The Role of Nitrogen-containing Substances in Food and Human Metabolism**

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The role of nitrogen-containing substances in human metabolism is attributed mainly to the class of amino acids. They are supplied by food protein and their main function is to synthesize body proteins. The protein requirement, the nutritional quality of food proteins, the methods of evaluating them, and the technologies adapted to preserve this nutritional quality are very well-known. The supply of adequate protein intake to the different groups of healthy populations for growth and maintenance is now more of an economic problem than a scientific one. – In addition to their role in protein synthesis, the amino acids, depending on the way to provide them (as free amino acids, peptides or proteins) or on their origin (vegetable or animal) or on their dietary level, or on their specific effect on metabolism or functions may be utilized by their modulation in the diet to correct or treat pathological situations relative to behaviour, allergy, infection, stress or several cases of metabolic diseases. Long-term studies are necessary to evaluate the biochemical evidences and control the effectiveness.

1. Introduction

Nitrogen enters in the composition of a great number of molecules of the living kingdom, participating in many biochemical and physiological functions. The privileged role of the dietary nitrogen compounds and particularly of proteins has been recognized very early on. The discovery of amino acids and of the essential role of some of them has contributed to establishing the basis of protein nutrition with the development of the notions of requirement, bioavailability, nutritional value and the simultaneous development of the methodologies to quantitatively evaluate them, by chemical and biological tests.

In parallel to this global approach of nutrition, the rapid development of bio-

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chemistry allowed the nutritionists to explain the mechanisms by which the nutrients are transformed, how they regulate the important metabolic pathways and influence cellular functions. On this basis, new concepts have been developed aiming at modifying some functions in modulating the dietary composition of nutrients. Since proteins possess twenty amino acids, the perspectives are great to act on functions in changing their dietary levels.

Those two aspects of protein nutrition will be discussed hereafter.

2. Foods as a Source of Nitrogen-containing Substances

Our foods contain a great number of nitrogen-containing substances which are synthesized in the living kingdom and which participate in the well-known «nitrogen cycle». Mineral nitrogen in the form of gaseous nitrogen, nitric acid or ammonia is transformed into organic nitrogen by bacteria and plants in the form of elaborated nitrogen substances which are necessary for the animal kingdom. The

metabolism residues of all living tissues return to the soil and are mineralized by the humification process.

Mankind also participates in the nitrogen cycle and takes the nitrogen-containing substances it needs in the plant and animal kingdom.

The different classes of nutrients possess nitrogen-containing substances: proteins are composed of amino acids, the lipid class contains phospholipids rich in ethanolamine and choline, carbohydrates contain amino sugars, and many vitamins contain nitrogen. In addition to the nutrient classes, many nitrogen-containing substances are also found belonging to the products of intermediary metabolism. For example, nucleic acids, amines, and polyamines.

The nutritional properties of those nitrogen-containing substances depend on both their «essentiality» and their level in the diet. The essential nitrogen-containing nutrients are some amino acids and some vitamins. The amino acids are provided by proteins which supply about 12% of our calories while vitamins are provided in trace amounts.

So, without neglecting the importance of the other nitrogen-containing substances, we will focus our interest on proteins which are the main source of nitrogen and possess twenty different amino acids each playing a particular role.

3. Foods as a Source of Proteins

3.1. Protein Resources

Among the food resources available in the plant and animal kingdoms, humans have selected during evolution, their food

to cover their needs. The needs in proteins were probably not the easiest to meet. Indeed, proteins are found to be distributed quantitatively in a very heterogeneous way, in a range varying between less than 1% for some green vegetables and up to 80% for some animal sources (Table 1)[1].

Human populations were able to adapt to ecosystems only producing a choice of foodstuffs rich enough in protein to cover their needs. However, it can be observed that although humans succeeded to adapt to extreme climatic conditions, many populations live in underprivileged conditions and suffer from calorie and protein malnutrition.

3.2. Requirement

Human populations with a low protein intake develop the following symptoms: growth retardation, loss of hair and of pigmentation, decrease of immune response, apathy, oedema, ... which disappear with a protein adequate diet. In order to avoid such nutritional deficiencies in at risk populations, expert committees have defined the protein requirements of different groups of population. The last ones have been edited in 1985[2]. The protein requirements called «safe level of protein intake» are expressed in g of protein/kg body weight/day (Table 2). The quantities of protein proposed relate to a protein source with a nutritional value similar to that of milk or eggs. Those values diminish with age showing that the needs for growth are more important than the needs for maintenance.

The notion of requirement is very much subject to and depends on the parameters used to evaluate them. For humans the parameters used are growth and nitrogen balance.

