Protein Research for The First Supper

Luke Comer and Rebecca Cox

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STRUCTURE, FUNCTION, & RELATIONSHIP

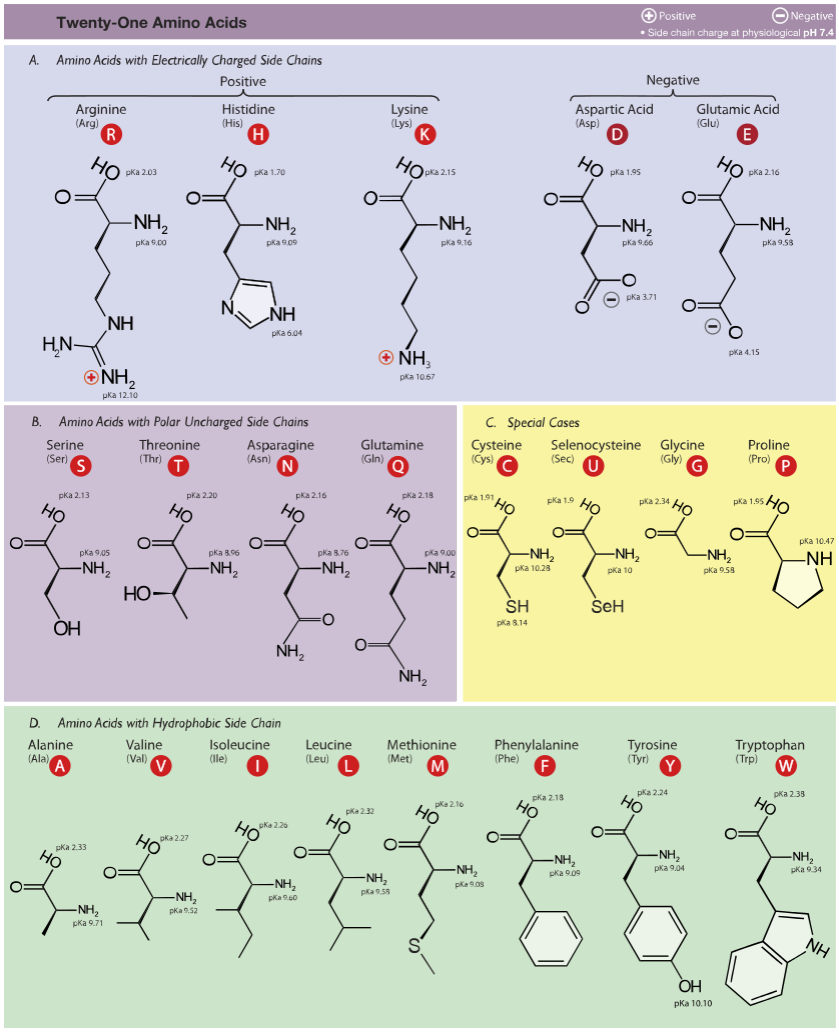
Proteins are macromolecules that differ from their lipid and carbohydrate counterparts by specifically possessing the element – nitrogen. These nitrogenous compounds are essential for life and execute a myriad of cellular functions. The term itself is derived from Greek origin –
"proteios", which translates to "prime" or "primary". This etymology effectively depicts the fundamental nature of protein as it is an indispensable component in mammalian physiology. The immune system is dependent upon antibodies, which are proteins, to neutralize pathogens of both viral and bacterial origin. Protein enzymes catalyze and mediate over 5,000 biochemical reaction types in living organisms. Neurotransmitters, such as serotonin and dopamine, communicate information throughout the body and are largely synthesized from amino acid precursors. Lipids, vitamins, minerals, and oxygen are transferred throughout the body via protein transporters. Hormones are often derived from proteinaceous origin (e.g., insulin) and serve as signaling molecules, which capacities span from facilitating digestion to reproduction and respiration. Amongst this host of biochemical undertakings – proteins also confer structural roles in the body. Structural proteins are referred to as scleroproteins, and provide rigidity to otherwise fluid biological components. This structural aspect is not in the least bit trivial: collagen binds bones and other tissues together; elastin permits organs, including arteries and lungs to recoil; keratin protects epithelial cells from damage and/or stress; and the reversible contraction of myosin in muscles allows for mobility to occur. Both functional and structural proteins are highly abundant in the body, with an estimated 1 – 3 billion proteins per cell.

At the molecular level, proteins are polypeptides, which consist of *linear polymers* of amino acids. When these polymers consist of <50 amino acids, they are termed – peptides. In contrast, when ≥50 amino acids are present, they are termed – proteins. These amino acids are linked together by condensation (i.e., loss of water) reactions to form *peptide bonds*. All amino acids possess what is termed an N-terminus (i.e., amine; $-NH_2$), a C-terminus (i.e., carboxyl; -COOH), an *aliphatic* proton (i.e., hydrogen; H^+), and a functional (i.e., -R) group. The functional groups differ by charge, polarity, and carbon chain type (i.e., *aliphatic* or *aromatic*). It is with this unique functional group that dictates amino acid specificity. That is to say, each amino acid is determined by the corresponding functional group that it possesses. When both the amine and carboxyl groups are attached to the first, or α-carbon (i.e., C^{α}), the amino acid is termed an α-amino acid. There are 21 α–amino acids that are encrypted by the genetic code (i.e., U, C, A, G; mRNA) in *eukaryotes* and these are referred to as *proteinogenic amino acids*. At the cellular level, nucleotides in DNA are transcribed by mRNA, which are then translated by ribosomes, in a process termed – protein synthesis. The ribosome translates three nucleotides at a time, which results in the formation of a specific amino acid pending this three-digit nucleotide sequence. In total, there are 64 possible combinations that result in the synthesis of the 21 proteinogenic amino acids. This transcription and translation process is coined the *central dogma of biology*, which provides the framework for life as we know it. The instructions for protein synthesis is contained within the DNA, located on the chromosomes, and ultimately gives rise to our biological traits i.e., our genotypic (genetic) and phenotypic (observable) makeup. Thus, the way we

look, feel, act, and our susceptibility to disease states are dependent upon proteins, and moreover, the specific order and structure of these proteins.

Humans possess the ability to synthesize 12 of the 21 proteinogenic amino acids (Figure 1)– but must consume the remaining nine through dietary means. These nine amino acids in which humans are unable to synthesize are termed – essential amino acids: histidine, leucine, isoleucine, valine, lysine, threonine, tryptophan, methionine, and phenylalanine. The remaining 12 amino acids that humans are able to synthesize are termed non-essential amino acids: glycine, proline, alanine, arginine, tyrosine, glutamine, glutamic acid, aspartic acid, asparagine, serine, cysteine, and *selenocysteine*. Although termed non-essential, these amino acids are contingent upon the presence of specific amino acid precursors (i.e., limiting amino acid), and can become conditionally essential, in which the rate they are synthesized becomes limited: arginine, cysteine, glycine, glutamine, proline, and tyrosine. Research has displayed that even when precursors are in abundance, that protein biosynthesis cannot satisfy metabolic demands, and synthesis of the aforementioned conditional amino acids may become compromised. When this lower limit of synthesis is attained – the non-essential amino acid(s) in question consequently becomes indispensable. From a physiological perspective, all amino acids are essential if normal growth and development is to be maintained. If the body lacks any one or more amino acid in sufficient quantity – protein synthesis will adversely be affected.

