux personnes âgées en bonne santé est aujourd'hui de 0,8 g/kg/jour – comme pour les jeunes adultes. En dépit de preuves contradictoires et de la controverse ayant divisé les experts au cours des dernières années à propos du caractère suffisant d'un tel apport, le comité d'experts OMS/FAO/UNU a récemment confirmé cette recommandation, indépendamment du sexe et de l'âge. On sait encore très peu de choses sur les besoins en protéines des personnes âgées fragiles et malades.

Les études du bilan azoté des personnes âgées ont abouti à des résultats contradictoires, certaines suggérant que tous les seniors ne parviennent pas à l'équilibre azoté avec 0,8 g/kg/jour, surtout si l'apport en énergie est insuffisant.. D'un autre côté, on ne dispose pas pour l'instant d'études montrant de manière convaincante que les besoins en protéines des personnes âgées différeraient fondamentalement de ceux

des jeunes adultes. Au-delà de l'équilibre azoté, un apport optimal en protéines s'avère également crucial pour la préservation de la masse musculaire, pour la santé et pour la prévention de la sarcopénie. Mais les études de longue durée ayant abouti à des résultats cliniques sont encore trop rares pour permettre d'en inférer des recommandations. Par ailleurs, de nombreuses incertitudes subsistent quant au métabolisme protéique des personnes âgées. Les spécialistes s'accordent certes à dire que l'anabolisme protéique peut être stimulé par des quantités bien dosées de protéines alimentaires, et que tant le modèle alimentaire que la production d'insuline, et l'activité physique ont une incidence sur lui. Mais il est difficile d'en tirer des conclusions précises, sachant que la plupart des données proviennent d'études de courte durée, réalisées dans des conditions expérimentales, et qu'elles sont contradictoires. Dans l'attente de preuves scientifiques supplémentaires, un apport en protéines d'au moins 0,8 g/kg/jour paraît indiqué pour les personnes âgées, notamment celles courant un risque accru de malnutrition (patients âgés fragiles et multi-morbides). Il est également crucial d'identifier précocement les difficultés nutritionnelles, pour pouvoir les éliminer. Outre le souci d'un apport adéquat de protéines et d'énergie, il faudrait également encourager l'activité physique, afin d'augmenter la dépense d'énergie – et donc la ration alimentaire – et de faciliter l'anabolisme protéique musculaire.

Seuls les résultats cliniques d'études de longue durée permettront de connaître précisément la quantité et la nature des protéines, ainsi que le moment où en ont besoin les personnes jeunes ou âgées, selon qu'elles sont bien portantes ou fragiles et malades, afin de prévenir toute carence et de préserver une masse corporelle svelte, le bon fonctionnement de l'organisme ainsi que la santé.

5.2. Specific situation of the elderly – age-related changes

Aging is associated with various physiological and metabolic changes that may contribute to altered dietary protein requirements in older adults.

One of the most pronounced and meaningful age-related physiological changes is the decrease of the whole body protein content with age, reflected in a decreasing lean body mass (LBM). The principal component of the decline in LBM is a decrease in skeletal muscle mass, which is the largest reservoir of protein in the body. The age-related loss of skeletal muscle mass and consequent fall in muscle strength, called sarcopenia, is strongly related to reduced physical function such as walking ability, gait speed or stair climbing capacity and is associated with a higher risk of falls, frailty and disability. Sarcopenia, thus, increases the risk of dependency from others and the need of institutionalisation and is of great public health concern. The aetiology of sarcopenia is complex, including hormonal and inflammatory changes, neurodegenerative processes and reduced physical activity. Inadequate nutritional intake may also contribute and aggravates the age-related loss of muscle mass. Vice versa adequate energy and protein intake are regarded as important factors for the preservation of muscle mass and prevention of sarcopenia [1;2].

Usually, food and energy intake of older adults are reduced, due to a combination of various factors. Physiological changes associated with age like decreased basal metabolic rate, reduced appetite, altered taste and smell sensation, slower gastric emptying and altered hormonal responses may contribute. Reduced intake in the elderly is also often observed as a consequence of chewing or swallowing problems, physical handicaps, mental impairment, loneliness or depression. In addition, chronic and acute diseases,

the presence of multiple diseases in many elderly, and accompanying inflammation, pain and multiple medication may substantially impair dietary intake [2;3].

These factors leading to a reduction in food intake in combination with the progressive loss of lean body mass predispose the elderly to energy-protein malnutrition (PEM). Sedentary, frail and ill elderly people are the population groups most at risk [4].

Regarding age-related changes of protein digestibility, turnover and efficiency of protein utilisation, there do not seem to be substantial impairments in the elderly. However, even small changes might have implications for protein requirements. Despite a large body of research in recent years, there are presently still many uncertainties.

Finally, when regarding the elderly, it is important to note that this population group covers a wide age range – from 65 to more than 100 years – that is characterised by a great heterogeneity with respect to functional, nutritional and health status, activity pattern and life-style.

5.3. Current recommendations

The current recommendation of the German, Austrian and Swiss Nutrition Societies [5] for protein intake of persons aged 65 years or older is 0.8 g of good quality protein per kg body weight per day both for men and women, and thus the same as for younger adults. This recommendation is in line with the current reference intakes from the Food and Nutrition Board of the Institute of Medicine for the U.S. and Canada [6], stating an Estimated Average Requirement (EAR) of 0.66 g/kg b.wt./d and a Recommended Dietary Allowance (RDA) of 0.8 g protein/kg b.wt./d for all adult age groups including the elderly. In their new report, the WHO/FAO/UNU expert committee recently confirmed this recommendation irrespective of sex and age [7]. Despite conflicting evidence and a controversial debate among experts in recent years about the adequacy of 0.8 g/kg b.wt./d for all elderly persons, this committee concluded that presently no convincing evidence exists for a change in the protein requirement with age. The report identified gaps in the knowledge base for the elderly population, in terms of both the minimum requirement for general health and well-being and the optimum intake for healthy aging, especially with respect to sarcopenia and osteoporosis [7].

In absolute amounts, the German, Austrian and Swiss Nutrition Societies [5] recommend 54 g/d for elderly men (68 kg) and 44 g/d for elderly women (55 kg). This is 4-5 g below the recommendation for young adults due to a decreasing body weight of the standard person with age.

As a result of the decrease in energy requirements with age and unchanged protein requirements, 0.8 g protein/kg b.wt./d represents a larger percentage of energy in elderly than in younger adults. Thus, elderly – particularly sedentary elderly women – need a more protein-dense diet than younger adults to meet both protein and energy requirements. This is reflected in a recommended protein density of 6.9 g/MJ for elderly men and 6.7 g/MJ for elderly women, corresponding to 28.9 and 28 g/1000 kcal respectively, that is about 0.5-1 g/MJ higher than in young adults [5].

The current recommendation of the German, Austrian and Swiss Nutrition Societies for protein intake of persons aged 65 years or older are summarised in **Tab. 1**.

Tab. 1: Recommendations of the German, Austrian and Swiss Nutrition Societies for daily intake of protein in persons aged 65 years or older [5] standard persons

	men	women
g/kg body weight	0.80	0.80
g for a standard person (68 kg man or 55 kg woman)	54	44
g/MJ	6.9	6.7

Because of the low food intake of many elderly persons, it is important to bear in mind that an insufficient intake of energy increases protein requirement. A negative energy balance promotes a negative nitrogen (N) balance, and neutral or positive N balance can only be obtained if energy intake is sufficiently high. Since even small discrepancies in energy balance can have major impact on protein metabolism and nitrogen balance [8], it is important for the elderly to ensure not only adequate protein but also appropriate energy intakes.

Regarding different physiological states and body composition in young-old, old-old and oldest old, there might be different requirements within the elderly age group. However, there are, at present, insufficient data to make differential recommendations neither by age, nor for elderly in different health or functional states.

5.4. Protein intake in the elderly

In accordance with the reduced energy expenditure with age, dietary intake of energy is usually reduced in elderly persons, and this decline in energy intake is accompanied by a decline in protein intakes. Data on protein consumption by elderly subjects in Switzerland are described in chapter no. 4.

In the recent German nationwide nutrition study (NVS II) median energy intake of participants in the oldest age group (65-80 years) was 2130 kcal in men and 1700 kcal in women and, thus, in men about 600 kcal and in women about 250 kcal/d lower than in young adults. Median protein intakes of the elderly – 76 g/d in men and 61 g/d in women – were about 5 g lower than in 51-64 year old participants. 14.5% of the elderly did not reach the recommended daily amount of 54 g for men and 44 g for women – irrespective of sex [9].

In a preceding nationwide study of German elderly living independently in their own household, protein intake was calculated per kg body weight resulting in a median intake of 1.2 g/kg b.wt./d. 6% of the participants consumed less than 0.66 g/kg b.wt./d, and 14% less than 0.8 g/kg b.wt./d with no sex difference and no difference between young-old (65-74 y.), old-old (75-84 y.) and oldest old (85+y.). Energy intake was below 1500 kcal/d in 17% of all participants – and in 11% of men and 21% of women, and in 26% of the participating women aged 85 years or older [10].

Finally, in 127 German nursing home residents a median energy intake of 1540 kcal/d was observed with 10% consuming less than 1000 kcal/d. Corresponding median protein intakes were 52 g/d or 0.90 g/kg b.wt./d. One fifth (19%) of the residents consumed less than 0.66 g/kg b.wt./d and one third (34%) less than 0.8 g/kg b.wt./d [11].

Thus, protein as well as energy intake does not reach the recommendation in considerable parts of the elderly population, especially in institutionalised elderly.

5.5. Nitrogen balance studies in the elderly: controversy about requirements

As for younger adults, current recommendations for protein intake for the elderly are based on analyses of available nitrogen (N) balance studies.

The major basis for setting the current protein allowances for elderly people is formed by four N balance studies, meanwhile already about 30 years old [12-15] (**Tab. 2**).

Tab. 2: Nitrogen balance studies in healthy elderly

First author	Study characteristics	Results and conclusion
Cheng (1978) [12]	7 men, 61-73 y, 62 kg; 8 men, 23-29 y, 67 kg. Energy intake 40 kcal/kg Wheat-soy-milk mixture Intake levels 0.4 / 0.8 / 1.6 g/kg Duration: 11 days	Negative balance at 0.4 g/kg, zero balance at 0.8 g/kg (with 8/15 subjects in negative balance) positive balance at 1.6 g/kg, no difference between age groups →0.8 g/kg high quality protein is adequate
Uauy (1978) [13]	7 women: 70-84 J, 69 kg, energy intake: 28 kcal/kg 7 women: 68-74 J, 74 kg, energy intake: 32 kcal/kg Egg protein Intake levels 0.52 - 0.85 g/kg/ Duration: 10 days	N balance at 0.7 - 0.85 g/kg in men and at 0.83 g/kg in women → Egg protein intake of 0.8 g/kg considered adequate.
Zanni (1979) [14]	6 men: 63-77 J, 83 kg Energy intake: 31 kcal/kg Egg protein Intake levels: 0.36 - 0.46 g/kg Duration: 15 d (following 17 days of protein-free diet)	Balance at 0.59 g/kg → FAO/WHO 1973 safe level of 0.57 g egg protein/kg considered adequate
Gersovitz (1982) [15]	8 women: 71-99 J, 62 kg, 7 men: 70-82 J, 71 kg, Energy intake: 30 kcal/kg Egg protein Intake level: 0.8 g/kg Duration: 30 days	Some persons couldn't reach balance. → 0.8 g/kg egg protein not adequate for long-term balance
Bunker (1987) [21]	11 men, 13 women, 70-86 y, Energy intake 27 kcal/kg Self-selected diet Intake level 0.94±0.19 g/kg Duration: 5 days	0.97 g/kg for N equilibrium overestimation, since no factor for miscellaneous N losses was used
Campbell (1994) [19]	8 men, 4 women, 56-80 J, Energy intake 30 kcal/kg Mixed lacto-ovo-vegetarian food Intake levels 0.8 or 1.62 g/kg Duration 11 days	Negative N-balance at low, positive N-balance at high intake level → estimated intake for N balance 1.0 g/kg; safe intake 1.0-1.25 g/kg high quality protein
Castaneda (1995) [22]	12 women 66-79 J, 68 kg Energy intake 27 kcal/kg Lacto-ovo-vegetarian foods Intake levels 0.45 / 0.92 g/kg Duration: 9 weeks	Negative N balance at 0.45 g/kg; positive N balance at 0.92 g/kg
Pannemans (1995) [23]	17 men, 11 women 65-80 J, 71 kg Energy intake ? Mixed diet Intake levels 0.9 / 1.5 g/kg Duration: 3 weeks	N balance not significantly different from zero during either diet
Pannemans (1998) [24]	12 women 69+4 J, 69+7 kg Energy intake ? Intake levels ~0.8 g/kg (50% animal, 50% vegetable) ~1.2 g/kg (75% animal, 25% vegetable) ~1.2 g/kg (25% animal, 75% vegetable) Duration: 2 weeks	N balance negative with low intake level and not significantly different from zero with higher intake levels (both diets)
Campbell (2001) [20]	4 men, 6 women 55-77 J, 68 kg Energy intake 35-39 kcal/kg Lacto-ovo-vegetarian foods Intake levels 0.80 g/kg Duration: 14 weeks	Maintenance of BC, leucin metabolism, decrease of urinary N excretion and mid-thigh muscle area → RDA not adequate to completely meet the needs of all older people
Morse (2001) [25]	11 women, 70-81 J, 73 kg Energy intake 31 kcal/kg Lacto-ovo-vegetarian foods Intake levels 0.5 / 0.75 / 1.0 g/kg Duration: 3 * 18 days	Requirement 0.70 g/kg in week 2; 0.56 g/kg in week 3; RDA 0.90 g/kg in week 2; 0.76 g/kg in week 3; decrease in urinary N excretion from wk 2 to wk 3 → protein needs are at or above current RDA → short-term studies are insufficient to establish RDA
Campbell (2008) [28]	23 young (21-46 y) 19 elderly (63-81 y) Lacto-ovo-vegetarian foods Intake levels 0.5 / 0.75 / 1.0 g/kg Duration: 3 * 18 d	Requirement 0.61 g/kg in young and 0.58 g/kg in elderly subjects. → requirement not different for healthy older than for younger adults and not different from current RDA

From these studies it was concluded that the protein need of elderly subjects are unlikely to be less than those of younger adults – as speculated earlier [16]. Based on these studies, in 1985, the FAO/WHO/UNU claimed a requirement of 0.6 g/kg b.wt./d and a suggested safe intake of 0.75 g/kg b.wt./d for the elderly [17]. A few years later, the NRC of the United States set their recommendation at 0.8 g protein/kg b.wt./d [18].

In 2003, a meta-analysis of all available N balance studies, commissioned by the FAO/WHO/UNU as a basis for new recommendations [26], included 6 studies performed in persons aged 63 years or older. No age effect was found, however only 14 old persons (from these only 3 – all women – were 75 y and older) entered the meta-analysis. The difference between young and old was not statistically significant, but translated into 0.17 g protein/kg b.wt./d that is about 10 g/d and 20% of the RDA [27].

In summary, the range in the level of protein intake recommended for elderly people derived from N balance studies performed in the last 30 years, ranges from 0.59 to 1.25 g/kg b.wt./d – a broad range, that could be explained by differences in study design and methods (e.g. study duration and protein source), difficulties in performing N balance studies in elderly persons and great heterogeneity of participants (e.g. age range from 55 to 99 years, energy intake between 20 and 40 kcal/kg b.wt./d, probably different activity levels). Some studies [12-14;28] support the adequacy of the current recommendation, whereas others [15;19;20;21] suggest that it should be higher, because the intake of 0.8 g protein/kg b.wt./d did not always result in a positive N-balance. Studies comparing young and older adults [12;23;28;29] did not find different results between the age groups, and, thus, do not provide evidence that protein requirements of the elderly differ from that of younger adults.

5.6. Effect of illness on protein requirement in the elderly

At present, three studies reporting N balance data in frail or ill elderly subjects are available (**Tab. 3**).

Tab. 3: Nitrogen balance studies in frail and ill elderly

First author	Study characteristics	Results
Bunker (1987) [21]	20 housebound elderly (7men, 13 women), 70-85 y, Energy intake 20 kcal/kg Self-selected diet Intake level 0.66±0.19 g/kg Duration: 5 days	Negative N balance at 0.66 g/kg overestimation, since no factor for miscellaneous N losses was used
Chevalier (2003) [30]	8 frail women, 84±2 y, Energy intake 30 kcal/kg Intake level - 0.87±0.03 g/kg for 9 days - 1.23±0.02 for the next 12 days Duration: 9 days + 12 days	Zero N balance at low intake level, positive balance at higher level
Gaillard (2008) [31]	36 geriatric patients (17 men, 19 women), 65-99 y. clinically stable (3-5 d after admission) Hospital diet Intake 0.99 +-0.24 g/kg Energy intake 23.5 +- 5.4 kcal/kg Duration: 3 days	Neutral mean N balance, half of patients in positive N balance mean protein intake to reach neutral N balance is 1.06±0.28 g/kg at energy intake of ≥ 1.31 * REE (regression approach)

Dietary recommendations are established for the healthy population not taking in account the presence of acute and chronic diseases, disability and limited mobility often present in the elderly.

Inflammation (including inflammaging), infections and wounds, for example, can rapidly deplete body nitrogen stores and may increase protein requirements, however, to which extent is difficult to assess.

Bunker et al., already in 1987, observed a negative N balance in 20 homebound elderly with a mean daily intake of 0.67 g protein/kg b.wt./d over 5 days, however, at a very low energy intake of only about 20 kcal/kg b.wt. [21]. In 2003, Chevalier et al. compared two protein intake levels in 8 frail 84-year old women and found a zero N balance during 9 days with a daily protein intake of 0.87 g/kg b.wt. and a positive balance during the following 12 days in the same women consuming 1.23 g protein/kg b.wt./d [30]. Only recently, nitrogen balance was measured in 36 clinically stable geriatric patients consecutively admitted to a short-stay or rehabilitation care unit in condition of spontaneous and ad libitum food intake [31]. A mean daily intake of 0.99 g protein and 23.5 kcal/kg b.wt./d resulted in a neutral mean N balance. Some patients were in negative N balance despite an intake of 0.8 g/kg b.wt./d. Using a regression approach the authors concluded that elderly hospitalized patients with an energy intake of 1.31 times resting energy expenditure (25 kcal/kg b.wt./d) require a minimal protein intake of 1.06 g/kg b.wt./d [31].

It is impossible to derive concrete figures for protein requirement of frail and ill elderly from these limited data. Protein requirements may be higher for frail elderly persons, however no N balance study that follows the WHO criteria is presently available to support this, and safe intake is difficult to assess in this specific group.

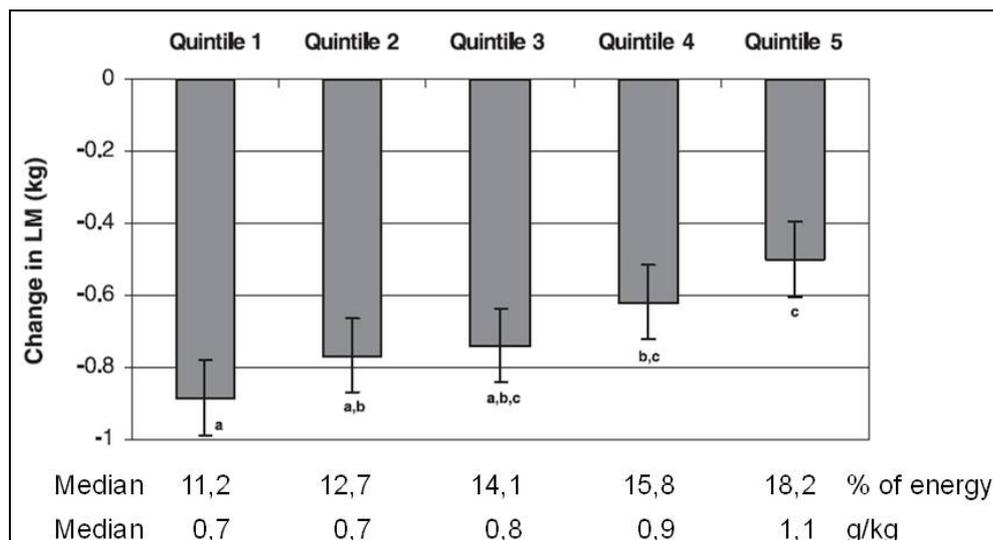
5.7. Protein intake and body composition, physical function and health

Nitrogen balance studies do not answer the question of optimal protein intake to minimize loss of LBM with age and preserve optimal function and health.

The relation between protein intake and muscle mass and function, and the detrimental effect of low protein intake in this respect has been reported by Castaneda et al. [22] in the already mentioned study comparing daily intake levels of 0.45 and 0.92 g protein/kg b.wt./d over 9 weeks in 12 elderly women. The low protein intake resulted in significant losses of LBM, especially muscle mass and muscle strength, whereas muscle mass and function were maintained at the higher intake level.

Only recently, some epidemiologic evidence for a relationship between the amount of protein consumed and the preservation of muscle mass emerged. In the Health, Aging and Body Composition study, a large cohort study of elderly men and women aged 70-79 years, three-year decrease in lean mass and in nonbone appendicular lean mass was found to be about 40% less in participants in the highest quintile of protein intake (median 1.1 g/kg b.wt./d or 18.2% of energy) compared to the lowest quintile (median 0.7 g/kg b.wt./d or 11.2% of energy) [32] (**Fig.1**).

Fig. 1: Adjusted lean mass (LM) loss by quintile of energy-adjusted protein intake (n=2066) [32]



Least squares with different superscript letters denote statistically significant differences ($p < 0.05$ or less).

In a small cross-sectional study of 38 healthy women aged 66 ± 5 years with a mean intake of 1.5 g protein/kg b.wt./d, significant correlations between muscle mass index and total protein intake ($r = 0.324$, $p < 0.05$) and between muscle mass index and animal protein intake ($r = 0.431$, $p < 0.01$) were observed. Stepwise regression analysis showed animal protein intake to be the only independent predictor of muscle mass index [33]. The same group, in another cross-sectional study of 50 healthy elderly aged 60-75 years with a similar intake level, found muscle mass index positively and significantly associated with total protein intake per day ($r = 0.44$, $p < 0.01$). However, no correlation with protein intake expressed per kg body weight ($r = 0.08$) or as percent of energy ($r = 0.10$) was observed [34].

In a report about changes of nutritional status and patterns of morbidity among participants of the New Mexico Aging Process Study, it is mentioned that women with protein intakes above 1.2 g/kg b.wt./d tended to have fewer health problems over the next 10 years than women with protein intakes below 0.8 g/kg b.wt./d, however, concrete figures, regrettably, are not given [35]. More recently, the InCHIANTI study, a cross-sectional study in Northern Italy among 802 elderly aged 65-93 years, reported a nearly doubled risk of frailty in the lowest protein intake quintile (< 66 g/d in men, < 55 g/d in women; corrected for energy intake) compared to participants with higher intake (OR 1.98, 95% CI 1,18 - 3,31) [36]. It can not be concluded from this study, however, whether a low protein intake is the cause or the consequence of frailty.

Thus, at present only few studies are available that examined the relationship between protein intake and body composition, function and health. These studies do not allow deriving definite conclusions for optimal protein intake in this respect. Long-term studies relating protein intake to clinical endpoints are clearly needed to elucidate the relationship between protein intake and health in the elderly.

5.8. Protein-anabolic effects of dietary proteins in the elderly

Sarcopenia is likely to be the result of a small imbalance between synthesis and degradation of proteins over years that is too small to be measured by N balance studies. Studying the effects of protein intake on protein digestibility, turnover, efficiency of protein utilisation, and age-related changes in this regard, may contribute to identify the optimal amount of protein needed to preserve muscle mass with aging.

As already mentioned, protein metabolism does not seem to be substantially impaired in the elderly. However, even small changes might have implications for protein requirements.

Measurements of protein metabolism in the post absorptive state have shown no change in whole body protein turnover per unit of active metabolic tissue with aging. However, a reduced contribution of muscle protein to whole body protein metabolism was reported in the elderly that can be explained by the reduced muscle mass. Consequently, the contribution of non-muscle lean tissues to whole-body protein metabolism, particularly that of visceral tissue is proportionally greater with ageing [27].

Metabolic response to the intake of protein or amino acids (AA) was examined in many studies in recent years. As in younger adults, in healthy elderly muscle protein synthesis can be stimulated by oral AA despite higher splanchnic extraction of dietary AA in the elderly [27]. However, muscle protein synthesis has been found to be less sensitive to small amounts of essential AA (EAA) than in young adults. According to Katsanos et al. [37] the aged skeletal muscle is unable to respond to low doses of EAA (7.5 g), whereas higher doses (10-15 g) are capable of stimulating muscle protein synthesis to a similar extent as in young persons. Consequently, the provision of sufficient amounts of EAA seems to be important.

Regarding different kinds of protein, it has been shown that a “fast” digested protein (whey protein) may be more beneficial in elderly persons than a “slow” one (casein) with respect to protein gain [38]. Interestingly, in a recent study, whey protein ingestion in elderly resulted in greater muscle protein accrual than ingestion of its constituent EAA [39]. Symons et al. [40] reported that also lean beef is able to stimulate the anabolic response in the elderly. A moderate serving of 113 g (30 g total AA, 12 g EAA) increased muscle protein synthesis by 50% in both young and elderly [41]. When 75% of 1.25 g protein/kg b.wt./d were derived from animal sources, inhibition of protein breakdown with meals and net protein synthesis measured in the post absorptive state were greater than when 75% of the protein were derived from a vegetable protein source [24].

Thus, it has been shown in a number of studies that moderate servings of EAA, whey protein and also common protein-rich foods like lean beef are able to promote muscle protein anabolism in healthy elderly – similar to healthy young.

On the other hand it has been shown that very large amounts of protein are not more effective. Paddon-Jones & Rasmussen [42] report that a large single serving of lean beef (340 g, containing 90 g protein) does not elicit a greater anabolic effect than a serving one-third the size. They conclude that ingesting more than 30 g protein at a single meal may be an inefficient means of stimulating muscle protein synthesis. This is in agreement with a recent study that showed no benefit of very large amounts of protein for 10 days (3.0 vs. 1.5 g/kg b.wt./d) in younger and older persons in terms of muscle protein synthesis [43].

In contrast to younger persons, the anabolic response to protein has been reported to be diminished in the elderly when protein is ingested together with carbohydrates or as part of a mixed meal. An age-associated resistance in the response of muscle proteins to insulin has been suggested as primary reason [42].

Besides the amount and source of protein, and the presence of other nutrients, the distribution of protein intake over the day has been shown to modify protein turnover different in the elderly than in the young. In contrast to younger persons, higher protein turnover and better N retention were observed, with three meals per day and providing 80% of the daily protein intake to elderly women at lunchtime (pulse feeding), compared with an equal distribution of protein in four meals (spread feeding) [44].

In summary, during the last years many studies have increased our knowledge and added many details about protein metabolism and the anabolic effect of amino acids and proteins in the elderly. There is general agreement that moderate servings of EAA and also common protein-rich foods like lean beef are able to promote muscle protein anabolism in healthy elderly similar to healthy young people, and that protein anabolism is furthermore affected by meal composition, eating pattern, insulin action and physical activity. However, results are from short-term studies under experimental conditions and partly conflicting. Presently, they do not allow drawing firm conclusions with respect to optimal protein intake in the elderly.

5.9. Protein requirement and resistance training in the elderly

The anabolic response of the skeletal muscle is – in addition to protein intake and insulin action – mainly determined by physical activity. There is compelling evidence that resistance training increases the rate of muscle protein synthesis and induces muscle hypertrophy until very old age, and is effective in maintaining or even increasing muscle mass and function.

Adequate dietary intakes of protein and energy to enable anabolic responses and avoid the loss of muscle mass are crucial; however protein needs for optimal training effects with respect to protein gain and prevention of sarcopenia are not established.

Several studies in recent years have shown that resistance exercise increased muscle strength at protein intakes of 0.8 g/kg b.wt./d or higher, and that such training responses are not improved by higher protein intakes [45]. Because of observed decreases in LBM in subjects who performed resistance training and consumed diets containing 0.8 g protein/kg b.wt./d in contrast to moderately higher protein intakes (0.9-1.2 g/kg b.wt./d), slightly higher protein requirements for the maintenance of LBM and muscle mass were suggested.

Several supplementation studies have consistently shown that in elderly subjects who habitually consume adequate amounts of protein (modestly above 0.8 g/kg b.wt./d), supplemental protein intake, either immediately after or separate from exercise, did not further augment the training-induced improvements in muscle mass and strength [45].

5.10. Negative effects of high protein intakes

There is no evidence that high protein intake (up to 2.0 g/kg b.wt./d) is detrimental to renal function in healthy elderly. However, renal function decreases with age, and high protein intake is contraindicated in individuals with renal disease. Assessment of renal function is recommended for older individuals before they adopt a high-protein diet [46].

5.11. Conclusions

Our knowledge concerning optimal protein requirements in the elderly is still incomplete, and many questions are open despite a large body of research in recent years.

N balance studies in the elderly revealed conflicting results and suggest that not all elderly can achieve N balance with 0.8 g protein/kg b.wt./d. On the other hand, there are, at present, no studies convincingly showing that protein requirements of elderly people differ from those of younger adults.

Beyond the amount of protein needed for N balance, the optimal protein intake for the preservation of body composition, in particular muscle mass, and for health and the prevention of sarcopenia is of paramount

interest. At present, there is insufficient long-term research with defined health outcomes to derive recommendations in this regard.

Until more evidence is available, it seems reasonable to ensure a protein intake of 0.8 g/kg b.wt./d in all elderly, particularly in the frail and ill and in those who regularly perform resistance training, because their requirements may be slightly higher. Since even small discrepancies in energy balance can impair N balance, it is at least equally important to ensure appropriate energy intakes. Early recognition of nutritional difficulties and subsequent elimination is crucial. In addition to securing adequate protein and energy intake, physical activity should be encouraged in order to facilitate muscle protein anabolism.

Some studies suggest that slightly increased protein intakes above 0.8 g/kg b.wt./d might be beneficial for some elderly persons and appear to be safe. There is, however, no definite evidence that such increases are in fact beneficial in all healthy subjects. In particular, training responses are not improved by supplemental protein intakes.

More research is needed to firmly establish protein needs for N balance as well as the optimum amount, kind and pattern of protein intake for the preservation of muscle mass, function and health in the elderly. Future studies should cover longer time periods, include clinical endpoints (e.g. functionality) and focus on the comparison of different age groups, also within the elderly, as well as on different health and functional states, as it is likely that requirements change as people get older and develop chronic illness.

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6. Role of dietary proteins in sports

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6.1. Summary/ Zusammenfassung/ Résumé

The previously separated dietary protein recommendations for strength and endurance athletes are no longer supported, and the daily intake for adult athletes suggested by most of the entities is about 1.5 g/kg b.wt./d with a range of perhaps 1.0 to 2.0 g/kg b.wt./d. This recommendation is a broad landmark that needs to be adapted to the individual circumstances of the athlete. Research of the past decade indicates a beneficial effect in respect of a positive net muscular protein balance if athletes ingest some protein before an exercise bout. The amount of protein to be ingested to elicit the highest benefit is about 10 to 20 g/h, but due to the insufficient amount of available data, it is not possible yet to rank different protein types or sources according to their anabolic potential. A simple way to translate the nutrient-based recommendations is the Swiss Food Pyramid for Athletes, which ensures a sufficient intake of energy, all macro- and micronutrients in relation to the volume and intensity of the daily exercise.

Zusammenfassung: Proteinzufuhr bei Sportlern

Bis anhin wurden für Kraft- und Ausdauersportler verschiedene Proteinzufuhrempfehlungen abgegeben. Dies wird heute nicht mehr unterstützt. Als tägliche Proteinzufuhr für erwachsene Sportler empfehlen heute die meisten Gremien 1.5 g/kg KG mit einem Bereich von 1.0-2.0 g/kg KG. Diese Empfehlung ist jedoch nur ein grober Richtwert, der auf die individuellen Gegebenheiten des Sportlers angepasst werden muss. Forschungsergebnisse aus dem letzten Jahrzehnt weisen auf einen förderlichen Effekt auf das positive Netto-Muskelprotein-Gleichgewicht hin, wenn die Proteineinnahme vor einer sportlichen Belastung erfolgt. Damit der grösste Effekt erzielt wird, sollte die eingenommene Proteinmenge ungefähr 10-20 g/h betragen. Die ungenügende Datenlage lässt es jedoch noch nicht zu, die verschiedenen Proteinarten und -quellen nach ihrem anabolischen Potential einzuteilen. In der Schweizerischen Lebensmittelpyramide für Sportler werden die nährstoffbasierten Empfehlungen auf einfache Art für die Praxis umgesetzt. Abhängig vom täglichen Sportumfang und der Intensität wird damit die ausreichende Zufuhr an Energie sowie allen Makro- und Mikronährstoffen sichergestellt.

Résumé : Apport en protéines chez les sportifs

Toutes sortes de recommandations ont été formulées, puis abandonnées, à propos de l'apport quotidien en protéines nécessaire aux sportifs de force ou d'endurance. La plupart des organisations préconisent aujourd'hui pour les athlètes une ration de 1,5 g/kg/jour, avec une fourchette allant parfois de 1,0 à 2,0 g/kg/jour. Cette recommandation n'a toutefois qu'une valeur indicative et doit être adaptée à chaque cas d'espèce. Il ressort des recherches des dix dernières années que l'absorption de protéines peu avant l'effort sportif aboutit à un gain net en termes de protéines musculaires. Pour obtenir un effet maximum, il faudrait en ingérer de 10 à 20 grammes par heure. Les données sont toutefois encore trop lacunaires pour permettre de classer les divers types ou sources de protéines en fonction de leur potentiel anabolique. La pyramide alimentaire suisse pour les sportifs contient sinon des recommandations faciles à suivre, basées sur les substances nutritives. D'où la garantie d'un apport suffisant en énergie ainsi qu'en macro- et micronutriments, selon l'intensité et la durée de l'exercice *quotidien*.

6.2. Introduction

The belief of meat being one of the most important nutritional factors for athletes is deeply rooted in history. Accordingly, already earliest accounts mention the ingestion of enormous amounts of meat by winners of the ancient Olympic Games [1]. Legendary in this respect is Milo, the Krotonian wrestler, who in the 6th century BC won 32 times at different ancient games, six of which at Olympia [1]. In his book "The Deipnosophists", the Greek Philosopher Athenaios quoted a now lost work of Theodoros of Hierapolis, according to which Milo was eating 20 minas of meat and the same amount of bread as well as three choes of wine per day [2]. Assuming a conversion factor of about 500 g per mina, this would result in each 10 kg of meat and bread. With an average protein content of 20 g/100 g meat and 9 g/100 g bread one gets a total of 2900 g of protein, or, assuming a body mass of 80 kg, a relative daily protein intake of about 36 g/kg b.wt. This figure is obviously unrealistic, but it could just be exaggerated by a factor of ten because of handing down the story through time. This emphasis on meat, and later specifically on protein, has remained a mainstay of diets for athletes: for example, according to the Official Report of the 1936 Olympic Games in Berlin, the average meat consumption of the athletes was 687 g/d [3]. With an average protein content of 20 g/100 g meat and this time an average body mass of all athletes of about 70 kg, this would correspond to a relative daily protein intake of about 2 g/kg b.wt. alone from meat consumption.