The figures of the safety levels of protein intake proposed by the experts correspond to the mean values experimentally measured to ensure normal growth for infants and a balanced nitrogen balance-sheet in adults plus two standard deviations to take into account the individual variations. As an example, the safety level for an adult of 70 kg is 52.5 g/day of protein.

Those requirements are increased during gestation by 6 g/day (mean values) and lactation by 17.5 to 13 g/day (mean values before and after 6 months) to take into account the increase of new tissues and the secretion of milk[2].

The organisms are able to adapt to protein intakes much higher than the recommended values. This provides advantages of a metabolic nature or of another nature. What has been observed in rats may be given as an example: To assure a normal growth, rats have to eat a diet containing 13% protein; for its maintenance an adult rat has to eat a diet containing only 4% protein[3]. But if adult rats have a free choice between their protein source (60% protein) in a cup and their calorie source (0% protein) in a second cup, they adjust

their protein intake to the level of $32.5 \pm 8.8\%$ [4]. We can imagine that the rat draws some advantages or that they do not get any disadvantage in spontaneously choosing a high protein diet.

The notion of needs has also to be extended and adapted to different kinds of situation. The requirements have been defined for the normal physiological situations (growth, maintenance, gestation, lactation) which do not correspond to the requirements for pathological situations in which not only the rate of tissue renewal is modified, but also the specific effects of certain amino acids are required.

The protein needs are actually a need for a certain amount of nitrogen to compensate the obligatory losses due to the renewal of tissues and to build new tissues. They are composed of a certain amount of essential amino acids plus a source of non-essential nitrogen provided as non-essential amino acids which are always present in food proteins.

4. Proteins as a Source of Amino Acids

4.1. Essential Amino Acids

Amino acids composing proteins can be classified in different categories according to their «essentiality», i.e. their capacity to be synthesized by the organism (Table 3). There exist *eight* essential amino acids which cannot be synthesized by the organism. Two other amino acids are essential for the baby, histidine and cystine. There exist two semi-essential amino acids which derive from an essential one: cystine from methionine and tyrosine from phenylalanine.

4.2. Non-essential Amino Acids

The other amino acids can be synthesized by the organism but some of them have a certain «essential» character as in some circumstances the organism is not able to produce them in enough quantity. This is the case for arginine and glycine. Those non-essential amino acids have been classified by *Rose* et al.[5] in a certain order with respect to their growth effects in the rat (Table 3).

4.3. Requirement for the Essential Amino Acids

In reality, the requirement for proteins can be defined more precisely into the requirements for the essential amino acids. Those have been estimated for the different

Table 1. Protein level of different classes of foodstuffs[1].

Foodstuffs		g of protein/100 g dry matter
Vegetables	tomato	0.2
	spinach	0.3
Starchy roots	cassava	1–2
	potato	8
Cereals	rice	8.6
	maize	11
	wheat	10–13
Legumes	peanuts	27
	soya	40
Animal sources	milk	28
	meat	40–60
	eggs	48
	fish	60–80

Table 2. Safe level of protein intake for different classes of age[2].

Class of Age		Safe Level of Intake (g/kg/day)
Children	1–3 years	1.26–1.13
	3–6 years	1.09–1.00
	6–10 years	1.01–0.99
Girls	10–18 years	1.00–0.80
Boys	10–18 years	0.99–0.86
Adults and Elderly		0.75

Table 3. Classification of amino acids with respect to their essential character in rats according to *Rose* et al.[5] with some modifications.

essential: lysine, tryptophan, phenylalanine, leucine, isoleucine, threonine, methionine, valine
essential for babies: histidine, cystine

semi-essential: cystine (derived from methionine), tyrosine (derived from phenylalanine)

non-essential: arginine, glycine, alanine, aspartic acid, glutamic acid, proline

Table 4. Comparison of suggested patterns of amino acid requirements with the composition of cow's milk[2].

Amino Acid (mg/g crude protein)	Suggested Pattern of Requirement				
	Infant mean[a]	Pre-school Child (2–5 years)[b]	Schoolchild (10–12 years)	Adult	Cow's Milk
histidine	26	(19)	(19)	16	27
isoleucine	46	28	28	13	47
leucine	93	66	44	19	95
lysine	66	58	44	16	78
methionine + cystine	42	25	22	17	33
phenylalanine + tyrosine	72	63	22	19	102
threonine	43	34	28	9	44
thryptophan	17	11	(9)	5	14
valine	55	35	25	13	64
Total including histidine	460	339	241	127	504
minus histidine	434	320	222	111	477

[a] Amino acid composition of human milk.
[b] Amino acid requirement/kg divided by safe level of reference protein/kg.
For adults, safe level taken as 0.75 g/kg; children (10–12 years), 0.99 g/kg; children (2–5 years), 1.10 g/kg.

groups of population and are expressed in mg/kg/day. Those values are complementary to the requirement for proteins as defined in Section 2.2 and Table 2 and can be utilized to calculate the ideal amino acid pattern for each class of population, expressed in g of amino acid/100 g of protein (Table 4)[2].