Figure 1. Amino Acid Primary Structure(s)



PROTEIN CONFORMATION

In order to understand protein functionality, it is vital to first grasp the various levels of protein structure. It is the particular three-dimensional conformation in which governs the biological fate of a protein. In total, proteins may occupy up to four levels of structure. The first structure, and

the simplest, is termed the primary structure (1°). The primary structure consists of the amino acid sequence that makes up the polypeptide chain – or backbone. This refers to the specific amino acids that are linearly adjoined via peptide bonds formed during protein biosynthesis. A single alteration in an amino acid within this primary sequence will have a significant net effect on overall physiological function(s). These genetic modifications are often the result of a *single nucleotide polymorphism* (SNP) and may result in various diseases. This effect is elucidated with the blood disorder, sickle-cell anemia, in which a single amino acid (i.e., glutamate) is replaced by another amino acid (i.e., valine) in the primary

sequence. With this disease, ability for the blood to carry oxygen is compromised, which may significantly truncate life-expectancy.

When specific groups within the polypeptide backbone interact, they form the secondary structure (2°). This secondary structure is the product of *hydrogen bonding* between amino acid residues and is independent of the aforementioned functional (-R) group. It is important to note that *covalent* bonds are the strongest of bond types – followed by ionic > hydrogen > hydrophobic > and Van der Waals, respectively. When the oxygen on the carbonyl group (i.e., C=O of the carboxyl) interacts with the hydrogen on an amine group ($-NH_3$) approximately four amino acid residues down the polypeptide chain – it forms a curled ribbon structure, known as an α-helix. Similarly, when two or more segments of a polypeptide chain line up laterally, they are also joined by hydrogen bonding between the carbonyl oxygen and amine hydrogen. This lateral orientation results in a sheet-like structure, termed β-pleated sheets. Unless denatured, proteins may contain only α-helices (e.g., myoglobin), only β-pleated sheets (e.g., antibodies and T-cell receptors) or a combination of both secondary folding types (e.g., hexokinase).

When functional groups (-R) between amino acid residues interact, they form the three-dimensional conformation known as the tertiary structure (3°). This tertiary structure is held together largely by non-covalent bonds. The corresponding three-dimensional conformation is dependent upon the attractive and repulsive forces of the functional groups on the amino acid residues. For example, functional groups with opposite charges will (I) form *ionic bonds* through attractive forces and (II) *hydrophobic* amino acid residues will coalesce together in order to exclude surrounding water molecules, which are *hydrophilic*, in a process known as the *hydrophobic effect*. The exception to non-covalent bonds present within the tertiary structure are *disulfide bonds*. These bonds adjoin neighboring cysteine groups in order to provide strength to the overall polypeptide structure. Up until this point, all levels of protein structures (i.e., 1°, 2°, and 3°) consist of a single polypeptide chain and the corresponding interactions within the amino acid residues of this polypeptide chain; all proteins consist of a 1°, 2°, and 3° structure. However, some proteins are composed of more than one polypeptide chain, which are termed *subunits*.

These subunits each acquire a tertiary structure of their own and these tertiary structures interact with each other to form a unique structure. The spatial arrangement of these subunits, and thus the corresponding conformation, is termed the *quaternary structure* (4°). There can be multiple subunits, as is observed within DNA polymerase (i.e., 10 subunits) and collagen (i.e., 3 subunits), or a complete lack of subunits, in which the protein does not possess a quaternary structure at all (e.g., myoglobin). Regardless of whether the quaternary structure is present or not, the final functional folded and/or assembled form of the protein is termed the *native conformation*. It is with this native conformation that permits functionality within the body.

There are physical, mechanical, and chemical means by which these native structures may become disrupted. Humans have manipulated these methods of disruption over the course of evolution to both harness the inherent nutritional aspects of food(s) in addition to

preserving them. When the native conformation is irreversibly disrupted, the protein reverts to its unstructured primary amino acid sequence, and the protein is accompanied by a loss of functionality. When this phenomenon occurs, the protein is said to have been *denatured.* Hydrochloric acid in the stomach is an example of *endogenous* chemical denaturation and is essential for nutrient assimilation. By denaturing, and thus unfolding the protein structure, surface area is increased. This increase in surface area permits *proteolytic* digestive enzymes to cleave the peptide bonds between amino acid residues more efficiently.

PROTEIN CLASSIFICATION AND NOMENCLATURE

PROTEIN DIGESTIBILITY CORRECTED AMINO ACID SCORE (PDCAAS)

The nutritional value of a protein is a vital piece of information when constructing a diet that supports optimal health. It was as early as the 1940s in which amino acids were separated based upon the ability to synthesize the carbon backbone *de novo*; amino acids in which the carbon backbone can be synthesized in the body were consequently termed "non-essential" and carbon backbones that cannot be synthesized in the body – "essential". This nomenclature resulted in the misconception that essential amino acids are more valuable in protein synthesis and other biological functions than non-essential amino acids. Although the scientist, W.C. Rose, who coined these definitions candidly stated on several occasions that this classification scheme is purely a matter of definition and is not designed to be taken in the literal sense – these terms have become muddled within the masses. This misnomer has survived the test of time and inevitably infiltrated into the realm of biological sciences, where it continues to serve as a standardization and assessment method for gauging the inherent quality of protein sources today.

This method is termed Protein Digestibility Corrected Amino Acid Score (PDCAAS) and measures the essential amino acid content present in protein sources compared to that of human requirements (specifically 2 to 5 years; most nutritionally demanding group). The scale ranges from 0 to 1.0, where proteins that are highly digestible and contain all the essential amino acids in specified amounts set by the Food and Agricultural Organization (FAO)/World Health Organization (WHO) receive a score of 1.0, and are deemed "high quality proteins" (e.g., egg white, milk, meat, and soy). It is possible to effectively formulate complementary proteins that individually rate very low according to PDCAAS, but together, receive a perfect rating. This is particularly evident in plant foods, which are often limited in one or more essential amino acid. For example, grains are low in lysine but provide a rich source of methionine – while legumes are a rich source of lysine but low in methionine. Alone, these commodities are incomplete proteins. However, when consumed together or

over the course of the day, they constitute a high-quality protein source in terms of supplying all nine essential amino acids.

Many scientists agree that the most accurate protein evaluation system that should be formed and used in the future will evaluate a protein beyond what was once deemed as the "essential amino acids" taking into account the other amino acids that contribute to many other metabolic processes critical in achieving and maintaining optimum health. As scientist R. Elango states "Normal growth and maintenance of health in humans requires all amino acids (i.e., essential, non-essential, conditionally essential) to be provided in appropriate quantity and form that is biologically utilizable. Optimal dietary protein intake will provide all the 21 amino acids (essential, conditionally essential, non-essential) in the correct proportions to meet the body's needs for metabolic functions including intestinal integrity, modulation of gene expression, protein synthesis, and regulation of cellular signal pathways.". Thus, a valid protein evaluation system that will represent the amino acid profile needed for optimum health rather and take into account all of the 20 amino acids.

Despite the current protein evaluations systems (PDCAAS) that only consider essential amino acids, the non-essential amino acids are just as important in protein synthesis and other metabolic processes. It seems as if Rose's classifications have been taken and applied too strictly, failing to acknowledge the gray areas that Rose himself once made reference to, and scientists today continue to suggest with their research. These gray areas highlight the fact that while some amino acids may be able to be synthesized by the body, the extent of their synthesis must have a limit, and this limit may not supply the body with the amount it needs.

When considering what proteins to include in one's diet that will support optimal health, it is important to take into account one's intake of essential, conditionally essential, and non-essential amino acids. By including what today are considered to be "high-quality proteins" (according to PDCAAS) such as egg white, milk, meat, and soy coupled with "lower quality" proteins such as gelatin or traditionally made broths, one can obtain a full spectrum of amino acids that includes all 20 of them. Furthermore, taking into account limiting amino acids in foods and finding a protein that may complement, as well as considering a protein's digestibility which may be altered by methods such as cooking, will further ensure that all sufficient protein is ingested.