By mid of the 19th century the chemical analysis of foods had progressed in a way that protein was identified as a major constituent of meat. This likely (mis)led the most influential chemist of the time, the German Justus Liebig, to the assumption that protein must be the main energy source for muscular work [4]. This notion was definitely proven wrong only with an 1866 experiment of the physiologist Fick and the chemist Wislicenus, both at the University of Zurich, where they clearly could ascribe the origin of the energy derived for muscular work to non-nitrogenous substances, i.e. carbohydrates and fats [5]. Nonetheless, the general emphasis on protein remained, with merely one difference: the focus switched from energy source to muscle growth.

6.3. Protein and body mass accretion in sports

Similar to Liebig's belief that protein must be the energy source for muscular work, today the general belief is that a high dietary protein intake is necessary for muscle-related body mass accretion because muscle contains high amounts of protein. This belief is mainly associated with resistance type sports, and only to a much lesser extent to endurance type activities. The current scientific evidence, however, shows a different and certainly much more sophisticated picture. Additionally, several factors independent of dietary protein are relevant for body mass accretion with athletes.

The general prerequisite for body mass accretion over a moderate to long period is a positive energy balance. However, a positive energy balance alone will not lead to a net increase in muscle mass. Recent reviews named the factors that influence or are necessary for muscle mass accretion: aging (i.e. becoming older), gender, exercise stimulus, co-ingestion of protein with other nutrients, and timing of protein intake [6-8]. Another factor sometimes discussed to be necessary for net muscular protein accretion is a sufficient hydration of the myocytes, although most of the evidence for this stems from in vitro studies with hepatocytes [9]. In any case, the sole consideration of dietary protein in respect to body mass accretion or net muscle mass gain would certainly be too simplistic. Furthermore, considering the factor "dietary protein" on its own, the focus moved away from the total amount of protein needed with athletes some ten years ago

[10] to aspects such as type of protein and plus timing of protein ingestion. But in spite of the progress of recent years, there are still many open questions, and the ideal regimen for optimal muscle mass accretion has yet to be identified.

The metabolic response to dietary proteins depends among others on the general delivery method of the protein (e.g. through entire foods such as milk and meat, or through protein isolates ingested as powder) and the type of the protein itself (e.g. casein, whey or soy protein) [11]. Furthermore, co-ingestion of other foods or nutrients might affect the metabolic handling of the protein ingested during exercise [12;13] and also the general state of the subject (e.g. fasted, non-fasted, exercise-trained, sedentary) impacts how dietary protein are used in the body [14]. Since obviously the amount of ingested protein influences protein metabolism too, it becomes clear that at present it is hardly possible to identify a single protein type, and even the total amount of protein that modulates body mass accretion in a best possible way. Nevertheless, some broad recommendations do exist, and they are discussed below.

6.4. Protein and body mass loss in healthy athletes

Obesity is a worldwide epidemic, and many consider loss of body mass a health promoting measure for the obese. The reason for body mass loss in sports is different and includes the wish to increase the force to mass ratio of an athlete, or to achieve a certain weight category such as in wrestling or rowing.

A fundamental prerequisite for body mass loss is a negative energy balance, and both, body fat and lean body mass contribute to total body mass loss. In obese subjects, an increased proportion of protein in the diet may increase body mass loss by increasing the loss of body fat, while the loss of lean mass may be reduced [15;16]. The same was recently reported for healthy young resistance-trained athletes, where the loss of lean body mass with a protein supply of about 1.0 g/kg b.wt./d even exceeded body fat losses [17]. In dieting obese, the lean body mass usually contributes less than 20% to total body mass losses, independent of the protein supply [15;16;18]. In contrast, the dietary supply seems to be relevant for athletes, as losses of lean body mass were significantly reduced when the athletes received a high protein supply of about 2.3 g/kg b.wt./d [17].

Body fat content seems to be a strong predictor for loss of lean body mass during weight loss, not only in man, but among different species [18]. The leaner a person or an animal is the more of the lost body mass comes from lean body mass. However, at least in human athletes a high protein supply may significantly reduce this lean body mass loss during a short-term hypo energetic weight loss intervention [17]. However, due to the paucity of data, it is difficult to establish a protein dose for an optimal body mass loss. The available information indicates that relatively high protein levels, possibly over 2.0 g/kg b.wt./d, are needed to optimally preserve lean body mass in lean athletes during hypo energetic body mass loss [17;19].

6.5. Protein ingestion during exercise

The investigation of protein or amino acid intake during exercise has recently regained some interest. The reason for this is that one might expect a reduction of net negative protein balance observed during exercise and perhaps even an improved performance due to sparing of glycogen stores. The available data, however, show a mixed picture, with some studies indicating no effect on exercise performance [20-22] and others reporting some exercise improvements with a protein plus carbohydrate co-ingestion [23;24].

Nevertheless, even if no direct performance effects might be apparent when comparing protein plus carbohydrate co-ingestion with sole carbohydrate ingestion during exercise, any effect on net protein balance might have long-term consequences and so indirectly influence performance through optimized long-term training effects. Some findings on the co-ingestion of protein plus carbohydrates during resistance exercise support this notion, as the addition of protein caused a switch from a net negative to a net positive whole body protein balance [12]. Further, a higher protein synthesis rate with carbohydrate plus protein co-ingestion occurred with during mixed endurance-resistance exercise [13].

6.6. Protein ingestion after exercise

The possible influences on post-exercise glycogen resynthesis and net muscle protein accumulation constitute the two main interests with protein ingestion after an exercise bout. Early data indicated an improved glycogen resynthesis after exercise with protein plus carbohydrate co-ingestion, but the situation now seems to be equivocal. Some authors reported no additional effect, while others still observed increased glycogen resynthesis rates with carbohydrate plus protein co-ingestion [25]. In any case, a sufficiently high carbohydrate provision of about hourly 1.5 g/kg b.wt. – with or without addition of protein – is certainly recommended from the perspective of an optimal glycogen recovery. If addition of protein does or does not lead to increased post-exercise glycogen resynthesis is not extremely relevant, as such addition is generally considered beneficial for an improved net protein balance and no negative effects are known. Interestingly, though increased amino acid availability is necessary for achieving a net positive protein balance in the post-exercise state, the presence of insulin might be more important [8]. This would mean that a protein plus carbohydrate co-ingestion might be indeed the general option of choice for the post-exercise macronutrient intake.

Because of the issues outlined above in relation to the optimal protein type for body mass accretion it is for the moment, however, not possible to indicate if one protein is superior to another in respect of achieving an optimal positive protein balance. Although there seems to be agreement that protein intake in the temporal proximity of an exercise bout elicits a more beneficial net anabolic muscle protein response [6], it is still an unresolved question if the protein intake shall best occur shortly before, during or shortly after exercise. Whatever time point might be best, a bolus of 20 g protein seems to be sufficient to maximize the net anabolic response over a period of a few hours [26].

6.7. Protein and amino acid supplements in sports

It is perhaps an intrinsic hope of humans to find a magic bullet that either leads to eternal health and longevity or, in case of athletes, to a better performance. The market of ergogenic substances is correspondingly enormous, and many protein, peptide, or amino acid supplements are available over the counter – most of them with no accompanying scientific evidence for their claimed ergogenic effects. The provision of any nutritional component out of its context of the original food matrix, in an unusual amount, or in a relation to other components that is not similar to the original food is questionable by principle. Nonetheless, the use of certain protein supplements can make the athlete's life easier. It is certainly much more convenient to use a protein shake during or directly after an exercise bout than preparing a steak or a slice of tofu in the dressing room. However, the usual athlete's diet very likely already provides a sufficiently high amount of dietary protein to meet his or her requirements and thus, one need to view protein "supplements" rather as a substitution of a part of the diet and not as an addition to it. As mentioned in the

previous paragraph, a bolus of 20 g protein seems sufficient for an optimal short-term metabolic response. Moreover, this amount might even be smaller for certain types of protein [27].

Some rationales exist according to which supplementations with single or mixtures of amino acids or protein hydrolysates could lead to higher absorption rates and so a more rapid delivery of the amino acids to the muscle. Due to the great number of possible hydrolysates and combinations of amino acid mixtures it is, however, also here not possible to pinpoint a single supplement or amino acid and attribute the label "optimal" in respect of net muscle balance to it. In a recent review on sports nutrition and ergogenic substances the only substances of amino acid or protein nature listed as apparently effective and generally safe were "protein" in general, essential amino acids, and β -alanine [28]. A question mark, however, needs to be set after β -alanine, as – in spite of being a non-essential amino acid – only little information is available concerning potential negative effects with a long-term use. The only reported side effect so far with short time supplementation is paresthesia (i.e. the sensation of numbness of the skin) [29] and this indicates that the supplementation affects neurobiochemical pathways and consequently side effects cannot be ruled out.

6.8. Protein recommendations in sports

The time of dietary protein recommendations for healthy athletes of much more than 2 g/kg b.wt./d has come to an end. Today, basically all existing recommendations revolve around 1.5 g/kg b.wt./d with a range of 1.0 to 2.0 g/kg b.wt./d [28;30]. More important than this general recommendation is the notion that such recommendations must be better defined individually for each athlete and only can be considered as broad indications [31]. Further, this broad recommendation applies to all athletes, and separate recommendations for strength and endurance athletes are, in general, no longer advised.

6.9. Conclusions

The general focus on dietary protein in sports is not accentuated as much as it was some time ago. Furthermore, one now considers dietary protein in a much more complex mode; a turning away from only looking at the total amount of protein intake was apparent during the last decade. As many aspects influence the metabolic fate of dietary protein in exercising subjects, it is extremely difficult to derive sound recommendations that have general validity. Though most entities recommend a daily intake of 1.0 to 2.0 g/kg b.wt./d, it is clear that such a recommendation needs to be adapted to the individual circumstances of the exercising athlete. It seems also a good choice to ingest protein, perhaps 10 to 20 g/h, in the temporal proximity of each exercise bout, allowing for a best possible net muscular protein balance.

One general problem with dietary recommendations is their practical usability. Because no simple, food-based dietary guidelines existed for adult athletes, the Swiss Forum for Sport Nutrition developed such a guideline based on the existing Swiss Food Guide Pyramid.

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7. Dietary proteins in obesity and in diabetes

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7.1. Summary/ Zusammenfassung/ Résumé

Dietary proteins influence body weight by affecting four targets for body weight regulation, namely satiety, thermogenesis, energy efficiency, and body composition. Protein ingestion results in more satiety than aequicaloric amounts of carbohydrates or fat. Their effect on satiety is mainly due to oxidation of amino acids fed in excess; this effect is higher with ingestion of specific "incomplete" proteins (vegetal proteins) than with animal proteins. Diet-induced thermogenesis is higher for proteins than for other macronutrients. The increase in energy expenditure is caused by protein- and urea synthesis and to gluconeogenesis. This effect is higher with animal proteins with larger amounts of essential amino acids than with vegetable proteins. Diet-induced thermogenesis after protein ingestion is increased by 20-30% versus 5-10% after carbohydrates and 0-5% after ingestion of fat.

Consumption of higher amounts of protein in obesity resulted in greater weight loss than during lower amounts of protein in the diets in studies lasting up to one year. During weight loss and decreased caloric intake, a relatively increased protein content of the diet maintained fat-free mass (i.e. muscle mass) and increased calcium balance, resulting in preservation of bone mineral content. This is of particular importance during weight loss after bariatric surgery because these patients are at risk for protein malnutrition.

Adequate dietary protein intake in diabetes type 2 is of specific importance since proteins are relatively neutral with regard to glucose and lipid metabolism, and they preserve muscle and bone mass, which may be decreased in subjects with poorly controlled diabetes. Ingestion of dietary proteins in diabetes type 1 exert a delayed increase in blood glucose levels due to protein-induced stimulation of pancreatic glucagon secretion.

Higher than minimal amounts of protein in the diet needed for nitrogen balance may play an important role in the increasing number of obese elderly subjects in our industrialised societies, since proteins exert beneficial effects on overweight, metabolic syndrome, cardiovascular risk factors, bone health and sarcopenia. Adverse effects of increased dietary proteins have been observed in subjects with renal impairment- this problem is frequently observed in the elderly, hypertensive and diabetic population.

Therefore, protein intakes of more than RDA amounts (0.8 g/kg b.wt./d) up to 1.5 g/kg b.wt./d appear to exert widespread health benefits excepting in cases where renal function is impaired. However, this should be a recommendation to be handled with caution because there are not enough long-term data to be able to assess the benefit/risk ratio with certainty. Nevertheless, dietary proteins deserve more attention in the future than they have received in the past.

Zusammenfassung: Proteinzufuhr bei Adipositas und bei Diabetes mellitus

Nahrungsproteine beeinflussen das Körpergewicht durch vier Mechanismen. Sie wirken auf Sättigung, Thermogenese, Energieeffizienz und die Körperzusammensetzung. Die Einnahme von Nahrungsproteinen führt zu einer höheren Sättigung als gleiche Energiemengen aus Kohlenhydraten oder Fetten. Ihre Wirkung auf das Sättigungsgefühl ist hauptsächlich auf die Oxidation von Aminosäuren zurückzuführen; dieser Effekt ist nach der Einnahme von bestimmten "unvollständigen" (pflanzlichen) Proteinen ausgeprägter als nach

tierischen Proteinen. Die nahrungsinduzierte Thermogenese ist höher bei Proteinen als bei anderen Makronährstoffen. Sie ist bedingt durch vermehrte Protein- und Harnstoff-Synthese und durch Gluconeogenese. Der Effekt ist stärker nach der Einnahme von tierischen Proteinen, die einen größeren Anteil an essentiellen Aminosäuren haben, als nach der Einnahme von pflanzlichen Proteinen. Die nahrungsinduzierte Thermogenese beträgt nach der Einnahme von Proteinen 20-30% des Energiegehalts, gegenüber 5-10% nach Kohlenhydraten und 0-5% nach der Einnahme von Fetten.

In Studien mit einer Dauer von bis zu einem Jahr führen proteinreiche Diäten bei Übergewicht und Adipositas zu grösserem Gewichtsverlust als bei „normalen“ Mengen von Proteinen. Während der Gewichtsabnahme durch verminderte Kalorienzufuhr bewirkte eine relativ proteinreiche Ernährung einen Erhalt der fettfreien Körpermasse (d.h. Muskelmasse) und eine erhöhte Kalzium-Retention, was dazu führte, dass der Knochenmineralgehalt erhalten blieb. Dies ist von besonderer Bedeutung bei der Gewichtsabnahme nach bariatrischer Chirurgie; diese Patienten haben ein erhöhtes Risiko einer Proteinmangelernährung.

Eine ausreichende Proteinzufuhr in der Ernährung ist bei Diabetes Typ 2 von besonderer Bedeutung, da Proteine relativ neutral sind in Bezug auf Glucose- und Fettstoffwechsel, und da sie die Muskel- und Knochenmasse, die bei Patienten mit schlecht eingestelltem Diabetes oft verringert sind, erhalten. Die Einnahme von Proteinen bei Diabetes Typ 1 bewirkt einen verzögerten postprandialen Blutzuckeranstieg durch eine proteininduzierte Stimulation der Glucagonsekretion des Pankreas.

Eine höhere Zufuhr von Proteinen als minimal nötig zur Aufrechterhaltung der Stickstoffbilanz spielt eine zunehmend wichtige Rolle im wachsenden Teil der übergewichtigen älteren Menschen in industrialisierten Gesellschaften, da Proteine eine positive Auswirkung auf Übergewicht, Metabolisches Syndrom, Herz-Kreislauf-Risikofaktoren, Knochengesundheit und Sarkopenie haben können. Negative Auswirkungen einer erhöhten Proteinzufuhr wurden bei Patienten mit eingeschränkter Nierenfunktion beobachtet. Dieses Problem besteht gehäuft in der älteren Bevölkerung sowie bei Diabetes und hohem Blutdruck.

Die Erhöhung der Proteinzufuhr von Recommended Daily Allowance (RDA)-Mengen (0.8 g/kg KG) bis zu 1.5 g/kg KG scheint keine nachteilige Wirkung auf die Gesundheit zu haben, es sei denn, die Nierenfunktion ist beeinträchtigt. Eine Empfehlung zur Steigerung der Proteinzufuhr sollte jedoch mit Vorsicht behandelt werden, weil es nicht ausreichend langfristige Daten zur Beurteilung des Nutzen-Risiko-Verhältnisses gibt. Dennoch sollten Nahrungsproteine in der Zukunft vermehrte Aufmerksamkeit erhalten.

Résumé : Apport en protéines en cas d'obésité et de diabète sucré

Les protéines alimentaires influencent le poids corporel en agissant sur quatre mécanismes : la satiété, la thermogénèse, l'efficacité énergétique et la composition corporelle. A calories égales, l'ingestion de protéines apporte une plus grande satiété que celle d'hydrates de carbone ou de graisses. Leur effet sur la satiété est principalement dû à l'oxydation des acides aminés excédentaires. L'effet sera encore plus marqué s'il s'agit de protéines spécifiques « incomplètes » (végétales) et non de protéines animales. La thermogénèse induite par l'alimentation est plus élevée pour les protéines que pour les autres macronutriments. L'augmentation de la dépense d'énergie est due à la synthèse accrue des protéines et de l'urée, ainsi qu'à la néoglucogénèse. L'effet sera d'autant plus grand s'il s'agit de protéines animales, renfermant de plus grandes quantités d'acides aminés essentiels, que de protéines végétales. En cas d'ingestion de protéines, la thermogénèse oscille entre 20 à 30% de l'apport énergétique, contre 5 à 10% pour les glucides et 0 à 5% pour les graisses.

En cas de surpoids ou d'obésité, la consommation de quantités élevées de protéines conduit à une plus grande perte de poids qu'un régime isocalorique moins riche en protéines, selon des études scientifiques s'étendant sur un an au maximum. En outre, le poids a beau diminuer sous l'effet du moindre apport calorique, la teneur relativement élevée en protéines du régime alimentaire maintient la masse maigre (p. ex., masse musculaire) et l'équilibre du calcium, préservant la masse minérale osseuse. Cela est particulièrement important dans la perte de poids après la chirurgie bariatrique, ces patients ont un risque accru de malnutrition protéique.

Un apport bien dosé en protéines alimentaires revêt une importance particulière en cas de diabète de type 2. En effet, les protéines sont relativement neutres par rapport au glucose et au métabolisme lipidique, et préservent de surcroît la masse musculaire et osseuse souvent réduite des sujets présentant un diabète mal contrôlé. En cas de diabète de type 1, l'ingestion suffisante de protéines alimentaires provoque une élévation retardée de la glycémie postprandiale, car les protéines stimulent la sécrétion du glucagon pancréatique.

Un apport accru de protéines dans l'alimentation (au dessus du minimum nécessaire pour un bilan azoté équilibré) peut s'avérer précieux pour la population vieillissante et souvent obèse du monde industrialisé. Les protéines exercent des effets bénéfiques sur le surpoids, sur le syndrome métabolique, les facteurs de risque cardiovasculaire, la santé osseuse et la sarcopénie. Un tel régime peut toutefois provoquer des effets secondaires chez les sujets présentant une insuffisance rénale, problème fréquent parmi la population âgée, souffrant d'hypertension et de diabète.

En résumé, un apport en protéines supérieur à l'apport journalier recommandé (0,8 g/kg), sans excéder 1,5 g/kg, semble présenter de multiples avantages pour la santé, à moins d'une altération de la fonction rénale. Les recommandations d'accroître l'apport en protéines seront toutefois émises avec prudence, les données à long terme étant trop peu nombreuses pour permettre d'évaluer avec certitude le rapport bénéfice/risque. Quoi qu'il en soit, les protéines alimentaires méritent qu'on leur accorde à l'avenir davantage d'attention que par le passé.

7.2. Introduction

Overconsumption of the energy containing macronutrients fats, carbohydrates and proteins is one of the major contributors to the development of obesity. However, compared to the adipogenic role of excess dietary carbohydrates and fats, the contribution of dietary proteins is generally considered to be less important, and the specific effects of proteins on body weight regulation and on postprandial metabolism and hormone signals is frequently ignored.

Although most people ingest only approx 1/6 of total energy intake as proteins, they have metabolic features which are anti-"obesogenic" as outlined below. Dietary proteins play a specific role in obesity during weight loss and during subsequent weight maintenance.

Dietary proteins in diabetes are often said to be metabolically "neutral", and nutrition recommendations like those of the American Diabetes Association [1] state that protein requirements in diabetes are "normal", however, there are several aspects of nutritional proteins which deserve special attention in this growing population.

Protein requirements in overweight and obese subjects have been discussed in a previous chapter (4, page 64).

7.3. Effects of proteins on satiety

A hierarchy prevails for the satiating effects of macronutrients, with proteins being the most and fat the least satiating per calorie [2,3].

In daily life, mixed proteins are consumed from meat, fish, dairy products and plants. A dose-dependent satiating effect of mixed protein has been shown, and persistent protein-induced satiety is shown when a mixed high-protein diet is given for 24 hours up to several days [3-5]. Using meals with an increased contribution of proteins (20% to 30% of energy rather than 10-15%) satiety and energy expenditure in healthy subjects were significantly increased. Casein, whey and soy protein similarly increased postprandial ghrelin, GLP-1, insulin and cholecystokinin. These are all candidate hormones contributing to the increased satiety after protein ingestion [6].

Protein-containing meals diminish caloric intake 2 to 3 hours later by 10-20% compared to isocaloric meals without proteins in normal subjects [7,8]. This effect was particularly shown with breakfasts containing milk proteins (whey, dairy fruit drinks or chocolate milk). It was also observed in obese boys [9].

There may be some specificity in the effects of individual amino acids on satiety since certain amino acids also serve as precursors for specific neurotransmitters involved in appetite or body-weight regulation or directly influence biochemical pathways involved in eating behaviour. For instance, the amino acid tryptophan may be involved since it may be converted to serotonin, and it has been suggested that brain serotonin is involved in appetite regulation, a hypothesis that is supported by the anorexigenic effects of serotonergic drugs in humans (review in [3]). Alternatively, tyrosine can be converted to dopamine and norepinephrine, both of which have shown to be involved in food-intake regulation. A third amino acid that functions as a precursor for a neurotransmitter is histidine, which can be converted into the anorexigenic neurotransmitter histamine.

Protein-induced satiety is also related to vagal feedback to (a) the nucleus tractus solitarius in the brainstem, where it signals satiety at almost a reflex level, and (b) the hypothalamus, where it suppresses feelings of hunger. It has been shown that at the molecular level, adenosine monophosphate (AMP)-activated protein kinase (AMPK) and the mammalian target of rapamycin (mTOR) are involved in protein-induced satiety [10]. When high-protein diets (20% to 30% of energy) from protein were ingested over prolonged periods of time, a higher satiety was shown throughout the day [2,3] than with normal protein diets.

These results are from relatively small studies with limited duration. They demonstrate that there increased protein intake is more satiating and promotes less energy intake, supported by increased plasma amino acid concentrations, anorexigenic hormones and energy expenditure, feeding back on the central nervous system.

7.4. Effects of proteins on energy expenditure

Protein Intake and diet-induced energy expenditure (DEE)

The main determinants of diet-induced energy expenditure (thermogenesis) are: 1. the energy content, and 2. the protein fraction in the food.

In healthy subjects consuming a mixed diet, DEE represents about 10% of total energy ingested over 24 hours (see [3]). The most common way to express DEE is to use the difference between energy expenditure after food consumption and basal energy expenditure, expressed as percent of caloric intake. Reported DEE values are 20 to 30% for proteins, 0 to 3% for fat, 5 to 10% for carbohydrates [11], and 10 to 30% for alcohol (in [3]). The effect of protein on DEE is clearly illustrated by the difference between the gross caloric (energy) value and the net metabolically energy value of 5.3-6 kcal/g protein vs. 3.1 kcal/g.

High-protein diets increase energy expenditure [2,4], which implies that oxygen consumption and heat production are increased. Potential mechanisms include the need for ATP required for the initial steps of metabolism, storage, urea and protein synthesis, and gluconeogenesis. This feature of proteins is also called “metabolic inefficiency”; it is due the low metabolic efficacy of protein oxidation compared to glucose; the calculated energy expenditure to produce ATP is 37 kcal/mole ATP for cysteine versus 22 kcal/ATP for glucose (in [3]).

The body has no storage capacity for excess proteins, and therefore needs to oxidize them. When amino acids are ingested in excess of the needs for protein synthesis, gluconeogenesis and consequently glycogenesis are increased since complete oxidation of all excessive amino acids would provide more ATP than the liver could utilize [12].

Protein Intake and sleeping metabolic rate

High-protein diets also increase also resting and sleeping metabolic rate (SMR) by 2-3% [4,13]. This occurred despite a 10% to 15% lower energy intake in these studies during the high compared to normal protein diets due to their higher satiating effect. The sustained slight increase in SMR after three days of high protein diets can be explained by stimulation of protein synthesis and protein turnover. The increases in thermogenesis and protein synthesis are higher with animal than with vegetable proteins [13] because animal proteins have an essential amino acid composition closer to human proteins than vegetable proteins. Animal proteins have therefore a higher “biological value” than plant proteins.

Relatively high-protein diets for weight loss and subsequent weight maintenance consist of at least 25% to 30% of energy from protein; thus, protein intake remains the same (in gr/d) as it was before the weight loss diet while total energy intake is decreased [3].

7.5. Protein turnover and effects of dietary proteins on insulin sensitivity

The main determinants of protein turnover are protein intake, fat free mass (for which body proteins play a major role), and age. Daily protein intake is essential for the maintenance of protein synthesis, and protein requirements (**Tab. 1**) are approx. 10-20% of total body protein turnover, depending on the type of protein.

Tab. 1: Recommended dietary protein intake in healthy, obese and diabetic subjects (references in [3]).

	Relative energy % of protein	Absolute g proteins/d
Weight-maintaining diets in healthy subjects		
-WHO*	10-15%	67-100 g/d
-DACH*		0.8 g/kg; ♂: 54-60 g/d ♀: 44-48 g/d
-DRI*	10-35% AMDR**	0.8 g/kg/d (RDA)
Diabetic Subjects (ADA*; [1])		DRI recommendations (exception: of diabetic nephropathy***)
During weight loss (negative energy-balance; e.g. 600 kcal/d)		
“Normal” protein intake	10-15%	11-17 g/d
“High” protein intake	40% (VLCD)*	52 g/d
Weight-maintaining diets after weight loss (e.g. 1900 kcal/d)		
“Normal” protein intake	10-15%	44-67 g/d
“High” protein intake	18-30%	80-120 g/d

***Abbreviations:**

WHO, World Health Organisation; DACH, German, Austrian and Swiss Nutrition Societies
DRI, Dietary reference intake (USA) ADA, American Diabetes Association
VLCD, Very low calorie diet FFM, fat free mass

RDA, recommended dietary allowance

** AMDR = Acceptable Macronutrient Distribution Range

***Reduction of protein intake to 0.8-1.0 g/kg b.wt./d in individuals with diabetes and an early stage of chronic kidney disease (CKD), and to 0.8 g/kg b.wt./d in late stage of CKD improves measures of renal function (urinary albumin excretion rate and GFR) and is recommended [1].

Since in obesity not only fat, but also fat free mass is increased, protein requirements are increased (when expressed as grams per day). Whole body protein turnover, plasma free amino acid concentrations and gluconeogenesis are increased in obese versus normal weight subjects in most studies [14]. However, when expressed per kg fat free mass, protein turnover (and protein requirements) in obesity are similar to those in normal-weight subjects.

Impaired sensitivity to insulin is a hallmark of obesity. This not only relates to glucose and lipid, but also to protein metabolism. Insulin resistance of protein metabolism indicates higher rates of amino acid oxidation and gluconeogenesis; this effect is counteracted by an increase in plasma insulin levels which is typically observed in non-diabetic obese subjects. This process is responsible for the fact that a higher fraction of postprandial hepatic glucose production is due to gluconeogenesis rather than to glycogenolysis. Excess dietary proteins may impair insulin sensitivity of glucose metabolism in obesity further, they promote gluconeogenesis and enhance insulin resistance (review in [15]).

Insulin resistance of obesity is associated with low grade inflammation. When overweight subjects were given supplements with dairy proteins, markers of inflammation and of oxidative stress were decreased, in contrast to supplements with soy proteins [16].

7.6. Protein-enriched diets for weight loss in obesity

Protein sparing diets poor in carbohydrates and fat were popular in the early 70ies of the last century (Protein Sparing Modified Fast). Drop-out rates were high and long-term weight results frequently poor, and cases with lethal outcome were reported, due to the poor protein quality of the diet, in particular to liquid proteins, and to hypokalaemia [17].

In the last decade there was a resurgence of interest in low carbohydrate diets; they contained “normal” [18,19] or increased (22% versus 17% of energy; [20]) amounts of proteins. All these studies showed increased weight loss after “low carb” compared to “low fat”, and serum lipids were also improved. However, the difference in weight loss was not maintained after 1 year [18]. Prolonged intake of high-protein weight loss diets (26% of total energy, or 1.2 g/kg b.wt./d compared to 19% of total energy during a low fat diet) appeared to confer not only a modest weight-loss benefit but also to improve cardiovascular disease risk factors and serum vitamins and minerals; all these secondary effects were due to weight loss and not to types of diet [21]. Most studies on weight loss diets with increased protein content (31% of total energy, i.e. 1.14 g/kg b.wt./d versus 18%, or 0.64 g/kg b.wt./d) showed not only decreased body weight but also an improved body composition (i.e., an increased fat free versus fat mass) and lower serum triglycerides [22]. Cardiovascular risk factors in women of the Nurses Health Study were lower in the highest vs. the lowest quintile of protein intake [23]. Satisfaction with the diet during weight loss was greater [24] or similar [25] during high-protein diets compared to low protein diets. Doubling the relative protein content of the diet from the normal level of 10 to 15 energy % to 20 to 30 energy % reduced food intake under ad libitum conditions [3].

The effect of high-protein diets was not always accounted for by differences in caloric intake or physical activity, suggesting a direct metabolic effect of dietary protein [3]. Nevertheless, most studies lasted only 12 months or less, and they showed a high attrition rate, the rates were similar during the different dietary interventions [26].

A recent large study compared 4 different weight loss regimens in 811 obese subjects. Weight loss was similar in the normal protein and the high protein groups (15% vs 25% of total energy). The diets were planned with an expected caloric deficit of 750 kcal per day. However, compliance to all diets must have been poor since mean weight loss after 2 years was only 4 kg [25].

The positive association of meat consumption with body weight was observed in a large prospective cohort study in a total of 103,455 men and 270,348 women (EPIC-PANACEA study) in 10 European countries. 250 g meat per day was correlated with a 2 kg higher body weight after 5 years [27].

Sufficient protein intake during rapid weight loss after gastric bypass for the treatment of morbid obesity is particularly important for the maintenance of lean body mass. Insufficient amounts of protein are frequently consumed by operated patients up to one year after surgery, and a further problem is that these patients complain frequently of impaired protein tolerance [28].

Dietary proteins for weight maintenance after weight loss

Weight maintenance after weight loss in obese subjects depends on (a) sustained control of satiety (b) sustained (and not reduced) basal energy expenditure (c) maintained (and not reduced) fat-free mass, the major determinant of basal energy expenditure (review in [3]). Diets with a relatively high-protein content act on all these three metabolic targets.

Weight regain after weight loss during a high-protein diet was 0.8 kg versus 3.0 kg during a normal protein diet ($p < 0.05$) after six months. The absolute amount of protein is of greater importance than the percentage of protein [3]. Protein malnutrition is a rare but severe complication of weight loss after gastric bypass surgery.

7.7. Protein intake after bariatric surgery for the treatment of obesity

Protein malnutrition, defined by hypoalbuminemia (serum albumin <3.5 mg/dl), remains the most severe macronutrient complication associated with malabsorptive surgical procedures. Less than 5% of patients receiving a gastric bypass operation develop protein malnutrition up to 43 months postoperatively [29].

When it occurs, protein malnutrition is generally observed at 3-6 months after surgery and is largely attributed to the development of food intolerance to protein rich foods. Protein-deficient meals are common after RYGB. Prevention of protein malnutrition requires regular assessment of protein intake and counselling regarding ingestion of protein from protein-rich foods and modular protein supplements. Protein needs are constant across all energy intakes. So at low energy intake, protein needs to be a higher percentage of total calories. Breakfast is an important meal for dietary protein because the body is in a catabolic state after an overnight fast. A meal with at least 30 g protein is required to initiate repletion of body proteins [29]. Protein at breakfast is also critical for regulation of appetite and daily food intake.

7.8. Dietary proteins in diabetes

Although there are no specific recommendations for protein intake for all patients with diabetes (**Tab. 1**), however, there are certain features of dietary proteins which deserve attention.

First, most subjects with type 2 diabetes are overweight or obese and therefore benefit from protein-induced satiety and energy expenditure; second, there are specific metabolic effects of proteins in diabetes due to hormone and substrate responses after meals. Third, diabetic subjects may be affected by nephropathy which impairs tolerance to dietary proteins [1]. In subjects with diabetes type 2, a high-protein weight-reduction diet (30% of energy) may in the long term have a more favourable cardiovascular risk profile than a low-protein diet with similar weight reduction [30].

Protein ingestion stimulates the secretion of the pancreatic hormones insulin and glucagons in healthy subjects and in patients with type 2 diabetes, resulting in counteracting effects on postprandial glucose metabolism. The stimulatory effect of proteins on insulin secretion is at least in part due to increases in gut-derived incretins (glucose-dependent insulinotropic peptide (GIP) and glucagon-like peptide 1 (GLP-1) [6,31], which both stimulate insulin secretion [32]. Postprandial plasma glucose levels were lowered in diabetic [33] and in obese subjects with insulin resistance [34]. In addition, dietary proteins decreased postprandial lipaemia in type 2 diabetes, and serum triglycerides [30] and LDL-cholesterol [34] were lowered. Whey protein outperformed other proteins in this respect, possibly because of the diminished formation and/or increased clearance of chylomicrons [31].

Protein turnover and catabolism is increased, and insulin resistance augmented in poorly controlled overweight diabetic subjects type 2, particularly in men [35]. Therefore, dietary protein requirements may be increased in hyperglycaemic type 2 diabetes to offset the reduced net protein balance [37].

In type 1 diabetes without residual insulin secretion, administrations of amino acids stimulates glucagon release from pancreatic β -cells while the hypoglycaemic stimulus of glucagon secretion is blunted [38]. The accelerated recovery from insulin-induced hypoglycaemia after amino acid infusion is due to the hyperglycaemic effect of glucagon release, in addition to the amino acid-induced increase in gluconeogenesis. Amino acids might also serve as substrates for the brain and therefore improve cognitive function during hypoglycaemia in insulin-treated diabetics [39]. When a protein-rich late night snack was given to subjects with type 1 diabetes, the risk of insulin-induced hypoglycaemia during the night was decreased [40].

Dietary proteins in diabetic nephropathy and proteinuria, should, however, be restricted [1]. Decreasing protein intake from 1.2 g to 0.9 g/kg b.wt./d, especially animal protein, beneficially influenced albuminuria, a risk marker for diabetic nephropathy [41].

7.9. Potential risks of high protein diets in obese and in diabetic subjects

Increased intake of dietary proteins leads to increased urinary excretion of nitrogenous waste products generated from protein catabolism, thereby increasing glomerular pressure and hyperfiltration [41]. Therefore, it was feared that high protein diets pose a risk of renal damage [43].

In subjects without renal impairment, changes in dietary protein intake caused adaptive increases in renal size and glomerular filtration rate without adverse effects [45]. In fact, the changes in renal function (induced by high dietary protein) are a normal adaptive mechanism, and there is little evidence that high-protein diets pose a serious risk to kidney function in healthy populations [42]. Long-term daily protein intakes up to 2.8 g/kg b.wt. were shown to have no negative effects on renal function in athletes [44].