4.4. Chemical Score

From these patterns of essential amino acids, it is possible to evaluate the nutritional value of every protein source for each group of population. For that, a comparison of the content in essential amino acids of the test protein relative to the reference amino acid pattern can be made. For each amino acid a value in % of the ideal level is obtained and the lower one is called «chemical score». This scoring reveals the limiting essential amino acid from each protein:

Score =

$$\frac{\text{mg amino acid in 1 g test protein}}{\text{mg amino acid in reference protein}} \times 100$$

Table 5 gives examples of amino acid scores of vegetarian diets for pre-school children[2].

4.5. Biological Tests

The disadvantage of the chemical score is that it does not take into account the physiology of digestion and the possible factors which can modify the absorption or the utilization of the amino acids. For many reasons (presence of trypsin inhibitors, undenatured proteins, unadapted technological process) a protein can be partially hydrolyzed by the intestinal proteolytic enzymes reducing the absorption and therefore the bioavailability of its amino acids. During processing and storage, some amino acids can be chemically transformed into derivatives which are not utilizable. So biological tests have to be made to control the real potential of proteins to cover the needs.

PER (Protein Efficiency Ratio) [6]

This is a growth test performed in weaning rats fed a diet adequate in every nutrient and containing the test protein at 10%. After 3–4 weeks' feeding, the ratio

$$\frac{\text{weight gain}}{\text{protein intake}} = \text{PER}$$

is calculated and compared to the PER value obtained from a group of rats fed the same basal diet but containing casein.

NPU (Net Protein Utilization) – Nitrogen Balance [7]

In this test, the rats fed the same diet as in PER determination are put in «meta-bolic cages» to separate the urines from the faeces. A balance sheet is calculated between nitrogen intake (I) and nitrogen excreted in the faeces (F) and in the urines (U).

The following factors are determined:

$$\text{apparent digestibility } D = \frac{I - F}{I}$$

$$\text{apparent biological value } BV = \frac{I - F - U}{I - F}$$

apparent net protein utilization

$$NPU = \frac{I - F - U}{I}$$

Table 6 gives some estimations on the nutritional value of food proteins by biological tests (PER and NPU).

Bioavailability of some Essential Amino Acids

Growth tests in rats have been developed to evaluate the bioavailability of lysine, methionine plus cystine, and tryptophan in food proteins. These tests are based on the property of the rate of growth to be proportional to the level of the limiting essential amino acid in the range of growth zero to maximum growth[8].

4.6. Effects of Processing and Storage

The chemical and biological tests have been utilized to evaluate the effects of processing and storage on the digestibility of food proteins and the bioavailability of the amino acids, the most sensitive to chemical modifications.

Several kinds of chemical modifications have been described involving proteins, sugars, oxidative agents, tannins etc. They concern a limited number of amino acids, mainly lysine, methionine, cystine, and tryptophan (Table 7). These chemical modifications occur during heat treatments, storage, alkaline treatments[9].

Food industries are very much aware of such problems and important efforts have been made to reduce or avoid these phenomena in order to preserve the nutritional value. However, in many cases, these chemical modifications cannot be avoided and a compromise has to be made between the technological constraints and the maintenance of the nutritive values[10].

5. Protein Turnover

The nitrogen balance sheet which is based on the measurement of the intake and of the excretions of nitrogen is a global and static approach to protein metabolism which does not take into account internal phenomena.

An individual with a nitrogen balance in equilibrium excretes as much nitrogen as

Table 5. Example of amino acid scores of vegetarian diets (percentage of adequacy)[2].

Diet	Pre-school Child			
	lysine	methionine + cystine	threonine	tryptophan
wheat-based	76	–	91	–
rice-based	81	–	–	73
mixed cereals	84	–	97	91
rice/beans/maize	97	–	–	–
maize 76%/beans 24%	66	–	–	64
cassava-based	72	96	83	–
sorghum-based	67	–	92	–

Table 6. Biological evaluation of different proteins from animal and vegetable sources.