This makes it imperative that Rose's classifications of essential and non-essential amino acids continue to be reevaluated and altered so that these gray areas may be more properly represented. In addition, the current most widely accepted protein evaluations system known as PDCAAS often over-estimates the protein quality of foods by neglecting to factor in anti-nutritive factors (e.g., trypsin inhibitors in soy protein), which render amino acids unavailable for protein synthesis. To reinforce these confines, numerous studies have elucidated that the current PDCAAS criteria is not capable of maintaining amino acid homeostasis and/or balance within the body and underestimates proper protein intake by as much as 40%. Indeed, the protein evaluation systems of the future will need to include all

20 amino acids, taking into account the functions of amino acids beyond their roles in the synthesis of proteins.

LIMITATIONS OF PDCASS

The PDCASS system does not account for anti-nutritive factors such as lectins, tannins, saponins, etc. that interfere with digestion. "Protein foods subjected to heat/alkali processing to improve food flavor and texture, or sterilization/pasteurization, may cause the formation of compounds that render the amino acids unavailable for protein synthesis. Trypsin inhibitors in soy protein, tannins in legumes and cereals, and phytates in cereals decrease the bioavailability of amino acids from the food sources. PDCAAS method doesn't take into account these anti-nutritional factors and tends to overestimate the protein quality of such products." (R. Elango et. al. p. 23).

"PDCAAS does NOT take into account the conditionally essential and non-essential amino acids, and does not take into account the nutritional value of high quality proteins, because values higher than 100% are truncated to 100%. Therefore, differences between two proteins such as milk and soy proteins are not distinguished, although the actual concentration of some essential amino acids and the capacity to complement other food sources are higher in milk than in soy protein." (R. Elango et. al. p. 22).

"Thus, the classic concept of "the ideal protein," which is based solely on ratios of EAA in tissue proteins, should be revised to include all NEAA. In other words, both EAA and NEAA should be taken into consideration in: 1) formulation of balanced diets to maximize growth performance in livestock species, poultry, and fish; 2) recommendation of AA requirements for humans to prevent growth retardation and chronic diseases; and 3) optimization of immune and reproductive functions in all species." (Wu, G. "Functional A.A...." p. 35).

ESSENTIAL, NON-ESSENTIAL, & CONDITIONALLY ESSENTIAL AMINO ACIDS

BACKGROUND

The nutritional value of a protein is a vital piece of information in constructing a diet to support optimal health. As mentioned, the foremost scientists to hold the torch on this scientific study of proteins was W.C. Rose in the 1940s. In W. C. Rose's defense of his controversial categorization of amino acids, he did write of the subjectivity underlying his proposed categories stating in the year 1947, "We have emphasized on several occasions. . . that the classification of an amino acid like arginine or glutamic acid as dispensable (non-essential) or indispensable (essential) is purely a matter of definition." It appears that W. C. Rose was not especially enamored with the way in which they were applied by others who essentially made the categories into black and white classifications when they were more flexible in nature with many gray areas yet to be elucidated.

Now more than ever the gray areas Rose once made reference to have been brought into light. It is clear from decades of research, especially in the last 20 years or so with the invention of more sophisticated technologies, that Rose's classifications are becoming increasingly ambiguous as more limits are found in the bodies' ability to synthesize adequate amounts of non-essential (or conditionally essential) amino acids needed for general health. Precursors are often needed for non-essential (or conditionally essential) amino acids, and even with adequate supplies of these precursors – limits in synthesis may occur – leading to possible impairments in growth or imbalances that disrupt normal metabolic processes. Furthermore, while it was previously thought that in addition to the essential amino acids, simple sources of nitrogen coupled with carbon sources would be sufficient to maintain nitrogen balance, there are now well-founded theoretical reasons to conclude that this is not likely in humans.

Another factor contributing to the increasing ambiguity of these categories is the fact that research on amino acids has progressed from studying amino acids solely for their abilities to be used in the synthesis of proteins towards a more holistic picture that includes amino acids' roles in a wide array of metabolic functions. As more of this research comes about, scientists are increasingly forced into expanding their viewpoint of the roles of amino acids to include much more than simply protein synthesis. In addition, this new approach has led many scientists to question the current essential amino acid requirements outlined by the Food and Agricultural Organization, the World Health Organization, and the United Nations that are presently the most widely accepted recommendations. These requirements were based upon earlier experiments that used methods that today are widely considered to be less accurate, leading to an overall underestimation of amino acid requirements. Newer, more sensitive methods using stable isotope tracers have proposed that the current protein requirements may be underestimated by as much as 40%, an outstanding underestimation.

Table 1. Amino Acid Essentiality

Essential	Non-essential	Conditionally Essential
Histidine	Alanine	Arginine
Isoleucine	Aspartic Acid	Cysteine
Leucine	Asparagine	Glutamine
Lysine	Glutamic Acid	Glycine
Methionine	Serine	Proline
Phenylalanine	-	Tyrosine
Threonine	-	-
Tryptophan	-	-
Valine	-	-

ESSENTIAL AMINO ACIDS

For obtaining essential amino acids, soy, egg white, milk, meat, and fish are all good sources of all the essential amino acids in their currently recommended amounts. Protein from meat provides all nine essential amino acids.

On the PDCAAS ranking system (that only takes into account essential amino acid content in food sources), animal meats like beef have a score of approximately 0.90, compared to values of 0.50 - 0.70 for most plant foods. The amino acid(s) glutamic acid and glutamine are present in meat in the highest concentrations (16.5%), followed by arginine, alanine, and aspartic acid. Meat contains a small amount of collagen (1-2% of muscle tissue). Meats with higher concentrations of connective tissue contain greater amounts of proline and glycine, and slightly more arginine and alanine, with an absence of tryptophan or cysteine. This amino acid pattern follows the general pattern seen with body tissues higher in connective proteins.

Some authors continue to reexamine the classification of amino acids into the "essential" group. For example, "An examination of the amino acids that are generally considered to be nutritionally essential indicates that each has a specific structural feature, the synthesis of which cannot be catalyzed by mammalian enzymes. In this regard, it is very important to note that the loss of the ability to carry out these biosyntheses appeared early in evolution and is a common feature of the metabolism of eukaryotic organisms in general, and not just of mammals. However, within this view, the important term is de novo synthesis [produced in the body]. This is because some essential amino acids can be synthesized from precursors that are structurally very similar. For example, methionine can be synthesized both by transamination of its keto acid analogue and by re-methylation of homocysteine. In this sense, then, the mammal is capable of synthesizing leucine, isoleucine, valine, phenylalanine and methionine. However, this is not new synthesis, because the branched-chain keto acids and homocysteine were originally derived from branched-chain amino acids and methionine, respectively. According to this restricted metabolic definition of essentiality, threonine and lysine (and perhaps tryptophan) are the only truly essential amino acids." (Reeds, Peter J. p. S1836). **Note: according to this author, there are not truly eight essential amino acids since five (or six) of them can be produced from a similar looking molecule already present in the body, and thus are not required in their whole and complete form in the diet.

NON-ESSENTIAL AMINO ACIDS

Strictly speaking, a truly nonessential amino acid (NEAA) is one that can be synthesized de novo (by the body) from a non–amino acid source of nitrogen (e.g., ammonium ions) and an appropriate carbon source. According to this metabolic definition, the only truly metabolically nonessential amino acids are glutamic acid and serine. (Reeds, Peter J. p. S1836). **Note: Thus, the other amino acids considered non-essential (besides glutamic acid and serine) require a precursor (or *part* of a precursor) of some sort in order to be synthesized so that their production is not entirely independent.

"The use of the word essential, however, does not mean that the other nonessential amino acids are not equally as essential for the formation of the proteins but only that the others are not essential in the diet because they can be synthesized in the body. For protein synthesis to take place, all the amino acids required must be available. If the diet lacks one or more of these essential amino acids, the body's ability to synthesize new protein is adversely affected." (DiPasquale, M.G. p. 148).