Sulfur containing amino acids may cause a blood pressure-raising effect by maintaining acid-base homeostasis through excretion of the excess acid load by the kidneys (26, 38), and this effect may lead to further nephron mass [46]. However, this did not translate to increased blood pressure in an intervention trial. On the contrary, subjects with prehypertension or hypertension showed a small but statistically significant reduction of blood pressure when a part of dietary carbohydrates were replaced by proteins (OMNI Heart trial [46]). Protein substitution lowered also serum triglycerides and increased HDL-C. However, subjects with subclinical renal injury, including elderly people, or subjects with low renal functional mass such as renal transplant recipients, and persons with obesity-related conditions, such as metabolic syndrome and type 2 diabetes, are susceptible for a blood pressure raising effect of dietary proteins [46],[48],[49].

A synergy between obesity, type 2 diabetes and low nephron number is typical [48] and may lead to an increased risk of protein-induced renal impairment. Therefore, in these subjects and in patients with renal disease high protein diets may have adverse effects [43].

Increased protein intake in childhood between the ages of 12-24 months has been associated with increased BMI at age 7 yrs (DONALD study) [49]. In a Danish study, protein intake at the same age was correlated with height at age 10 yrs [51]. Protein intake, particularly animal proteins, correlated significantly with the risk for type 2 diabetes in the EPIC-NL study [52] and with the risk of gestational diabetes in women [53].

Opponents of high-protein diets are also concerned about the interference of high protein intake with calcium homeostasis. Indeed, several short-term studies demonstrated increased renal calcium excretion and negative calcium balance at intakes of 2.0 g/kg b.wt. protein daily versus 0.7-1.0 g/kg b.wt. daily [43]. The acid load generated by high-protein diets is usually blamed for this effect, since it would be partially buffered by bone, which results in bone resorption and hypercalciuria unless alkali-rich foods such as fruits and vegetables are consumed [54]. Whether high protein diets are deleterious for bone health is discussed in detail elsewhere in this report.

There are also concerns regarding cardiovascular health during high protein diets. The American Heart Association stated in 2001 that high-protein diets are not generally recommended because they restrict healthful foods that provide essential nutrients and do not provide the variety of foods needed to adequately meet nutritional needs [55]. A possible illustration for this apprehension is the fact that the large Greek epidemiological EPIC study showed that adults adhering to a low carbohydrate - high protein diet had an increased mortality ([56]. This may have been due to associated adverse effects of the increased dietary fat, particularly saturated fat, on mortality.

To conclude, increased protein intake (up to approx. 1.5 g/kg b.wt./d in healthy subjects) appears to be safe unless there is an impairment of renal function.

7.10. Conclusions

Dietary proteins should receive more attention in nutrition recommendations in obesity and diabetes than they have in the recent past.

Layman et al proposed that the 2010 American Dietary Guidelines should emphasize more protein intake [57]. With the advancing age of the population, we are confronted with increasing risks for age-related problems such as obesity, type 2 diabetes, osteoporosis, metabolic syndrome and sarcopenia. All these conditions appear to be influenced in a favourable direction by an increase in dietary protein above the RDA amount. Whether these promises to improve the health state of the population hold true, remains to be examined in future large and long-term studies.

7.11. References

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8. Protein intake and bone health

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8.1. Summary/ Zusammenfassung/ Résumé

Adequate nutrition plays an important role in the development and maintenance of bone structures resistant to usual mechanical stresses. In addition to calcium in the presence of an adequate supply of vitamin D, dietary proteins represent key nutrients for bone health and thereby in the prevention of osteoporosis. Several studies point to a positive effect of high protein intake on bone mineral density or content. This fact is associated with a significant reduction in hip fracture incidence, as recorded in a large prospective study carried out in a homogenous cohort of postmenopausal women. Low protein intake (<0.8 g/kg b.wt./d) is often observed in patients with hip fractures and an intervention study indicates that following orthopaedic management, protein supplementation attenuates post-fracture bone loss, tends to increase muscle strength, and reduces medical complications and rehabilitation hospital stay. There is no evidence that high protein intake per se would be detrimental for bone mass and strength. Nevertheless, it appears reasonable to avoid very high protein diets (i.e. more than 2.0 g/kg b.wt./d) when associated with low calcium intake (i.e. less than 600 mg/d). In the elderly, taking into account the attenuated anabolic response to dietary protein with ageing, there is concern that the current dietary protein RDA, as set at 0.8 g/kg b.wt./d, might be too low for the primary and secondary prevention of fragility fractures.

Zusammenfassung: Einfluss der Proteinzufuhr auf die Knochengesundheit

Eine ausgewogene Ernährung spielt eine wichtige Rolle bei der Entwicklung und Erhaltung von Knochenstrukturen und ihrer Resistenz gegen mechanische Belastungen. Neben Calcium und einer ausreichenden Versorgung mit Vitamin D sind Nahrungsproteine wichtig für die Knochengesundheit und damit für die Prävention der Osteoporose. Mehrere Studien deuten auf darauf hin, dass eine höhere Proteinzufuhr einen günstigen Effekt auf die Knochenmasse hat. Eine große prospektive Studie mit einer homogenen Gruppe von postmenopausalen Frauen zeigte bei einer erhöhten Proteinzufuhr eine signifikante Abnahme von Hüftfrakturen. Eine tiefe Proteinzufuhr (<0.8 g/kg KG/Tag) wurde oft bei Patienten mit Hüftfrakturen beobachtet. Eine Interventionsstudie konnte zeigen, dass eine Proteinsupplementierung nach einem orthopädischen Eingriff die postoperative Abnahme der Knochenmasse vermindert und die Muskelkraft steigert. Zudem wurde die Rekonvaleszenz beschleunigt. Es gibt keinen Beweis dafür, dass eine erhöhte Proteinzufuhr per se nachteilig für die Knochenmasse wäre. Dennoch scheint es vernünftig, zu hohe Protein-Diäten zu vermeiden (d.h. mehr als 2.0 g Protein/kg KG/Tag), vor allem wenn diese mit einer geringen Calcium-Zufuhr (<600 mg/Tag) einhergeht. Bei älteren Menschen wurden unter Berücksichtigung der abgeschwächten anabolischen Reaktion auf Proteine Bedenken geäußert, dass die derzeitige Empfehlung zur Proteinzufuhr (RDA) von 0.8 g/kg KG/Tag für die primäre und sekundäre Prävention von osteoporotischen Frakturen womöglich zu tief ist.

Résumé : Influence de l'apport en protéines sur la santé osseuse

L'alimentation adéquate joue un rôle important dans le développement et la préservation de structures osseuses résistantes aux contraintes mécaniques. Outre le calcium et un apport suffisant en vitamine D, les protéines alimentaires sont essentielles à la santé osseuse et donc à la prévention de l'ostéoporose. Diverses études ont relevé l'effet positif d'un régime riche en protéines sur la masse osseuse. Il ressort ainsi

d'une vaste étude prospective, réalisée sur une cohorte de femmes postménopausées, que le risque de fracture du col du fémur diminue significativement en cas d'apport élevé en protéines. A contrario, une faible consommation de protéines (<0,8 g/kg/jour) s'observe souvent chez les victimes d'une fracture du col du fémur. Comme l'indique une expérimentation réalisée sur des patients ayant subi une intervention orthopédique, une supplémentation protéique atténue la diminution de masse osseuse durant la phase postopératoire. Elle tend en outre à accroître la force musculaire, réduit le risque de complications médicales et accélère la convalescence à l'hôpital. Rien ne prouve qu'un apport accru en protéines puisse être préjudiciable à la masse osseuse et à la solidité des os. Il paraît certes raisonnable de proscrire les régimes particulièrement riches en protéines (>2,0 g/kg/jour), a fortiori s'ils sont pauvres en calcium (<600 mg/jour). Mais comme les réactions anaboliques aux protéines diminuent avec l'âge, il se peut que l'apport nutritionnel conseillé (ANC) en protéines, à savoir 0,8 g/kg/jour, soit insuffisant chez les personnes âgées dans une optique de prévention primaire et secondaire des fractures liées à l'ostéoporose.

8.2. Proteins as a bone matrix constituent

Bone is a composite tissue, made up of mineral, organic matrix, water – by weight: 60, 30 and 10%, respectively – and cells. The major constituent of bone mineral is an impure form of hydroxyapatite ($\text{Ca}_{10}[\text{PO}_4]_6[\text{OH}]_2$). Other mineral constituents are: Magnesium, Carbonate, Citrate, and Sodium. It consists of small crystals located within and between collagen fibrils. Collagen Type I represents about 98% of total bone proteins. The main non-collagenous proteins are osteocalcin, osteopontin, sialoprotein, and osteonectin.

In the process of bone modelling, mainly during growth, and remodelling during adulthood, the organic matrix is formed and resorbed. Molecular products of these two processes, particularly from Type I collagen, are released into the systemic extra cellular compartment. They can be chemically analyzed and used as markers of bone formation and resorption. Other non-collagenic bone proteins such as specific bone alkaline phosphatase or osteocalcin are also released during the process of bone remodelling. They are detectable within the systemic extra cellular compartment, and are also used to estimate the rate of bone remodelling, as well as its changes in response to either pharmaceutical or nutritional interventions.

8.3. Effect of protein intake on calcium-phosphate economy and bone metabolism

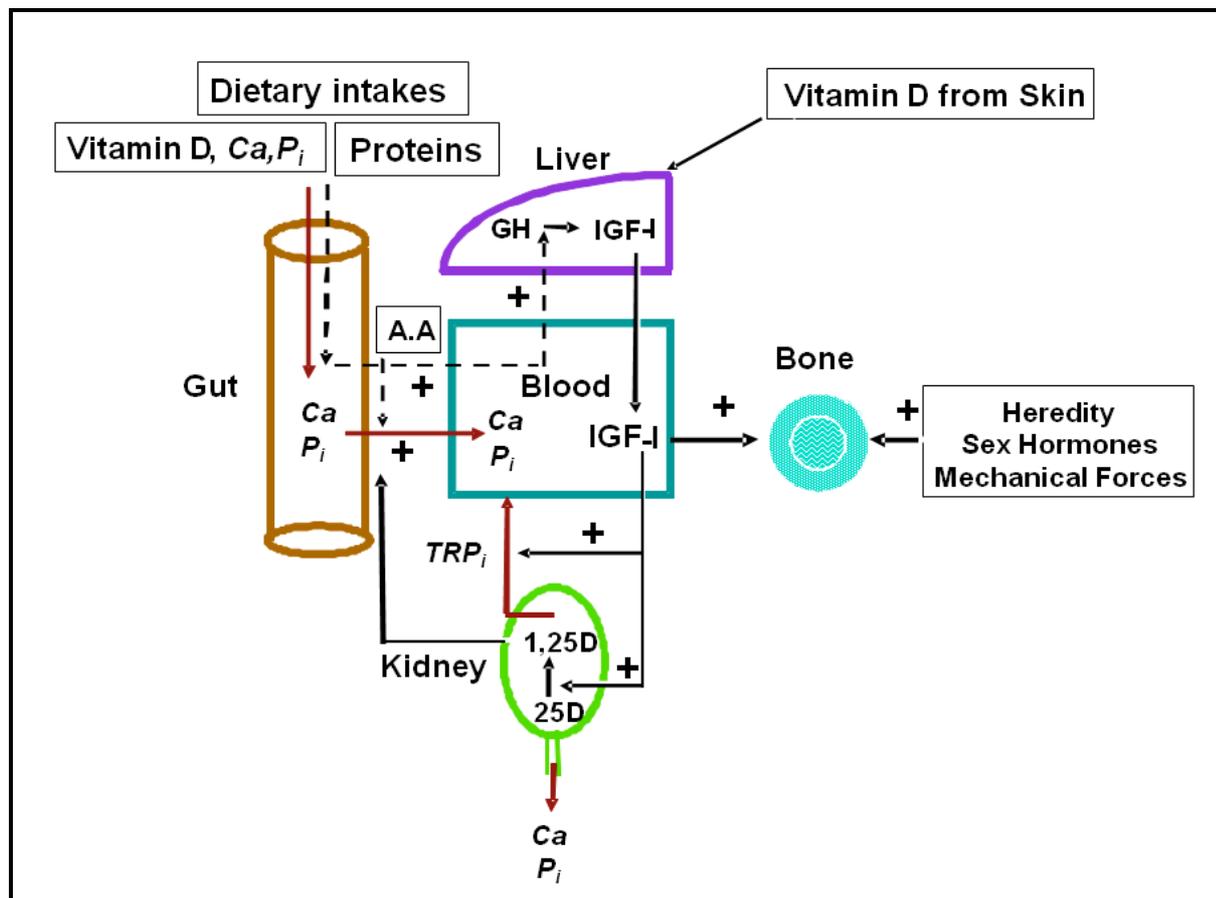
Protein supply from foods is required to promote bone formation. As for any other organs of the body, amino acids are required for the synthesis of intracellular and extra cellular bone proteins, and other nitrogen-containing compounds. Besides this role as "brick supplier", proteins through their amino acid content can influence calcium-phosphate economy and bone metabolism. Thus, dietary proteins stimulate the formation of insulin-like growth factor-I (IGF-I) from hepatic cells, which are the main source of this circulating growth factor (**Fig. 1**).

Increment in the circulating level of IGF-I can be observed in response to increased protein intake. This effect can be observed in absence of any difference in dietary energy supply.

Stimulation of IGF-I by food proteins can also exert a favourable impact on bone mineral economy by a dual renal action. IGF-I enhances the production of 1,25 dihydroxyvitamin D (1,25D), the active form of vitamin D. 1,25D, in turn, stimulates the intestinal absorption of both calcium and inorganic phosphate (Pi). The second action of IGF-I at the kidney level is to increase the tubular reabsorption of Pi. Through this dual activity of

IGF-I, the concentration of calcium and Pi in the systemic extra cellular compartment rise and thereby positively influence the process of bone mineralization.

Fig. 1: Role of dietary protein on calcium and inorganic phosphate (Pi) economy, and bone health.



This indirect positive effect of proteins on intestinal calcium absorption, via the IGF-I – 1,25D link, is associated with a direct stimulatory effect of amino acids such as arginine and lysine on calcium translocation from the luminal to the contra-luminal side of the intestinal mucosa (see below). The overall effect of protein intake is enhanced intestinal calcium absorption, and this accounts for the associated increased calciuria. In fact, the increased urinary calcium excretion associated with high protein diet does not result in negative skeletal calcium balance that would reflect bone loss (1).

In sharp contrast to experimental and clinical evidence, it has been alleged that proteins, particularly those of animal sources, might be deleterious for bone health by inducing chronic metabolic acidosis leading eventually to osteoporosis. Over the last decades, this apparently attractive hypothesis has prompted several investigators to explore in epidemiologic studies whether consumption of high animal protein intake would be associated with either decreased areal (a) bone mineral density (BMD) or content (BMC), or increased incidence of fragility fractures, particularly those occurring at the level of the proximal femur (see below). However, evidence-based scientific arguments against this theory have been developed in reviews and meta-analysis of the acid-ash hypothesis on calcium balance and osteoporosis (2-4).

As previously reviewed (2), there is no consistent evidence for superiority of vegetal over animal protein on calcium metabolism, bone loss prevention and risk reduction of fragility fractures.

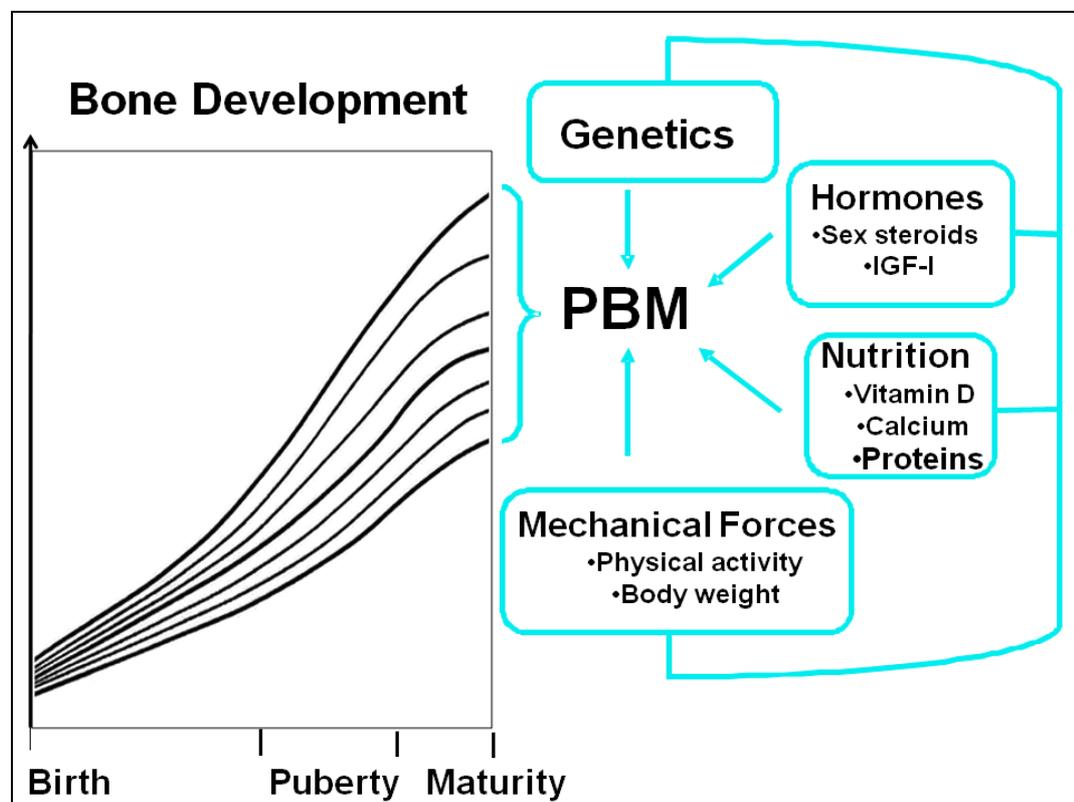
At the bone level some amino acids such as arginine can exert a stimulatory effect on the local production of IGF-I by osteoblastic cells (5). This effect is associated with increased osteoblastic cell proliferation and collagen synthesis (5). IGF-I is probably the main mediator of the anabolic effect of subcutaneous administration of parathyroid hormone (PTH), as documented in randomized controlled trials (RCTs) carried out in osteoporotic women. In relation with this mediating role of IGF-I, there is evidence from animal experiments that low protein diet may attenuate the anabolic effect of PTH.

8.4. Protein intake and determinants of bone development

Bone mass and strength achieved by the end of the growth period, simply designated as “peak bone mass (PBM)”, plays an essential role in the risk of osteoporotic fractures occurring in adulthood. It is considered that an increase in PBM by 1.0 standard deviation would reduce by 50% the fragility fracture risk. As estimated from twin studies, genetics is the major determinant of PBM, accounting for about 60 to 80% of its variance. Before puberty there is no substantial gender difference in aBMD when adjusted to age, nutritional factors and physical activity. During pubertal maturation, the size of the bone increases whereas the volumetric bone mineral density remains virtually constant in both genders. At the end of puberty, the sex difference is essentially due to a greater bone size in male than female subjects. This is achieved by larger periosteal deposition in boys, thus conferring at PBM a better resistance to mechanical forces in men than in women. Sex hormones and the IGF-I system are implicated in the bone sexual dimorphism occurring during pubertal maturation.

The genetically determined trajectory of bone mass development can be modulated, to a certain extent, by modifiable environmental factors (Fig. 2).

Fig. 2: Determinants of bone mass and strength development from birth to maturity.



Among these factors physical activity and nutrition are key players for the acquisition of bone mass during growth. Growing bones are usually more responsive to mechanical loading than adult bones. However, the impact seems to be stronger before than during or after the period of pubertal maturation. Among nutrients that can specifically interact with bone metabolism, calcium supplementation has been extensively studied from infancy to the end of pubertal maturation. Much less consideration has been given to protein intake, although this macronutrient is essential for adequate accumulation of bone tissue during growth, as well as maintenance of the skeletal structural integrity throughout life (see next sections).

8.5. Protein intake and bone acquisition

Both animal and human studies indicate that low protein intake *per se* could be particularly detrimental to bone acquisition. Undernutrition, including inadequate supplies of energy and protein during growth, can severely impair bone development (6). An inadequate protein supply appears to play a central role in the pathogenesis of the delayed skeletal growth and reduced bone mass that is observed in undernourished children.

Low protein intake could be detrimental to skeletal integrity by lowering the production of IGF-I (7). Variations in the production of IGF-I could explain some of the changes in bone and calcium-phosphate metabolism that have been observed in relation to dietary protein intake. Indeed, the plasma level of IGF-I is related closely to the growth rate of the organism. In humans, circulating IGF-I rises progressively from 1 year of age to reach peak values during puberty. As mentioned above, this factor appears to play a key role in calcium-Pi metabolism during growth by stimulating two kidney processes: tubular Pi reabsorption and the production of 1,25D. Furthermore, IGF-I is considered as an essential factor for bone longitudinal growth, as it stimulates proliferation and differentiation of chondrocytes in the epiphyseal plate. It also plays a role on trabecular and cortical bone formation. IGF-I also affects bone mass positively, increasing the external diameter of long bone, probably by enhancing the process of periosteal apposition. Therefore, during adolescence, a relative deficiency in IGF-I or a resistance to its action may result in a reduction in the skeletal longitudinal growth, and impaired width- or cross-sectional bone development.

In "well" nourished children and adolescents, the question arises whether or not variations in the protein intake within the "normal" range can influence skeletal growth and thereby modulate the influence of genetic determinants on peak bone mass attainment (8). In the relationship between protein intake and bone mass gain, it is not surprising to find a positive correlation between these two variables (8). As for the calcium intake, the association appears to be particularly significant in pre-pubertal children. These results suggest that relatively high protein intakes could favour bone mass accrual during childhood. Interventional studies testing different levels of protein intakes in otherwise isocaloric diets could eventually determine the quantitative relationship between protein intake and bone mass acquisition during childhood and adolescence. Furthermore, calcium requirement for optimal bone mass accrual could vary according to the protein intake. The possible positive interaction between protein and calcium intake deserves to be investigated in the perspective of increasing peak bone mass by modifying bone trophic nutrients.

8.6. Interaction of protein intake and physical activity

Growing bones are usually more responsive to mechanical loading than adult bones. Increased physical activity was shown to stimulate mineral mass accumulation in children and adolescents. The positive impact on bone acquisition might be greater before than during or after the period of pubertal maturation (8),

although this pubertal maturation modulation may depend upon the skeletal site (axial vs. appendicular) and/or structural (cortical vs. trabecular) examined components. Adequate nutritional supply can be expected to sustain the anabolic effect of mechanical loading on bone tissue as it does on skeletal muscle development. Among nutrients, high calcium intake was shown to enhance the response to physical activity in healthy children aged 3-5 years (9). Long term protein consumption exerts a stronger impact than calcium intake on bone mass and strength acquisition in healthy children and adolescents aged 6-18 years (10). That high protein intake may enhance the bone response to increased physical activity has been recently reported in 8-year-old prepubertal boys (11). At the femoral neck level, the increased aBMD and BMC was associated with a wider external perimeter (11), a macro-architecture feature that should confer greater resistance to mechanical load (12).

8.7. Deficient protein intake in anorexia nervosa

A positive correlation between protein intake and bone mass has been found in premenopausal women (13). In women on a low-calorie diet, insufficient protein intake could be particularly deleterious for bone mass integrity. In athletes or ballet dancers intensive exercise can lead to hypothalamic dysfunction with delayed menarche and disruption of menstrual cyclicality and bone loss (14, 15). The combination of an eating disorder, menstrual dysfunction and osteopenia has been called “female athlete triad”. Nutritional restriction can play an important role in the disturbance of the female reproductive system resulting of intense physical activity. The propensity to nutritional restriction is more common when leanness confers an advantage for athletic performance. Insufficient energy intake with respect to energy expenditure is supposed to impair the secretion of GnRH and thereby leads to a state of hypoestrogenism. However the relative contribution of insufficient protein intake with low IGF-I remains to be assessed, since it is frequently associated with reduced energy intake.

Anorexia nervosa is a frequent condition in young women. Reduced aBMD can be measured at several skeletal sites in most women with anorexia nervosa (16). It is not surprising that young women with anorexia nervosa are at increased risk of fracture later in life. Body weight, but not estrogen use, is a significant predictor of aBMD in women with anorexia nervosa. With estrogen and calcium deficiency, low protein intake very likely contributes to the bone deficit observed in anorexia nervosa. In this condition, serum osteocalcin and bone specific alkaline phosphatase, two biochemical markers of bone formation, are significantly reduced (17). Interestingly, IGF-I was the major correlate of bone formation markers in mature adolescents with anorexia nervosa (17). Furthermore, IGF-I level changes were dependent of variations in the nutritional state (17).

8.8. Epidemiological studies on protein intake in women and in the elderly

An early, small but often quoted, cross-sectional study suggested that high protein diet might be detrimental on forearm area bone mineral density (aBMD) in limited number of healthy young women (18). However, in several later reports this negative association between protein intake and aBMD or content (BMC) was not confirmed in both premenopausal and postmenopausal women. Furthermore, in a large number of studies, a positive relationship between protein intake and aBMD or BMC has been found (see for review: (2, 3)). In the Framingham Osteoporosis Study, increased protein intake was protective against spinal and femoral bone loss in a large cohort of elderly women and men prospectively followed over a period of 4 years (19). As in hospitalized elderly patients, those with a higher protein intake had a greater aBMD, particularly at the

femoral neck level (20). Whereas a gradual decline in caloric intake with age can be considered as an adequate adjustment to the usual progressive reduction in energy expenditure, the parallel reduction in protein intake is certainly detrimental for maintaining the integrity and functioning of several organs or systems including skeletal muscle and bone. As mentioned above dietary protein is crucial for bone and muscle development. Recent evidence suggests that increasing protein above the Recommended Dietary Allowance (RDA) may help prevent the loss of bone and muscle mass in elderly (3).

There is evidence that the favourable effect of increasing protein on aBMD or BMC is better sustained when the supply of both calcium and vitamin D are adequate (21-23). Reciprocally, in postmenopausal women with low calcium intake (600 vs. 1500 mg/d), a relatively high protein consumption (20 vs. 10% of energy intake) enhanced calcium retention. Likewise, in healthy older women and men, protein supplements increasing the daily intake from 0.78 to 1.55 g/kg b.wt./d, when exchanged isocalorically for carbohydrates, was associated with higher circulating levels of IGF-I and lowered levels of urinary N-telopeptide, a marker of bone resorption (24). These results are compatible with a preventive effect of relatively high protein intake on bone loss in elderly.

8.9. Association of protein intake with risk of osteoporotic fractures

Some cross-cultural studies comparing protein intake and hip fracture incidence in women living in various countries have been interpreted as suggesting that high protein intakes from animal source exert deleterious effects on bone health (25, 26). However, the way both terms of this putative relationship between protein intake and hip fracture incidence were derived is highly questionable. First, the use of per capita food supplies provided by the FAO of the United Nations is not a reliable estimate of the protein intake of the population at risk of hip fracture. It is derived from the total amount of animal protein available for the whole population, i.e. the amount produced plus the amount imported minus the amount exported by a given country, divided by the number of inhabitants. In this rough average estimate of the whole population intake, any selective decline in protein consumption with aging is not taken into account as reported in several reviews (3, 22, 27, 28). Second, as expected, countries with the highest incidence of hip fracture are those with the longest life expectancy. Age adjustment to the 1977 or 1987 distribution of US women population (25, 26) does not correct for marked difference in life expectancy between populations of various socio-economic conditions.

In contrast to this “negative” aspect of protein intake hypothesized from cross-cultural analysis, several prospective observational studies have rather shown either a protective effect of relatively high protein consumption or, at least, no detrimental effect on hip fracture incidence.

Low protein intake has been documented in elderly subjects at risk of fragility fractures, and more so in those experiencing hip fracture (see for review (27)). It is associated with low body mass index (BMI) as clearly documented in a meta-analysis including 12 prospective worldwide multicenter studies including 60'000 men and women with a total follow-up of 25'000 persons year (29). In elderly, low BMI is correlated with protein undernutrition, that in turn is associated with low bone and skeletal muscle mass (3, 28).

In a large prospective study (Iowa Women's Health Study) including about 32'000 women aged 55-69, total protein intake was inversely associated with the risk of hip fracture (30). Thus, the risk reduction in hip fracture incidence was 67 and 79% for the highest vs. the lowest quartile in total and animal protein intake, respectively, representing 1.3 versus 1.0 g protein/kg b.wt./d (30). The risk reduction remained significant

after adjustment for various potential confounding factors including body mass index, smoking, alcohol intake, estrogen use, and physical activity (30). In a smaller case-control study including both women and men residing in Utah, higher total protein intake was associated with a significant reduced risk of hip fracture in 50-69 years old subjects (31). In older, 70-89 years old residents of this county, however, protein intake was not significantly associated with a decreased or an increased risk of hip fracture (31). As discussed by the authors, it is unclear whether the lack of protective effect in the 70-89 years group would reflect a functional difference in nutritional protein metabolism or merely an artefact due to methodological limitations of the case-control study design in the oldest subjects (31). In both the Iowa and Utah studies, calcium intake did not modify the risk evaluation of hip fracture in relation with protein intake (30, 31). These observations contrast somewhat with an analysis (32) of results obtained in a large French postmenopausal women cohort study initiated in 1990 to identify most frequent cancer-associated risk factors (33). Overall, no association was found between fracture risk and either total protein (from animal or vegetable sources) or calcium intake (32). However, further cross-tabulation analysis that subdivided the population in 4 subgroups revealed a slightly but significant increased risk when the highest quartile of protein intake was combined with the lowest quartile of calcium intake (32). Of note, in this population of relatively young postmenopausal women with mean age about 57 years, the daily protein intake was normal to high (mean about 1.45 g/kg b.wt.) and the calcium intake fairly high (mean about 1045 mg/d) (32). Therefore, this epidemiological study does not concern elderly women at risk of undernutrition as observed in hip fracture patients (34). In another relatively young cohort aged between 35 and 59 years, the "Nurses' Health Study", a trend for hip fracture incidence inversely related to protein intake has been reported (35). In the same prospective epidemiological study, however, forearm fracture incidence was slightly increased (RR = 1.18, 95% CI 1.01-1.38) in the highest (>95 g/d) as compared to the lowest (<68 g/d) quintile of age-adjusted total protein intake (35). The reason for this skeletal site difference in the recorded association might be related to physical activity and mode of falling that differs for hip vs. forearm fracture (12). In contrast to the French study discussed above (32), as well as to a retrospective Norwegian survey (36), no significant relation with the calcium/protein ratio was found with either hip or forearm fracture incidence in the "Nurses' Health Study", (35).

Studies reported from 1966 to 2008 on the relation between protein and bone integrity in healthy human adults were systematically reviewed and meta-analyzed (37). From the 18 studies that could be quantitatively analyzed a significant positive pooled correlation between protein intake and aBMD or BMC measured at the main clinically relevant skeletal sites was found among 18 cross-sectional surveys (37). Likewise, a significant positive influence of protein supplementation on lumbar spine BMD was computed in the meta-analysis of 6 randomized placebo-controlled intervention trials (37). Four suitable hip fracture studies (30, 35, 36, 38) were also meta-analyzed (37). In contrast to cross-cultural ecologic studies mentioned above (25, 26), no negative association with the relative risk of hip fracture was found with the total protein intake, or separately analyzed from animal or vegetable sources (37). Thus, this meta-analysis made on cohorts of either gender and of various age at baseline, ranging from 35 to 74 years (30, 35, 36, 38) at least rules out a detrimental effect of high protein intake on hip fracture risk. Large heterogeneity and the relatively young age of a substantial number of included women, may explain why the recorded increase in aBMD or BMC did not translate into a significant reduction in hip fracture risk after pooling these four disparate studies. Of note, the only cohort showing a clear-cut reduction in hip fracture risk with increased protein intake, thereby in keeping with the significant increased aBMD or BMC recorded in the other meta-

analyzed reports (37), was the prospective study that included only post-menopausal women with an age at baseline ranging from 55 to 69 years (30).

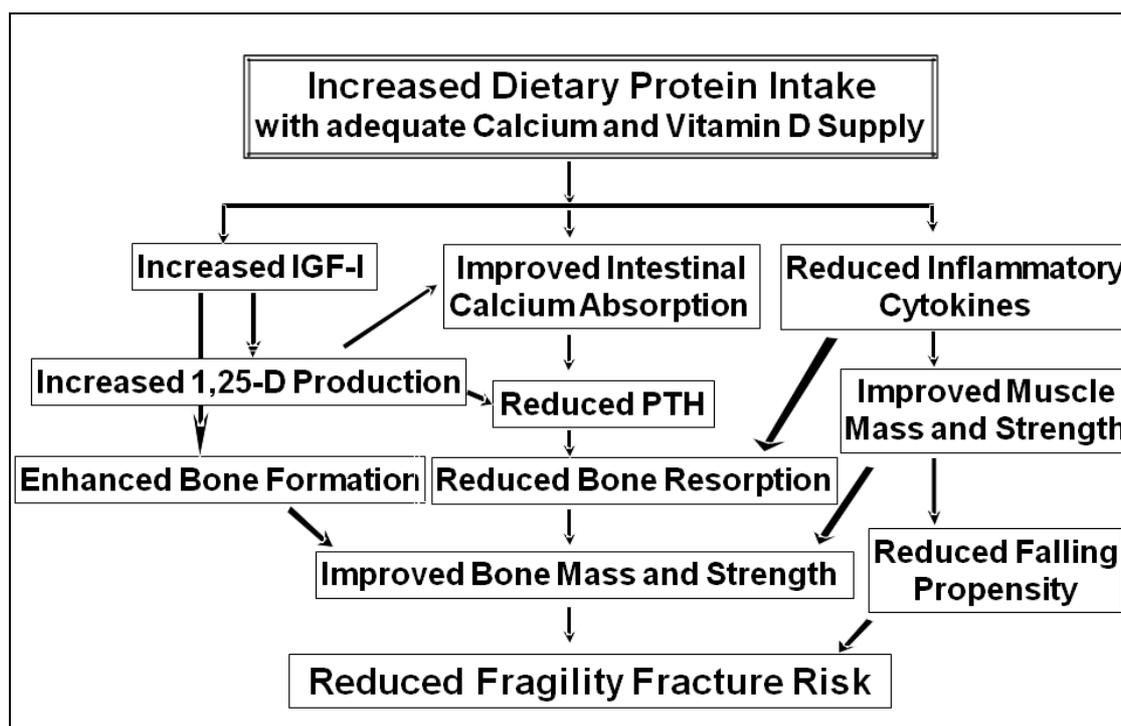
In relation with protein undernutrition and fragility fractures, the risk of spinal and hip fractures was associated with low circulating levels of IGF-I (39, 40). Furthermore, in the elderly at risk of osteoporotic fractures, marginal dietary protein intake results in loss of muscle mass, which is associated with reduced IGF-I plasma, level (41). Muscle mass and strength are important determinants of the risk and consequence of falling in elderly (27). There is evidence that the anabolic response of muscle to dietary protein is attenuated in elderly and consequently, the amount of protein required to enhance muscle mass is greater (3). Several epidemiological and clinical studies point to a beneficial effect of increasing the protein intake in elderly above the current RDA of 0.8 g to approx. 1.2 g/kg b.wt./d; short-term studies indicated beneficial effects of protein intake up to 1.6-1.8 g/kg b.wt./d (3).