Protein	PER		Digestibility (D)	Biological Value (BV)	NPU	Limiting Amino Acid(s)
	absolute	% of casein				
egg	4.02	126	99.1	98.7	98.3	
whey protein	3.88	121	99.8	91.4	91.2	
beef muscle	3.58	112	92	87.5	80.4	
casein	3.2	100	93.1	83.9	77.3	methionine
soya meal	2.2	69	79.4	74.1	58.8	methionine
peanut meal	1.1	34	90.9	50.8	46.1	lysine, methionine
wheat gluten	0.31	9.7	98.5	48.5	47.7	lysine, threonine
zein (maize)	−1.44	0	48.3	26.7	12.9	lysine, tryptophan
yeast protein	1.72	54	89.1	50.4	44.9	methionine

Table 7. Types of reactions between proteins and food components.

Types of Reaction	Amino Acids Involved
Protein-Protein	
→destruction	cystine, arginine
→lysinoalanine	lysine, cystine
→racemization	serine, aspartic acid
→isopeptides	glutamic acid, lysine
Protein-Sugar	
→«early» Maillard	lysine
→«advanced» Maillard	lysine, methionine, tryptophan
Protein-Oxidizing Agent	
– H_2O_2, polyphenols	methionine, cystine, lysine
– oxidizing lipids	
Protein-Tannin	
– cocoa	(Digestibility)
– sorghum	

he eats and therefore does not increase his protein storage (Fig. 1). In reality this means that he synthesizes as much protein that he breaks down and that he renews his tissues without any loss or gain. When an individual has a negative nitrogen balance of 10g/day, this means that he breaks down more protein than he synthesizes and that the deficit is 10 g of nitrogen. Several cases can occur[11]:

a) Only the synthesis is modified (increased or diminished);

b) Only the breakdown is modified (diminished or increased);

c) Both synthesis and breakdown are modified to obtain a global deficit of 10 g nitrogen per day.

All these cases can correspond to specific physiological situations. For example, during the early period of fasting, only the protein synthesis is reduced while under prolonged fasting both synthesis and breakdown are diminished. In contrast, stress (effect of cortisol) increases the protein breakdown. To the static approach of the nitrogen balance, it is better to substitute a dynamic approach involving the notion of flux or of turnover (Fig. 2).

Tissue proteins are synthesized from a pool of free amino acids which is supplied on one hand by the amino acids coming from digestion of the dietary proteins and on the other hand by the amino acids coming from the breakdown of the tissue proteins. The flux (Q) is the quantity of nitrogen crossing the pool of free amino acids. This flux, also called turnover, is equal to:

$$Q = A + D = E + S$$

A = nitrogen intake;
D = nitrogen coming from protein breakdown;
E = excreted (urinary + fecal + tegumental) nitrogen;
S = nitrogen utilized for protein synthesis.

This equation can be transformed as follows:
$$A - E = S - D$$
Nitrogen balance = difference between synthesis and degradation.

Practically nitrogen flux is measured with amino acids isotopically labeled with ^{15}N, ^{13}C or [14]C. Table 8 gives some figures of protein turnover in infected well- and under-nourished people[12].

In many pathological situations, a rapid turnover is beneficial as it increases the metabolic adaptation to any change and increases the rate of tissue repair. This can be achieved with a high protein diet. It appears that the dietary proteins contribute to about 20% of the nitrogen flux.
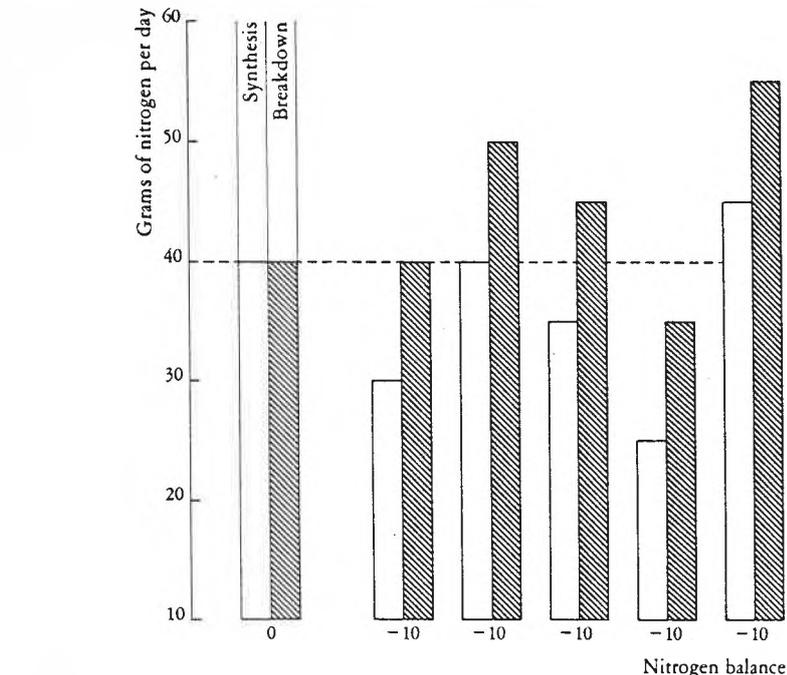