"While the presence of essential amino acids is critical to protein synthesis, there is some evidence that lack of the nonessential amino acids can result in lower plasma levels of these amino acids, which may ultimately compromise protein synthesis in situations where there is rapid growth [or tissue repair]." (DiPasquale, M.G. p. 148).

"In the definition of protein requirements, much emphasis has been placed on the need for indispensable (essential) amino acids; however, attention needs to be given to the amount of dietary protein that allows adequate endogenous formation of amino acids that are dietarily dispensable (non-essential)." (Jackson, Alan A.).

CONDITIONALLY ESSENTIAL AMINO ACIDS

"...the synthesis of these amino acids can become limiting for growth and other physiological functions..." (Reeds, Peter J p. S1837).

"There are some amino acids that are synthesized by more complex pathways than the simple transamination of an appropriate keto acid. These amino acids are frequently termed 'conditionally' essential, the term being used to imply that there are measurable limitations to the rate at which they can be synthesized. When this limit is attained, the amino acid in question becomes an essential component of the diet. The limitations can result from a number of factors. First, the synthesis of these amino acids requires the provision of another amino acid, either as the carbon donor or as a donor of an accessory group, such as the sulfur group of cysteine. Thus, the ability of the organism to synthesize a given conditionally essential amino acid is set by the availability of its amino acid precursor (i.e., limiting amino acid), a point that was emphasized by Rose in his studies of the interactions among glutamate, proline and arginine nutrition." (Reeds, Peter J. p. S1837).

"...most evidence suggests that even in the presence of abundant quantities of the appropriate precursors, the quantities of conditionally essential amino acids that can be synthesized may be quite limited, so it can be argued that there are circumstances, especially stressful circumstances, under which the metabolic demands for the amino acids rise to values that are beyond the biosynthetic capacity of the organism." (Reeds, Peter J. p. S1837).

Some amino acids may be synthesized in only a limited number of tissues. For example, the synthesis of both proline and arginine is crucially dependent on intestinal metabolism. Moreover, in the case of these two amino acids, the available evidence suggests that dietary, as opposed to systemic, amino acid precursors are obligatory. (Reeds, Peter J. p. S1837). **Note: Even though the amino acid may not be required in its whole form, parts of it (in the form of precursors) may need to be acquired through diet.

"it appears that the synthesis of these [conditionally essential] amino acids can become limiting for growth and other physiological functions..." (Reeds, Peter J. p. S1837).

"despite the longevity of the convention, as more information has become available, the distinctions between dispensable and indispensable amino acids, at least at the metabolic level, have become increasingly blurred [as more research work has been done]. Indeed, W. C. Rose, who was responsible for the initial definition of the two terms, was not especially enamored with the way in which they were applied by others and wrote the following (Womack and Rose, 1947):

"We have emphasized on several occasions. . . that the classification of an amino acid like arginine or glutamic acid as dispensable or indispensable is purely a matter of definition." (Reeds. Peter J. p. S1835).

"From a nutritional perspective, it is quite clear that some amino acids are absolute dietary necessities if normal growth is to be maintained. Even so, growth responses to deficiencies of dispensable (non-essential) amino acids can be found in the literature. From a strictly metabolic perspective, there are only three indispensable amino acids (lysine, threonine and tryptophan) and two dispensable amino acids (glutamate and serine). In addition, a consideration of in vivo amino acid metabolism leads to the definition of a third class of amino acids, termed conditionally essential, whose synthesis can be carried out by mammals but can be limited by a variety of factors. These factors include the dietary supply of the appropriate precursors and the maturity and health of the individual. From a functional perspective, all amino acids are essential..." (Reeds, Peter J. p. S1835).

Table 2. Precursors to GAP (Glycine, Arginine, & Proline)

AMINO ACID	PRECURSOR AA
Glycine	Threonine, Serine
Arginine	Glutamine and/or glutamate/aspartate
Proline	Glutamate

Glycine and serine are produced from one another in a reversible reaction that requires folate (B9) (Gropper, Sareen S. p. 220).

Threonine is the precursor of glycine and serine, two other amino acids in the threonine group (Braverman, Eric R.).

Glutamate is the precursor to proline (Gropper, Sareen S. p. 189).

Glutamate or glutamate/aspartate are precursors to arginine. (Gropper, Sareen S. p. 189).

"The nutritionally indispensable (essential) amino acids alone do not maintain body nitrogen balance; a source of nonspecific nitrogen from dispensable (non-essential) amino acids, such as glycine and alanine or other N compounds, is also required." (DiPasquale, M.G. p. 161).

ALPHA-AMINO NITROGEN

More Ambiguity in Modern Amino Classification

There now appears to be a requirement for pre-formed alpha-amino nitrogen in the form of glutamate, alanine, or aspartate, for example. It was previously thought that, in addition to the essential amino acids, simple sources of nitrogen such as urea and diammonium citrate together with carbon sources would be sufficient to maintain nitrogen homeostasis (FAO/WHO, 1965). However, there are now good theoretical reasons to conclude that this is not likely in humans. The mixture of dispensable and conditionally dispensable amino acids as supplied by food proteins at adequate intakes of total nitrogen will assure that both the nitrogen and specific amino acid needs are met." (Institute of Medicine - IOM p. 594).

Animals are dependent on alpha-Amino Nitrogen as an Essential Nutrient: "Experiments by Rose & colleagues from the 1940s established the basic view of amino acid nutrition that mammals, including humans, do not nutritionally require the nonessential amino acids. Their results only indicated that animals can synthesize the carbon skeletons of the non-essential amino acids... Rose's nutritional experiments were only concerned with whether C skeletons of particular amino acids can (nonessential) or cannot (essential) be synthesized from common intermediates. This pertains to carbohydrate metabolism only...Clearly, of primary importance in amino acid synthesis should be the means for obtaining the alpha-amino groups and not whether their carbon skeletons can or cannot be synthesized de novo..." (Katagiri, Masayuki et. al. p. 125-6).

"...recent research indicates the dietary significance of the various amino acids [as essential or non-essential] is more elaborate than this earlier classification might suggest...research now has shifted away from the earlier, narrow focus on N balance towards a more comprehensive evaluation of the consequences of altered amino acid levels and balance of intake on the metabolic and functional status of individuals...[leading to the classification of conditionally indispensable amino acids: arginine, proline, glutamine, cysteine, and glycine.]" (Young, Vernon R.).

"the question as to the optimum ratio of essential to non-essential amino acids is not known...in rats, the optimum ratio of total to essential amino acids per gram of dietary N was between 3 and 4." (Young, Vernon R.).

"nutritional experiments performed by others indicated that if only minimal amounts of essential amino acids are provided to humans, pigs, and rats, a striking growth stimulus results from the further provision of nonessential amino acids." (Katagiri, Masayuki et. al. p. 126).

"Many nutritional studies have shown that body weight gain and Nitrogen retention in humans, rats, cats, chicks, and other animals are optimal in the presence of moderate amounts of both essential AND nonessential amino acids." (Katagiri, Masayuki et. al. p. 127).

"Normal growth and maintenance of health in humans requires all amino acids (essential, non-essential, conditionally essential) to be provided in appropriate quantity and form that is biologically utilizable. Optimal dietary protein intake will provide all the 20 amino acids (essential, conditionally essential, non-essential) in the correct proportions to meet the body's needs for metabolic functions including intestinal integrity, modulation of gene expression, protein synthesis, and regulation of cellular signal pathways." (R. Elango et. al. p. 20 & 21).