8.10. Intervention study on the impact of protein repletion after hip fracture

In a randomized, double-blind, placebo-controlled trial, oral protein supplement providing 20 g of casein/d during 6 months, as compared to an isocaloric supplement given to patients with a recent hip fracture, improved clinical outcomes and muscle strength, and lessened loss of bone mineral mass at the contra lateral proximal femur with a trend for less vertebral fracture (42). Both the protein supplemented and the placebo-controlled groups were vitamin D repleted, and received daily 500 mg of elemental calcium. The protein supplemented group displayed a significantly greater increase in plasma IGF-I level and reduced length of stay in rehabilitation hospital (42).

Thus, in the primary or secondary prevention of osteoporosis, protein repletion in frail elderly, by positively influencing both bone mineral and muscle mass and strength could contribute to prevent falls and the consecutive occurrence of fragility fractures (Fig. 3).

Fig. 3: Positive influences of dietary proteins on bone and skeletal muscle health in elderly at risk of fragility fractures.



The hepatic production of insulin-like growth factor-I (IGF-I), which is under the positive influence of growth hormone (GH), is also stimulated by amino acids (a.a.). IGF-I exerts a direct action on bone anabolism. In addition, at the kidney level, IGF-I increases both 1,25-dihydroxyvitamin D (1,25D) formation from 25-hydroxyvitamin D (25D) and the tubular reabsorption (TR) of Pi. By this dual renal action, IGF-I favours a positive balance of calcium and Pi. Amino acids can still directly stimulate the intestinal absorption of calcium that can account for the increased urinary calcium excretion observed with high protein diet. 25D is formed in the liver from vitamin D, which is supplied from both dietary and cutaneous sources.

In healthy human subjects four main determinants including genetics, hormones, nutrition and mechanical forces, influence bone mass and strength from birth to the end of the second decade. At this time maximal value, the so-called peak bone mass (PBM,) is virtually attained. As depicted on the right these four factors are interconnected, as for instance an increased protein intake enhances the positive impact of physical activity on bone acquisition during growth. The curves of the diagram on the left illustrate the wide range of individual PBM values that can be assessed at maturity among young healthy subjects of both genders. The genetically predetermined trajectory can be modified by environmental factors including nutrition and physical activity.

In the elderly, dietary proteins by impacting on both bone and skeletal muscle anabolism play a key role in the prevention of bone loss and sarcopenia, thus reducing the propensity to fall and the risk of fragility fractures. The positive action of dietary proteins requires adequate supply of both vitamin D and calcium.

8.11. References

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9. Protein catabolism and protein requirements in severe illness

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9.1. Summary/ Zusammenfassung/ Résumé

Reduced total body protein mass is a marker of protein-energy malnutrition and has been associated with numerous complications. Severe illness is characterised by a loss of total body protein mass, mainly from the skeletal muscles. Studies on protein turnover describe an increased protein breakdown and, to a lesser extent, an increased whole-body protein synthesis, as well as an increased flux of amino acids from the periphery to the liver. Appropriate nutrition could limit protein catabolism.

Nutritional support limits, but does not stop the loss of total body protein mass occurring in acute severe illness. Its impact on protein kinetics is so far controversial, probably due to the various methodologies and characteristics of nutritional support used in the studies. Maintaining calorie balance alone in the days after an insult does not clearly lead to an improved clinical outcome. In contrast, protein intakes between 1.2 and 1.5 g/kg b.wt./d with neutral energy balance minimise total body protein mass loss. Glutamine and possibly leucine may improve clinical outcomes, but it is unclear whether these benefits occur through an impact on total body protein mass and its turnover, or through other mechanisms.

Present recommendations suggest providing 20-25 kcal/kg b.wt./d over the first 72-96 hours and increasing energy intake to target thereafter. Simultaneously, protein intake should be between 1.2 and 1.5 g/kg b.wt./d. Enteral immunonutrition enriched with arginine, nucleotides and omega-3 fatty acids is indicated in patients with trauma, Acute Respiratory Distress Syndrome and mild sepsis. Glutamine (0.2-0.4 g/kg b.wt./d of L-glutamine) should be added to enteral nutrition in burned and trauma patients (ESPEN guidelines 2006) and to parenteral nutrition, in the form of dipeptides, in ICU patients in general (ESPEN guidelines 2009).

Zusammenfassung: Proteinabbau und Proteinbedarf bei schweren Erkrankungen

Der Verlust an Körperproteinmasse ist ein Zeichen einer mangelhaften Protein- und Energiezufuhr und wurde mit vielen Komplikationen in Verbindung gebracht. Eine verminderte Proteinmasse, vor allem der Skelettmuskulatur, ist charakteristisch für schwere Krankheiten. Studien über den Proteinturnover beschreiben einen erhöhten Proteinabbau und, in geringerem Ausmasse, eine verstärkte Proteinsynthese im ganzen Körper, sowie einen gesteigerten Fluss von Aminosäuren von der Peripherie zur Leber. Eine angepasste Ernährung kann den Proteinabbau beschränken.

Während schweren Erkrankungen kann eine Ernährungstherapie den Verlust von Körperproteinen beschränken, jedoch nicht völlig aufhalten. Ihr Einfluss auf die Proteinkinetik ist bisher umstritten, wahrscheinlich aufgrund der unterschiedlichen methodischen Ansätze und Eigenschaften der verwendeten Nahrungszusätze, die in den Studien angewendet wurden. Die alleinige Zufuhr ausreichender Mengen an Energie am Folgetag nach dem Auftreten einer schwerwiegenden Erkrankung führt nicht klar zu einem besseren klinischen Erfolg. Im Gegensatz dazu kann eine Proteinzufuhr von 1.2 bis 1.5 g/kg KG/Tag bei einer ausgeglichenen (neutralen) Energiebilanz den Verlust von Körperproteinen minimieren. Zusatz von Glutamin und eventuell auch Leucin zur künstlichen Ernährung können den Verlauf solcher schwerkranker Patienten verbessern. Es ist jedoch nicht klar, ob diese Verbesserung durch einen Einfluss auf die Körperproteine, ihren Umsatz oder durch andere Mechanismen zustande kommt.

Die aktuellen Ernährungsempfehlungen bei schweren Erkrankungen lauten daraufhin, dass mit einer Gesamtenergiezufuhr von 20-25 kcal/kg KG/Tag in den ersten 72-96 Stunden begonnen werden soll, und anschliessend die Energiezufuhr entsprechend dem Bedarf erhöht werden soll. Gleichzeitig soll eine Proteinzufuhr von 1.2-1.5 g/kg KG/Tag erfolgen. Eine enterale Immunernährung, die mit Arginin, Nukleotiden und Omega 3-Fettsäuren angereichert ist, wird bei Patienten mit Trauma, Acute Respiratory Distress Syndrome („akutes Atemnot-Syndrom“) und leichter Sepsis empfohlen. Glutamin (0.2-0.4 g/kg KG/Tag L-Glutamin) sollte der enteralen Ernährung von Patienten mit Verbrennungen und Trauma beigefügt werden (ESPEN-Leitlinien 2006). Bei der parenteralen Ernährung von Patienten auf der Intensivstation wird diese Zusatzernährung in Form von Dipeptiden empfohlen (ESPEN-Leitlinien 2009).

Résumé : Catabolisme des protéines corporelles et besoins en protéines en cas de maladie grave

Une faible masse protéique de l'organisme est un signe de dénutrition protéino-énergétique et a été associée à de nombreuses complications. Les maladies graves comportent typiquement une perte de masse protéique, au niveau de la musculature squelettique notamment. Les études consacrées au renouvellement protéique décrivent une augmentation significative de la dégradation protéique et, dans une moindre mesure, une intensification de la synthèse protéique au sein de l'organisme ainsi qu'une augmentation du flux d'acides aminés de la périphérie vers le foie. Un régime approprié pourrait limiter ce catabolisme protéique.

Une alimentation ciblée permettrait de limiter, à défaut de l'empêcher, la perte de masse protéique corporelle en cas de maladie aiguë sévère. Son impact sur la cinétique protéique demeure toutefois controversé, probablement en raison de différences d'approche méthodologique et des compléments alimentaires utilisés dans les études. A lui seul, le maintien de la balance énergétique les jours qui suivent l'apparition d'une maladie grave n'améliore pas clairement le devenir clinique du patient. Par contre, des apports protéiques compris entre 1,2 et 1,5 g/kg de poids corporel/jour, avec une balance énergétique neutre, limiteront la perte de masse protéique. Une supplémentation en glutamine, voire en leucine, semble améliorer le devenir clinique d'un patient gravement malade, sans qu'on ait pu établir si ce changement découle d'un impact sur la masse protéique corporelle et du renouvellement protéique, ou alors d'autres mécanismes.

Les recommandations actuelles préconisent des apports énergétiques de 20 à 25 kcal/kg/jour pendant les 72 à 96 premières heures, avant d'augmenter les apports caloriques jusqu'à la cible énergétique. Les apports protéiques devraient se situer entre 1,2 et 1,5 g/kg/jour. Une immunonutrition entérale enrichie avec de l'arginine, des nucléotides et des acides gras omega-3 est indiquée pour les patients avec syndrome de détresse respiratoire aiguë, sepsis léger ou trauma. Une supplémentation en glutamine (0,2 à 0,4 g/kg/jour de L-glutamine) devrait être ajoutée à la nutrition entérale chez les patients brûlés ou traumatisés (recommandations ESPEN 2006) et à la nutrition parentérale, sous forme de dipeptides, chez les patients de soins intensifs en général (recommandations ESPEN 2009).

9.2. Introduction

Proteins are stored in visceral tissue and muscle, mainly skeletal muscle. Along with water, minerals and glycogen, protein mass is often grouped under the term of fat-free mass, as opposed to fat mass. A low protein or fat-free mass is a marker of protein-energy malnutrition (PEM). Since PEM has a high prevalence

among hospitalised patients and has been associated with numerous complications, methods to prevent or limit PEM and the associated loss of protein mass are suitable.

The literature describes several types of PEM, i.e. cachexia, starvation and/or sarcopenia, which frequently overlap in severely ill patients. Cachexia represents a complex metabolic syndrome associated with underlying illness, characterized by an accelerated loss of muscle mass with or without loss of fat mass and often associated with anorexia, inflammation, insulin resistance and increased muscle protein breakdown. In contrast, starvation results from a pure deficit of all macro- and micronutrients, as seen for instance in hunger strikers and persons with anorexia. Sarcopenia describes the depletion of skeletal muscle mass occurring mostly in older or immobilized subjects but it is not quite sure whether it reflects a third type of PEM, as it is associated with increased plasma concentrations of inflammatory cytokines.

Since there is no universal definition of “severe illness” and studies use various severity scores, if any, to describe their patients, we decided to define arbitrarily severely ill patients as intensive care, burned, septic or trauma patients.

This article reviews the impact of acute severe illness and nutritional intake on protein mass and turnover and the subsequent protein requirements in human adults.

9.3. Protein mass in severe illness

Protein mass can be evaluated *in vivo* by measurements of body composition. *In vivo* neutron activation (IVNA) is usually considered the gold standard but available only at a small number of centres worldwide and relies on administration of radioisotopes to humans. It measures directly the nitrogen content of the body and allows calculation of total body protein mass (TBP) by the relationship: Protein (g) = 6.25 x nitrogen (g). The other methods determine fat-free mass directly or indirectly. Bedside methods are of limited accuracy in case of overhydration, as is often seen in intensive care unit (ICU) and critically-ill patients.

Studies reporting TBP changes in severe illness are summarized in **Tab. 1**.

Tab. 1: Studies on the impact of severe illness on total body protein mass measured by *in vivo* neutron activation

Authors	Patients (n)	Severity Score	Duration of follow-up	ΔTBP	Nutrition during follow-up
Plank LD et al. 1998	Generalized peritonitis (12)	Mean APACHE II Score = 21±7	21 days	-13%	Energy balance: -318±264 kcal/d during first 10 days
Streat SJ et al. 1987	Postoperative sepsis (8)	Acute Physiology Score 22±5	10 days	-12.5%	Energy intake: 43 non protein kcal/kg FFM/d and 2.3 g/kg FFM/d
Monk DN et al 1996	Blunt trauma (10)	Mean Injury Severity Score = 36±6	15 days	-16%	Energy balance: -1293±695kcal/d
Finn PJ et al 1996	Blunt trauma (9), severe sepsis (11)	Not available	21 days	-15%	Not available
Chandrasegar am MD et al. 2005	Severe acute pancreatitis (15)	Atlanta criteria met in 13 patients	14 days	+2%*	Energy prescription: 40 kcal/kg/d with calorie to nitrogen ratio of 150:1 Energy balance: not available
Sevette A et al 2005	Surgery for upper GI malignancy (15)	Not available	14 days	-3.5%*	Energy intake: 26.5±1.4 kcal/kg/d and 0.25±0.04 g N/kg/d

ΔTBP: change of total body protein mass compared to baseline

*non significant compared to baseline

Four of these studies showed an important loss of TBP. Two of them found that TBP can be maintained in severe illness but likely included subjects who were less ill. Indeed, Chandrasegaram et al. performed the measurements of TBP and started parenteral nutrition at different stages of illness and not specifically on the day of diagnosis, and thus patients may already have been in their recovery phase (1). In the study by Sevette et al., the authors had excluded patients who became septic or required admission to the ICU (2). The changes of total body protein mass over 3 weeks are similar between patients with severe sepsis and major trauma (6). Similarly, energy and macronutrient balances during 7 days did not differ between septic (APACHE III score of 70±11) and non septic patients who received a similar amount of daily parenteral nutrition, providing an energy supply of 25% above the measured resting energy expenditure (7). Reeds et al. even showed that the kinetics of nitrogen loss after an insult and the peak of nitrogen loss were similar between numerous stressors (8).

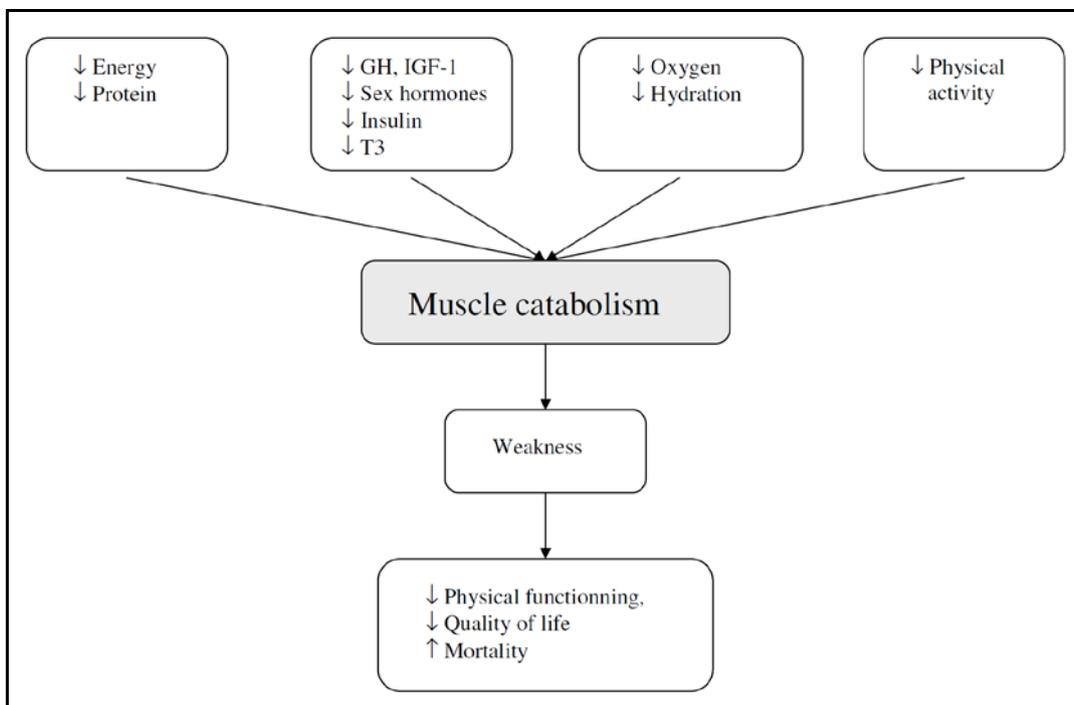
These studies demonstrate the loss of protein mass during severe illness. This loss of protein mass cannot be overcome by nutritional support in case of severe sepsis or trauma and occurs mainly in skeletal muscle and is similar between patients with a variety of stressors.

9.4. Protein turnover in severe illness

The understanding of mechanisms underlying protein catabolism requires insight into protein turnover, which is a dynamic process. It is ideally measured by incorporation of isotope-labelled tracer amino acids into protein or dilution of tracer amino acids in the free amino acid pool by protein breakdown and performing tissue or limb balances. During severe illness, there is an increased protein breakdown (25-127%) and, to a lesser extent, an increased whole-body protein synthesis (16-47%) (acute phase response, wound repair, immune response...) (9), leading to a negative protein balance, and an increased flux of amino acids from the periphery to the liver.

The accelerated protein breakdown results from a generalized stress response associating complex neuronal, inflammatory and hormonal interactions (**Fig. 1**). It occurs mainly in skeletal muscle, as mentioned earlier, and involves the activation of the ubiquitin-proteasome proteolytic pathway. The protein breakdown is likely not disease-specific since an animal study showed that muscle atrophy related to multiple systemic diseases share a common set of transcriptional adaptations (10). The resulting amino acids can be either used for protein synthesis or for non-protein physiological and metabolic functions (peroxidative protection, lymphocyte proliferation, energy production). Noteworthy is the fact that there is a mismatch between the amino acids provided by muscle protein breakdown and the amino acids needed for protein synthesis during the acute phase response. Indeed, 2.6 g of muscle protein have to be catabolised to synthesise 1 g of fibrinogen. Thus, the type of amino acids needed for the acute phase response may determine the severity of muscle catabolism.

Fig. 1: The decreased fat-free mass occurring in severe disease originates from the complex association of neuronal, inflammatory and hormonal interactions.



Although whole-body protein synthesis is increased in severe illness, protein synthesis varies between individual tissues. Muscle protein synthesis decreases in severely ill patients, which results from impairments at the transcriptional and posttranscriptional levels. In contrast, as shown in animal models of sepsis, protein synthesis increases in numerous other tissues. Liver contributes mainly by an increased synthesis of secreted proteins as part of the acute phase response. In humans, studies have described an increased synthesis of specific proteins, originating mainly from the liver (**Tab. 2**) or an increased protein synthesis of specific tissues during severe illness. For instance, studies have shown an increased protein synthesis in circulating blood lymphocytes of critically-ill patients (11), in the colonic mucosa of patients with a benign or malignant colorectal tumour or inflammatory bowel disease (12) or of phenylalanine in wounds of burned patients (13).

Tab. 2: Human studies comparing fractional synthesis rate (turnover rate) of specific proteins or glycoproteins between severely ill patients and healthy controls

Authors	Patients (n)	Isotope used	Mean fibrinogen FSR	Mean albumin FSR	Mean fibronectin FSR
Essen et al, 1998	ICU patients (15)	² H ₅ -L-phenylalanine		12.8*	
Preston et al., 1998	Pancreas cancer (6) Controls (7)	² H ₅ -L-phenylalanine	39.3 21.9	- -	- -
Mansoor et al., 1997	Head-injury (8) Controls (5)	[¹³ C]leucine	28.1 15.1	11.4 1.0	- -
Thompson et al., 1989	Burn + trauma (6) Controls (9)	[¹⁵ N]glycine	32.8 10.8	- -	22.0 14.7
Moshage et al., 1987	Inflammatory disease (4); Controls (20)	[¹⁴ C] arginine	- -	16.8 16.2	- -

FSR: fractional synthesis rate (%/24h)

*Compared to a reported control value of 6.7%/24h

The transport of amino acids is altered in severely ill patients. In skeletal muscle of 19 severely burned patients, at 14±5 post-burn days, Biolo et al. studied transmembrane transport by calculating the rate of net movement of the essential amino acids phenylalanine, leucine, and lysine from the muscle to the vein. When comparing the results to healthy controls, they found that outward transmembrane transport of these amino acids are higher in patients, presumably to promote the export of amino acids to other tissues such as liver, spleen, kidney, skin or digestive tract. In contrast, inward transport to the muscle is impaired relatively to the increased delivery of circulating amino acid to skeletal muscle secondary to accelerated blood flow (14). Noteworthy is also that intensive care patients have lower plasma amino acid concentrations in their basal state, defined as a state where patients receive glucose infusion but no nutritional support for at least 12 hours, compared to volunteers, which in view of the negative protein balance is probably due to increased amino acid oxidation (15).

9.5. Albumin synthesis in severe illness

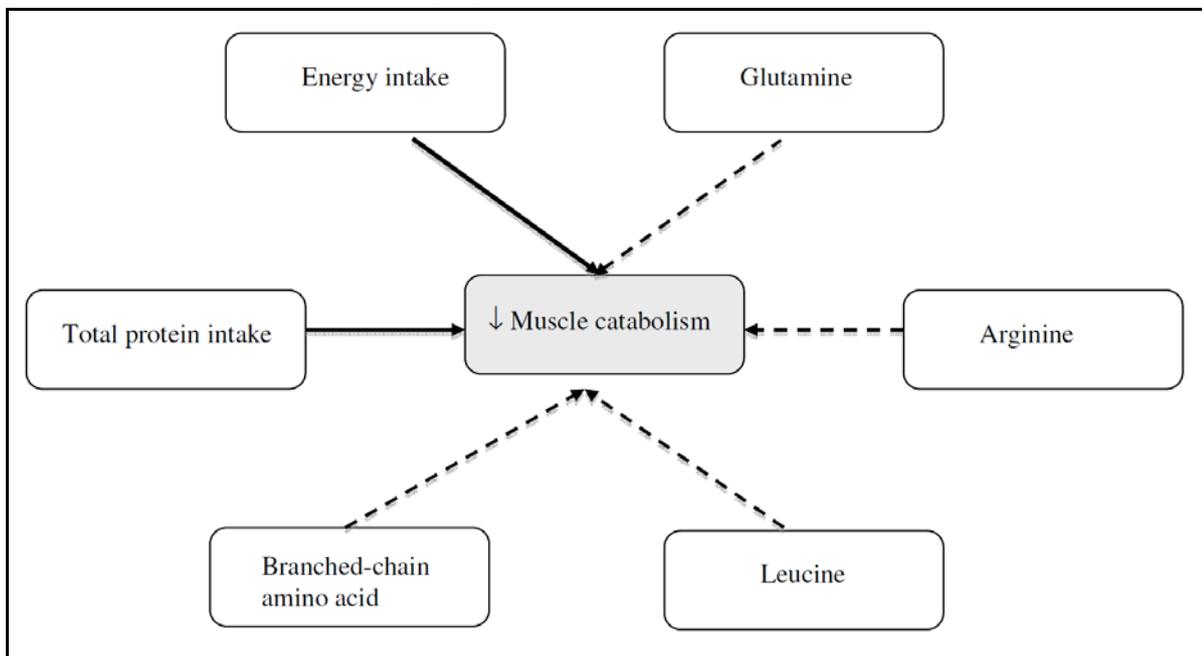
The effect of severe illness on albumin synthesis is unclear. Moshage et al. found a decrease in absolute albumin synthesis in 4 patients with inflammatory diseases, compared to healthy controls (16). The patients were fed orally with about 1800 kcal/d (60 g protein/d). The authors of this study examined the possible molecular mechanisms in rats and found a reduced concentration of albumin mRNA in the liver. This contrasts with the results of two more recent studies, which both found an increase in the absolute synthesis rate of albumin in their patients compared to healthy controls. Essen et al. had included ICU patients between day 2 and 30 after ICU admission, who received parenteral nutrition (~1800 kcal/d, 58 g protein/d) and albumin (32±10 g) the day before and glucose the night before measurement of albumin synthesis (11). Mansoor et al. had included 6 head-injured patients on day 8 after admission, fed by continuous enteral nutrition (~39 kcal/kg b.wt./d, 1.5 g protein/kg b.wt./d) (17). The latter authors hypothesized two mechanisms for the increased albumin synthesis. First, they suggest a biphasic pattern in acute inflammation, where albumin synthesis is first decreased and later increased, as described in animal models. Second, they suggest that high leucine concentrations resulting from accelerated proteolysis may stimulate albumin synthesis. Indeed, branched chain amino acids formulas were shown to stimulate albumin synthesis in cancer patients. The reason why these two studies show contradictory results with the one of Moshage et al. is unclear. It may be related to different severity of inflammation diseases or energy balances. High-energy

infusions for instance have been shown to increase albumin synthesis in postoperative patients. In any case, the hypoalbuminemia related to acute severe illness is in all likelihood not related to decreased albumin synthesis but rather to transcapillary escape as demonstrated earlier by Fleck et al. (18).

9.6. Impact of energy intake on protein mass and turnover

Energy and protein intakes may limit muscle catabolism in severe disease (Fig. 2). As mentioned in the section “Protein mass in severe illness”, studies lasting several days or weeks showed that energy intake did not prevent the loss of total body protein mass in patients with severe sepsis or major trauma. For obvious ethical reasons, none of these studies compared patients with and without nutritional intakes. However, one study looked at the impact of energy provision by nutritional support on IVNA-derived TBP. In patients after major upper gastrointestinal surgery, the administration of full-strength parenteral nutrition (26.5 ± 1.4 kcal/kg b.wt./d and 0.25 ± 0.04 g N/kg b.wt./d) compared to partial parenteral nutrition (20.2 ± 1.5 kcal/kg/d and 0.14 ± 0.03 g N/kg b.wt./d) preserved TBP (2).

Fig. 2: Nutritional elements which limit muscle catabolism (—) or are under investigation for prevention of muscle catabolism (---)



With regard to protein turnover, it is unclear whether short-term nutritional support promotes protein synthesis. In 6 burned patients, Wolfe et al. measured protein turnover by leucine kinetics and nitrogen excretion and described a stimulation of protein synthesis through feeding, as compared to a 10 to 12 hour fast (19). Shaw et al. compared the rates of net protein catabolism in 18 septic patients receiving either glucose (3.8 ± 0.6 mg/kg b.wt./min) or parenteral nutrition (glucose 3.8 ± 0.3 mg/kg b.wt./min, fat 20%: 9.8 ± 0.6 ml/kg b.wt./d, protein 1.5 ± 0.3 mg/kg b.wt./min) over 6 hours. Patients who received parenteral nutrition and thus higher amount of calories and protein showed a lower rate of net protein catabolism than those treated with glucose infusions (20). In contrast, in patients undergoing laparoscopic cholecystectomy, total parenteral nutrition infused over 8.6 ± 1.0 h and providing 17.5 nonprotein kcal/kg b.wt. compared to no nutritional support did not influence fractional synthesis rate of total liver protein (21).

Only few human studies looked at the impact of nutritional support over several days on protein kinetics. Frankenfield et al. randomized 30 trauma patients into 3 groups, i.e. 1) non-protein energy intakes

corresponding to measured energy expenditure 2) total energy intakes corresponding to measured energy expenditure 3) or hypocaloric intakes. On day 4 of nutritional support, they measured urinary urea, nitrogen production and 3-methylhistidine excretion during 24 h. They showed that achievement of energy balance did not attenuate the negative nitrogen balance (22). A more recent study allocated patients after gastrointestinal surgery to four different types of parenteral nutrition, administered for 7 days after surgery (23). The authors determined protein kinetics preoperatively and at day 2, 4 and 7 after operation. Already on the second postoperative day, the group fed with total parenteral nutrition (TPN) showed decreased protein breakdown and synthesis compared with those on a hypocaloric TPN formula and those receiving only carbohydrates and amino acids. Protein synthesis and breakdown rates in the TPN group remained in the preoperative range and were in favour of positive net protein synthesis.

A negative energy balance exerts a negative effect on outcome in ICU patients. It was correlated with a higher rate of infectious complications (24). More studies focussed on the effect of early vs. delayed enteral nutrition. Early enteral nutrition resulted in higher mean energy intakes the days after the insult. One meta-analysis found that early enteral nutrition was associated with a lower risk of infections, a reduced length of hospital stay in patients with severe illness (25) but another study was inconclusive (26).

Noteworthy is the fact that studies evaluating the impact of energy intake on protein mass and turnover vary with regard to the type of disease, nutritional state and type of feeding, which precludes comparisons between studies. Furthermore, a thorough study on the impact of energy intake necessitates taking into account energy expenditure. However, measurements of energy balance in patients with severe illness are not easy to perform and may not be accurate. Energy expenditure is generally measured by indirect calorimetry, but this method extrapolates 24 h energy expenditure from short periods of measurements and may give false results in case of gas leaks through endotracheal or chest tubes and high O₂ requirements. Some studies extrapolate total energy expenditure from body composition changes but this requires at least two measurements, is performed retrospectively and consequently cannot be used for everyday's practice. Thus, these studies have to be interpreted with caution as they include numerous confounding factors.

To summarize, it seems that nutritional support may limit but not stop the loss of total body protein mass occurring in acute severe illness.

9.7. Impact of protein and amino acid intake on total body protein mass and turnover

In many studies, the impact of protein intakes on total body protein is obscured by energy balance. Ishibashi et al. studied 18 trauma and 5 sepsis patients who were hemodynamically stable (27). They measured fat-free mass by dual-energy x-ray absorptiometry and allocated them to enteral intakes of 1.1, 1.5 or 1.9 g protein/kg fat-free mass/d with similar non-protein calorie content relative to fat free mass. They measured changes in TBP over 10 days by IVNA. While the average loss of TBP was 10%, the loss was significantly higher in the group receiving only 1.1 g protein/kg fat-free mass/d (-1.8 ± 0.8 kg) than the groups who received 1.5 (-0.8 ± 0.6) or 1.9 g/kg FFM/d (-1.0 ± 0.5 kg).

With regard to whole-body protein turnover, Shaw et al. included 18 septic patients and separated them into three groups who received intravenously 1.1, 1.5 or 2.2 g protein/kg b.wt./d with similar total energy intakes relative to body weight during 6 hours (20). They found that the optimal protein sparing effect was lowest with an infusion of about 1.5 g protein/kg b.wt./d. Similarly, another study in septic patients receiving parenteral

nutrition for 6 days described no advantage in nitrogen balance between patients who received 1.2 vs. 2.3 g protein/kg b.wt./d (28). In 6 burned patients submitted in a crossover study to either a 1.4 or a 2.2 g protein/kg b.wt./d isocaloric regimen during 3 days intravenously or enterally there was no benefit of a higher N provision on whole-body protein balance (19). Finally, in trauma patients who were randomized into five groups receiving from 0-1.9 g protein/kg b.wt./24h intravenously as well as fat and glucose in isocaloric amounts, the negative nitrogen balance improved with intakes up to 1.3 g protein/kg b.wt./d compared to the no nitrogen group (29).

The protein synthesis rate in response to nutritional support likely varies between individual tissues. Rittler et al. compared, in two different studies, the effect of a 4-hour amino acid infusion (1.6 g/kg b.wt./24h) containing glutamine vs. saline in 16 patients recovering from major abdominal operations. They showed that the amino acid infusion increased small-bowel protein synthesis but not albumin (30) or colon protein synthesis (31). They explain that the low synthesis rate in the colon may be related to a lower percentage of proliferative cells and metabolic turnover than in the small-bowel, making the colon less sensitive to parenteral substrates.

Protein intake affects circulating free amino acids. In healthy volunteers, an infusion of amino acids at a rate of 0.2 g N/kg b.wt./d is sufficient to switch net outflow to inflow of amino acids into peripheral tissues as skeletal muscle. Interestingly however, this amount of N does not seem sufficient to increase arterial concentrations of amino acids and reverse the flow in severe illness, as suggested by Iresjö et al (15). They compared 3 types of amino acid formulations in ICU patients who received parenteral nutrition providing 20 kcal/d of non-protein calories and 0.2 g N/kg b.wt./d for 3 days. The infusion of these formulas increased the overall arterial amino acids concentrations only to the level of fasting healthy subjects. They concluded that the composition of the available amino acid formulations may not be optimal to increase arterial amino acid concentrations and promote amino acid inflow.

Diminished FFM loss and improved nitrogen balance may ameliorate outcome in acute severe illness, although it is unknown whether achievement of a neutral nitrogen balance is desirable. In burned patients, growth hormone and insulin, considered as anabolic hormones, decreased mortality (32) and hospital length of stay, respectively (33) but in critically ill patients in general, it worsened outcome (34). In an interesting review, Strout et al. hypothesized that anorexia and limitation of food and protein intake accompanying catabolism in severely ill patients may even exert a protective effect. Indeed, provision of high amounts of protein leads to increased amino acid oxidation and formation of urea. This can be problematic in severely ill patient as high levels of urea may aggravate renal failure and reduce the capacity of salt and water elimination in patients with oedema. A high level of protein supply could also stimulate protein synthesis but since it may not provide the amino acids needed for the acute phase response, it could force the metabolism away from pathways maximizing survival (35).

Consequently, administration of amino acids or of protein improves total body protein mass and protein turnover in severe illness. The effect seems to be maximal at doses around 1.2 and 1.5 g/kg b.wt./d, but most of the studies did not detail how they measured body weight. It is variable according to individual tissues. It is not clear whether forcing the body to preservation of muscle mass in severely ill patients improves outcome.

Impact of specific amino acids on total body protein mass and turnover

There is growing interest in the role of specific amino acids on total body protein mass and protein turnover. In 2006, De Bandt et al. carefully reviewed the utility of branched-chain amino acids (BCAA) in burn, trauma and sepsis (36). They found 7 studies performed in trauma and ICU patients, among which 3 claimed an improvement in muscle protein balance, and 2 in septic patients, which showed an improvement in prealbumin, retinol-binding protein and a reduction of muscle catabolism and mortality with BCAA. They concluded that BCAA did not show any clear benefit in these medical conditions. They mentioned however that leucine-supplemented amino acid solutions deserve further attention, as they showed anticatabolic effects in animal studies. Since 2006, one published study included intensive care patients after radical cancer surgery (37). Patients received intravenously, in a crossover design, isonitrogenous amino acid solutions over 3 hours (246 mg/kg b.wt.) with a ratio of leucine to total amino acids of 0.09 (22 mg/kg b.wt.) or 0.22 (54 mg/kg b.wt.) on the first two post-operative days. The solution with the higher leucine content stimulated protein synthesis and increased glutamine release from skeletal muscle.

Conditionally essential amino acids, as glutamine and arginine, also received considerable attention in severe illness. In a meta-analysis including critically ill patients, high arginine-content enteral formulas led to a decreased length of hospital stay, a trend toward a reduction in infectious complications but no effect on mortality compared with other immunonutrition formula (38). However, the authors recognized that these effects of arginine relied on speculations as all high arginine-content enteral formulas contained more than one specific nutrient. Noteworthy is the fact that the authors found a higher mortality with immunonutrition when considering studies with high methodological scores.

Surgical, trauma and sepsis patients show low plasma and intramuscular glutamine levels (39), which have been related to high severity illness scores and hospital mortality. In ICU patients who randomly received for five days increasing doses of intravenous glutamine (0, 0.3, 0.6, 0.9 g/kg b.wt./d) as part of an isocaloric isonitrogenous parenteral diet, plasma glutamine normalized in a dose-dependent manner but free muscle glutamine, muscle protein synthesis and muscle protein content did not change (40). Similarly, in critically ill patients, parenteral nutrition with glutamine (0.4 g/kg b.wt./d) vs. without glutamine for 3 days did not affect protein balance (41). Regarding outcome, two meta-analysis found that supplementation of glutamine in enteral and parenteral nutrition reduced infectious complications and lengths of stay in surgical patients as well as complications and mortality in critical illness (42, 43). Possible mechanisms of these beneficial effects are decreased oxidant damage and inflammatory cytokine production, reduction of gut bacterial translocation and improvement of nitrogen balance.