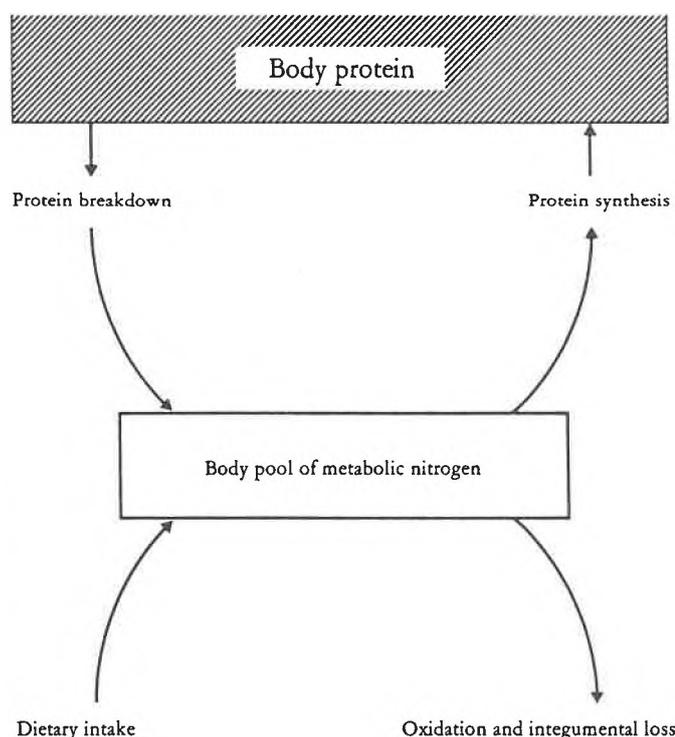*Fig. 1. Mechanisms of nitrogen imbalance* [11].

Fig. 2. Components of whole-body protein metabolism [11].

This means that the organism synthesizes about five times more protein than is eaten or that each amino acid is recycled five times.

6. Amino Acids as Precursors of Neurotransmitters

Neurotransmitters are chemical mediators which intervene in the process of communication between the neurons of the central nervous system and are therefore involved in many aspects of behaviour. Some amino acids are neurotransmitter precursors: glutamic acid is the precursor of GABA (gamma-aminobutyric acid), tryptophan is the precursor of serotonin, and tyrosine is the precursor of dopamine

Table 8. Infection and protein turnover[12].

Group	Protein Turnover [g/kg/9h]	
	Synthesis	Breakdown
well-nourished acute infection	3.8 ± 1.4	5.1 ± 1.9
malnutrition + infection	4.7 ± 1.3	4.5 ± 1.5
kwashiorkor + infection	2.5 ± 0.6	2.4 ± 0.5
kwashiorkor + no infection	1.3 ± 0.7	1.2 ± 0.7

and norepinephrine. The activity of a neurotransmitter depends on the concentration in the synapses and therefore on the concentration of the amino acid precursor where the neurotransmitter is produced. Since the amino acid precursors are provided by the diet, it is tempting to think that it is possible to modify the synthesis of the neurotransmitters in modifying the intake of their precursors and consequently to influence some type of behaviour.

The utilization of L-DOPA ((−)-dihydroxyphenylalanine), a metabolite of tyrosine to treat Parkinson's disease, was the first clinical application of a neurotransmitter precursor. This success had a profound effect on subsequent research on neurotransmitter precursors and on the research related to diet and behaviour.

Many studies have been made on the effect of dietary tryptophan which is the precursor of serotonin involved in the regulation of sleep, appetite, body temperature, sensitivity to pain, and aggressivity. In the case of tryptophan, there is a chain of causality to establish and to control before any application can be made[13]:

dietary tryptophan → plasma tryptophan → brain tryptophan → brain serotonin → change of behaviour

This type of causality seems to be evident but the biological processes are not so simple and obey their own laws which have to be discovered. For example:

- tryptophan enters the brain via the large neutral amino acid transport system and it must compete with valine, leucine, isoleucine, tyrosine, phenylalanine, and methionine;
- animal experiments have shown that the level of brain tryptophan depends not on the plasma tryptophan level but on the ratio tryptophan/large neutral amino acids;
- tryptophan binds reversibly to albumin and only free tryptophan is directly available for transport;
- animal experiments have shown that the rate-limiting step in the formation of serotonin is the hydroxylation of tryptophan into 5-OH-tryptophan and that this enzyme is unsaturated at the tryptophan concentration normally found in the brain;
- human experiments have shown that the ratio tryptophan/large neutral amino acids in the plasma increases by the ingestion of free tryptophan at the same time as the ingestion of carbohydrate. The effect of carbohydrates seems to be due to the increase of plasma insulin which enhances the entry of the large neutral amino acids in the muscles.