"Studies [up to 2008] have shown that currently accepted 1985 criteria for amino acid requirements pattern are not capable of maintaining body amino acid homeostasis or balance. Thus, it is hypothesized that current international estimations of essential amino acid requirements are far too low and must be modified in light of present research." (DiPasquale, M.G. p. 148).

"FAO diet is not capable of maintaining amino acid homeostasis...we've concluded that the results of earlier nitrogen-balance experiments[see next quote] should not be used to establish the quantitative needs for specific essential amino acids in healthy human adults...estimate the physiological requirements for the essential amino acids to be two to three times those currently proposed by FAO/WHO/UNU...[the new requirements will be based upon the results of a series of stable-isotope tracer studies]." (Marchini, Sergio J. p. 670-78).**

Primary nitrogen data is now largely questioned since the technique initially used by Rose et. al. to define essential and nonessential amino acids overestimates N intake and underestimates N excretion, leading to an overly positive balance and therefore underestimation of the requirement. (Young, Vernon R.). "Tentative estimates based on the use of [New] tracer techniques are generally higher than earlier determinations based on the Nitrogen balance approach." (Young, Vernon R.).

New methods for defining individual amino acid requirements, such as the Indicator Amino Acid Oxidation (IAAO) model (a stable isotope based method), suggest that current protein recommendations are underestimated by around 40%. (R. Elango, 2008).

SCLEROPROTEINS

A look at what amino acids the body is largely composed of further suggests the essentiality of all amino acids. While the human body does indeed carry around a fairly large amount of muscle tissue, a major part of the proteins used to form the bodies structure are actually connective tissue proteins. Two of the main connective tissues of the body are referred to as "collagen" and "elastin," each one forming a considerable amount of the whole-body protein. These two tissues form the matrix of the body, keeping everything glued together, and helping to protect vital organs. Thus, they can be found in every nook and cranny of the body. Elastin is known to comprise around 28% of the body, and collagen forms 25-35% of the whole-body protein. Thus, the body requires ample amounts of the amino acids that compose both of these connective tissues. Although highly abundant in the body, these proteins receive a 0 according to the PDCAAS rating system and are thus considered to be inferior, or "low quality", sources of dietary protein.

Mammalian elastin, which constitutes approximately 28% of whole body protein, is predominately 30.8% (glycine), 23.5% (alanine), and 12.7% (proline) (Mecham 2008). The preponderance of amino acids with small functional groups (e.g., glycine, proline, alanine) is characteristic of structural proteins. With such a high percentage of connective tissue protein in the body, it becomes biologically taxing to supply all of the non- and conditionally essential amino acids necessary for adequate maintenance and biosynthesis. Scientific literature demonstrates that humans are unable to synthesize non-essential amino acids in the amounts necessary to satisfy metabolic requirements under stressful *or* standard physiological conditions. A deficit in glycine alone has been implicated in the development of connective tissue diseases. Additionally, healthy humans are only able to satisfy 30% of daily glycine synthesis. Moreover, approximately 10 grams per day (for a 70-kg individual) of glycine are required for all metabolic uses – including collagen synthesis. The approximate 3.0 grams per day that are generated via protein turnover, in addition to the 1.5 – 3.0 grams per day assimilated through dietary means, is not commensurate with this metabolically imperative metric.

Apart from collagen and elastin, there are other types of connective tissue such as keratin, gelatin, and cartilage. Table 3 summarizes the different types of scleroproteins while Table 4 illustrates the AA composition of various Scleroprotein sources.

Table 3. Scleroprotein Overview

Structural Protein/Connective Tissue	Primary Locations	Amino Acid Composition
Collagen	bone matrix, skin, tendons	30% glycine, 20% proline/hydroxyproline; very little cysteine (sulfur a.a.)

Elastin	**lungs, blood vessels, ligaments**	**30% glycine, rich in alanine, valine, proline, & lysine in descending order**
Gelatin	**hydrolyzed (melted down) collagen**	**same as collagen**
Cartilage	**joints between bones, the rib cage, the ear, the nose, the bronchial tubes and the intervertebral discs**	**same as collagen of which it is largely composed of; also contains some elastin higher in some areas such as the outer ear**
Keratin	**hair, skin, nails, horn(s), beaks, wool, outermost layer of skin or fibrous outer covering of an organism**	**rich in cysteine (sulfur) amino acids - up to 24%, dominated by glutamic acid, serine, glycine, alanine, leucine; differs upon location but quite similar between species**

Table 4. AA Composition of Various Scleroprotein Sources

Amino Acid	α-Keratin (Wool)	Fibroin (Silk)	Collagen (Bovine Tendon)	Elastin (Pig Aorta)
Gly	8.1	44.6	32.7	32.3
Ala	5.0	29.4	12.0	23.0
Ser	10.2	12.2	3.4	1.3
Glu + Gln	12.1	1.0	7.7	2.1
Cys	11.2	0	0	—[e]
Pro	7.5	0.3	22.1[a]	10.7[c]
Arg	7.2	0.5	5.0	0.6
Leu	6.9	0.5	2.1	5.1
Thr	6.5	0.9	1.6	1.6
Asp + Asn	6.0	1.3	4.5	0.9
Val	5.1	2.2	1.8	12.1
Tyr	4.2	5.2	0.4	1.7
Ile	2.8	0.7	0.9	1.9
Phe	2.5	0.5	1.2	3.2
Lys	2.3	0.3	3.7[b]	3.6[d]
Trp	1.2	0.2	0	—[e]
His	0.7	0.2	0.3	—[e]
Met	0.5	0	0.7	—[e]

Note: The three most abundant amino acids in each protein are indicated in red. Values given are in mole percent.

[a]About 39% of this is hydroxyproline.

[b]About 14% of this is hydroxylysine.

[c]About 13% of this is hydroxyproline.

[d]Most (about 80%) is involved in cross-links.

[e]Essentially absent.

(Matthews, Van Holde, & Ahern).

ELASTIN VS. COLLAGEN MAIN DIFFERENCES

"The amino acid composition of elastin is both similar and different from collagen. It [elastin] has one-third glycine and is rich in proline (like collagen). Though 1/3 of the amino acid residues are glycine, this amino acid is not regularly spaced." (Boonshoft School of Medicine-Wright State University Online Education p. 141).

[Elastin has fewer lysine cross-links than collagen, making it more elastic and less rigid than collagen]. (Matthews, van Holde, Ahern).

"The triple helix of the (unrelated) structural protein collagen likewise has a high percentage of glycine, as does the connective tissue protein elastin, which also has a high percentage of alanine." (New World Encyclopedia contributors)

"The principal constituents of the blood vessels are the fibrous proteins collagen and elastin...It has been suggested that type I collagen is largely responsible for the strength of the wall and so a change in its proportion in the stressed tissue would not be unexpected." (Ryan, Patricia A.).

ELASTIN

The human body is largely made up of elastin, comprising 28% of the body. Elastin fibers are present in virtually all tissues of vertebrates, but is in appreciable amounts in the arteries, some ligaments, and the lung. As the name implies, elastin has an elastic nature that is also resilient, allowing for body tissues to resume their shapes after being stretched or twisted. Elastin's unique amino acid composition, containing high amounts of glycine and proline, gives elastin its unique properties.

Elastin "has intrinsic elasticity that can stretch and undergo elastic recoil when necessary." (B.C. Starcher).

Remodeling of Elastin is very slow...once the elastin fiber is formed, normal remodeling processes are extremely slow...suggest that less than 1% of the total body elastin pool turns over in one year. (B.C. Starcher). "From data on elastin content in different tissues, the total elastin in the human body can be estimated as about 75 g though there have been reports of turnover as high as 455 mg/day in aortic tissue, which would mean an average lifetime of about 11 days." (Meléndez-Hevia, Enrique p. 867).