Thus, glutamine and potentially leucine are important for clinical outcome, but it is unclear whether these benefits occur through an impact on TBP and turnover or through other mechanisms.

9.8. Protein requirements in severe illness

The essential role of nutritional support in severe disease is to protect lean tissue mass and function. In ICU patients, the adequate amount of protein supply is still debated but intakes between 1.2 and 1.5 g/kg ideal b.wt./d are usually advised (44, 45).

According to the ESPEN guidelines, if enteral nutrition is indicated, it should be given at a dose of 20-25 kcal/kg b.wt./d during the first 72-96 hours and then up to 25-30 kcal/kg b.wt./d during stabilization and recovery (46). They do not mention protein requirements by enteral nutrition. There is no advantage of using

peptide-based formula compared to whole protein formula. Immunonutrition enriched with arginine, nucleotides and omega-3 fatty acids should be given to patients with trauma, ARDS, mild sepsis (APACHE II<15) but not to patients with severe sepsis and those who do not tolerate more than 700 ml of enteral nutrition per day. No position is taken for immunonutrition in burned patients. Glutamine should be added in burned and trauma patients but there is insufficient data to support its use in surgical or heterogeneous critically ill patients.

If parenteral nutrition is indicated in ICU patients, a balanced mixture of amino acids and should be infused at about 1.3 to 1.5 g/kg ideal b.wt./d with 0.2-0.4 g/kg b.wt./d of glutamine (ESPEN guidelines, (44)). Energy should be supplied at a dose of 25 kcal/kg b.wt./d in the absence of indirect calorimetry and increased to target over the following 2-3 days. It remains unclear whether actual or anamnestic weight should be taken into account since these patients are often overhydrated. In addition, energy requirements may be affected by sex and age. In ICU patients, glutamine should be added as 0.2-0.4 g/kg b.wt./d of L-glutamine.

In obese patients, the weight to be used seems to be the actual rather than the ideal body weight (47). In these patients, several studies support the use of hypocaloric (11-14 kcal/kg actual b.wt./d or 22-25 kcal/kg ideal b.wt./d) hyperproteinic ≥ 1.5 g protein/kg actual b.wt./d) parenteral nutrition, but so far no specific nutritional guidelines have been provided for them. Noteworthy is however that hypocaloric hyperproteinic feeding is contraindicated in patients with renal insufficiency, hepatic encephalopathy, diabetic ketoacidosis, hypoglycaemia, age greater than 60 yrs or severe immunocompromise (48).

In undernourished patients, the resting energy expenditure is 25% higher than predicted by the Harris-Benedict formula (49). In the absence of clinical data, and based on mathematical approximations, Hoffer et al. estimated that protein requirements may be higher by 25%, thus at 1.9 g/kg b.wt./d, in neutral or positive energy balance, in undernourished patients (50). Studies including specifically underweight patients with acute severe illness are needed to confirm these estimations before recommendations on requirements can be given.

9.9. Conclusions

Severe illness is characterized by increased protein turnover leading eventually to loss of total body protein mass, mainly in the skeletal muscle. Energy and protein intakes limit muscle catabolism and improve outcome. Current guidelines suggest the administration of 20-25 kcal/kg b.wt./d during the acute phase (e.g. 1-3 days after ICU admission) and 25-30 kcal/kg b.wt./d once the condition is stabilized. The optimal protein requirement has been set at 1.2-1.5 g/kg b.wt./d.

However, these recommendations rely on two types of study design, which have to be interpreted with caution. In the first study design, study groups have received different protein intakes (in g/kg b.wt.) and similar amounts of glucose or fat and, in this case, the highest protein supply led also to the highest calorie supply. In the second study design, patients have received different protein intakes with isocaloric diets and consequently the subjects with the lowest protein supply received more calories as glucose or fat. Consequently, it is impossible to study the impact of protein intakes alone on total body protein mass and protein turnover. Nutritional supply of study groups either differ in energy or macronutrient composition.

9.10. References

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10. Dietary proteins and atherosclerosis

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10.1. Summary/ Zusammenfassung/ Résumé

More than 100 years ago the hypothesis "protein" of the pathogenesis of atherosclerosis and its association with cardiovascular disease was put forward on the basis of animal experiments; however, it has so far never been verified in humans. This theory was soon replaced by the "lipid hypothesis", which was confirmed in humans as of 1994. Epidemiological ecological studies in the 1960s showed significant associations between dietary animal protein and mortality from cardiovascular disease. However, animal protein intake was also significantly correlated with saturated fatty acid and cholesterol intake. In the last decades two prospective cohort studies demonstrated a decreased cardiovascular risk in women during high versus low protein intake when adjusting for other dietary factors (e.g. saturated fats) and other cardiovascular risk factors. A direct cholesterol lowering effect of proteins has not been shown.

Despite earlier research indicating that soy protein has cardioprotective effects as compared to other proteins, these observations have not been confirmed by randomised placebo-controlled trials. However, most experts recommend the consumption of foods rich in plant proteins as alternatives to meat and dairy products rich in saturated fat and containing cholesterol.

There are no scientific arguments to increase the daily protein intake to more than 20% of total energy intake as recommended by the guidelines, in order to improve cardiovascular health.

Zusammenfassung: Proteinzufuhr und Atherosklerose

Vor über 100 Jahren wurde auf der Basis von Tierversuchen die Hypothese aufgestellt, dass Proteine einen Einfluss auf die Pathogenese von Atherosklerose und kardiovaskuläre Erkrankungen haben. Beim Menschen wurde diese Hypothese allerdings bisher noch nicht bestätigt. Bald wurde sie durch die „Lipid-Hypothese“ überholt, die beim Menschen 1994 bestätigt wurde. Epidemiologische Studien aus den 1960er Jahren zeigten einen signifikanten Zusammenhang zwischen dem Verzehr tierischer Nahrungsproteine und der Mortalität an kardiovaskulären Erkrankungen. Allerdings war die Einnahme von tierischen Nahrungsproteinen auch signifikant mit der Einnahme von gesättigten Fettsäuren und Cholesterin korreliert. Zwei in den letzten beiden Jahrzehnten durchgeführte prospektive Kohortenstudien konnten nach sorgfältiger statistischer Korrektur für kardiovaskuläre Risikofaktoren und Ernährungsfaktoren (z.B. gesättigte Fettsäuren) bei Frauen ein vermindertes Risiko für koronare Herzkrankheiten bei einem hohen Proteinverzehr im Vergleich zu einem geringen Proteinverzehr. Eine direkte cholesterinsenkende Wirkung von Proteinen konnte nicht nachgewiesen werden.

Trotz vielversprechenden ersten Ergebnissen über kardioprotektive Effekte von Soja-Proteinen gegenüber anderen Proteinen, konnten randomisierte, Placebo kontrollierte Studien der letzten Jahre diesen Vorteil nicht bestätigen. Allerdings sind sich die meisten Experten einig, dass die Einnahme von Lebensmitteln, die reich an pflanzlichen Proteinen sind, als Alternative zu Fleisch- und Milchprodukten, die reich an gesättigten Fettsäuren und Cholesterin sind, gefördert werden sollte.

Basierend auf dem heutigen Kenntnisstand gibt es keine wissenschaftlichen Argumente, dass für eine Verbesserung der kardiovaskulären Gesundheit eine höhere Proteinzufuhr als maximal 20% der gesamten Energiezufuhr empfohlen werden sollte.

Résumé : Protéines nutritionnelles et athérosclérose

L'hypothèse « protéique » de l'athérosclérose et son lien avec les maladies cardiovasculaires, avancée il y a plus de 100 ans sur la base de l'expérimentation animale, n'a jamais été vérifiée chez l'homme à ce jour. Par contre, elle a laissé rapidement place à l'hypothèse lipidique « cholestérol », confirmée chez l'homme en 1994. Dans les années 60, les études épidémiologiques plaidaient en faveur d'une augmentation du risque cardiovasculaire liée à la consommation de viande et de produits laitiers. Cependant, la consommation de protéines animales était aussi significativement liée à l'apport en acides gras saturés et en cholestérol. Par la suite, deux études prospectives de cohorte soigneusement conduites ont même démontré une diminution du risque cardiovasculaire chez les femmes suivant un régime riche en protéines plutôt qu'un régime pauvre en protéines, après ajustement pour les facteurs confondants diététiques (p. ex., graisses saturées) et les autres facteurs de risque cardiovasculaire. Toutefois, aucun effet hypercholestérolémiant des protéines per se n'a pu être démontré.

Malgré des résultats initiaux prometteurs sur les effets cardioprotecteurs des protéines de soja en comparaison des autres protéines, les études randomisées de type placebo-contrôle entreprises au cours de ces dernières années n'ont pas permis de confirmer ces observations. Cependant, la plupart des experts s'accordent à promouvoir la consommation d'aliments riches en protéines végétales comme substitution aux produits carnés et laitiers riches en graisses saturées et contenant du cholestérol.

En l'état des connaissances actuelles, il n'y a pas d'arguments scientifiques suggérant d'augmenter la part protéique à plus de 20% de l'apport énergétique total journalier, dans le but de la prévention des maladies cardiovasculaires.

10.2. Introduction

In 1909, the pathologist Ignatowski was the first to demonstrate the link between diet and atherosclerosis [1]. He observed that rabbits fed eggs, meat and milk developed atherosclerosis of the aortic tree, which he attributed to the protein content of these foods. In 1913, Anitschkov Chalatov produced atherosclerosis in rabbits with a diet based on vegetable oil containing up to 1% cholesterol. They also show that if one removes cholesterol from the diet, there is a regression of initial lesions [2].

In subsequent years, some authors observed the onset of atherosclerosis in rabbits when they consumed a casein diet rich in lean beef, but not with a diet rich in soy protein [3-4]. In general, cholesterol levels rose in animals fed with animal protein, and vice versa they dropped under the influence of plant proteins. Moreover, in most animal studies, there was a direct correlation between the amount of animal protein consumed and degree of hypercholesterolemia, and conversely, between vegetable protein intake and hypocholesterolaemia. These findings have strengthened the theory of R. Virchow dating from 1856, for whom atherosclerosis was "a modified form of chronic inflammation induced by lipids". It should be noted that the injuries produced in these animals were not exactly the same as those observed in humans. Moreover, experimental data revealed that while a number of animal species (rabbits, chickens, pigeons) were prone to atherosclerosis induced by the addition of dietary cholesterol to protein, other species were

resistant, such as dog, guinea pig, rat or monkey [3]. For these reasons animal testing is mainly focused on lipid disorders induced by diet as atherogenic factors, rather than the by proteins themselves.

In humans, the scientific interest on the relationship between nutrition and atherosclerosis started only in the 1960s, alongside with attempts to prevent ischemic heart disease as a major public health issue in industrialized countries. Early studies in humans were conducted by De Langen in the island of Java in 1916. They advanced the idea that the inhabitants of the island "have little atherosclerosis because their traditional diet contains little cholesterol and fat. But when they adopted a European-style diet, their cholesterol rose" [5].

Mjassnikow in Leningrad in 1925 observed that subjects with aortic and coronary atherosclerosis often have high cholesterol levels which can be reduced with a diet rich in vegetables [6]. It also appeared that man belongs to a species not susceptible to hypercholesterolemia induced by pure dietary proteins [3]. This is probably why the protein hypothesis of atherosclerosis stated by Ignatowski was soon replaced by the lipid hypothesis.

This review therefore aims to summarize current knowledge on the role of dietary proteins in the development of atherosclerosis in humans.

10.3. Atherosclerotic diseases and their determinants

Atherosclerosis is defined as a chronic inflammatory disease of the arterial wall, characterized by the formation of atherosclerotic plaques that are focused and scattered throughout the arteries of medium and large calibre. Inflammation induces the formation, progression and rupture of plaques unpredictably. This results in instant formation of a thrombus at the site of rupture which may lead to arterial occlusion. This is the origin of major clinical complications such as acute coronary syndrome, angina pectoris, cerebral ischemic attack or peripheral arterial disease [7-8].

The disease is silent for decades until the onset of the first clinical manifestations which are usually sudden. To date, it is well established that among the major risk factors for atherosclerosis are dyslipidaemia, hypertension, smoking, diabetes and obesity.

Apart from smoking, each of these risk factors is influenced by eating habits and can even be regarded as a marker, although imperfect, for certain eating habits. So far, we have no accurate epidemiological data on the early development of atherosclerosis; this gap is mainly due to the silent nature of the disease and the lack of imaging techniques applicable to large-scale, non-invasive investigations without radiation hazard and at acceptable costs. Thus, there are indirect criteria of atherosclerosis which are taken into account in studies, namely the cardiovascular morbidity and / or mortality.

10.4. Dietary protein and human atherosclerotic cardiovascular diseases

Although few in number, some epidemiological studies performed since the 1950s in humans have examined the association between diet and cardiovascular disease. Overall, these studies showed a strong correlation between the consumption of animal proteins and cardiovascular mortality. Whereas animal protein consumption was clearly correlated with coronary heart disease (CAD; $r = 0.78$), the reverse was true for the consumption of vegetable proteins ($r = -0.40$) [9].

However, these observations were flawed by various confounding factors such as socioeconomic status, lifestyle factors or lipid composition of the diet. In this regard, the consumption of animal proteins was also significantly associated with intake of saturated fat and cholesterol, factors known to be hypercholesterolaemic and atherogenic [10]. Moreover, in countries with low protein intake, there was conversely a higher fibre intake.

Considering the cohort studies cited by F. Hu et al. [11] totalling 33'289 men followed for 5-20 years, only one of them showed a positive association between protein intake and risk of CAD, but it had not been adjusted for fat intake.

More recently, the "Nurses' Health Study" is of considerable interest because it used more refined methodology and had a large size. There were 80'082 healthy women included; they were aged 34-59 years, with no history of cardiovascular disease, cancer or hypercholesterolemia [11]. The 14-year follow-up was conducted by sending a questionnaire relating to risk factors and the occurrence of diseases every two years, and by sending every four years a standardized food questionnaire. The results were adjusted for age, cardiovascular risk factors, total daily energy intake, and the specific type of fat consumed (**Tab. 1**).

Tab. 1: Relative risks (RR) of ischemic heart disease and 95% CIs according to quintiles of protein intake [11]

Protein intake	Quintile 1	Quintile 5
Total protein intake:		
Median (% of energy)	14.7	24
Number of cases	211	169
Multivariate Relative Risk (RR) for CAD	1	0.72 (0.57, 0.91)
RR with additional adjustment for specific fats	1	0.74 (0.59, 0.95)
Animal protein intake:		
Median (% of energy)	11.6	20.6
Number of cases	195	172
Multivariate Relative Risk (RR) for CAD	1	0.86 (0.68, 1.09)
Further adjustment for vegetable protein	1	0.84 (0.65, 1.07)

Unlike other studies mentioned above, this study showed an inverse association between protein intake and coronary risk. Comparing the first and fifth quintile of protein intake (14.7% vs. 24% of AETJ) shows a significant reduction in relative risk of CAD (multivariate adjusted RR = 0.72, 95% CI = 0.57-0.91). Interestingly, subgroup analysis revealed no significant further reduction of coronary risk in the high protein intake group when total fat intake was low compared to high (low fat intake RR of CAD: 0.76, 95% CI = 0.55-1.06) versus high fat intake RR CAD: 0.72, 95% CI = 0.52-1.01). The same was true for subjects in the high protein intake quintile regarding saturated fat intake – high or low saturated fat intake had no additional effect on CAD.

The beneficial effect of higher protein intake was observed in both, animal and plant-derived proteins. In the opinion of the authors, this study has the same limitations as earlier ecological studies and it is possible that the observed inverse relation between protein intake and cardiovascular risk was due to unmeasured factors, such as the socio-economic status. Other limitations inherent to this type of study include possible errors of

actual food intake based on self-assessment questionnaires. However, It should support the conclusion of authors claiming that their "data do not support the hypothesis of an increased coronary risk in case of high protein", contingent upon the fact that intake did not exceed 20% of total energy intake as recommended by experts [12].

In 2005, the "Iowa Women's Health Study" reported a decreased risk of CAD among 29'017 women aged 55-69 years followed prospectively for 15 years during iso-energetic replacement, of carbohydrates with animal and vegetable proteins [13]. By comparing the fifth and the first quintile of total protein intake (22.0% vs. 14.1% of total energy intake, respectively), and after adjusting for several factors (age, cardiovascular risk factors, hormonal status, socioeconomic status, dietary cholesterol), there was a no significant reduction of coronary risk of 16% (95% CI = 0.39-1.79) when more animal proteins were consumed (-12%, 95% CI = 0.42-1.86) compared to a significant reduction in CAD risk during more plant proteins (-51%, 95% CI = 0.49-0.99).

In order to strengthen the conclusion of these two prospective studies, the "Cholesterol Lowering Atherosclerosis Study" should also be quoted [14]. This randomized double-blind trial aimed to demonstrate the evolution of angiographic coronary atherosclerosis in 162 patients who were undergoing surgery for coronary revascularization surgery and who were treated with lipid lowering drugs. Dietary habits were assessed by a dietary recall questionnaire of 24 hours. Multivariate logistic regression analysis showed a protective effect defined by the lack of appearance of new coronary lesions when dietary proteins were increased. The combination of lean meats, and low fat dairy products was also protective (OR = 0.82, 95% CI = 0.69-0.96). Other protein sources were not associated with development of new lesions. These results are consistent and of great interest, but they deserve to be validated in other studies with expanded collectives, using imaging techniques of atherosclerosis more accurately than angiography and tools to assess dietary intake more suitable than the 24-h dietary recall. Also to be noted is the fact that the average protein intake in the protected group was 17.4% of total energy intake, and thus below the 20% upper limit currently recommended.

Therefore, on the basis of existing data, it is reasonable to conclude that the hypothesis of atherosclerosis induced by dietary protein has not been demonstrated. However, the evidence for the opposite is still insufficient to recommend a high protein diet for prevention of atherosclerosis.

10.5. Protein, atherosclerosis and stroke

A few ecological and cohort studies suggest that the rate of stroke mortality is higher in Asian regions such as Japan and China than in Europe or North America. This could result in eating habits characterized in Asia by a very low intake of fat and animal protein and high consumption of salt, a constellation that may be associated with higher rates of stroke, particularly hemorrhagic stroke [15]. The gradual reduction of stroke observed in Japan since the early 60s has been attributed to improved treatment of hypertension and changes in eating habits. Increased consumption of animal products including meat, eggs, dairy products associated with elevated blood cholesterol has been suggested as potentially beneficial to the risk of hemorrhagic stroke, not only in Japan but also China [16-17]. However, it is known that vegetarians who consume little protein and animal fat have an increased risk of stroke [18].

The U.S. "Nurses' Health Study [19] involving the same cohort as that cited in [10] also shows an inverse association, but non-significant, between the risk of hemorrhagic stroke and animal protein consumption

(RR = 0.47, 95% CI = 0.2-1.11), vegetable protein intake (RR = 0.81, 95% CI = 0.4-1.63) or with the consumption of saturated fats.

In the prospective "Hiroshima / Nagasaki Life Span Study" 40'349 subjects followed between 1980 and 1996 using validated questionnaires, including 24-h dietary recalls, C. Sauvaget et al. [20] identified an inverse association between high vs. small consumption of animal products (beef, pork, chicken, dairy products, eggs, fish) and the risk of stroke mortality (RR = 0.88, 95% CI = 0.77-1.0) by hemorrhagic stroke (RR = 0.76, CI 95% = 0.58-0.99) and ischemic stroke, (RR = 0.89, 95% CI = 0.73-1.09), again suggesting a beneficial effect of dietary proteins. However, this analysis was adjusted for all confounding factors, but without the fat content of protein-rich food.

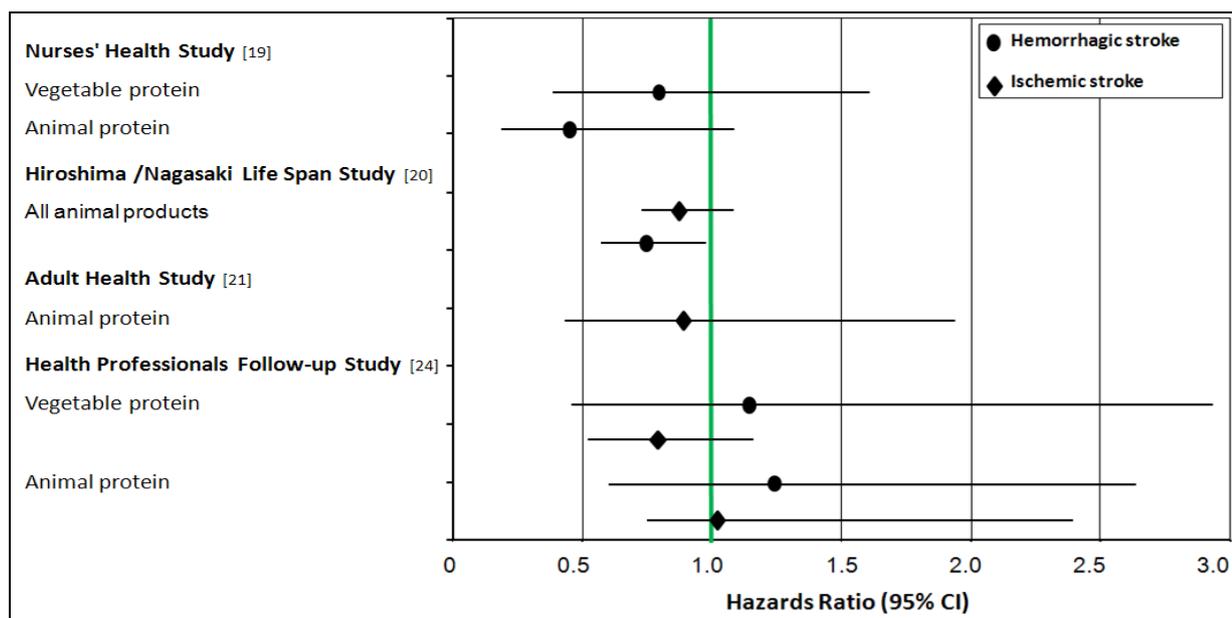
But one year later, the same authors reported that animal protein intake was not significantly associated with cerebral infarction mortality after adjustment for animal fat and cholesterol (RR = 0.92, 95% CI = 0.43-1.95) in the "Adult Health Study", a sub-group of the "Hiroshima / Nagasaki Life Span Study [21].

Similarly, for each 3% increment of daily energy intake from total fat, a significant reduction in risk of stroke-I (RR = 0.85, 95% CI = 0.78 - 0.94) was observed in a group of 832 participants the 'Framingham Heart Study "reviewed bi-annually from 1966 to 1969 [22].

These results were reversed in the recent publication of the Health Professionals Follow-up Study, another prospective cohort study totalling 43'960 men initially aged 40-75 years and followed from 1986 to 2004 using validated questionnaires [23]. After 18 years of follow-up, no significant association was found between the risk of total stroke (RR = 1.14, 95% CI = 0.94-1.43) and consumption of proteins. Unlike other studies, this analysis included a complete adjustment for confounding factors, including the type of fats.

The summary of the association between risk of stroke and levels of protein intake observed in these several cohort studies are illustrated in **Fig. 1**.

Fig. 1: Relative hazards and 95% confidence intervals of stroke according to consumption level and types of proteins



10.6. Protein foods of animal origin and cardiovascular diseases

In recent years, American and European recommendations for the prevention of cardiovascular disease advocated moderate consumption of red meat, sausages and processed meat products (PMP) [12, 24]. These recommendations stem primarily from changes induced by these products on the blood lipid profile and atherogenic potential, as well as the consequences of excessive calories and fat intake.

Definition and classification of meat

According to the "Vulgaris-medical" encyclopédie [25], meat is defined as the flesh, in terms of food, from mammals and birds. We distinguish:

- a) red meat from beef, pork, veal, sheep and horse
- b) white meat from poultry and rabbits.
- c) dark meat from game

However, in their important review of the literature on the effects of red meat and PMPs, Micha et al. [26] used a different classification:

- a) red meat = beef, pork and lamb,
- b) processed meat products = ham, hot dogs, salami, sausages, meats.

Red meat and processed meat products

To this day, there are no randomized controlled trials to assess the impact of meat products on human health, given the difficulties of feasibility, methodology (e.g. double-blind, compliance, long duration and / or very large group to detect a number of events for statistical significance) or costs. In fact, despite their limitations already mentioned, prospective cohort studies, and to a lesser extent case-control studies provide today the best level of evidence. Rather than review the various epidemiological studies, only the results of the systematic review and meta-analysis by Micha et al. are presented here [26]. Of the 1598 abstracts identified by computerized systematic research, only 17 prospective cohort studies and 3 case-control studies were selected because they contained the information necessary to judge the effect of red meat consumption on CAD risk, on stroke and diabetes. These 20 studies totalling 1'218'380 persons there were 23'389 subjects with CAD, 2'280 with stroke and 10'797 with type 2 diabetes. The analysis of results took into account the necessary adjustments and assigned a quality score to each of the studies identified, the score of between 3 and 5 / 5 (mean = 3.8). As shown in **Tab. 2**, the consumption of PMP over 50 g/day was associated with a significantly increased risk of CAD (RR = 1.42, 95% CI = 1.07-1.89) and diabetes (RR = 1.19, 95% CI = 1.07-1.27). There was also a significantly increased risk of ischemic stroke (RR = 1.24, 95% CI = 1.08-1.43) correlated to the total consumption of meat products, this increase was not significant for consumption of red meat or PMP. However, there was no evidence of an increased risk of CAD (RR = 1.0, 95% CI = 0.81-1.23), or reducing the risk of stroke (RR = 1.16, 95% CI = 0.92-1.46), linked to the consumption of red meat (> 100 g/d), which corresponds to 20% of total energy intake calculated for a diet of 2000 Kcal.

Tab. 2: Risk of clinical events associated with the consumption of red meat, processed meat and meat products (Meta-analysis of R. Micha et al. [26].)

	Red meat	Processed meat products	Total meat products
Coronary heart disease (CAD)			
No. & type of studies	2 CS*+ 1 CCS	4 CS + 1 CCS	5
No. of subjects	56'312	614'063	658'696
No. of cases with CAD	766	21'308	24'437
RR (CI 95%)	1.00 (0.81-1.23)	1.42 (1.07-1.89)	1.27 (0.94-1.72)
Stroke			
No. & type of studies	2 CS	2 CS	2 CS
No. of subjects	108'898	108'898	115'500
No. of cases with stroke	1'600	1'434	601
RR (CI 95%)	1.17 (0.40-3.43)	1.14 (0.94-1.39)	1.24 (1.08-1.43)
Diabetes			
No. & type of studies	5 CS	7 CS	3 CS
No. of subjects	298'982	336'291	142'851
No. of cases with diabetes	7'349	8'888	5'904
RR (CI 95%)	1.16 (0.92-1.46)	1.19 (1.07-1.27)	1.12 (1.05-1.19)

*CS = cohort study, CCS = case control study

*RR = Relative Risk for 100 g/d red meat, for 50 g/d of processed meat and for 100 g/d of total meat products; Statistical significance: $p < 0.05$ when the 95% CI do not cross the value 1.00

These data strongly suggest that a high consumption of total or processed meat products increases the risk of cardiovascular disease and of diabetes. Although not significant, it also appears that consumption of red meat (> 100 g/d) tends to adversely affect the risk of stroke and diabetes.

White meat

As it stands, the data are conflicting on lean meats whose characteristics are essential to their content and fat composition, illustrated in **Tab. 3** [15, 27].

Fish

In 1975, Bang and Dyerberg [28] showed a lower incidence of cardiovascular mortality in Eskimos living in Greenland compared to those living in Denmark, mortality rates being inversely correlated to the consumption of fish and marine mammals. Since then, most studies have demonstrated a cardioprotective effect of fish, specifically of its omega-3 polyunsaturated fatty acids. The latest meta-analysis of 13 cohort studies totalling 222'364 persons followed for an average of 11.8 years showed an inverse correlation between fish consumption and CAD [29]. Compared to those consuming less than 1x/month, 5x/week consumption of fish reduced the risk by 38% (RR = 0.62, 95% CI = 0.46-0.82). For fish consumption 1x/wk vs. 1x/month the difference was less, but there was still a significant decrease in risk of 15% (RR = 0.85, 95% CI = 0.76-0.96). This study demonstrated that each 20 g/d of fish consumption was associated with a 7% reduction in coronary mortality. The same authors also conducted a meta-analysis of 8 cohort studies looking at stroke incidence; it demonstrated a reduced risk of ischemic stroke only in people who consumed fish 1x/week compared to those eating less than 1x/week (RR = 0.68, CI 95% = 0.52-0.88). Consumption of 5x/week vs. less than 1x/week was associated with a further decrease of risk of ischemic stroke (RR = 0.65, 95% CI = 0.46-0.93). The effect on hemorrhagic stroke was not significant (RR = 0.80, 95% CI = 0.44-1.47). Given these data, it appears that the minimum consumption of fish could result in reduced risk of

cardiovascular mortality was at least 1x fish consumption per week. Current recommendations advocating 2x/week seem adequate, especially if one takes also into account ecological considerations, especially the fact that overfishing can cause important alterations in the structure and dynamics of a large marine ecosystem.

10.7. Plant proteins and cardiovascular diseases

Many animal studies have shown a reduction of serum cholesterol during ingestion of plant proteins, especially for soybeans compared to animal proteins. In humans, epidemiological studies showed that in Asian countries soy consumption was much higher than in Western countries, associated with a lower incidence of ischemic cardiovascular diseases [31].

When animal and vegetable proteins are compared (**Tab. 3**, see below.), plant proteins contain more carbohydrates, less total fat, more polyunsaturated fats, no cholesterol and more fibre.

Plant proteins with high quality because of their amino acid contents are found in legumes, whole grains and products imitating meat such as tofu and seitan. However, the main scientific interest during the past 30 years has focussed on soy protein and its influence on cardiovascular disease.

Tab. 3: Comparison of nutrient composition between plant and animal proteins [27]

Foodstuff	Energy, Kcal/100 g	CHO, g	Prot., g	Fat, g	Fats, SFA. %	Fats, MUFA %	Fats, PUFA. %	P/S ratio	Cholesterol, mg	Fibre, g
Red meat										
Beef: steak	138	0.0	22.3	5.4	46	48	7	0.14	63	0
Beef: filet	105	0.0	21.9	2.0	50	44	6	0.11	35	0
Pork chop	180	0.0	20.6	10.9	41	47	11	0.29	77	0
Pork tenderloin	128	0.0	22.3	4.4	38	51	10	0.27	60	0
Lamb chop	213	0.0	18.3	15.6	46	49	6	0.13	66	0
Lamb leg	173	0.0	19.6	10.5	49	42	9	0.18	84	0
Average	156	0.0	20.8	8.1	45.1	46.9	8.0	0.19	64.2	0
White meat										
Chicken thigh with skin	184	0.0	17.6	12.6	32	51	17	0.54	80	0
Veal: roast	124	0.0	20.2	4.8	42	42	15	0.36	81	0
Veal: cutlet	103	0.0	22.2	1.6	36	45	18	0.50	65	0
Average	137	0.0	20.0	6.3	36.9	46.3	16.9	0.47	75.3	0.0
Fish										
Cod, raw	79	0.0	18.1	0.7	20	60	20	1.0	43	0
Pike, raw	81	0.0	18.4	0.8	33	33	33	1.0	63	0
Salmon, smoked	171	0.3	22.5	8.9	21	44	35	1.63	70	0
Average	110	0.0	19.7	3.5	24.9	45.8	29.3	1.21	58.7	0.0
Sausage and processed meat products										
Saveloy	259	1.3	13.1	22.7	41	51	8	0.20	37	0.0
Pies	292	0.9	13.1	26.0	39	48	12	0.32	111	0.1
Roast pork sausage	253	0.4	15.7	21.0	38	50	12	0.31	53	0.1
Salami	424	0.3	25.2	35.2	39	50	11	0.28	61	0.0
Ham	248	0.4	29.4	14.3	40	12	49	0.30	70	0.0
Average	295	0.7	19.3	23.8	36.8	52.9	10.3	0.28	66.4	0.0
Plant proteins										
Soy milk	32	0.8	2.9	1.9	17	61	61	3.7	0	0.0
Tofu	78	0.7	8.1	4.8	3	69	69	27.0	0	1.2
Soybeans, dry beans	347	15.8	35.9	18.6	15	61	61	3.96	0	15.7
Soy flour, whole	453	23.5	36.8	23.5	15	61	61	3.93	0	13.3
Soy bruised	194	0.6	45.2	1.2	18	64	64	3.50	0	10.7
Chickpeas, dry	306	44.3	19.0	5.9	14	57	57	4.0	0	15.5
Dry bean, white	261	41.4	21.1	1.2	33	56	56	1.67	0	18.1
Lens, dry	308	50.4	24.0	1.2	25	50	50	2.0	0	11.2
Average	247	22.2	29.0	5.0	17.6	59.8	59.8	6.22	0.0	10.7

Soy proteins

Soy is a plant of the Fabaceae family, close to the bean, widely cultivated for its seed oil which makes it the major edible oil consumed in the world. It contains a large amount of protein, carbohydrates, lipids, vitamins A and B, potassium, calcium, magnesium, zinc and iron. In addition, its proteins contain all essential amino acids. Furthermore, soy contains isoflavones or phytoestrogens with weak estrogenic activity.

Reports on anti-atherosclerotic effects of diets containing soy have been published repeatedly, but there is no clear evidence of such an effect in humans [32-34].

Nevertheless, in 1999 the U.S. Food and Drug Administration approved the labelling of foods containing soy protein as a cardiovascular protective. This was based on the fact that 25 g/d of soy would lower serum total and LDL cholesterol levels [33]. In 2000, a scientific committee of the American Heart Association (AHA) concluded that "it is prudent to recommend the inclusion of foods containing soy protein in a diet low in saturated fat and cholesterol" [35]. A new position paper of a scientific committee of the AHA published in 2006 on the basis of a rigorous assessment of new scientific knowledge on soy protein and isoflavone compound [36] based on a meta-analysis of 22 randomized controlled trials (RCTs) stated that only a minor decrease of LDL-cholesterol (approx. 3%) was observed when extracts of soy protein plus isoflavones (25-135 g/d) were added to the diet compared to casein, milk protein or wheat, and a mixture of animal proteins. These authors concluded: "Previous studies demonstrating a clinically favourable effect of soy protein compared to other proteins has not been confirmed. Nevertheless, many soy products should be beneficial to cardiovascular and general health because of their high content of polyunsaturated fatty acids, fibre, vitamins, minerals and their low content in saturated fatty acids. In the recommendations of the AHA that followed, therefore, experts summarized their opinion as follows: "A large amount of soy protein covering more than half the daily protein intake may lower blood levels of LDL-cholesterol by a few percent when they replace dairy protein or a mixture of animal proteins. Eating foods rich in soy protein may indirectly reduce cardiovascular risk if it replaces animal protein and dairy products containing saturated fats and cholesterol. Recently, a new meta-analysis of 30 randomized controlled trials that included 2913 subjects indicated that consumption of 25 g/d or more of soya protein resulted in a significant mean decrease of LDL-C of 6% ($p < 0.001$) and a nonsignificant increase in HDL-cholesterol [37].

10.8. Conclusion

Initiated more than 100 years ago on the basis of animal experiments conducted in rabbits, the hypothesis "protein" of atherosclerosis and its association with cardiovascular disease has not been proven in humans. However, the studies conducted so far show that for the prevention of cardiovascular diseases, proteins have no clear beneficial effects.