The last step is to demonstrate the efficacy on one aspect of behaviour. This was done on human volunteers with long sleep latencies. They received 500 mg of free tryptophan, corresponding to 140% of the minimum requirement, in a chocolate bar supplying a small carbohydrate load. Using a double-blind cross-over design, it was shown that after tryptophan administering, sleep latency was significantly shorter.

Similar studies are in progress to study the effect of dietary tyrosine on mild hypertension and depression[14].

7. Protein Sources and Cholesterol Metabolism

Several studies performed on animals and humans have shown that the dietary protein level as well as the protein source (animal or vegetable) may affect plasma cholesterol and the development of arteriosclerosis[15].

7.1. Influence of the Dietary Protein Level

In general, a protein-poor diet decreases body weight and increases plasma cholesterol both in animals and humans. This can be explained by various metabolic changes including hypertriglyceridemia, hepatic synthesis of fatty acids, and sulfur amino acid deficiency.

7.2. Influence of the Nature of the Dietary Proteins

The origin of the dietary proteins, animal or vegetable, has been shown to have an important effect on cholesterol metabolism. A diet rich in animal protein increases plasma cholesterol and favors the development of arteriosclerosis. In contrast, plant proteins, particularly soya, are able to decrease plasma cholesterol and mainly LDL cholesterol in type II hypercholesterolemia. Soya was also found to have no effect on normo-cholesterolemic subjects.

7.3. Hypotheses on the Mechanism

A diet containing a mixture of free amino acids of the same composition as casein has a hypercholesterolemic effect similar to that of casein while a diet containing a mixture of free amino acids of the same composition as soya protein has a hypocholesterolemic effect lower than that of soya (Table 9)[16]. The hypercholesterolemic effect of soya can be attributed to various factors: presence of phytosterols and of saponins, the carbohydrate composition, the mineral content, and also the low methionine and choline content.

The hypercholesterolemic effect of casein cannot be attributed to the presence of minor components like with soya and there is important evidence that the hypo- or hyper-cholesterolemic effects of soya and casein, respectively, are due to their amino acid profile or other factors resulting from their structure, for example the rate of amino acid absorption or size and sequence of the peptides absorbed.

Table 9. Serum cholesterol levels in rabbits fed diets containing casein and soya or mixture of free amino acids of the same composition[16].

Protein and amino acid (AA) source	Amino acid composition equivalent to	Serum cholesterol [mmol/L]
casein	casein	5.72 ± 0.98
AA	casein	5.52 ± 1.09
soya	soya	1.75 ± 0.36
AA	soya	3.21 ± 0.78

Table 10. Differences in the amino acid composition of casein and soya.

Amino Acids [g/16 gN]	Casein	Soya
lysine	8.2	6.6
arginine	4.0	7.5
lysine/arginine	2.05	0.88
methionine	3.0	1.3
cystine	0.4	1.3
methionine/cystine	7.5	1.0
glycine	1.6	4.2

Lysine/Arginine Ratio

Casein and soya differ by their levels of lysine and arginine with a ratio lysine/arginine of 2.04 and 0.88%, respectively (see Table 10).

In some animal experiments, the addition of arginine to casein decreases its hypercholesterolemic effect while in others, the addition of lysine to soya reduces its hypocholesterolemic effect. Therefore, either lysine or arginine or their ratio seem to be involved in cholesterol metabolism. The exact mechanism is not known but several hypotheses have been proposed: For instance, the inhibitory effect of lysine on arginase which increases the level of arginine available for the arginine-rich apolipoprotein, or the insulin stimulating effect of arginine which modifies the activity of the enzymes involved in the synthesis and the degradation of cholesterol, HMG-CoA reductase and cholesterol-7-α-hydroxylase, respectively[15].

Sulfur Amino Acids

The levels and the proportions of sulfur amino acids, methionine and cystine, are very different in soya and casein. Rat experiments have shown the hypercholesterolemic effect of the methyl moiety of methionine and the hypocholesterolemic effect of cystine[17,18] which could be partly attributed to its ability to produce taurine precursor of taurocholic acids[17].