"As in mice, the turnover of elastin in normal humans is best estimated in years.... Elastin is an unusual protein from a biological point of view because of its long biological half-life. It would appear that in normal situations elastin fibers are meant to function for exceedingly long periods." (Rucker, Robert B. et. al. p. 189).

COMPOSITION

Elastin, which typically coexists with collagen in the body's connective tissue, contains high amounts of glycine (33%) and proline (12%) similar to collagen. However, the glycine within elastin is typically irregularly spaced, unlike collagen that contains a glycine at about every third residue. Elastin also contains significant amounts of the simple amino acids valine and alanine (Table 4). Elastin is composed of cross-links formed through lysine, similar to

collagen, but with fewer cross-links than collagen, making elastin more elastic and less rigid than collagen.

1/3 of amino acid residues are glycine, 12% are proline, and over 40% of the remaining amino acids contain hydrophobic [water-fearing] side chains, making this one of nature's most non-polar proteins.

"The primary structure [of elastin] contains significant amounts of lysine residues that are involved in inter fiber cross-linking." (Khan, M.Y. & Sangeeta Saxena). Lysine is important in the formation of cross-links between tropoelastin protein molecules. Lys-pro/Lys-ala; Tropoelastin is the building block For elastin.

PHYSIOLOGICAL FUNCTION(S)

Elastin is a protein in connective tissue that allows many tissues in the body to resume their shape after stretching or contracting. Elastin helps skin to return to its original position when it is poked or pinched. Elastin is also an important load-bearing tissue in the bodies of vertebrates and used in places where mechanical energy is required to be stored.

"Elastin fibers are present in virtually all vertebrate tissues, although it is only within a few, such as arteries, some ligaments, and the lung, that elastin comprises an appreciable % of the total protein." (B.C. Starcher). They endow connective tissues such as blood vessels, lungs and skin with the critical properties of elasticity and resilience. (Kielty, Cay M.). Elastin is an essential connective tissue component of tissues that must be deformable and resilient for proper biological function, such as the aorta & large arteries. (Foster, J.A. & Sandra W. Curtiss).

LUNGS

"It has long been recognized that elastin plays a critical role in the mechanical action of the lung as a gas-exchange organ. Because of the intrinsic elasticity of elastin, its presence in the alveolar wall imparts the essential stretch and recoil integral to respiration dynamics....Elastin comprises approximately 2.5% of the dry weight of the lung...." (Foster, J.A. & Sandra W. Curtiss p. L13).

"Elastin is found in lung pleura, parenchyma, blood vessels, bronchi, and trachea. Since elastic fibers are concentrated around the mouths of the alveoli, it is speculated that alveolar shape results from the molding influence of elastic fibers. It is generally accepted that elastic fibers are of crucial importance in the maintenance of lung structure and normal lung compliance, and that alteration in lung elastin metabolism can lead to a variety of pathological changes in the lung." (Rucker, Robert B. et. al.).

"Elastin protein levels are a vital modifier affecting normal lung development and susceptibility to emphysema... the quantity of functional elastin in the lung is an important

modifier of both lung development & response to injury... Animals with intermediate elastin levels exhibit normal alveolar structure but develop worse emphysema than normal mice following cigarette smoke exposure....[suggests that] humans with altered levels of functional elastin could have relatively normal lung function while being more susceptible to smoke-induced lung injury." (Shifren, A, Durmowicz A.G. et. al.)

"A critical elastin protein level is required for normal lung development. Elastin is known to have a pivotal role in lung development." (Shifren, A, Durmowicz A. G. et. al.).

COLLAGEN

The other connective tissue protein found in ample amounts in the body is collagen, comprising 25-35% of the whole-body protein, making it the most abundant protein in mammals and in the human body. Present in virtually every tissue in the human body, collagen functions to hold everything together as its name that comes from the Greek word colagené, (i.e. "glue forming") implies. Collagen maintains shape and mechanical rigidity with its tensile strength that bears structural stresses well. Collagen, in the form of elongated fibrils, is mostly found in fibrous tissues such as tendon, ligament, and skin, and is also abundant in cornea, bone, blood vessels, the gut, fibrous cartilage, organ capsules, and the intervertebral disc. It can be found in significant amounts in the vascular walls, contributing strength to these walls.

COMPOSITION

"There are at least nineteen different types of collagens which have been found in humans and reported in literature. Types I through V are the chief ones. Type I is the most abundant collagen, the principal element of ligaments, tendons, skin, and bones, sclera, dentin, fibrous cartilage, and organ capsules. Type II characterizes more than 50% of the protein in cartilage. Type III supports the walls of hollow areas such as the fetal skin, intestinal tract, uterus, and blood vessels. Type IV collagens provide the filter for the blood capillary system as well as the glomeruli of kidneys and lens capsule. Type V is found in the basement laminae of blood vessels and smooth muscle cells, and exoskeleton of fibroblasts and other mesenchymal cells." (Stuart, Paula S. et. al. p. 393).

The characteristic sign in the composition is the amino acid glycine in every 1/3 position which makes for the unique 3D structure and content of an unusual amino acid hydroxyproline. Collagen contains a considerable amount of glycine (330 moles for 1000 moles of amino acids), and a high content of hydroxyproline and proline is characteristic for the amino acid composition of collagen along with hydroxyl serine. Collagen is low in methionine, leucine, and isoleucine.

Interestingly, glycine is the only achiral amino acid that is small enough to fit in the restricted space accessible down the central core of the triple helix. Many of the remaining

chains are filled by proline or hydroxyproline. Proline and hydroxyproline give stiffness to the collagen molecule. (Stuart, Paula S. p. 393

"The mammalian gelatins & collagens form a compact group in regard to their amino acid composition. The placental land mammals man, ox, and pig show few differences in composition..." (Eastoe, J.E. p. 599).

Collagen is deficient to varying degrees in all essential amino acids – which is reflected by the assignment of 0. Despite this low rating, which suggests that collagen is devoid in complete nutritional value, collagen encompasses 25 – 35% of whole body protein. While over 27 total collagen types have been discovered to date – Type(s) I, II, and III constitute 80 – 90% of collagen present in the body. Type I representative tissues comprise skin, tendon, bone, ligaments, dentin, and interstitial tissue; Type II representative tissues comprise cartilage and vitreous humor; and Type III representative tissues comprise skin, muscle, and blood vessels Although differing by anatomical region, the general repetitive tripeptide sequence of Gly-X-Y, where X and Y are proline and hydroxyproline, respectively, holds true for the distinguishable types of collagen. To better conceptualize the composition of collagen, specific amino acid percentages are detailed in Table 5 below in descending order. Table 5a. illustrates a summary of the predominant amino acid found in collagen, glycine. Table 6 additionally breaks down AA composition of bone collagen.

Table 5. Amino Acid Composition of Mammalian Skin Collagen

Amino Acid	Composition % (Descending)
Glycine	32.9
Proline	12.6
Alanine	10.9
Hydroxyproline	9.5
Glutamic acid	7.4
Arginine	4.9
Aspartic acid	4.7
Serine	3.6
Lysine	2.9
Leucine	2.4
Valine	2.2
Threonine	1.9
Phenylalanine	1.3
Isoleucine	1.1
Hydroxylysine	0.6
Methionine	0.6
Histidine	0.5
Tyrosine	0.3
Cysteine	0.1
Tryptophan	0

***Non-essential amino acids are highlighted in gray-field to provide a visual aid**

Table 5a. Summary of Glycine Function

Amino Acid	Functions	Deficiency	Notes
Glycine	Synthesis of bile salts, heme, creatine, nucleic acids, glutathione (liver detoxification), glucose; associated with a strongly reduced risk of asthma; stimulates the secretion of gastric acid; wound healing;	Limit the synthesis of heme, glutathione, purines, and creatine (Yu, 1985)	Higher requirements with certain conditions, including sickle-cell anemia and pregnancy. Pregnancy requires a glycine intake 2 – 10x greater to support fetus development, and 2 -10X greater than other proteinogenic amino acid requirements

Table 6. Percentage Breakdown of Amino Acids in Bone Collagen

Amino acids	Percentage
Proline/Hydroxyproline	18.3%
Glycine	26.2%
Glutamic acid	5.9%
Arginine	15.4%
Alanine	9.3%
Other essential amino acids	14.19%
Other non-essential amino acids	20.9%

PHYSIOLOGICAL FUNCTION(S)

Collagen, in the form of elongated fibrils, is mostly found in fibrous tissues such as tendon, ligament, and skin, and is also abundant in cornea, bone, blood vessels, the gut, and the intervertebral disc. In muscle tissue, it serves as a major component of the endomysium. Collagen constitutes 1 to 2% of muscle tissue, and accounts for 6% of the weight of strong tendinous muscles.