10.9. References

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11. Protein intake in renal and hepatic disease

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11.1. Summary/ Zusammenfassung/ Résumé

The kidney and the liver play a central role in protein metabolism. Synthesis of albumin and other proteins occurs mainly in the liver, whereas protein breakdown and excretion are handled through an intricate interaction between these two organ systems. Thus, disease states of either the liver and/or the kidney invariably result in clinically relevant disturbances of protein metabolism. Conversely, metabolic processes regulated by these two organs are directly affected by dietary protein intake. Of particular importance in this respect is the maintenance of acid/base homeostasis. Finally, the amount and composition of ingested proteins directly impacts on renal function, especially in a state of diseased kidneys.

Consequently, dietary protein intake is of paramount importance in patients with chronic nephropathy and renal insufficiency. Limitation of ingested protein, particularly from animal sources, is crucial in order to slow the progression of chronic kidney disease and impaired renal function. In contrast, patients with chronic renal failure undergoing renal replacement therapy by hemo- or peritoneal dialysis, have an increased protein demand. The syndrome of “protein-energy malnutrition” is a relevant factor of morbidity and mortality in this population and requires early detection and vigorous treatment.

Protein intake in patients with cirrhosis of the liver should not be diminished as it was earlier suggested but rather increased to 1.0-1.2 g/kg b.wt./d, in order to prevent protein malnutrition. Only in patients with advanced hepatic encephalopathy moderate restriction depending on protein tolerance (0.5-1.2 g/kg b.wt./d), with the possible addition of branched chain amino acids (BCAA) has been recommended. Proteins of plant origin are theoretically superior to animal proteins.

Zusammenfassung: Proteinzufuhr bei Nieren- und Leberkrankheiten

Niere und Leber spielen eine zentrale Rolle im Metabolismus von Proteinen. Dabei Albumin und andere Proteine hauptsächlich in der Leber synthetisiert. Proteinabbau und -ausscheidung finden im engen Zusammenspiel der beiden Organe statt. Erkrankungen mit Fehlfunktion von Leber und/oder Niere führen daher fast immer zu gesundheitlich relevanten Störungen des Proteinmetabolismus. Umgekehrt hat die Proteinzufuhr bedeutenden Einfluss auf metabolische Prozesse, welche durch diese beiden Organe reguliert werden. Von Bedeutung ist dabei insbesondere die Steuerung des Säure-/Basenhaushaltes. Schliesslich beeinflusst die Höhe und Zusammensetzung der diätetischen Proteine die Funktion der Nieren, insbesondere wenn diese krankheitsbedingt geschädigt sind.

Entsprechend kommt der Proteinzufuhr bei chronischen Nierenerkrankungen mit eingeschränkter Nierenfunktion eine wichtige Rolle zu. Die Einschränkung des Verzehrs von Nahrungsproteinen, insbesondere tierischer Proteine ist bedeuten, um die Progression von Nierenschäden und Nierenfunktionsstörungen zu verlangsamen. Im Gegensatz dazu haben Patienten mit chronischem Nierenversagen, welche dialysiert werden, einen erhöhten Proteinbedarf. Eine „Protein-Energie Mangelernährung“ ist ein wichtiger Auslöser für die Morbidität und Mortalität dieser Patientengruppe und bedarf einer intensiven Behandlung.

Bei Patienten mit Leberzirrhose sollte die Proteinzufuhr nicht, wie früher empfohlen, gesenkt werden, sondern auf 1.0 bis 1.2 g/kg KG/Tag, erhöht werden, um eine Proteinmangelernährung zu verhindern. Allerdings sollte bei Patienten mit fortgeschrittener hepatischer Enzephalopathie eine moderate Einschränkung des Proteinverzehrs empfohlen werden (0.5-1.2 g/kg KG/Tag), abhängig von ihrer Proteintoleranz, allenfalls mit Zusatz von verzweigt-kettigen Aminosäuren (BCAA). Proteine pflanzlichen Ursprungs sind theoretisch tierischen Proteinen überlegen.

Résumé: Apport en protéines en cas de maladie des reins ou du foie

Les reins et le foie jouent un rôle central dans le métabolisme protéique. La synthèse de l'albumine et d'autres protéines se fait principalement dans le foie, tandis que la dégradation et l'excrétion des protéines requièrent une étroite coopération de ces deux organes. Par conséquent, les maladies entraînant un dysfonctionnement du foie et/ou des reins affectent presque toujours le métabolisme protéique. Inversement, l'apport en protéines alimentaires a un impact direct sur les processus métaboliques réglés par ces deux organes. Dans ce contexte, le maintien constant de l'équilibre acido/basique s'avère d'une grande importance. En définitive, la quantité de protéines absorbées et leur composition ont une incidence directe sur la fonction rénale, notamment en cas de maladie des reins.

Par conséquent, l'apport protéique est crucial pour les patients atteints de néphropathie chronique ou d'insuffisance rénale. Un régime pauvre en protéines – d'origine animale surtout – contribuera à ralentir la progression des maladies chroniques ou des dysfonctionnements des reins. Par contre, les patients souffrant d'insuffisance rénale chronique et subissant un traitement de substitution (hémodialyse ou dialyse péritonéale) ont un besoin accru en protéines. La « malnutrition protéino-énergétique » constitue dans ce groupe de population un facteur de morbidité et de mortalité, qu'il s'agit donc d'identifier précocement et de traiter de façon ciblée.

L'apport en protéines chez les patients atteints de cirrhose du foie ne doit pas être diminuée comme il a été suggéré précédemment, mais plutôt augmenté à 1.0-1.2 g/kg/j, afin de prévenir la malnutrition protéique. Seulement chez les patients avec une encéphalopathie hépatique avancée, une restriction modérée en fonction de la tolérance aux protéines (0.5 à 1.2 g/kg/j), avec l'ajout possible d'acides aminés à chaîne ramifiée (BCAA) a été recommandée. Les protéines d'origine végétale sont théoriquement supérieures aux protéines animales.

11.2. The role of kidney and liver in protein metabolism in health and disease

Whereas a detailed review of systemic protein metabolism is given in chapter 2, the following synopsis will focus on the hepatorenal interplay in protein synthesis and breakdown.

11.3. Albumin and total protein synthesis and deficiency in disease states

Albumin is the most abundant circulating protein, produced only by the liver, in a quantity between 12 and 25 g daily (1). This may account for up to 50% of total hepatic protein synthesis under extreme conditions, but less than 10% of total protein production by the liver in one day under normal conditions. Approximately 6% of daily nitrogen intake is required for albumin synthesis. Total calorie and protein intake are the main dietary factors regulating albumin production, and have far greater effects on its synthesis than on that of other proteins. Subnormal serum concentrations of albumin may result from inadequate protein intake, decreased hepatic synthesis and/or renal loss in patients with certain forms of kidney disease. Relevant

structural damage of the liver, such as in cirrhosis, negatively impacts on protein synthesis in general, and on that of albumin in particular. Decreased serum albumin concentrations are also a hallmark of many severe diseases (see also chapter 9).

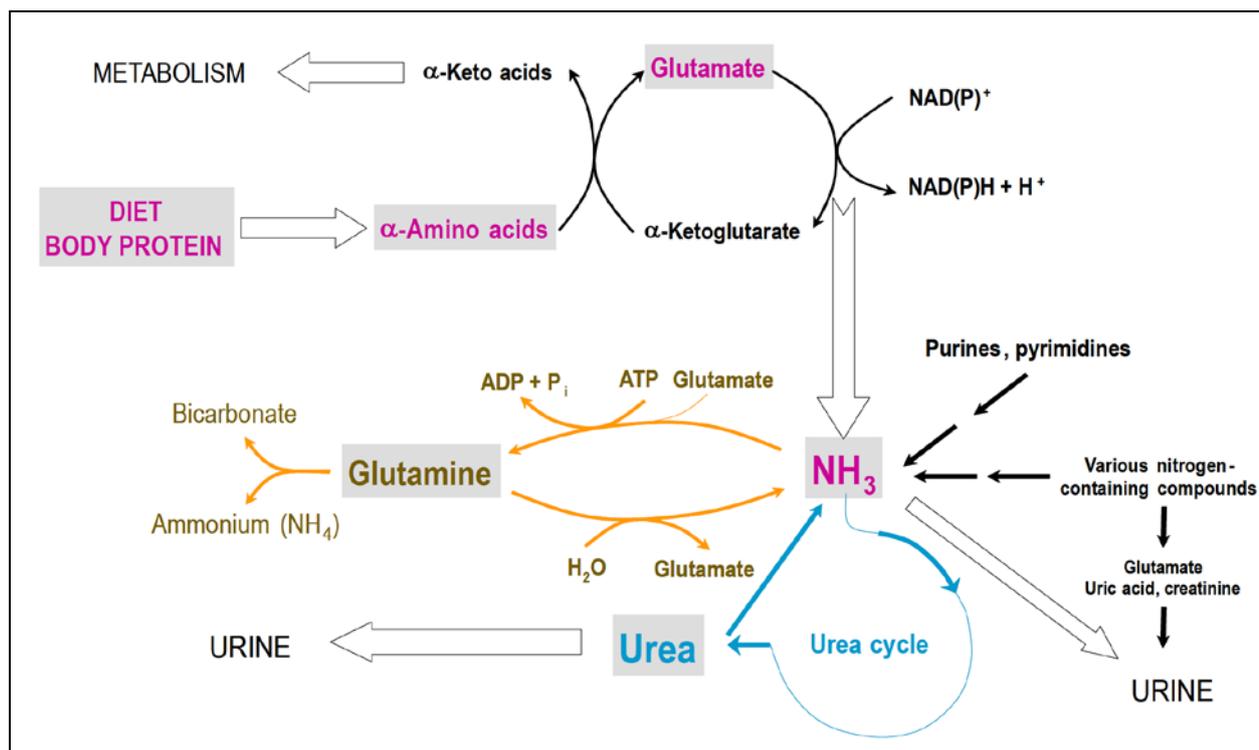
Albumin regulates fluid distribution through the body by its colloidal properties, being responsible for 75% of the normal oncotic pressure. Permanently low serum albumin concentrations may result in generalized edema formation due to low oncotic pressure. In addition, albumin plays a key role in substrate binding and transport, thereby having a major impact on the pharmacokinetics of drugs. Thus, the dosage of drugs with a substantial binding to albumin may have to be adjusted in order to ensure their therapeutic efficacy. Apart from albumin, muscle proteins account for approximately one half of the total protein pool in the body. Obviously, a quantitative and/or qualitative deficit of muscle protein may result in relevant changes in muscle mass and function and may have a profound impact on strength, mobility, control of posture, etc.

11.4. Nitrogen metabolism

Turnover of both, ingested and endogenous proteins involves nitrogen generation and disposal, which are accomplished in a concerted effort by the liver and the kidney.

Amino acids (AA) are the basic elements of all proteins and their common structure is made up by a nitrogen (\rightarrow amino, NH_2) and a carboxyl (\rightarrow COOH) group. Protein intake from food is the most abundant source of nitrogen entering the body. As nitrogen cannot be stored, and amino acids in excess of the biosynthetic needs of the cells are immediately degraded, the elimination of protein breakdown products is of paramount importance. Nitrogen is primarily metabolized to ammonia (NH_3), which, in small amounts, is excreted in the urine (Figure 1). The major part of ammonia, however, is used in the synthesis of urea and glutamine. While ammonia- and ureagenesis take place in the liver, glutamine and urea are quantitatively excreted with the urine along with other nitrogen metabolites such as glutamate, uric acid and creatinine. Obviously, the functional capacity and the interaction of these two organ systems are crucial for the maintenance of a balanced turnover and elimination of dietary and endogenous proteins.

Fig. 1: Nitrogen generation and disposal from dietary protein metabolism by the liver (ureagenesis) and the kidney (ammoniogenesis)



11.5. Effects of protein disposal on systemic acid/base homeostasis

Ammonia (NH₃) resulting from hepatic protein degradation is the precursor of urea, the major nitrogen containing compound in urine, and of urinary ammonium (NH₄) which is produced by excess acid following animal protein ingestion. Thus, it is obvious that nitrogen and acid excretion may interfere with each other by competing for ammonia (Fig. 1). Basically, high protein turnover may result in metabolic acidosis, as it diverts ammonia from the kidney to the liver for ureagenesis. This effect could even be accentuated, as urea production consumes substantial amounts of bicarbonate, which is the major extracellular buffer for excess acid. Vice versa, metabolic acidosis might impair nitrogen disposal by using ammonia for renal acid excretion. It has only been shown recently that metabolic acidosis in humans leads to stimulated rates of ureagenesis (2). Thus, the rate of urea production is not directed towards maintenance of acid/base homeostasis but rather driven by the need to remove or retain nitrogen, depending on net protein balance, resulting from protein catabolism or anabolism, respectively. As a consequence, high protein intake may contribute to the development of metabolic acidosis, in addition to the net acid load of an average Western diet rich in animal protein.

11.6. Effects of protein intake on kidney function

Glomerular filtration is the renal process responsible for the removal of fluid and solutes, including metabolic end products, from the circulation and their disposal into urine. The quantitative measure of this process is termed glomerular filtration rate (GFR), expressed as the volume of fluid filtered through the renal glomerular capillaries into urine per unit time (ml/min).

In healthy individuals, many factors influence GFR, such as age, gender, body size and pregnancy. In addition, acute protein loads and changes in habitual protein intake of specific amino acids significantly alter GFR through indirect effects on the hormonal milieu as well as direct effects on renal processes (3).

The quantitative effect of variable amounts of protein in the diet on GFR can be substantial, especially, when maintained over prolonged periods of time. Increasing protein intake from low (0.1-0.4 g/kg b.wt./d), to medium (1.0-1.4 g/kg b.wt./d), to high (2.6 g/kg b.wt./d) for 2 weeks is associated with increases in GFR by 9 and 22%, respectively (4). In studies of subjects in whom the dietary pattern was maintained for months to years the differences in GFR are more pronounced. In vegetarians, GFR was 40% lower than in omnivores. Similarly, in patients with chronic malnutrition, GFR was 27 to 64% lower than after repletion of nutritional status.

The delayed response of GFR to changes in habitual protein intake raises the possibility of structural as well as hemodynamic alterations. Indeed, renal enlargement and hyperfiltration have been noted in patients receiving total parenteral nutrition. Conversely, the kidneys of Jamaican children who died with malnutrition were smaller than those of age matched children who died of other causes. In addition, kidney sizes of well-nourished Jamaican children were smaller than those of their age matched American counterparts, perhaps reflecting the higher protein content of the American diet.

The effects of dietary protein intake and glomerular filtration rate are of particular interest with regard to the preservation of renal function in diseased kidneys, and will be discussed extensively in section 11.7.

11.7. Protein intake as a modifier of kidney disease progression

Kidney disease is associated with variable degrees of renal tissue damage, which may subside spontaneously or be controlled by medical treatment. A substantial proportion of patients, however, will experience a continued disease process resulting in progression of organ damage. This course is termed “chronic kidney disease” (CKD) and is invariably accompanied by the development of renal insufficiency. The latter describes impairment in renal function, defined mainly as a decrease in glomerular filtration rate (GFR) and, thus, in the excretory capacity of the kidney. This process usually is continuous in nature with a linear and progressive decline in GFR over months to years. Once residual renal function reaches approximately 10 percent of its normal capacity the implementation of renal replacement therapy by dialysis or kidney transplantation becomes inevitable to prevent substantial medical complications or death. As the kidney also fulfils multiple metabolic and endocrine functions, chronic renal insufficiency impacts on systemic regulation beyond the excretion of excess fluid and waste products. In particular, impaired kidney function is associated with arterial hypertension, anaemia, disorders of mineral and bone metabolism, and metabolic acidosis, among others. Medical therapy is directed towards slowing the progression of CKD, in order to delay dialysis therapy or transplantation.

A much debated modifier of progressive renal disease is dietary protein intake. As elaborated in the previous chapter, specific amino acids do have a modulatory effect on glomerular filtration rate (GFR). Sustained glomerular hyperfiltration from various causes, including high protein intake, has been postulated to be a promoting factor for accelerated loss of renal function (5). Moreover, structural damage of renal tissue results in urinary protein loss (or “proteinuria”), and has invariably been associated with progression of kidney disease (6). A causal relationship exists between protein nutrition and proteinuria independent of the hemodynamic effects of proteins mentioned earlier. Therefore, modification of diet with regard to protein

intake in patients with chronic kidney disease (CKD) has been considered for many decades to be a major measure to slow progression of renal insufficiency.

The original rationale for the restriction of protein in chronic uraemia (clinical term for “renal insufficiency”) was to lower blood urea concentration, thereby limiting the symptoms associated with this condition, such as nausea and vomiting. Beyond lowering production of nitrogenous compounds, decreased nutritional protein content could limit sodium and phosphate intake and optimize serum levels of bicarbonate and potassium. Moreover, it may prevent the development of severe secondary hyperparathyroidism, and reduce proteinuria. Whereas symptom reduction actually may be achieved with this strategy, concerns were raised that reducing protein intake below 0.8 g/kg b.wt./d might confer an increased risk of malnutrition. Thus, common recommendations did not limit nutritional protein content. In patients with signs of malnutrition and difficulties to increase protein intake, dialysis was advocated based on the notion that it improves dietary intake and nutritional status.

It was not until the early 1990s that a large prospective controlled randomized trial was conducted to examine these opposite concepts and the effect of modified protein intake in non-diabetic patients with chronic kidney disease (7). In the MDRD (Modification of Diet in Renal Disease) study by Klahr et al. 585 patients with glomerular filtration rates of 25 to 55 ml/min (moderate to mild renal insufficiency) were randomly assigned to a usual-protein diet or a low-protein diet (1.3 or 0.58 g of protein/kg b.wt./d). An additional 255 patients with GFR of 13 to 24 ml/min (severe to moderate renal insufficiency) were randomly assigned to the low-protein diet (0.58 g per kilogram per day) or a very-low-protein diet (0.28 g per kilogram per day) with a keto acid-amino acid supplement. The mean follow-up was 2.2 years.

In those patients with only moderate to mild renal insufficiency (GFR 25-55 ml/min) the projected mean decline in the glomerular filtration rate at three years did not differ significantly between the diet groups. In patients with more severe renal insufficiency at baseline (GFR 13-24 ml/min), the very-low-protein group had a marginally slower decline in glomerular filtration rate than did the low-protein group. The difference, however, did not reach statistical significance, even when longer follow-ups of up to 6 years were evaluated later on. Also, there was no delay in the time to the occurrence of end-stage renal disease or death.

In one of several post-hoc analyses to this study the effects on nutritional status among the different dietary regimens as *per protocol* (considering only patients adhering to the prescribed diet) were analyzed. Various indices of nutritional status remained within normal range during follow-up in each diet group. However, in the low-protein and very-low-protein diet groups, serum albumin rose, while serum transferrin, body weight, percent body fat and arm muscle area declined. It was cautioned that these declines are of concern because of the adverse effect of protein calorie malnutrition in patients with end-stage renal disease. Physicians who prescribe low-protein diets were advised to carefully monitor patients' protein and energy intake and nutritional status. Of note, analysis of the subgroups of patients achieving prescribed protein intakes revealed a more rapid drop in GFR of borderline statistical significance in the group who averaged 0.69 g protein/kg b.wt./d than in the 0.46 g/kg b.wt./d plus ketoacid group.

The only other prospective controlled and randomized trial published since 1994 examining the effect of dietary protein modification in 423 patients with CKD was performed in Italy from 1999 to 2003 with a mean follow-up of 48 months (8). Unlike the MDRD trial the study compared protein diets with 0.8 and 0.55 g/kg b.wt./d. Again, the differences with regard to decline in renal function and time to dialysis or death

were not significant between groups. However, progression of kidney disease, incidence of renal failure and mortality were all rather low in this cohort and, thus, the study may have been underpowered to detect an effect. Another difficulty with this study was the achieved protein intakes, which were 0.73 and 0.90 g/kg b.wt./d in the respective groups (instead of 0.55 and 0.8 g/kg b.wt./d). The fact that less than one third of the patients strictly adhered to the low protein diet indicates the problems of compliance with such regimens. Of note, only 3 patients developed relevant signs of protein-calorie malnutrition.

Positive results from restricted protein intake were found in several other studies. However, most studies examining the effects of low protein intake in patients with chronic renal insufficiency were clearly limited by rather low numbers of participants. Nevertheless, based on secondary analyses of the MDRD trial results along with several meta-analyses supporting the role of supervised low-protein diets (ranging from 0.6-0.75 g/kg b.wt./d), the National Kidney Foundation in its Clinical Practice Guidelines for Nutrition in Chronic Renal Failure recommended in the year 2000 consideration of a planned low-protein diet in non-dialyzed patients with chronic kidney failure (9). In addition to retard kidney disease progression, they argued, this strategy would potentially ameliorate metabolic complications and preserve nutritional status.

In a meta-analysis data from 1494 patients were analyzed. A 39% reduction in renal death was observed ($P < 0.001$) in patients on a low-protein diet (10). When examining the effect of low protein intake on the GFR of more than 1900 patients, Kasiske et al. detected a protective effect in those with the lowest protein intakes; GFR was 'spared' significantly by 0.53 ml/min/year (11).

Additional support for lowering protein intake in CKD came from large population based epidemiological studies. In a prospective cohort of 1624 women enrolled in the Nurse's Health Study Knight et al. found that the relationship between the quantities of protein ingested and change in estimated GFR varied with baseline renal function (12). Whereas no association between protein consumption and GFR change in women with normal function at enrolment (defined as a GFR of at least 80 ml/min) was found, each 10 g/d increase in consumption was linked with an adjusted decrease in GFR of 1.69 ml/min in the subset of 489 women with mild renal insufficiency (GFR 56-80 ml/min). The effect was even more pronounced assessing those women with renal insufficiency in the highest quintile of protein intake which experienced an average adjusted decline in GFR that was 4.77 ml/min greater than those in the lowest quintile. Further analyses of the findings by Knight et al. revealed that the effect was restricted to non-dairy animal protein, but was not found for dairy and vegetable protein. Animal protein appears to have the most pronounced effect on renal hemodynamics, followed by dairy protein and, finally, plant protein. Thus, reducing the proportion of animal protein may be a suitable therapeutic strategy when total protein restriction is not feasible.

► Recommendations on protein intake in chronic kidney disease

Available data suggest a beneficial effect of limiting dietary protein intake in patients with chronic kidney disease. In general, the benefit seems to be inversely related to renal function, being increasingly greater in patients with more advanced renal insufficiency (GFR <50 ml/min). Moreover, protein restriction may (indirectly) improve metabolic control of patients with CKD, such as serum phosphorous levels, parathyroid function, metabolic acidosis, insulin resistance, and arterial hypertension. However, as proof of this concept from prospective controlled randomized trials is still lacking so far, there are several caveats that have to be raised before recommending protein restriction to every patient with CKD:

- If dietary protein intake is limited, it has to be ensured that energy intake meets the recommended levels (i.e. 35 kcal/kg b.wt./d for patients aged less than 65 years and 30-35 kcal/kg b.wt./d for those aged 65 years or over)
- Protein composition in restricted diets has to be of high biological value.
- In general, protein intake less than 0.75 g/kg b.wt./d should be recommended with caution and only if signs of protein-energy malnutrition (PEM) are absent. If lower levels of protein intake are to be prescribed, supplements of essential amino acids or ketoacids should be considered so as to prevent essential amino acid deficiency.
- Of note, nutritional requirements of children with CKD are not covered by this review and need to be considered separately and with special emphasis on growth requirements.
- Finally, any dietary interventions depend on the compliance of the patient and require continuous counselling and close surveillance, especially with regard to signs of malnutrition.

11.8. Protein malnutrition in patients with end stage renal disease (ESRD)

Whereas current evidence suggests that limiting protein intake is beneficial in patients with chronic kidney disease not undergoing dialysis, the situation is different altogether in the setting of renal replacement therapy. Surveys using classic measures of nutritional status indicate that approximately 18-75% of patients with CKD undergoing maintenance dialysis therapy show evidence of wasting. Malnutrition in uremic patients is characterized by insidious loss of somatic protein stores and visceral protein concentrations. Most importantly, multiple prospective and retrospective studies have demonstrated that the presence of malnutrition in chronic dialysis patients sharply increases mortality and morbidity in this population. An important aspect of the pathogenesis in this regard is the chronic inflammatory process that is highly associated with CKD. The combination of malnutrition, protein-energy wasting and inflammation in the context of renal failure has also been coined “Malnutrition Inflammation Complex Syndrome” (MICS) (13). It accounts for many derangements and pathologic conditions typically inherent to patients on chronic renal replacement therapy, such as loss of body weight, reduced BMI, atherosclerotic cardiovascular disease, and vascular calcification. Thus, MICS is viewed as a major cause of increased morbidity and mortality in chronic renal failure (14). The present review will focus on the aspects of protein malnutrition contributing to this syndrome.

Causes of impaired nutritional status in patients with ESRD

The pathogenesis and causes of impaired nutritional status in patients on renal replacement therapy are complex and multifactorial. *First*, dialysis treatment by itself is accompanied with substantial losses of nutrients into the dialysate. During haemodialysis, amino acid losses average about 6 to 12 g per treatment. With peritoneal dialysis, protein losses range from about 8 to 12 g/d and amino acid losses are about 3 g/d. Assuming maximal losses, in a 70 kg patient, the calculated additional protein needs for haemodialysis patients would be about 0.06 g/kg b.wt./d (9 g of amino acids per session, 27 g/week, or 3.8 g/d) and about 0.2 g/kg b.wt./d for peritoneal dialysis patients (15 g of protein and amino acids per day) (15).

Second, protein and energy intake in chronic dialysis patients is clearly inadequate. Typically, patients undergoing maintenance dialysis therapy have reduced intake of both protein and energy. In our own analysis of a Swiss haemodialysis cohort population we found the average energy intake to be only 81% of

daily allowance (16). Accordingly, carbohydrate intake and nutritional protein content met only 69 and 84%, respectively, of calculated daily requirements. Typically, estimated protein intake from calculated normalized protein catabolic rate (nPCR) is less than 1.0 g/kg b.wt./d, namely 0.83 ± 0.19 g/kg b.wt./d in our analysis of 60 Swiss HD patients. The causes of impaired protein-energy intake in patients with end stage renal disease are multifactorial. One major reason is anorexia, presumably from induction and accumulation of cytokines. An additional factor is age, which is known to be associated with both lesser appetite and reduced protein-energy intake. This is of relevance, as dialysis patients represent an older population with a median age of 71 years in Switzerland.

Third, the uremic milieu in patients with chronic renal failure is considered a potentially maladaptive state for balanced protein turnover (17). It has been argued extensively whether uraemia is a net protein catabolic state by itself. However, more recent data convincingly demonstrate that renal insufficiency does not induce net protein breakdown as shown by nitrogen balance studies as well as whole-body amino acid turnover kinetic studies. In fact, there is a concomitant decrease in both protein synthesis and degradation in patients with advanced uraemia due to low protein turnover rate. Consequently, net nitrogen balance is not different from matched healthy controls. Thus, patients with severely impaired renal function seem to be able to compensate for reduced protein intake and synthesis by a slowdown of protein breakdown. However, this balance is fragile and limited to clinically stable patients. At times of accelerated protein degradation due to increased metabolic needs, such as acute illnesses or stress conditions, it is likely that the appropriate compensatory mechanisms, such as increased protein synthesis, fail.

Finally, the haemodialysis procedure has been shown to be a protein catabolic or, rather, anti-anabolic state with an imbalance between protein breakdown and synthesis, the net result being a substantial loss in both whole-body and muscle protein during haemodialysis.

An additional factor contributing to negative protein balance in renal failure is metabolic acidosis, a consequence of impaired renal function being highly prevalent in dialysis patients. In experimental animal studies, chronic metabolic acidosis causes increased nitrogen excretion despite the same dietary protein intake as control animals (18), and, in humans, profound acidemia causes cachexia. Accordingly, correction of acidosis decreases proteolysis and amino acid oxidation in chronic renal failure patients and results in normalization of muscular essential amino acid content.

11.9. Nutritional requirements and prevention of protein malnutrition in haemodialysis patients

The findings from many studies that maintenance haemodialysis patients have a high incidence of PEM underscore the importance of maintaining an adequate nutrient intake. Few studies have directly assessed the dietary protein requirements for HD patients. No randomized long-term clinical trials have been conducted to assess different dietary protein levels with regard to morbidity, mortality, or quality of life. Thus, recommendations for dietary protein intake in HD patients are somewhat circumstantial. However, from the facts mentioned before, and the available literature on outcomes in patients on renal replacement therapy, it can be concluded that protein-energy intake in maintenance haemodialysis patients clearly needs to be higher compared to those with predialysis chronic kidney disease.

► **Nutritional recommendations for patients on haemodialysis (based on (9)):**

- Protein intakes of less than 0.75 g/kg b.wt./d are inadequate for most maintenance HD patients. Ingestion of 1.1 g of protein/kg b.wt./d may maintain good protein nutrition in some haemodialysis patients but is not sufficient to maintain good nutrition in the great majority of clinically stable patients ingesting 25 or 35 kcal/kg b.wt./d. It is therefore recommended that a safe dietary protein intake that will maintain protein balance in almost all clinically stable MHD patients is 1.2 g protein/kg b.wt./d.
- At least 50% of the protein ingested should be of high biological value. Protein of high biological value has an amino acid composition that is similar to human protein, is likely to be an animal protein, and tends to be utilized more efficiently by humans to conserve body proteins. The increased efficiency of utilization of high biological value protein is particularly likely to be observed in individuals with low protein intakes.
- From experience it is difficult for most HD patients to maintain this level of daily protein intake. Techniques must be developed to ensure this level of intake for all patients. Education and dietary counselling should be the first steps in attempting to maintain adequate protein intake. If this approach is unsuccessful, nutritional support, such as that outlined below in the section below should be considered.

11.10. Nutritional requirements in chronic peritoneal dialysis patients

Whereas haemodialysis provides disposal of metabolic endproducts over an artificial filter (=hemodialyzer), peritoneal dialysis (PD) is performed via the natural surface of the peritoneal membrane (i.e. the membrane confining the intestinal organs against the abdominal cavity) against a glucose-containing solution (=dialysate). Due to these differences, loss of endogenous protein is more pronounced in PD patients compared to those undergoing HD treatment. Moreover, anorexia due to glucose absorption from dialysate may also contribute to reduced dietary intake and malnutrition. As a consequence, the nutritional recommendations for PD patients with regard to protein intake differ from those for haemodialysis patients.

► **Nutritional recommendations for patients on peritoneal dialysis (based on (9)):**

- dietary protein intake in clinically stable PD patients should be at least 1.2 g protein/kg b.wt./d, as it is almost always associated with neutral or positive nitrogen balance. A dietary protein intake of 1.3 g/kg b.wt./d probably increases the likelihood that adequate protein nutrition will be maintained in almost all clinically stable individuals. At least 50% of the protein should be of high biological value.
- Patients who do not have an adequate dietary protein intake should first receive dietary counselling and education. If dietary protein intake remains inadequate, oral supplements should be prescribed.
- If the oral supplements are not tolerated or effective and protein malnutrition is present, consideration should be given to use tube feedings to increase protein intake.
- Amino acids may be added to dialysis solutions to increase amino acid intake and to replace amino acid losses in dialysate.

11.11. Nutritional supplements in patients on chronic renal replacement therapy

The high prevalence of uremic malnutrition in ESRD patients indicates that the attempts to increase dietary protein intake by dietary counselling alone is not always successful to maintain neutral nitrogen balance in this patient population. In subjects with obvious signs of uremic malnutrition, other forms of nutritional intervention have also been proposed, such as oral, tube fed, and parenteral nutritional supplementation.

Oral nutritional supplements

Only a limited number of (controlled) studies are available on the effects of added protein intake from nutritional supplements given by the oral route. Nevertheless, dietary prescriptions given during haemodialysis sessions in the form of a combination of yogurt, cream, and protein-enriched milk powder, or oral amino acid supplements improve measures of nutritional status as well as muscle strength and mental health (19-21). Although preliminary, with findings that warrant larger, randomized clinical trials, oral nutritional supplementation as a practical measure should be attempted in malnourished dialysis patients if the problems that could be responsible for reducing nutrition intake cannot be resolved. However, oral nutritional supplements are not considered as “medication” and, therefore, not covered by the basic health insurance (“Grundversicherung”) in Switzerland. A reassessment of the reimbursement practice should be strongly considered by the responsible health authorities (i.e. the BAG).

Intradialytic parenteral nutrition (IDPN)

Nutritional supplementation by IDPN capitalizes on the availability of a permanent vascular access in haemodialysis patients. Thus, substantial amounts of protein and energy can be administered during each HD session without the need of an additional central venous catheter and additional treatment time. Also, unlike oral supplements, IDPN is reimbursed by health insurance despite its considerably higher costs. IDPN has been shown to promote a 96% increase in whole-body protein synthesis and a 50% decrease in whole-body proteolysis during a HD session compared to no treatment. In addition, it provides a change from negative (muscle loss) to positive (muscle accretion) balance in forearm protein synthesis. Unfortunately, treatment costs are high and longterm studies with relevant clinical endpoints are scarce. Indeed, the largest prospective controlled trial conducted so far with a total of 186 malnourished haemodialysis patients comparing oral nutritional supplements with or without 1 year of IDPN was negative without improvement in 2-year mortality, hospitalization rate, Karnofsky score, body mass index, or laboratory markers of nutritional status in patients supplemented with IDPN (22).

In fact, both groups demonstrated improvements in body mass index and in the nutritional parameters serum albumin and prealbumin, which were associated with a substantial decrease in 2-year mortality, as well as reduced hospitalizations and improved general well-being.

In conclusion, there are no data to show that aggressive enteral nutritional supplementation is inferior to parenteral supplementation in dialysis patients. Until controlled studies comparing various forms of nutritional supplementation in similar patient groups are completed, one should be cautious in choosing very costly nutritional interventions.

11.12. Protein intake in chronic liver disease

Obviously, performing its role as the central machinery of protein synthesis, the liver very much depends on the availability of the necessary precursors to perform this task. However, unlike for the kidney, the literature is scarce with regard to the specific effects of dietary protein intake on hepatic growth and function. A major role in this respect is attributable to growth hormone (GH), which stimulates production of insulin-like growth factor 1 (IGF-1) in the liver, which is a major target organ of GH itself. GH secretion can be stimulated by either a protein meal or infusion of arginine. From these observations it can be inferred that proteins are not only synthesized predominantly in the liver, but also are a major determinant of liver growth and function.

Protein malnutrition and restriction in patients with chronic hepatopathy

Moderate to severe malnutrition has been found to be prevalent in more than 50% of patients with liver cirrhosis from different aetiologies (23). Thus, malnutrition is a common complication particularly in advanced stages of liver disease and progressively increases with the severity of liver failure (as classified by the Child-Pugh score). Different patterns of malnutrition were found with muscle-mass depletion being more prevalent in males and fat depletion in females (24). Whereas the pattern of malnutrition in female patients is similar to that observed in other chronic diseases or starvation, the pattern in male patients with cirrhosis resembles that of critical illnesses. The reduction of muscle mass in malnourished male cirrhotic patients is attributable in part to hormonal alterations. Moreover, protein energy malnutrition (PEM) is a common finding in cirrhotic patients, which also may contribute to muscle wasting (23). Multiple factors have been considered in the aetiology of PEM in chronic liver disease, with a particular emphasis on metabolic alterations induced by impaired liver function. Specifically, insulin resistance and impaired glucose utilization, which have been documented in patients with liver cirrhosis, are of particular pathogenetic relevance and may be instrumental in skeletal muscle and adipose tissue catabolism.