Glycine

Its hypocholesterolemic effect could be attributed to its ability to detoxify an excess of methyl groups of methionine in producing sarcosine[17,19].

8. Free Amino Acids – Peptides – Proteins

Food proteins are hydrolyzed in the intestine by proteolytic enzymes into peptides and free amino acids. Before getting

into the bloodstream, amino acids are transported through the gut either as free amino acids via specific transport mechanisms or as peptides which are hydrolyzed in the enterocyte. This second transport system is more efficient and more rapid, so that feeding free amino acids is nutritionally and physiologically (high osmotic pressure) less adequate than feeding peptides or intact proteins.

When the hydrolysis of food proteins in the gut is no longer adequately ensured, as it is the case in severe malnutrition, pancreatic insufficiency, chronic diarrhoea, intestinal infection etc., feeding hydrolyzed proteins is recommended. When the cases are severe, the permeability of the intestinal membranes towards large peptides or protein fragments increases with a risk of immunological reactions and of allergy to food proteins.

Adequately hydrolyzed proteins can therefore resolve the problems of the amino acid absorption, of the osmotic pressure, and of the allergenic response.

It is now possible to prepare on an industrial scale enzymatically hydrolyzed proteins in quantities large enough to provide products for a large market. Such products are foreseen to be utilized by at-risk populations, i.e. premature or full-term infants suffering from chronic diarrhoea. For the development of such products, a certain number of problems had to be solved:

- The effect of the Maillard reaction on the nutritional value of milk-type products containing free amino acids, peptides, and reducing sugars has to be controlled. The risk is important as each peptide linkage which is hydrolyzed liberates an additional free amino group susceptible to reaction with sugars.
- The hypoallergenicity has to be checked for each production batch[20].
- The exocrine pancreatic response has not to be impaired. It is well known that the synthesis and pancreatic secretion of the proteolytic enzymes, trypsin and chymotrypsin, are stimulated by food proteins present in the duodenum via a negative feedback regulation mechanism.

Studies in rats fed diets containing either whey proteins, intact or hydrolyzed by trypsin, chymotrypsin or pancreatin, or a free amino acid mixture of the same composition have shown that the stimulation of the pancreas (i) increases with the dietary nitrogen level and (ii) is similar with intact or enzymatically hydrolyzed proteins and lower with the mixture of free amino acids[21].

Feeding peptides does not therefore disturb the exocrine pancreatic response.

9. Amino Acids and the Detoxification Process

Our organism is equipped to detoxify many kinds of molecules coming from our food or from endogenous origins such as free radicals. A certain number of amino acids are involved in the detoxification processes.

9.1. Detoxification of Xenobiotics

It is well known that glycine is involved in the detoxification of aromatic compounds which are excreted as hippuric acid. Recently, it has been demonstrated that hydroxymethylfurfural (HMF) produced in processed foods via the Maillard rection was excreted as hydroxymethylfurfurylic acid free and combined with glycine. It was also shown that the proportion of the glycoderivative increases with the increased intake of glycine[22].

Cysteine and reduced glutathione (a tripeptide formed by glutamic acid, cysteine, and glycine) are able to bind xenobiotics to detoxify them and accelerate their elimination via the kidney. This is the case for terpenes (cafeol and cafestol) and for bromobenzene[23].

9.2. Detoxification of Free Radicals

Free radicals (superoxide ion, hydroxyl and peroxyl radicals) are generated in cells submitted to stimulation by xenobiotics, microorganisms, allergens, etc.) or to injuries (such as reoxygenation after hypoxia). Each cell is equipped to detoxify those free radicals and among the mechanisms involved, sulfur amino acids play an important role. Reduced glutathione is able to destroy peroxides via the glutathione-peroxidase activity. Taurine, a catabolite of cystine present to a high level in muscles, is able to detoxify hypochlorite ions which have been found to be produced by the reaction of endogenously generated hydrogen peroxide and chloride ion[24].

The possibility of increasing the pool of glutathione and of taurine by dietary means may be envisaged to improve the efficiency of the detoxification of free radicals.

10. Proteins and Amino Acids in Infections

The resistance of the organism to infections depends on many factors and among them dietary proteins and amino acids are greatly involved.

10.1. Dietary Protein Levels

The dietary proteins level considerably influences the immune status. In protein malnutrition immune defence is drastically affected which facilitates every type of infection (bacteria, virus, parasites) and slows down recovery.

When the infection is established, its evolution can depend not only on the efficiency of the immune system, but also on the capacity of the host to feed the parasite either from its endogenous reserves or from food. This is the case of intestinal parasites and also of parasites circulating in the blood as those of malaria.