In order for collagen turnover to proceed and collagen synthesis to occur some of its amino acid precursors are needed. These precursors will activate the cells (fibroblasts) that synthesize collagen. "Collagen synthesis is, therefore, efficiently maintained only when those specific amino acids are continuously available and are present in a specific ratio." (Dioguardi, Francesco Saverio, MD. p. 638). If these precursor amino acids are not supplied, over time diseases of the connective tissue known as collagen diseases may ensue, afflicting joints, heart vessels, lungs, heart, bones, and skin where collagen is found in high amounts.

"Because of its tensile strength, this extracellular protein bears structural stresses either as the connective tissues of any parenchymal organ (such as liver, kidneys, intestines, lungs, and heart) or as the protein matrix of the skeleton and its related structures (bone, teeth, cartilage, tendons, and ligaments)..." (Dioguardi, Francesco Saverio, MD. p. 636).

COLLAGEN DISEASES

Evidence continues to emerge validating the invaluable nature of NEAAs, which further denotes the importance of procuring these compounds by virtue of the diet. If any of the connective tissue-forming precursors are present in quantities lower than physiological measures – resulting disease of the connective tissues, or "collagen diseases" (e.g., osteoporosis and osteoarthritis), may ensue. These diseases may impact any region abundant in connective tissue, including arteries, tendons, skin, bones, gut, around the organs, eyes, and the lungs, which results in an extensive list of associated ailments. Because connective tissue proteins significantly decrease with age – individuals over the age of 50 are notably at risk. To avoid the onset of such maladies, and to promote the health of connective tissues overall, consumption of collagenous-rich food sources will provide augmented supplies of the non- and conditionally essential amino acids in quantities proportional to those outlined in Table 3.

With such a high percentage of connective tissue proteins in the body, it may become difficult for the body to supply all of the non-essential (or conditionally essential) amino acids necessary for the maintenance and synthesis of these connective tissues. If the needs are not met, over time, diseases of the connective tissue often referred to as "collagen diseases" may ensue. These diseases may occur in any tissues of the body that contain high amounts of connective proteins. This leads to a long list of tissues that may be susceptible to such diseases. Therefore, the incorporation of extra non-essential/conditionally essential amino acids in the diet ensures that adequate turnover and synthesis of connective tissues will take place, keeping these tissues healthy and free from many of the diseases that plague our society today.

Surprisingly, these tissues are not largely composed of those amino acids considered essential. A majority of the amino acids making up collagen and elastin include the non-essential (or at times conditionally essential) amino acids. Elastin contains very high amounts of non-essential/conditionally essential glycine and proline similar to collagen,

which contains considerable amounts of glycine and proline in addition to arginine, and the unusual amino acid hydroxyproline. Thus, all of these amino acids are abundant in arteries, ligaments, lung, tendon, skin, bone, gut, around organs, eyes, and even in the little crevices between the circular shaped bones forming the spinal cord.

Both of these tissues' synthesis and integrity are contingent upon ample stores of these amino acids (and their precursors) that can be synthesized in the body to a certain extent. In the case of collagen, its renewal is a significant part of the daily protein turnover, often requiring super normal protein intakes to ensure that the turnover is able to occur, and the integrity of the collagen and the tissue it is within are maintained. This is particularly important in your lungs, tissues surrounding your teeth, and your bones, where collagen turnover is the highest.

When these amino acids (or their precursors) are not available in sufficient amounts, the integrity of the tissue will diminish over time, ultimately leading to disease. Diseases of the connective tissue known as 'collagen diseases' may ensue, afflicting joints, heart vessels, lungs, heart, bones, and skin where collagen is found in high amounts. In order to avoid such diseases and promote the health of the collagenous tissues, it is possible to consume collagen in the form of traditional broths made from slowly boiling down collagenous tissues, or in the form of gelatin pills that are simply hydrolyzed collagen. Both of these options would contain large amounts of conditionally essential/non-essential amino acids proline, hydroxyproline, glycine, glutamine, and arginine, which are all important in driving the synthesis of collagen. Hydrolyzed collagen has a glycine and proline concentration that is as much as 20 times higher than other food sources of protein.

HEART

Collagen and elastin may form up to 60% of the dry weight of aortic tissue...the rest may be largely smooth muscle. (Cattell, M. Anne et. al. p. 81).

"total collagen & elastin in the tissue decrease [with age, particularly after the age of 50]" (Cattell, M. Anne et. al. p. 83)… a very significant loss of wall extracellular matrix structure with aging process... in turnover or remodeling, degradation must predominate over synthesis." (Cattell, M. Anne et. al. p. 83).

LUNGS

"Because ventilation imposes mechanical stress, the lungs are perhaps more dependent on connective tissue proteins for the maintenance of normal structure than other vital organs." (Ryan, Patricia A.)

"TYPE I COLLAGEN is a major structural protein in the lung interstitium (the tissue and space around the air sacs of the lungs).... and is the most abundant collagen in pulmonary tissues." (Goldstein, R.H. p. L29).

"The amount of collagen decreases in the lungs of rats fed a protein-deficient diet in the neonatal period." (Kucharz, Eugene J.).

BONE

"Proteins in bone include primarily collagen (about 85-90% of proteins)..." (Gropper, Sareen S. p. 436).

SKIN

"Glycine-proline content is 22% (1/8 of body's total protein)...collagen makes up for 70% of the total N content of skin proteins." (Dioguardi, Francesco Saverio, M.D., 2008).

AGING

"The cross-links are non-reducible and increase in number with age...cross-links between collagen fibers provide strength and stability to the muscle..." (Eklund, Krystin p. 39).

"Skeletal muscle ECM becomes stiffer with age. Collagen is the most abundant extracellular fibrous protein... It [collagen] allows penetration of nerves and blood flow to all of the parts of the muscle." (Eklund, Krystin p. 38).

"Aging is associated with significant changes in the connective tissue compartment of skeletal muscle. An increase in both concentrations of collagen and the extent of nonreducible cross-linking occurs with aging in both skeletal muscle and heart. The age-related changes are thought to be the result of decreased collagen turnover rates. The biochemical changes of collagen have been correlated with changes in muscle stiffness, i.e. stress/strain, such that stiffness increases in muscles of old animals." (Gosselin, Luc E. et. al.).

EXERCISE

"both muscle stiffness & the concentration of the mature collagen cross-link hydroxyproline increases in muscle of aging rats. However, regular exercise training can alter the passive mechanical properties of senescent (aging) soleus (calf) muscle, and this change is associated with concomitant changes in collagen cross-linking." (Gosselin, Luc. E. et. al.).

"...it appears that exercise acts as a positive regulatory stimulus on muscle ECM (extracellular matrix) synthesis...physical exercise seems to decrease the level of these cross-links in old animals." (Kragstrup, T.W. et. al.).