Several studies have shown malnutrition to be related with poorer survival in patients with (alcoholic) hepatitis and liver cirrhosis. Although the presence of nutritional alterations should not be considered as a consequence of chronic liver disease only, it nevertheless seems to accelerate the natural history of the disease and adversely affect the patients' outcome.

With regard to dietary measures, oral refeeding has been proven to retain nitrogen at rates increased above normal in malnourished cirrhotic patients, similarly to that of underweight individuals without organ diseases, and to induce a significant increase in protein synthesis. At any rate, in these patients, a regimen of chronic protein restriction, by favouring progressive protein depletion, may be harmful. In addition, adequate protein diet is suggested because muscle tissue may substitute for the failing liver in ammonia detoxification, which is impaired in patients with cirrhosis due to the inability of hepatic urea synthesis.

Apart from liver cirrhosis hepatic encephalopathy (HE), as a potential complication of liver failure, merits special consideration. It has been estimated that at least 25% of patients with liver cirrhosis will experience HE during the natural history of the disease (25). Although pathogenetically complex, accumulation of ammonia is considered a major contributor to the condition, which presents with neurological symptoms ranging from sub-clinical cognitive dysfunction to overt changes in the behaviour and the state of consciousness that may reach a state of deep coma. HE is more frequent in patients with more severe liver insufficiency and in those with spontaneous or artificially created porto-systemic shunts. The treatment of HE

was traditionally based on the correction of the precipitating factor, and the administration of non-absorbable disaccharides or non-absorbable antibiotics to decrease intestinal generation or ammonia absorption through the intestinal tract. Moreover, the restriction of protein intake has long been considered a mainstay for the treatment of HE. Convincing evidence from more recent studies, however, clearly suggests maintaining protein intake in patients with hepatic encephalopathy. Several authors have shown that protein restriction rather worsens the clinical condition of HE, whereas higher protein intake was associated with improvements in mental status. Proteins of vegetable origin have some theoretical benefits over animal proteins in the dietary regimen of patients with HE, their clinical usefulness, however, is controversial. Branched chain amino acids, finally, are associated with better recovery from HE, although no advantage could be proven in patient survival.

11.13. Recommendations on energy and protein supply in chronic liver disease

Below are described the recommendations of the 1997 ESPEN consensus group (26)

Clinical condition	Non-protein energy Kcal./kg/day	Protein or amino acids g/kg/day
Compensated cirrhosis	25-35	1.0-1.2
Complications, inadequate intake, malnutrition	35-40	1.5
Low-grade encephalopathy	25-35	Transient 1.0-1.5 if protein intolerance: vegetable protein or BCAA supplement
High-grad encephalopathy	25-35	0.5-1.2 BCAA enriched amino-acid solution
Oral and enteral routes are preferred and parenteral nutrition is used only when enteral feeding is not possible or impracticable		

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12. Allergic reactions to food proteins

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12.1. Summary/ Zusammenfassung/ Résumé

Four to eight percent of the population is estimated to be food allergic and recent investigations point towards an increase in the prevalence rate of food allergies during the past years. These figures render food allergy a significant health problem in Switzerland. Most food allergies in adolescents and adults are acquired on the basis of cross-reaction to pollen allergens. These allergens are ubiquitous in the plant kingdom. Therefore pollen-allergic patients might acquire a multitude of different plant food allergies and even react to novel foods, to which they have never previously been exposed.

A curative therapy for food allergy does not yet exist. Food-allergic patients have to rely on strict avoidance diets. The widespread use of industrially processed foods poses a general problem for food allergic patients. Although the most frequent allergens must be declared openly in the list of ingredients, involuntary contamination with allergy-provoking compounds can occur. The precautionary labelling "may contain.." is sometimes applied even if the chance of contamination is very low; on the other hand, foods not declared to contain possible traces of allergenic foods may actually contain relevant amounts of allergenic proteins. Switzerland is the only country in Europe with legal regulations on contamination by allergenic food; however, the allowance of 1 g/kg is too high to protect a relevant proportion of food allergic individuals. This regulation allows e.g. a peanut contamination of up to 100 mg in a 100 g milk chocolate bar. Recent investigations indicate that 10% of all peanut allergic individuals react to an average dose of 8.4 mg peanut with allergic symptoms, which clearly demonstrates the insufficiency of our directives. The legal situation in terms of cross-contact of foods with allergenic ingredients should be revisited based on threshold dose data.

Zusammenfassung: Allergische Reaktionen auf Nahrungsproteine

Vier bis acht Prozent der Bevölkerung leiden unter einer Lebensmittelallergie. Aufgrund von neuen, zum Teil noch unpublizierten Daten gehen wir von einer Prävalenzzunahme der Lebensmittelallergien in den letzten Jahren aus. Diese Zahlen weisen darauf hin, dass Lebensmittelallergien auch in der Schweiz zu einem relevanten Gesundheitsproblem geworden sind. Lebensmittelallergien bei Jugendlichen und Erwachsenen wird hauptsächlich über eine Kreuzreaktion zu Pollenallergenen erworben. Da die verantwortlichen Allergene im Pflanzenreich weit verbreitet sind, können Pollenallergiker auf eine breite Palette von pflanzlichen Nahrungsmitteln allergisch werden und reagieren sogar allergisch auf neue Lebensmittel, die sie zuvor noch nie konsumiert haben.

Eine kurative Therapie von Lebensmittelallergien gibt es bis heute nicht. Lebensmittelallergiker müssen strikt auf allergenhaltige Lebensmittel verzichten. Die weit verbreitete Verwendung von industriell hergestellten Lebensmitteln stellt für Personen mit Lebensmittelallergien ein Problem dar. Obwohl die häufigsten Allergene deklariert werden müssen, können ungewollte Kontaminationen von Lebensmittel-Allergenen auftreten. Die Kennzeichnung „kann Spuren von... enthalten“ bedingt für den Lebensmittelallergiker häufig einen unnötigen Verzicht. Obwohl die Wahrscheinlichkeit einer Verunreinigung vernachlässigbar ist. Andererseits findet sich auf Lebensmitteln, die relevante Mengen an Verunreinigungen mit allergenen Proteinen enthalten, kein entsprechender Warnhinweis. Die Schweiz ist das einzige Land in Europa, das die Kontamination von abgepackten Lebensmitteln mit allergenen Nahrungsmitteln regelt. Diese müssen bei Mengen über 1 g/kg

zwingend deklariert werden. Diese Regelung ist gegenüber der EU ein Fortschritt, schützt allerdings Lebensmittelallergiker noch nicht genügend. So kann gemäss Schweizer Gesetzgebung zum Beispiel eine Tafel Milkschokolade à 100 g bis zu 100 mg Erdnüsse enthalten. Neuste Untersuchungen haben aber gezeigt, dass 10% aller Erdnussallergiker bereits auf eine durchschnittliche Dosis von 8.4 mg Erdnüsse mit allergischen Symptomen reagieren. Die Rechtssituation in der Schweiz sollte aufgrund solcher "Schwellenwert-Studien" in Zukunft überdacht werden.

Résumé : Réactions allergiques aux protéines alimentaires

Au moins 4 à 8% de la population souffre d'intolérance alimentaire et, selon des recherches récentes, la prévalence des allergies alimentaires est en augmentation ces dernières années. Ces chiffres montrent qu'en Suisse aussi, les allergies alimentaires sont devenues un réel problème de santé. La plupart des allergies alimentaires développées à l'adolescence ou à l'âge adulte résultent d'une réaction croisée avec des allergènes polliniques. Et comme ces allergènes sont très répandus dans le monde végétal, les patients allergiques aux pollens peuvent devenir allergiques à quantité d'aliments d'origine végétale et même réagir à des produits qu'ils n'avaient encore jamais consommés.

Il n'existe pas à ce jour de thérapie curative aux allergies alimentaires. Les patients souffrant d'allergies alimentaires doivent par conséquent se soumettre à un régime strict d'éviction. L'utilisation répandue d'aliments transformés industriellement pose un problème général pour les patients souffrant d'allergies alimentaires. Bien que les allergènes les plus fréquents doivent être déclaré ouvertement dans la liste des ingrédients, la contamination involontaire par des composés allergie-provoquant peut se produire. La déclaration « peut contenir... » est souvent imprimée par précaution, même quand le risque de contamination est négligeable. D'un autre côté, on trouve des aliments qui, sans le mentionner, renferment d'importantes quantités de protéines allergènes. La Suisse est le seul pays d'Europe à réglementer la contamination des denrées alimentaires préemballées par des aliments allergènes. Toutefois, la tolérance de 1g/kg est un progrès comparé à l'UE mais reste trop élevée pour offrir une réelle protection aux personnes souffrant d'allergies alimentaires. A titre d'exemple, une plaque de chocolat au lait de 100 g peut contenir, selon le droit suisse, jusqu'à 100 mg d'arachides. Or des travaux récents ont montré que 10% des individus allergiques aux arachides développent des symptômes d'allergie à partir d'une dose moyenne de 8,4 mg seulement, ce qui prouve que les directives en vigueur laissent à désirer. D'où la nécessité de revoir la législation, à la lumière de telles études consacrées aux doses seuil.

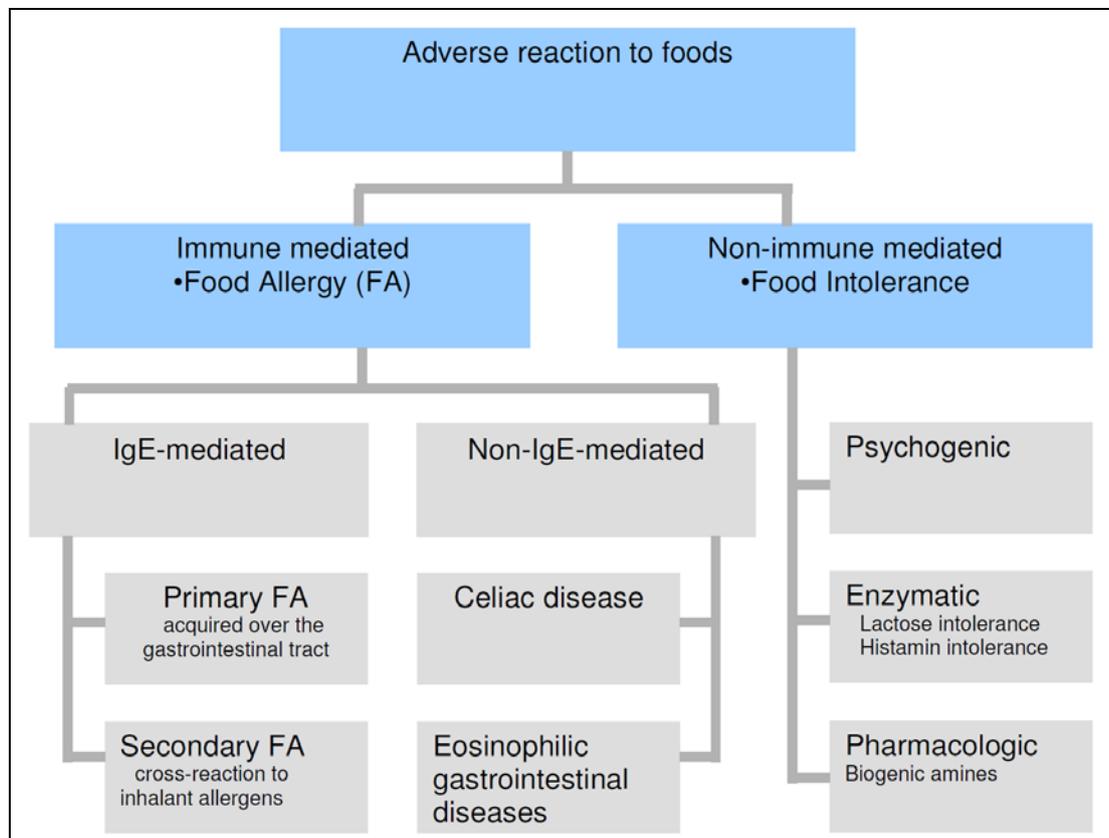
12.2. Introduction

The term "adverse reaction to food proteins" applies to any clinically abnormal response induced by a food protein. It comprises a wide spectrum of clinical entities with different pathological mechanisms, diagnostic procedures and therapeutic options. The European Academy of Allergy and Clinical Immunology (EAACI) proposed a classification of adverse reactions to foods based on mechanisms, which is shown in an adapted form in **Fig. 1** [1]. Allergy is defined as an immune-mediated reaction induced by proteins derived from plants or animals. Allergic reactions to foods are mainly mediated by food specific IgE antibodies. Non-IgE-mediated immune reactions affect in particular the gastrointestinal tract such as in eosinophilic gastrointestinal diseases or in protein-entero- or proctocolitis.

Food intolerance, however, is mainly due to factors inherent in a food such as its pharmacological properties (eg histamine in spoiled fish, tyramine in well matured cheese), or due to enzyme deficiency such as in lactose or histamine intolerance.

The present report will focus on IgE-mediated food allergies.

Fig. 1: Classification of adverse reaction to food proteins (adapted from Bruijnzeel-Koomen 1995)



12.3. Epidemiology of food allergy

Up to 30% of the general population perceives food allergy as a major health problem, although only a part of the claims can be confirmed after a full clinical evaluation including controlled oral challenges [2-3]. Recent population surveys have provided some insight into the prevalence of food allergy [3-7]. The disease affects at least 1 to 3% of the general population. It is estimated to be more frequent in children, most often under 3 years of age, in whom the prevalence of food allergy may be up to 8%. In the adult population, however, the prevalence is very likely underestimated since up to 8% of the population suffer from a birch pollen allergy and up to 80% of birch pollen allergic patients develop a food allergy to plant foods (see pathogenesis of food allergy). Recent data indicate that the prevalence of food allergy is increasing. A 2008 *Centres for Disease Control and Prevention* report indicated an 18% increase in childhood food allergy from 1997 to 2007 [8]. However, differences in study designs and diagnostic criteria render comparisons between the different studies difficult and make it impossible to date to derive validated epidemiological data from currently available investigations. A large international epidemiological study on food allergy including an objective diagnosis of the disease by means of IgE tests and oral challenges is actually performed within the EU supported multicenter project EUROPREVALL. The evaluation of the EUROPREVALL results will provide comparable point prevalences, will allow to study the contribution of population-related factors in the

near future, and hopefully will provide prevalence data for the Swiss population, that are largely lacking so far.

12.4. Clinical manifestations

Allergic reactions to food generally appear within minutes to 2 hours following the food ingestion, and may involve one or more target organs, including the skin, the gastrointestinal and upper/lower respiratory tracts and the cardiovascular system (see **Tab. 1**). The most frequent symptom of food allergy is oral contact urticaria (i.e. a swelling and itching of the oral mucosa immediately after the contact with the allergenic food), followed by skin manifestations such as urticaria or angioedema.

Anaphylaxis is the most severe manifestation of food allergy and a medical emergency. It is defined as a generalized potentially lethal allergic reaction caused by the massive release of mast cell mediators that may involve multiple organ systems. In food-dependent exercise-induced anaphylaxis, the intake of a specific food or (more rarely) of any food, induces a generalised reaction only if the patient exercises in the 2-4 hours following the ingestion. Other factors which might enhance allergic reactions to foods are concomitant intake of nonsteroidal anti-inflammatory drugs, beta-blockers and alcohol.

Tab. 1: Symptoms of IgE mediated food allergy

Affected organ	Symptom
Skin	urticaria, angioedema, flush, exacerbation of atopic eczema
Gastrointestinal tract	oral contact urticaria, nausea, vomiting, diarrhea, cramps
Respiratory tract	rhinitis, laryngeal edema, cough, asthma attack
Cardiovascular system	vertigo, tachycardia, drop of blood pressure, shock

12.5. Pathogenesis of food allergies

Despite the evolution of an efficient barrier system in the mature gut, i.e. the gastrointestinal mucosal barrier, about 2% of ingested food antigens are absorbed and transported into the body in an immunologically intact form. Even though these foreign proteins penetrate the gastrointestinal tract, they generally do not elicit adverse reactions since most individuals acquire tolerance to them. Sensitisation to food proteins may occur, however, in predisposed individuals and particularly in young children with a not fully mature gastrointestinal mucosal barrier or immune system. This form of food allergy is considered to be a primary food allergy. Such infantile primary food allergies often disappear in preschool age due to a delayed acquired tolerance (see natural course of food allergy).

In older children and adults from northern and central Europe, the majority of IgE-mediated food allergies are caused by cross-reacting allergen structures which are present in pollen as well as in allergenic foods of plant origin. The development of primary food allergy in this age is rare due to the phenomenon of gastrointestinal food tolerance. Recent investigations, however, suggest that the use of antacids that might interfere with the degradation of the allergenic proteins resulting into exposure to more intact proteins could facilitate the sensitisation to primary food allergens in adults [9].

In the secondary food allergy the primary sensitization is directed to an inhalant allergen (i.e. pollen). The food allergen is recognized by these inhalant allergen specific IgEs due to a high structural homology

between the food and the inhalant allergen on the basis of cross-reaction [10]. Pollen related food allergy is frequently encountered in patients with a sensitization to birch pollen. **Tab. 2** summarises potential food allergies to plant foods in patients with pollinosis.

The allergenic proteins of the two forms of food allergy are differentiated by cardinal characteristics. The majority of primary food allergens are heat stable and resistant to degradation or proteolytic digestion, whereas allergens inducing a secondary food allergy usually are labile proteins, which are easily degradable [11]. The stable primary food allergens have the potential to induce severe reactions up to life-threatening anaphylaxis, whereas easily degradable allergens in secondary food allergy tend to induce often – but not always – milder reactions frequently limited to oral contact urticaria [12]. These differences are important in regard to the potential of processed food to induce allergic reactions.

Tab. 2: Allergy to plant foods in pollinosis patients.

Pollen	Food
Birch	Apple, pear, cherry, peach, nectarine, apricot, plum, kiwi, hazelnut, other nuts, almond, celery, carrot, raw potato, soy, peanut, mung bean
Mugwort	Celeriac, carrot, spices, melon, honey, lychee, mango, peach, cashew nuts, pistachio, sunflower seeds, grape, pepper, camomile, cucumber
Ragweed	Watermelon and other melons, banana, zucchini, cucumber
Plane	Hazelnut, peach, apple, melon, kiwi, peanuts, maize, chickpea, lettuce, green beans

12.6. Food allergens

More than 160 foods are known to cause food allergies, however, with varying prevalence rates. The foods most frequently involved in allergic reactions are cow's milk, hen's egg, peanut and tree nuts, fish, shellfish, soy, fruits and vegetables. The focus of this report is on IgE mediated food allergies and does not include aspects of celiac disease, which are however subject of a separate expert report (EEK 2010). The relative importance of allergenic foods varies widely with the age of the patients and the geographical location. Cow's milk and egg are the most prevalent food allergies in infants and children a consequence of the worldwide consumption of these foods by this age group. Local differences in the prevalence of some specific foods are well known, i.e., peanut allergy is frequently observed in early childhood in the USA, allergy to fish is common in Spain or sesame allergy in Israel. Altogether, these studies suggest that, when the sensitisation is produced through the oral route (in the so called "primary food allergy"), the timing of exposure and dietary habits play a significant role in determining the prevalence of specific food allergies. Most food allergies with onset in adulthood, however, are linked to inhalant allergies, and are mainly directed to fresh fruits, tree nuts and vegetables. In this age group apple and hazelnut are the most prevalent allergenic foods in Switzerland, but also in whole Central and Northern Europe [4]. A real newcomer among the allergenic foods in Europe is kiwi. Kiwi has only been consumed in Europe in the past ca. 30 years, but currently kiwi is among the 5 most prevalent food allergies in our country [13]. In a recent study performed in collaboration with the Department of Life Science and Facility Management of ZHAW we investigated the allergenicity of three novel vegetables, i.e. water spinach, hyacinth bean and Ethiopian eggplant, to which the included pollen-allergic patients had not been previously exposed [14]. We observed allergic reactions to the first intake of these novel vegetables in a subpopulation of the pollen-allergic patients due to the fact that they contained proteins

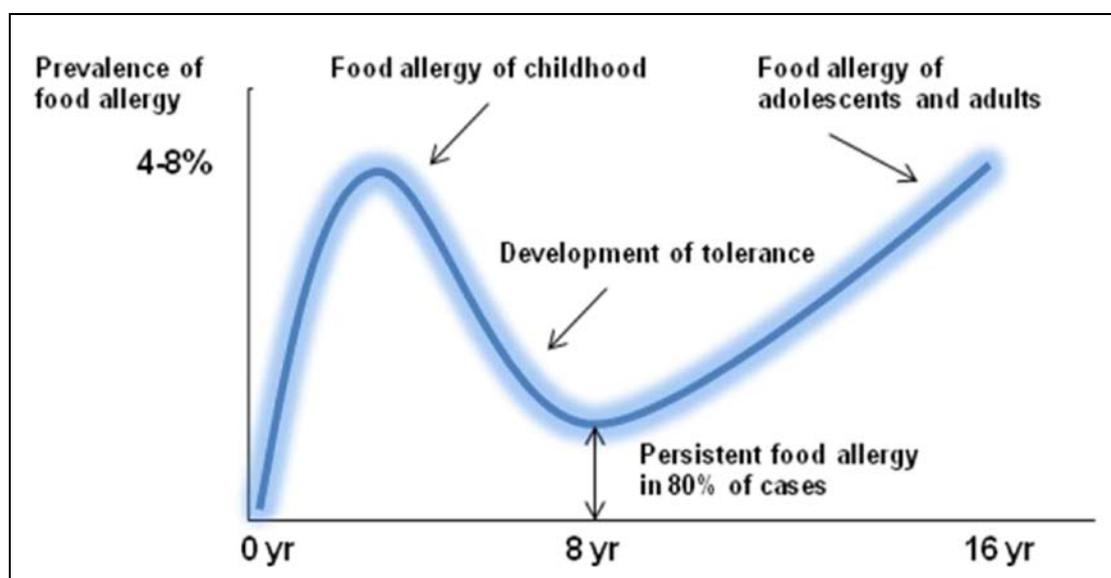
homologous to known allergens in endemic vegetable foods. Thus, globalisation of the food market will inevitably lead to a broadening of the spectrum of allergenic foods in the future.

About 90% of the most serious, i.e. anaphylactic reactions, to foods worldwide are considered to be elicited by milk, egg, fish, crustacean shellfish, tree nuts, peanuts and soybean. In Switzerland celeriac is still the most prevalent elicitor of anaphylactic reactions.

12.7. The natural course of food allergies

The prevalence of allergies to specific foods changes from childhood to adults since up to 80% of food-allergic children develop tolerance to the formerly allergenic protein with the maturation of the gastrointestinal mucosal barrier and the gastrointestinal immune system. At a later stage when they become allergic to inhalant allergens, in particular pollen, they may, however, develop a secondary food allergy (**Fig. 2**).

Fig. 2: Age dependent course of food allergy



The ability to acquire tolerance to a formerly allergenic food varies from food allergy to food allergy. In a Danish cohort of 1749 newborns followed for 15 years, the prognosis of cow's milk allergy/intolerance was good with a total recovery of 56% at 1 year and 97% at 15 years [15]. Egg allergy has been considered to be lost in 75% of children by the age of 7 years, although recent investigations suggest a longer duration with tolerance rate of 61-86% at the age of 14 years [16]. Fish allergy may be outgrown, although to a lesser extent. However, tolerance to peanut, tree nuts and shellfish is rare, and these food allergies are generally considered life long. Recent studies suggest that peanut allergy may be lost by 20% of allergic children. **Tab. 4** summarises the usual age at onset and at resolution of selected food allergies (adapted according to [17]).

Tab. 4: The natural history of food allergy

Food	Usual age at onset	Usual age at resolution
Hen's egg white	6-24 months	7 yr (75% of cases resolve)
Cow's milk	6-12 months	5 yr (76% of cases resolve)
Peanut	6-24 months	persistent in 80% of cases
Tree nuts	Children: 1-7 years Older children and adults after birch pollen sensitisation has occurred	persistent in 91% of cases
Sesame seeds	6-36 months	persistent in 80% of cases
Fruits, vegetables	Older children and adults after birch pollen sensitisation has occurred	Unknown, most likely persistent in major part of cases

12.8. Allergenicity of processed foods

Food and food ingredients are subjected to a large variety of food processing conditions which might influence the allergenic potential of proteins. In general, heat treatments are considered to lower the IgE reactivity of allergens. This concept, however, does not hold true for all allergenic proteins. Degradation of the allergenic potential by heat processing has particularly been demonstrated for birch pollen-related allergenic fruit proteins and to a lesser extent for nut proteins [18]. In birch pollen-related soy allergy, however, the allergenicity is preserved even in heat processed soy products [19]. Another exemption is celeriac, a particularly Swiss food allergen that is often related to mugwort pollen sensitisation, which is widely used in dried and powdered form as ingredient of spice mixtures or constituent of sauces and soups. Even though processed, this powder is still allergenic for patients allergic to celeriac root [20].

The Maillard reaction between free amino acids and aldehyde or ketone groups of sugar is a chemical reaction that may affect the allergenicity of food proteins by inducing the formation of aggregates that are more resistant to gastric digestion and that might bind more effectively IgE antibodies. The high temperature when roasting peanuts leads to such Maillard reactions that apparently increases stability and allergenicity of peanut allergens [21]. This might contribute to a higher rate of peanut allergy in westernized countries where peanuts are consumed roasted compared to lower prevalence rates in China where peanuts are consumed fried or boiled.

The role of many food processed ingredients derived from major food allergens in food allergy has not yet been fully elucidated. For instance lecithin is often derived from soybeans and might contain residual protein that could elicit allergic reactions in highly sensitive individuals [22]. The real risk associated with such protein residues, however, has not been studied to date. Partially hydrolyzed protein ingredients such as partially hydrolyzed casein in sausages can elicit allergic reactions. Again, the risk has not been studied to date in controlled trials. The risk, however, might be substantially lower for extensively hydrolyzed proteins.

Edible oils are mainly derived from major food allergens such as soy and peanut. They may contain varying levels of protein. The consumption of highly refined oil seems not to induce reactions in allergic individuals. The risk is much higher to develop an allergic reaction to unrefined or cold pressed oil that contains higher concentrations of proteins [23]. For instance, crude peanut and sunflower oil contains protein concentrations of 100 to 300 µg/ml but highly refined oils only 0.2 to 2.2 µg/ml [22]. Correspondingly, in a study including

60 peanut allergic patients, none responded to the ingestion of highly refined peanut oil, but six to crude peanut oil [23].

12.9. Treatment of food allergies

The primary therapy is the avoidance of the allergenic foods. For accidental reactions the food allergic patient has to be equipped, however, with emergency drugs.

In order to avoid exposure to an allergen in a processed food, patients need to check the labels and in particular the ingredient list. They should be taught how to read this list and which foods may contain the ingredients in question. Therefore, a correct avoidance diet needs constant vigilance, and may be a source of stress; it has a negative impact on the quality of life of food allergic patients and their families. Furthermore, food allergic patients have to take particular care when consuming foods in restaurants, schools or canteens since they may contain allergens.

12.10. Food labelling; legal situation

Accurate labelling is crucial for allergic consumers to enable them to strictly avoid specific foods to prevent allergic reactions. The European and the Swiss directives on food labelling require that allergenic ingredients and ingredients that are derived from allergenic foods have to be listed in the ingredient list of food labels. The list of such major food allergens is summarised in **Tab. 5**. This directive applies within the EU for deliberately added ingredients but does not regulate accidental cross-contact. In Switzerland, however, contaminations exceeding 1 g/kg (0,1%) have to be declared “may contain ...”, and contaminations below 1g/kg can voluntarily be declared with the same phrase. Cross-contact may occur, when residues of allergenic foods are present in the manufacturing environment and are unintentionally incorporated into a food that is not intended to contain that food allergen and therefore is not declared as an ingredient on the food label. Cross-contact may occur for instance when multiple foods are produced on the same processing line, or from the use of shared storage, transportation or production equipment. Recent investigations have clearly demonstrated that undeclared allergens in foods can reach levels that are relevant and can lead to reactions in allergic consumers. Recently, hundreds of different cookies and chocolates from different European countries were analysed for peanut and hazelnut proteins. 11% and 25% of the analysed cookies contained hazelnut or peanut protein without corresponding declaration. For chocolate these figures were 25% and 53% [24]. On the other hand, many producers label their products voluntarily or even precautionary even if the chance of contamination is negligible which leads to unnecessary limitation of the allergic consumer's choice. Due to this unsatisfactory situation allergic consumers are increasingly ignoring advisory labelling and put themselves at risk to experience an allergic reaction [25]. Therefore, an important scientific issue would be to know threshold levels below which it is unlikely that most allergic consumers would develop an allergic reaction. It would also be desirable to have improved knowledge on the allergenic proteins responsible for clinical reactivity and to dispose of tools to detect allergens in processed foods. This would then require a concerted action of regulatory agencies and of the processing industry to improve the situation. Allergic consumers must be aware, however, that cross-contamination is a general problem in the industrial processing of foods, which will be difficult to solve entirely in the near future.

Tab. 5 : Mandatory labelling of allergenic food ingredients (Directive 2003/89/EC and Swiss LKV, Anhang 1, http://www.admin.ch/ch/d/sr/c817_022_21.html)

- Celeriac
- Cereals containing gluten
- Crustaceans
- Egg
- Fish
- Lupine
- Milk
- Molluscs
- Mustard
- Peanut
- Sesame seed
- Soy beans
- Sulphite > 10 mg/kg
- Tree nuts

12.11. Threshold levels for allergenic proteins

Toxicology defines a threshold dose as “a limit below which a stimulus causes no reaction”. For practical purposes the term is used in food allergy synonymously with the “lowest observed adverse effect level” (LOAEL), an amount of a specific food that would elicit mild, objective symptoms in highly sensitive individuals [26]. Titrated double-blind placebo-controlled food challenges starting with low doses of the investigated food are the standard procedure to determine threshold level doses.

Knowledge on threshold doses is needed to assess the risk posed by residues of allergenic foods for the individual patients and more importantly to determine appropriate risk management strategies at the population level. The population threshold is defined as the largest amount of an allergenic food that would not cause an adverse reaction in any individual within the total population of individuals with that specific allergy. Such a figure is obviously absolutely unrealistic to determine, since all those patients would have to be challenged. However, an approach to calculate population thresholds is to include individual threshold data from different studies in a statistical model, i.e. into a dose distribution model.

To date, the most reliable data on threshold doses result from studies performed in peanut allergic patients. Determined individual LOAEL doses highly differ from study to study and span from 0.5 mg up to 10 mg of whole peanut [26]. Including individual threshold data for 185 peanut-allergic subjects obtained from 12 published clinical studies in a dose distribution model resulted in an ED₁₀ (the dose predicted to provoke a reaction in 10% of the peanut-allergic population) of 8.4 mg of whole peanut. In a recently published study on soy allergy we determined individual threshold doses ranging from 10 mg to 50 g soy. By statistical analysis of our experimental data we calculated that 11.2% of soy allergic patients are predicted to react at a dose of 10 mg of soy flour [27]. **Tab. 6** summarises published LOAELs (in mg protein) for some food allergens. The EUROPRVALL project will provide in the future much more information on threshold doses for many different foods. Within this project we have found individual threshold values are generally in the lower microgram range. These data are unfortunately not yet published.

Tab. 6: Published LOAELs for some food allergens [22]

Food	LOEAL (mg of protein)
Egg	0.13-1.0
Peanut	0.25-10
Milk	0.36-36
Tree nuts	0.02-7.5
Soy	88-522
Fish	1-100

12.12. References

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13. Bioactive proteins and peptides in foods

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13.1. Summary/ Zusammenfassung/ Résumé

Increasing amounts of data demonstrate a bioactive role of proteins and peptides above and beyond their nutritional impact. The focus of the investigations has mainly been on vitamin- and mineral-binding proteins, on antimicrobial, immunosuppressing/-modulatory proteins, on proteins with enzyme inhibitory activity as well as on hormones and growth factors from different food proteins; most research has been performed on milk proteins. Because of their molecular size, intact absorption of proteins in the human gastrointestinal tract is limited. Therefore, most of the proteins with biological functions show physiological activity in the gastrointestinal tract by enhancing nutrient absorption, inhibiting enzymes and modulating the immune system to defend against pathogens.

Peptides are released during fermentation or digestion from food proteins by proteolytic enzymes, such peptides have mainly been found in milk. Some of these released peptides exert biological activities such as opiate-like, antihypertensive, mineral-binding, antioxidative, antimicrobial, immuno- and cytomodulating activity. Intact absorption of these smaller peptides is more likely than that of the larger proteins. Consequently, other organs than the gastrointestinal tract are possible targets for their biological functions. Bioactive proteins as well as bioactive peptides are part of a balanced diet. It is possible to accumulate bioactive peptides in food, for example by using specific microorganisms in fermented dairy products. Although bioactive peptides have been the subject of several studies *in vitro* and *in vivo*, their health potential is still under investigation. Up to now, the Commission of European Communities has not (yet) authorized any health claims for bioactive proteins and peptides from food.

Zusammenfassung: Bioaktive Proteine und Peptide in Lebensmitteln

Eine zunehmende Anzahl wissenschaftlicher Daten zeigt, dass Nahrungsproteine und -peptide in der Ernährung nicht nur als Nährstoffe, sondern auch als bioaktive Stoffe von Bedeutung sind. Zu den bioaktiven Proteinen gehören Vitamin- und Mineralstoff-bindende Proteine, antimikrobielle, immunsupprimierende oder -modulierende Proteine und Proteine mit enzymhemmender Aktivität sowie Hormone und Wachstumsfaktoren. Am meisten Forschung wurde mit Milchproteinen durchgeführt. Aufgrund ihrer molekularen Grösse ist die intakte Absorption der Proteine im menschlichen Gastrointestinaltrakt limitiert. Deshalb zeigen die meisten Proteine mit biologischen Funktionen ihre physiologische Wirkung im Gastrointestinaltrakt durch die Erhöhung der Nährstoffabsorption, durch Enzyminhibition und Modulierung des Immunsystems in der Abwehr von Pathogenen.

Peptide werden während der Fermentation oder der Verdauung von Nahrungsproteinen durch proteolytische Enzyme freigesetzt. Solche Peptide wurden vor allem in Milch gefunden. Einige dieser freigesetzten Peptide weisen biologische Aktivitäten wie opiat-ähnliche, blutdrucksenkende, mineralstoffbindende, antioxidative, antimikrobielle, immun- und zellmodulierende Aktivität auf. Die intakte Absorption dieser kleineren Peptide ist eher gewährleistet als jene der grösseren Proteine. Folglich kommen neben dem Gastrointestinaltrakt andere Organe im Körper für ihre biologische Wirkung in Frage. Bioaktive Proteine und Peptide sind Bestandteil einer ausgewogenen Ernährung. Es ist möglich, bioaktive Peptide in Lebensmitteln anzureichern, z.B. indem spezielle Mikroorganismen in fermentierten Milchprodukten eingesetzt werden.

Obwohl bioaktive Peptide in verschiedenen *in vitro* und *in vivo* Studien untersucht wurden, ist deren möglicher Einfluss auf die Gesundheit immer noch ungeklärt und wird weiter erforscht. Bis zum heutigen Zeitpunkt hat die Kommission der Europäischen Union noch keine gesundheitsbezogene Anpreisung (health claim) für bioaktive Proteine und Peptide in Lebensmitteln zugelassen.