Studies performed in rats infected with *Plasmodium berghei* have shown that a protein-rich diet increases the number of circulating parasites while a protein-free diet decreases the circulating parasites but without completely eliminating them (Table 11).

Table 11. The effect of dietary protein and amino acids on the development of malaria in rats[25].

Casein % in diet	Diet added amino acids	Relative Infection
0		0.1
4.2		1.0
8.5		1.7
17.0		7.5
4.2	Phe, Lys, His, Try	0.4
4.2	Thr, Met, Phe, Lys, Try	2.5
4.2	Thr, Ile	2.3
4.2	Thr, Val	2.1
4.2	Thr, Val, Ile	3.0
4.2	Thr, Val, Ile, Leu, Met	6.7

10.2. Effect of Specific Amino Acids

The malaria parasites circulating in the blood stream develop from the nutrients present in the blood. They have specific requirements for essential amino acids. They find them in the blood and their development should be modified by changing the amino acid blood profile.

Rat experiments have shown the importance of five amino acids, namely threonine, valine, isoleucine, leucine, and methionine on the severity of the infection. This shows that parasites are in competition with the host for the supply of amino acids and that the requirements of parasites have a pattern different from that of the host[25].

Similarly, arginine has been found to be essential for the replication of the Herpes Simplex (HSV) virus in tissue culture and that in the presence of arginine lysine inhibits the replication of the virus by a mechanism of antagonism. In humans the intake of free lysine at the dose of 1 g/day was able to decrease both the severity of the symptoms and the healing time[26]. However, those studies are contested by others that did not find any effect.

11. Dietary Treatment of Metabolic Diseases

A certain number of metabolic diseases affect the metabolism of amino acids. The genetic defect results from the absence of a key enzyme essential for the normal metabolism (phenylketonuria) of from the absence of the transport system (cystinury).

Some of those diseases can be treated with an adequate protein diet.

11.1. Phenylketonuria

In this disease, there is a lack of phenylalanyl-hydroxylase activity. This results in the accumulation of phenylalanine and of phenylpyruvic acid which is neurotoxic. Babies suffering from this disease have to ingest an adequate amount of phenylalanine to build up their proteins and to avoid any toxicity. For each patient, a balanced proportion has to be adapted between a normal source of protein to provide the exact requirement for phenylalanine and a mixture of free amino acids containing no phenylalanine.

11.2. Cystinose

Children suffering from this disease excrete, in their urine, large amounts of cystine, lysine, and arginine. The treatment which has been proposed is a diet poor in sulfur amino acids, methionine and cystine. The protein source which is utilized is lentil which is very poor in sulfur amino acids. The poorer varieties are selected to this purpose.

12. Other Pathologies

Many other pathologies have a dietetic approach which concerns the protein intake. Among them:

– Celiac disease which necessitates a diet free of gluten.
– Liver insufficiency which results in the decrease in hepatic oxidation of phenylalanine and tyrosine. The level of both amino acids increases in plasma and brain leading to encephalopathy. The perfusion of branched-chain amino acids which compete with aromatic amino acids entering the brain reduces the brain disfunction.
– Renal failure which results in the impossibility for the kidney to eliminate urea. A low-protein diet rich in essential amino acids reduces the production of urea. The utilization of keto-analogs would be a means of reducing the ingestion of nitrogen.

13. Conclusion

Among all the nitrogen-containing substances present in our foods, proteins are the most important as they provide the amino acids that we need.

The amino acids are utilized first of all for the synthesis of our body proteins, to build new tissues and to renew the existing ones. The renewal process gives 20% loss which has to be compensated by dietary protein. The protein requirements have been established for the different groups of population and have to be met by a judicious choice of foods which differ by the level and the nutrition value of their proteins. The nutritive value of dietary proteins depends on their essential amino acid composition and on their bioavailability which can be modified by the culinary and industrial processes. The nutritional quality can be quantitatively evaluated by chemical and biological tests. The basic knowledge and the technological skills exist to avoid protein malnutrition.

Amino acids are also involved in many metabolic pathways which influence a large range of functions such as sleep (tryptophan), cholesterol metabolism (plant/animal protein, methionine, cystine, lysine/arginine), resistance to infections (protein level, specific role of amino acids), detoxification of xenobiotics (cysteine, glycine) and of free radicals (cysteine, glutathione, taurine), allergy to food proteins (protein hydrolysates), and the treatment of many metabolic diseases and organ insufficiencies (liver, kidney).

Nutrition is now evolving from the classical sense in which the aim was the avoidance of deficiency states to a new field in which the aim is the modulation of metabolic functions.

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