Résumé : Protéines et peptides bioactifs dans les aliments

Toujours plus de données scientifiques montrent qu'au-delà de leur impact nutritionnel, les protéines et les peptides jouent un rôle bioactif important. Les travaux de recherche se sont concentrés jusqu'ici sur les protéines de liaison de vitamines et de minéraux, les protéines antimicrobiennes, immunosuppressives ou immunomodulatrices, celles ayant une activité inhibitrice des enzymes, ou encore les hormones et les facteurs de croissance de différentes protéines alimentaires. La plupart de ces recherches ont porté sur les protéines du lait. En effet, la grande taille de leurs molécules limite les possibilités d'absorption intacte de telles protéines dans le tractus gastro-intestinal humain. Par conséquent, la plupart des protéines possédant des fonctions biologiques déploient une activité physiologique dans le tractus gastro-intestinal, en améliorant l'absorption des substances nutritives, en inhibant les enzymes et en agissant sur le système immunitaire afin de lutter contre les agents pathogènes.

Au cours de la fermentation ou de la digestion de certaines protéines alimentaires, les enzymes protéolytiques libèrent des peptides. Les protéines en question se trouvent principalement dans le lait. Certains des peptides libérés déploient toutes sortes d'activités biologiques (effets similaires à ceux des opiacés, action anti-hypertensive, liaisons entre minéraux, activité antioxydante, antimicrobienne, immunomodulatrice ou cytomodulatrice). Ces peptides de faible taille ont de plus fortes chances d'être absorbés intacts que les protéines plus volumineuses. Par conséquent, d'autres organes que le tractus gastro-intestinal constituent des cibles possibles pour leurs fonctions biologiques. Tant les protéines bioactives que les peptides bioactifs font partie d'une alimentation équilibrée. Il est possible d'enrichir des aliments de peptides bioactifs, par exemple en utilisant certains microorganismes présents dans les produits laitiers fermentés. Les peptides bioactifs ont beau avoir fait l'objet de diverses études *in vitro* et *in vivo*, leur influence possible sur la santé continue à faire l'objet de recherches. La Commission européenne n'autorise pas (encore) les fabricants des aliments à apposer des allégations de santé sur les emballages des produits alimentaires riches en protéines ou peptides bioactifs

13.2. Introduction

Proteins, along with carbohydrates and fats, are one of the three main macro-nutrients in food. Over the last century, protein research mainly investigated the importance of essential amino acids and their relevance for nutrition and health. As part of this work, nutritional science focused on other issues related to the biological value of proteins. Specifically, the biological function of certain dietary proteins was investigated in greater detail. Bioactive proteins are referred to dietary proteins with special bioactivities that have the potential to influence health, mainly in a beneficial way. This statement, however, does not include the potentially damaging effects on human physiology such as toxicity, allergenicity and mutagenicity, which are undoubtedly examples of "bioactivity" in its broadest sense [1]. Furthermore, it has been recognized that peptides with particular amino acid sequences which are inactive in the intact protein may exert biological functions after their release from the intact molecule. Such bioactive peptides have been defined as

“peptides with hormone- or druglike activity” that eventually modulate physiological functions through binding interactions to specific receptors on target cells, leading to induction of physiological responses [2].

13.3. Bioactive proteins

Most of the dietary proteins demonstrating biological activity which have been investigated to date originated from milk (immunoglobulins, caseins, whey proteins). However, proteins from other animal sources as well as plant proteins have been reported to exert specific bioactivities. A broad spectrum of proteins shows physiological activity in the gastrointestinal tract. These activities range from enhancement of nutrient absorption, inhibition of enzymes, enzyme activity, growth stimulation to modulation of the immune system in defending against pathogens.

A number of proteins have been suggested to facilitate the uptake of essential nutrients. For example α - and β -caseins are thought to enhance calcium uptake by forming soluble casein phosphopeptides during digestion; lactoferrin appears to facilitate iron uptake, whereas other proteins such as vitamin B₁₂-binding protein (haptocorrin) and folate-binding protein improve the availability of vitamins. The bioactivities observed for a number of milk proteins such as immunoglobulins, vitamin- or mineral-binding proteins indicate that bioactive proteins are of importance for the development and protection of newborn mammals [3].

Vitamin-binding proteins

Folate and vitamin B₁₂ from bovine milk are strongly bound to whey proteins. The involved proteins may favour the transition of these vitamins from the blood plasma into milk of the animal and facilitate their direct absorption in the gut. Additionally, the binding of vitamins prevents their degradation or uptake by the intestinal microflora and, as a result of the limited availability, inhibits the growth of pathogens. Bovine milk is not a rich source of folate but does contain an excess of folate-binding proteins. In combination with folate-rich foods, these proteins may improve the bioavailability of folate [4]. The protein β -lactoglobulin is able to bind small hydrophobic molecules like retinol and fatty acids. Its binding capacity is high at pH levels above 7.4 and decreases until a pH of 5.5 is reached. A reduction of pH to below 5.5 is accompanied by structural changes of β -lactoglobulin from an open to a closed conformation, whereby no further substances can be bound [3].

Vitamin-binding proteins for thiamine, riboflavin, biotin, cobalamin, retinol, and cholecalciferol have been found in egg yolk. Similarly, binding proteins for thiamine, riboflavin, biotin and cobalamin are present in egg white. Among these latter proteins the glycoprotein avidin, a component of raw eggs strongly binds to biotin. Therefore the consumption of raw eggs may reduce the bioavailability of biotin. This problem does not occur in boiled eggs, because avidin is denaturated during the heating process, biotin remaining intact. Since vitamin-binding proteins are usually not saturated with respect to their ligands, they are able to scavenge nutrients and are, therefore, thought to have both nutritional and antimicrobial functions [5].

Mineral-binding and metal-binding proteins

Minerals in bovine milk are present either in free ionic form or in complexes with various components such as proteins, peptides, amino acids, or carbohydrates. Within a protein, the side chains of the amino acid residues can interact with ions. For example, milk proteins such as α - and β -casein strongly bind bivalent and trivalent cations like calcium, manganese, zinc, copper, and iron, whereas the affinity is highest for calcium (Ca²⁺) and lowest for iron (Fe³⁺). Similarly, whey and egg proteins are able to bind minerals:

α -lactalbumin mainly binds calcium but also zinc, and lactoferrin as well as ovotransferrin iron. From animal studies but not yet from human studies some beneficial effects of these mineral-binding proteins on bone health could be reported.

Metal-binding proteins like metallothioneins, phytochelatins, metallochaperons, and several animal fibrous proteins are thought to play a role in metal homeostasis of zinc and copper, and to aid with the detoxification of heavy metals like cadmium and mercury. These proteins are broadly distributed among mammals, plants, yeasts, and microorganisms. Studies in mouse models have suggested that administration of metal-binding proteins has anti-inflammatory and neuroprotective effects and can reduce both the symptoms and incidence of multiple sclerosis and collagen-induced arthritis. Other reported effects of metallothioneins are protection against pathological damage caused by cadmium, arsenic, and anticancer agents, as well as *Helicobacter pylori* induced gastritis. Accumulated trace elements like iron, zinc, manganese, and selenium in plants achieve greater soluble concentrations and, therefore, a higher bioavailability than those provided in free form in supplements. The biochemical complexation between the metals and the metal-binding compounds maintains the metals in a soluble form that is readily available for the organism [7].

Antimicrobial proteins

A broad spectrum of antimicrobial proteins protects the gastrointestinal tract against pathogenic bacteria and viruses. They act indirectly by stimulating the growth of beneficial microorganisms in the gut or directly by exerting an antimicrobial activity or neutralizing the mechanisms of attachment or invasion of pathogens. Further, bioactive proteins can inhibit the growth of pathogens by withholding nutrients which are essential for the proliferation of bacteria.

Lactoferrin is an iron-binding glycoprotein that forms the antibiotic fragment lactoferricin during digestion. Another mechanism of the antimicrobial activity of lactoferrin is the growth inhibition of pathogens by iron scavenging. Furthermore, the bactericidal effect of lactoferrin has been related to direct interaction between the protein and the membrane of gram-negative bacteria. Although only a few clinical studies are found in the literature, it seems that bovine lactoferrin is not as effective as human lactoferrin. This is probably due to the failure of the human receptor for lactoferrin to recognize bovine lactoferrin [8].

Lactoperoxidase is one of the most prominent enzymes in bovine milk and catalyzes the peroxidation of thiocyanate and some halides such as iodide and bromide to generate oxidizing agents like hypochlorite. This acts by oxidizing the cell membrane of microorganisms, which results in a loss of structure and leads to cell lysis and death [9].

Lysozyme (muramidase, EC 3.2.1.17) is an ubiquitous enzyme present in human serum, urine, tears, seminal fluid, as well as in milk and, in higher concentrations, in egg white. Lysozyme hydrolyzes β -(1-4)-glucosidic linkages between N-acetylmuramic acid and N-acetyl-D-glucosamine residues present in the mucopolysaccharide cell wall of a variety of microorganisms. This enzyme is effective against gram-negative bacteria by degrading the cell wall of these bacteria and is widely used as a food additive. It also improves the antimicrobial activity of lactoferrin and specific antibodies. However, its concentrations in bovine milk and the colostrum are very low [8].

Haptocorrin (vitamin B₁₂-binding protein) binds strongly to vitamin B₁₂ stabilizing it and preventing its breakdown in the low-pH environment of the stomach. Furthermore, it demonstrates antimicrobial activity through vitamin B₁₂ binding, thereby preventing its use by bacteria that need it as an essential nutrient.

Immunoglobulins (Igs) are present in elevated concentrations in the colostrum whereas only low concentrations are found in milk. The primary biological function is to protect the offspring against microbial pathogens and toxins and to prevent infections in the mammary gland. Due to their antigen-binding properties, immunoglobulins can directly bind and neutralize bacteria and viruses and make them non-infectious. In mammals, five major classes of Igs have been characterized: IgG, IgM, IgA, IgD and IgE. IgG and IgM can activate bacteriolytic reactions and augment recognition and phagocytosis of bacteria by leucocytes. IgA agglutinates antigens, neutralizes viruses and bacterial toxins, and prevents attachment of enteropathogenic bacteria to mucosal epithelial cells (9). Immune protection is mainly restricted to the gastrointestinal tract, but they may also protect against dental caries due to their activity against cariogenic mutans streptococci. IgG₁ is relatively resistant to gastric acids and proteolytic enzymes such as trypsin. Various studies showed that 10-30% of orally administered bovine Igs can be recovered intact from the stool of human infants and adults (3). However, the uptake of bovine milk antibodies is of limited use in human immune defence since possible interactions with pathogens are restricted to the oral and gastrointestinal area [10].

Immunosuppressing or immunomodulatory proteins

No clear answer exists as to whether and how milk influences the human immune system [11]. Neonatal ruminants are born with a poorly developed immune system and therefore need to build it up so that it suits their own requirements. During this development, the maternal milk plays a central role. Several studies indicate that bovine milk may influence the human immune system. Intact casein may only modulate B-lymphocyte function, whereas κ -casein and its subfractions have the potential to affect T- and B-lymphocytes. Kappa-casein and caseinomacropeptides (CMP) could suppress production of the cytokine interferon- β , whereas α - and β -casein enhance production of this cytokine [11]. Lactoferrin can suppress the cytokine interleukin-6 in monocytic cell lines and inhibit the cell proliferation in bovine mammary epithelial cell lines. Furthermore, lactoferrin, and also bovine serum albumin, showed anticarcinogenic activity inducing apoptosis in tumour and transformed cells *in vitro* [6].

However, bovine milk contains more than 25 protein components that may induce specific antibody production in humans. These antibodies are most frequently directed against β -lactoglobulin, followed by casein, α -lactalbumin, γ -globulin and serum albumin. Genetic predisposition and short or no breastfeeding may increase the risk of a cow's milk allergy in infancy. However, 85% will outgrow the milk allergy by the age of five [12].

Hormones and growth factors

A wide range of hormones have been identified in milk, including prolactin, somatostatin, insulin, and melatonin. Another group of hormones, known as growth factors, usually consist of proteins or steroid hormones. Many families of growth factors exist, but milk mainly contains insulin-like growth factor-1 (IGF-1) and epidermal growth factor (EGF). They can directly influence newborns' metabolism, and promote growth and differentiation of several organs and target tissues. Furthermore, they have a cytoprotective effect against toxic substances and reduce the risk of necrotizing enterocolitis. The amino acid composition of growth factors in human and bovine milk are rather similar, and recent studies support the idea that bovine growth factors may contribute to human body functions such as increased protein synthesis during and after physical exercise [3]. Bovine growth factors extracted from cheese whey reduced small bowel damage after oral ingestion in methotrexate-treated rats [13]. Similar to lactoferrin, IGF-1 and EGF stimulate the growth

and proliferation of the mucosa that results in a larger surface area for nutrient absorption in the gut as well as a more developed barrier function. IGF-2, another growth factor present in bovine milk, exhibits anabolic activity, whereas transforming growth factor (TGF- α) is a protein that helps to maintain the normal epithelial function in the mucosa and TGF- β controls proliferation, cellular differentiation, and other functions in most cells [3].

Proteins with enzyme inhibitory activity in milk and other foods

Proteins from fish, wheat germ, and flour as well as soybean cotyledons exhibited a lipase-inhibitory effect in *in vitro* and in animal studies. Reduced lipase activity in the gut can result in reduced and/or delayed assimilation of fat. The consequences are lower postprandial triglyceride and LDL levels, and a better ratio of HDL to total cholesterol. These changes may be associated with improved insulin sensitivity and a lower risk for atherosclerosis, obesity, and other symptoms of the metabolic syndrome [6]. Feeding different sources of protein together with a diet containing cholesterol to rats showed a greater hypocholesterolemic effect for whey proteins in comparison to casein or soybean protein. The cholesterol lowering effect of soybean protein seems to correlate with the bile-acid-binding capacity of these proteins whereas whey proteins affect the cholesterol absorption and the serum cholesterol level by influencing intestinal emulsification and the nature of the resulting micelles [14].

The cystatins are a family of cysteine protease inhibitors that typically comprise about 115 amino acids. They inhibit most cysteine endopeptidases, which are widely expressed in animals and plants. These peptidases are involved in a number of physiological processes, such as intracellular protein degradation, bone remodelling, control of antigen presentation; their activities are also increased in pathophysiological conditions, such as cancer metastasis and inflammation [15]. The presence of cysteine proteases inhibitors in food may influence the activity of digestive proteases.

13.4. Bioactive peptides

Bioactive peptides generally consist of between 3 and 20 amino acids and are encrypted within the primary structure of a dietary protein. Bioactive peptides are produced using dietary proteins by means of the following four mechanisms [16]: 1) during the fermentation of food using proteolytic starter cultures; 2) during the manufacture of protein hydrolysates; 3) as a result of the degradation of dietary proteins by digestive enzymes *in vivo*; or 4) as a result of the enzymatic action of digestive enzymes *in vitro*. For example lactic acid bacteria such as *L. helveticus* can release the tripeptides VPP² and IPP during the fermentation of milk. To demonstrate which peptides can result during digestion, β -casein was subjected to a two stage *in vitro* model of mammalian gastrointestinal digestion with pepsin, chymotrypsin / trypsin, pancreatin (named stage I digestion) and with brush-border peptidases on intestinal epithelial cells (stage II digestion with Caco-2 cells) [17]. The results of the *in vitro* study showed that the dipeptides NV, IV, QD, SK, VK, HK, PV, QS, VE, QS, QA, QE, PV and PI as well as the tripeptides PGE, INK, TED, IHP, FPP, YQE, PVL and GPF were released after the first stage of digestion. In the following stage II of the *in vitro* digestion the remaining casein-framework and the resulting peptides were degraded to only a few additional dipeptides and tripeptides (AQ, QS, PQ, VM, MP and HLP). The results of this study indicate that the

² One-letter abbreviations of amino acids: A = alanine, C = cysteine, D = asparagic acid, E = glutamic acid, F = phenylalanine, G = glycine, H = histidine, I = isoleucine, K = lysine, L = leucine, M = methionine, N = asparagine, P = proline, Q = glutamine, R = arginine, S = serine, T = threonine, V = valine, W = tryptophan, Y = tyrosine.

resistance of peptides to gastrointestinal digestion is an important prerequisite in order to obtain physiological effects *in vivo* after oral administration of bioactive peptides.

Depending on their functionality bioactive peptides are divided into various groups such as opioids or casomorphins, angiotensin-converting enzyme (ACE = dipeptidyl carboxy peptidase; EC 3.4.15.1)-inhibitory peptides, phosphopeptides, antimicrobial peptides, immuno- and cytomodulating peptides, and peptides with antithrombotic activity [18]. Some sequences of bioactive peptides exhibit multifunctional effects (**Tab. 1**).

Tab. 1: Sequences of bioactive peptides (without ACE-inhibiting peptides) derived from food proteins [19;20]

Protein	Primary structure or peptide fragment
Opioids	
α_{s1} -casein	α_{s1} -casein exorphin (90-96)
β -casein	casomorphin 4, 5, 7, 8 (60-63, -64, -66, -67)
κ -casein	casoxin A, B, C (25-34: YIPIQYVLSR, 35-42: YPSYGLNN; 58-61:
β -lactoglobulin	β -lactorphin (102-105)
α -lactalbumin	α -lactorphin (50-53)
serum albumin	serorphin (399-404: YGFNA),
bovine hemoglobin	hemorphin-4, -5, -6 (34-37, -38, -40: YPLSTQEF),
gluten	exorphin A4, A5, B4, B5 (GYYP, -T and YGGW, -L),
spinach	rubiscolin-5, -6 (YPLDL, -F)
soybean	soymorphin-5 (YPFVV)
Mineral-binding peptides	
α_{s1} -casein	CPP (59-79, 64-84)
α_{s2} -casein	CPP (1-21, 46-70)
β -casein	CPP (1-25)
κ -casein	CPP (147-153)
Antioxidative peptides	
α_{s1} -casein	YFYPEL (144-149)
β -casein	VKEAMAPK (89-105), AVYPYQR (177-183), KVLVPVEK (169-, 170-176)
β -lactoglobulin	WYSLAMAASDI (19-29), MHIRL (145-149), YVEEL (42-46)
fermented milk	ARHPHPLSLFM (κ -casein 96-106)
soybean	peptides with LLPHH sequence
Antimicrobial peptides	
α_{s1} -casein	isracidin (1-23), (99-109)
α_{s2} -casein	casocidin-I (150-188), (164-179, 164-, 175-, 181-, 182-207)
β -casein	(184-210)
κ -casein	(18-24, 30-32, 139-146), CMP (106-169)
β -lactoglobulin	(15-20, 25-40, 78-83, 92-100)
lactoferrin	lactoferricin (17-41)
Immuno- and cyto-modulatory peptides	
α_{s1} -casein	(1-23, 23-34, 90-95, 90-96, 194-199)
β -casein	(60-66, 60-70, 63-68, 177-183, 191-193, 193-202, 193-209)
κ -casein	(17-21, 38-39)
α -lactalbumin	YG
lactoferrin	lactoferricin

The numbers or letters in brackets indicate the amino acid sequence of the related protein.

Opioids

The late 1970s saw the first report detailing how a bioactive peptide released from food proteins was isolated. The protein in question was the bovine β -casomorphin-7 (YPFPGPI), an opioid peptide from a casein hydrolysate. Recently, the European Food Safety Agency (EFSA) published a comprehensive review on casomorphins, which are classified as opioids [19]. Unlike endomorphins found in human organisms, exorphins such as casomorphins from casein are also found in other milk proteins (β -lactoglobulin, α -lactalbumin, lactoferrin), in cereal proteins such as wheat (gluten, gliadin), barley (hordein, avenin, secalin, zein), rice (albumin), in vegetables such as soybeans (α -protein), spinach (rubisco protein), and in meat/poultry (albumin, hemoglobin, γ -globulin), egg (ovalbumin) (**Tab. 1**).

Homologous sequences have also been identified in both human and goat milk. In addition to the presence of casomorphins in hydrolyzed casein, casomorphins have been found in fermented milk products and in cheese, although the quantities found in cheese are significantly less than 1 mg/kg. Exorphins primarily affect the intestinal lumen and mucosa by regulating gastro-intestinal motility as well as gastric and pancreatic secretions. A discussion of the effect of exorphins on cerebral processes is not necessary in this context as these peptides must be supplied parenterally [19].

Antihypertensive (ACE-inhibitory) peptides

Angiotensin-converting enzyme (ACE)-inhibitory peptides represent an additional group of bioactive peptides. These are peptides which inhibit the activity of the angiotensin-converting enzyme *in vitro*. In humans, the peripheral blood pressure is regulated, amongst others, by the renin-angiotensin system of which ACE is a part of. Inhibiting this enzyme *in vivo* results in a reduction in blood pressure. ACE-inhibitory peptides with a chain length between 2 and more than 10 amino acids were first obtained from a range of milk proteins such as α_{s1} -, β -, κ -casein, β -lactoglobulin, α -lactalbumin, and serum albumin, although they have been found as well in other animal (non-milk) and plant proteins (**Tab. 2**).

Actually, the sequences of more than 150 ACE-inhibiting peptides obtained from cow's milk protein have been identified. The most studied peptides are the two tripeptides VPP and IPP, which are released by *L. helveticus* during the fermentation of milk. Similarly, various ACE-inhibitory peptides have been discovered in cheese [23]. Recently, seven ACE-inhibitory peptides from cooked eggs were subjected to an *in vitro* gastrointestinal digestion. The study included five tripeptides (VDF, LPF, MPF, IPF, and TTI) and two pentapeptides (YTAGV, ERYPI) [24]. In addition, ACE-inhibitory peptides originating from plant proteins were found in water-soluble extracts of broccoli, mushroom, garlic, buckwheat, and wine as well as in protein hydrolysates of soybean, mung beans, sunflower, rice, corn, wheat, buckwheat and spinach [21]. For example in the enzymatic hydrolysate of glycinin, the major storage protein of soybean, an ACE-inhibitory peptide with the sequence VLIVP was identified.

Tab. 2: Sequences of ACE-inhibitory peptides mainly derived from food proteins (compiled according to [21] and [22])

Protein	Primary structure or peptide fragment
Bovine milk	
α_{s1} -casein	23-34, 23-, 24-, 25-27, 27-30, 28-, 32-34, 104-109, 142-147, 143-148, 157-164, 194-, 197-, 198-199
β -casein	48-, 49-, 50-, 52-, 53-, 54-, 55-, 56-, 57-, 58-, 59-61, 59-64, 60-66, 74-76, 84-86, 108-113, 169-174, 169-175, 177-179, 177-, 179-181, 177-, 181-183, 193-198, 193-202
κ -casein	25-34 (YIPIQYVLSR), 35-41 (YPSYGLNY), 58-59 (YP), 108-110 (IPP)
β -lactoglobulin	9-14, 15-20, 102-103, 102-, 104-105, 142-, 146-, 147-148, 146-, 148-149
α -lactalbumin	50-51, 50-, 52-53, 105-110
serum albumin	208-216 (ALKAWSVAR)
Animal (non-milk)	
chicken muscle	LKP, FKGRYYP, IVGRPHQG, LAP, LKA, FQKPKR
sardine	LKVGKQY, KVLGM, HQAAGW, VKAGF, LKL
tuna muscle	IF, VWIG, LTF, IFG
porcine myosin	79-81, 80-82, 79-, 81-83 (MNPPK)
porcine hemoglobin	GKKVLQ, FQKVVA(K), FQKVVAG
ovalbumin	(F)FGRCVSP, ERKIKVYL, LW, FCF, NIFYCP
Plant	
broccoli	YPK
buckwheat	GPP
garlic	FY, NY, NF, SY, GY, SF
mushroom	VIEKYP, GEP
pea albumin	LGP, YW, VY, DG, LY, MF, GP, GS, GK
pea vicilin	LKP, IY, VK, AF, GYK, IR, QK, FG, SG, GK
α -zein	LRP, LSP, LQP, LAQ, IRA, VSP, LAA, VAA, VAY, FY, LNP, LLP, LQQ, IRAQQ
soybean protein	DLP, HHL, DG

Mineral-binding peptides

Similarly to mineral-binding proteins, casein-derived phosphopeptides, that are reported as caseinophosphopeptides (CPP) show mineral-binding properties (**Tab. 1**). This effect is related to the presence of the phosphorylated serine residues that can form salts with minerals such as calcium [16]. These peptides are involved in the remineralisation of tooth enamel as well as in the increased absorption and bioavailability of calcium and other minerals such as zinc, copper, manganese and iron in the intestine.

Antioxidative peptides

Bioactive peptides which have an antioxidative effect have previously been obtained from various dietary proteins after enzymatic hydrolysis. The presence of such peptides, derived from hydrolyzed food proteins such as caseins, whey proteins, soybean (**Tab. 1**), rice bran, quinoa seed protein, buckwheat protein, egg-yolk protein, porcine myofibrillar proteins and aquatic by-products proteins, has been investigated in a number of studies [20]. They are effective against enzymatic and nonenzymatic peroxidation of lipids and essential fatty acids, as free radical scavengers, in metal ions chelation and in adduct formation. The inhibition of oxidative processes is of particular importance for the survival of cells in an organism. However,

undesired oxidative processes also occur in foods. The formation of free radicals results in a deterioration of food quality, for example rancid flavour, unacceptable taste, and shortening of shelf life.

Antimicrobial peptides

In addition to antimicrobial proteins such as lactoferrin, lysozyme, lactoperoxidase and immunoglobulins, antimicrobial peptides are also known to exist. The best investigated antimicrobial peptide is the fragment 17-41 of lactoferrin, more commonly known as lactoferricin (**Tab. 1**). Antimicrobial peptides are effective against different bacteria and yeasts but only a few *in vivo* studies have been carried out to date [25].

A protection against pathogens has been attributed to α -lactalbumin and involves the release of peptides, which support the immune function in humans [9]. Different antimicrobiological functions have been attributed to the CMP that is formed during cheese manufacture or digestion from κ -casein (106-169). It binds enterotoxins (cholera and *E.coli*), modulates the immune system response, inhibits bacterial and viral adhesion, suppresses gastric secretions and promotes bifidobacterial growth [26].

Immuno- and cytomodulatory peptides

As previously mentioned, proteins can have an immunomodulatory effect and bioactive peptides from caseins and whey proteins are also known to have such an effect (**Tab. 1**). These peptides can modulate the proliferation of human lymphocytes, down-regulate the production of certain cytokines, and stimulate the phagocytic activities of macrophages. As a result, they can regulate the development of the immune system in newborn infants [27]. In addition, cytomodulatory peptides exist, such as the fragments 1-18 and 105-117 from β -casein, which have been shown to influence the viability as well as the proliferation, differentiation, and apoptosis of different cell types [16].

Other bioactive peptides

The CMP and human lactoferrin also contain peptide sequences which have an antithrombotic effect. These antithrombotic peptides can inhibit blood clotting and aggregation of platelets. A hypocholesterolemic peptide has been identified from the tryptic hydrolysate of β -lactoglobulin (IIAEK), from soybean glycinin (LPYPR), and from fish protein. Additionally, antiobesity peptides (or bioactive appetite suppressants) in β -conglycinin derived from soybean protein (VRIRLLQRFNKRS) and in the CMP as well as hypotriglyceridemic peptides from blood (globin) (VVP; VYP; VTL) are believed to exist [28]. There are also indications that the β -casein fragment 177-183 exhibits a cell growth-stimulating effect as it stimulates DNA synthesis in mouse fibroblast cells [16]. Other dietary proteins also promise additional surprising results. For example, marine organisms are a rich source of bioactive peptides with antihypertensive, antioxidant, anticoagulant and antimicrobial properties [29].

13.5. Examples of bioactive peptides with physiological effects in biological systems

The presence of the tripeptides VPP and IPP in milk which had been fermented using *L. helveticus* and *Saccharomyces cerevisiae* has been known since 1995. VPP is located in the sequence 84-86 and IPP in 74-76 both in the β -casein as well as in 108-110 of κ -casein. These studies were the starting point for the development of hypotensive milk-drink products, such as *Ameal S*TM (Calpis Company, Japan) and *Evolus*[®] (Valio, Finland), and several patents have been filed in order to protect their commercial use. In our own in-depth studies, new insights into the prevalence and ripening-dependent formation of the two lactotripeptides

VPP and IPP in various Swiss cheeses are being compiled. The results show that soft cheeses contain only traces of VPP and IPP whereas large differences in the content of the two tripeptides were obtained in samples of semi-hard, hard, and extra-hard cheeses. The total concentration of VPP and IPP varied from 1.6 mg/kg in a sample of Sbrinz cheese up to 424.5 mg/kg in a sample of Bernese Alpkäse. However, high levels of variation were found even within the samples of the same cheese variety: in Bernese Alpkäse the concentration varied between 10.7 and 424.5 while in Bernese Hobelkäse the concentration varied between 6.8 and 353.0 mg/kg [23].

The resistance of peptides to gastrointestinal digestion is an important prerequisite in order to obtain physiological effects *in vivo* after oral administration of bioactive peptides. Synthetic VPP and IPP were highly resistant to the treatment with different digestive enzymes in a two-stage *in vitro* model, and thus would reach the small intestine in intact form. Current knowledge indicates that absorption is only possible with dipeptides and tripeptides. In humans, IPP but not VPP was absorbed intact into the circulation after consumption of a lactotripeptide-enriched milk beverage, but bioavailability was low, and the elimination half-life from plasma rather short [23]. Only very little is known about the absorption mechanism for peptides longer than three amino acids. It would appear possible that passive diffusion takes place through the intestinal mucosa, although the quantities absorbed are extremely small. Additionally, absorbed peptides are further degraded by peptidases in the blood. In the case of opioids, a passage through the blood-brain-barrier is necessary in order to allow activity in the central nervous system to take place. This type of passage is regarded as rather unlikely and if it does occur it is likely to be at very low levels [19].

Various animal studies concluded that consumption of fermented milk containing VPP and IPP results in a reduction in blood pressure. These observations were also confirmed in human studies including subjects with mildly elevated blood pressure. According to two meta-analyses, one including 12 randomized controlled trials published between 1996 and 2005 with a total of 623 participants, and the other including 15 placebo-controlled clinical studies, systolic blood pressure decreased by 4.8 and 5.13 mmHg, respectively, while diastolic blood pressure decreased by 2.2 and 2.42 mmHg respectively. However, three other placebo-controlled studies recently published found no evidence of this effect after administration of a dairy drink containing these lactotripeptides; as a result, the antihypertensive effect of fermented milk is still debatable [23].

The demineralising effect of cheese consumption on tooth health due to its high calcium and phosphorus content is well known. There is evidence, that casein itself has the same effect. In Western countries tooth loss is a growing problem among children and adolescents a consequence of their increased consumption of erosive foods such as citrus fruits and soft drinks. Caseinomacropeptide, a bioactive peptide, shows an inhibiting effect to tooth erosion. In an *in vitro* study hydroxyapatite was selected as a tooth model system. It was pretreated with CMP and then exposed to an acidic solution, a citrate buffer at three different pH levels (2.3, 3.5 and 4.5). The conclusion of this study is a reducing potential of whole CMP and its fractions (a glycosylated and phosphorylated fraction and a non-glycosylated but phosphorylated fraction) against the erosive effect of acidic foods and drinks by 30 to 40% [30].

13.6. Safety aspects

If new, pharmacologically active substances are identified in human food, it is essential that both, potential benefits and risks of these substances are evaluated. For this reason, intensive toxicological studies have been carried out on VPP and IPP. The following toxicological methods were used: Short-term studies, single-dose and 4-week repeated-dose toxicity in rats, 8-week studies in dogs and rats, 13-week toxicity, fertility and reproductive performance in rats, micronucleus test in rats and mice, and evaluation of cytotoxicity, clastogenicity, as well as mutagenicity (*Salmonella-E.coli* microsome incorporation assay). Similarly, a commercial milk protein hydrolysate containing IPP was investigated in three *in vitro* genotoxicity tests and in a 90-day repeated-dose oral toxicity study in rats. Overall, these studies found no adverse effects as a result of the administered lactotripeptides [23]. According to a comprehensive review by the EFSA, casomorphin-7 does not pose any health risks. After its oral consumption of this or related bioactive peptides did not demonstrate any cause-effect relationship with the aetiology or course of any suggested non-communicable diseases. Consequently, a formal EFSA risk assessment of food-derived peptides has not been recommended [19].

13.7. Application and regulation of bioactive protein and peptides in food

Intensive research on bioactive peptides being carried out around the world has already led to the introduction of a wide range of commercial products. Eleven of them are functional foods or food ingredients containing casein-derived bioactive peptides, such as the fermented milk Calpis or Evolus, identified by Phelan et al. [16], five claims to have hypotensive properties, four claims to aid mineral absorption, one claim to improve athletic performance, and one claim to reduce stress. Since 1991, the Ministry of Health and Welfare in Japan has awarded the status of Food of Specific Health Use (FOSHU) to foods with scientifically validated health claims. Since then, antihypertensive peptides such as VPP, IPP, VY and CPP have obtained FOSHU approval, according to Phelan et al. [16].

As a result of the regulation of health claims made on foods passed by the European Parliament which subsequently took effect, the EU can permit health claims (1) referring to reduction of disease risk (article 14(1)(a)), (2) referring to children's development and health (article 14(1)(b)), and (3) based on newly developed scientific evidence and/or including a request for the protection of proprietary data (article 13(5)). As the highest regulatory authority, the EFSA is responsible for verifying all applications submitted for permission for products to carry health claims. Regarding the applications already processed, the Commission of European Communities has not yet authorized any claims relating to the effect of bioactive peptides in foods. For example, the claim that Evolus reduces arterial stiffness in mildly hypertensive subjects, and consequently the risk of cardiovascular disease was rejected, as was the health claim related to dairy foods (milk and cheese) and dental health as well as the health claim related to the effects of a dairy product enriched with milk peptide and magnesium on the reduction of anxiety (Regulation (EC) No 1924/2006; http://ec.europa.eu/food/food/labellingnutrition/claims/index_en.htm).

In addition to the formation of bioactive peptides in fermented foods previously mentioned, it is quite possible to enrich foods with bioactive peptides derived from the hydrolysates of various food proteins. An example in this direction would be the development of antioxidant-rich peptides from milk protein using microbial proteases which would then be used in cooked beef to prevent lipid peroxidation, or the addition of CPPs to soluble fractions of fruit beverages in order to improve iron transport in Caco-2 cells.

13.8. Conclusions

Bioactive proteins are a part of our daily food intake and their effects on the human body mainly take place in the lumen and mucosa of the digestive tract. Bioactive peptides, which are encrypted in native peptides, are primarily found in fermented foods, especially in fermented dairy products. The quantities in which they are present are highly dependent on the specific effects of the lactic acid bacteria involved, which can result in substantial variations in traditional dairy products, as our studies on cheese have shown. In addition, bioactive peptides may be formed or degraded in the digestive tract by proteases and peptidases. The issue of whether bioactive peptides or proteins can have an effect outside the intestinal tract is questionable as their absorption is limited or impossible due to the size of their molecules.

In recent years, new peptides demonstrating biological activity have steadily been discovered in different foods. Bioactive peptides from milk proteins have been studied most intensively so far. Nowadays, the application of proteolytic enzymes in combination with new technologies such as chromatographic and membrane separation techniques as well as the use of specific cultures allow the large scale production of bioactive peptides from various food proteins. This enables the enrichment of selected foods with bioactive peptides or the development of new functional foods. Although a large number of physiological effects of bioactive peptides have been described in *in vitro* assays, no clinical studies involving humans have been performed yet, with the exception of those on ACE-inhibitory peptides from milk proteins. For this reason, randomized controlled trials are needed in order to evaluate the health potential of bioactive peptides and proteins in the diet.

13.9. References

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