"Collagen provides a scaffold for maintaining muscle-tendon integrity...as the muscle is stretched beyond L_0 (optimal length), the elastic properties of collagen are responsible in part for the passive force developed...several factors related to collagen may contribute to the passive viscoelastic properties of skeletal muscle..." (Gosselin, Luc E. et. al.).

"skeletal muscle collagen proteins increase their synthesis rate acutely (by two fold) after exercise." (Holm, Lars et. al.).

"Part of collagen's toughness arises from cross-links between lysine residues of adjacent chains. This cross linking reaction occurs throughout life and makes bones, skin, and tendons less elastic - properties we associate with aging." (Matthews, van Holde, Ahern).

"Collagen is unusual not only in having modified amino acid residues, such as hydroxyproline and hydroxylysine, but also in having so many of them. Hydroxyproline helps to stabilize the triple helix via hydrogen bonds. Hydroxylysine functions to form attachment sites for polysaccharides. Hydroxylation of proline requires ascorbic acid (vitamin C). Deficiency of vitamin C reduces hydroxyproline production, leading to weakened collagen fibers and the condition known as scurvy." (Matthews, van Holde, Ahern)

While the mechanism behind the regulation of cross-linking in collagen is currently unknown, research up to this point indicates that aging increases the number of crosslinks, exercise decreases them, and nutrition has not yet shown any effects. Thus, with aging, muscles become less flexible and more stiff largely due to an increase in crosslinks within the collagenous tissue. The amount of collagen also increases in aging muscle, also contributing to stiffer muscles. This increase along with the increase in crosslinks is believed to be from a decrease in the turnover of collagen.

Exercise increases collagen turnover, acutely increasing the rate of synthesis of skeletal muscle collagen proteins two-fold. Overall, this appears to decrease the level of these crosslinks in old animals, thus acting as a positive regulatory stimulus on the connective tissue compartment of skeletal muscle.

Contrary to what would be expected, nutritional collagen supplements have not yet shown any effects upon collagen synthesis other than hair, skin, and joints when protein intake is recorded at adequate amounts.

BIOSYNTHESIS

"...collagen synthesis is different because some of its amino acids should be provided in the precursor form to activate the synthetic drive by fibroblasts. Collagen synthesis is, therefore, efficiently maintained only when those specific amino acids are continuously available and are present in a specific ratio." (Dioguardi, Francesco Saverio, MD. p. 638).

"Collagen turnover was long believed to be very slow...[today] it is recognized [that collagen] is a very significant proportion of the whole daily protein turnover." (Melendez - Hevia, Enrique et. al. (2009) p. 854).

"The highest rates of collagen turnover are observed in weight-bearing bones, lungs, and in periodontal tissues. Collagen synthesis is elevated under conditions requiring remodeling and replacement of tissues. Collagen is also synthesized at elevated rates in pathological conditions such as fibrosis in lungs & in the liver." (Talwan, G.P. & L.M. Srivastava p. 81).

"Collagen related problems are typically found in large animals, not in small ones. These problems accumulate with age." {small animals can normally synthesize the small amount of collagen they need regardless of age & amino acid availability. Whereas a large animal has such a large amount of collagen to produce that an improper amino acid intake (& increased age) may limit collagen synthesis.} (Melendez-Hevia, Enrique et. al. p. 855).

"Super normal protein intakes are often required for collagen formation, enzyme activity, and cell replication." (Stuart, Paula S. et. al.)

"The amount of collagen, the extent of cross-linking and the architectural organization of the collagen fibrils contribute to the viscoelastic property of skeletal muscle, and allow for the flexibility seen within the tissue." (Eklund, Krystin p. 38-9).

"Little is known, however, about the mechanisms that regulate collagen cross-linking in skeletal muscle." (Gosselin, Luc. E. et. al.).

GELATIN (HYDROLYZED COLLAGEN)

Gelatin is collagen that has been irreversibly hydrolyzed."....[GELATIN amino acid composition] "is closely similar to that of the collagen preparations, suggesting that gelatin is representative of the main protein constituent of collagenous tissues in amino acid composition." (Eastoe, J.E. p. 600). It contains no tryptophan and is deficient in isoleucine, threonine, and methionine.

Several Russian researchers offer the following opinion regarding certain peptides found in gelatin: "gelatin peptides reinforce resistance of the stomach mucous tunic to ethanol and stress action, decreasing the ulcer area by twice."

"Gelatin has also been claimed to promote general joint health. A study at Ball State University sponsored by Nabisco, the former parent company of Knox gelatin, found that gelatin supplementation relieved knee joint pain and stiffness in athletes."

"Oral gelatin consumption has been claimed to have a beneficial therapeutic effect on hair loss in both men and women. In addition, there are scientific publications that present

evidence that consumption of oral gelatin has beneficial effects for some fingernail changes and diseases."

While hydrolyzed collagen does contain all 20 amino acids, the overall amino acid composition of collagen is low in all 9 essential amino acids, and does not meet the current human essential amino acid requirements. According to the PDCAAS system, hydrolyzed collagen scores "0" labeling collagen as having very poor protein quality. As previously discussed, PDCAAS does not take into account non-essential or conditionally essential amino acids, nor does it account for nitrogen contributions of which collagen contains a high proportion of on a gram for gram basis. In addition, collagen has proven to have a high digestibility and absorption, with measurable accretion in blood, cartilage, and skin.

"The bioavailability of hydrolyzed collagen was demonstrated in a 1999 study; mice orally administered C hydrolyzed collagen digested and absorbed more than 90% within 6 hours, with measurable accumulation in cartilage and skin. A 2005 study found hydrolyzed collagen absorbed as small peptides in the blood [of humans!. Iwai, K.et. al. (2005).

Even though the PDCAAS system does not account for them in its evaluation of a protein's quality, the conditionally essential amino acids glycine, proline, and arginine (found in high amounts in connective tissue proteins) are important in the maintenance and renewal of many tissues in the body, and plays an important role in many metabolic processes. While these amino acids may be produced to a degree in the body, limitations upon their synthesis makes acquiring them in the diet vital. Without a dietary intake of these non-essential/conditionally essential amino acids, the body may survive, but may suffer from reduced growth or metabolic imbalances due to a lack of sufficient amounts of these amino acids.

Table 7. Gelatin *vs.* Collagen Hydrolysate

	Gelatin	**Collagen Hydrolysate**
Source	Bones, skin and scales of animals	
Amino Acid Profile	Identical	
Dissolves In	True gelatin only dissolves in hot water	Dissolves in both hot and cold water
How It Reacts to Liquid	Causes liquids to gel	No reaction

Gelatin is derived from collagen, and thus contains the same amino acid pattern as collagen, completely lacking in tryptophan and deficient in isoleucine, threonine, and methionine. Gelatin has a PDCAAS of 0 reflecting the total lack of tryptophan (note the PDCAAS score reflects the amount of the amino acid in lowest concentration). The sulfur containing amino acid cysteine is the 2nd limiting amino acid. Despite the score of "0" assigned to gelatin, that would appear to indicate gelatin's amino acid profile as lacking any nutritional value, gelatin has been found to be beneficial for joints, stomach, ulcers, hair loss, fingernail changes, and diseases.

CARTILAGE

"Cartilage is a form of connective tissue which is chemically abundant in collagen, proteoglycans [i.e.sugar protein molecules], acidic polysaccharides [i.e. long strings of bonded sugar molecules], and water." (P. Kittiphattanabawon et. al.).

Amino acids of collagens from shark cartilages - "All collagens had glycine as the major amino acid and were rich in alanine, proline, and hydroxyproline." (P. Kittiphattanabawon et. al. p. 797).

The connective tissue, cartilage, is largely formed of collagen with some elastin content as well, especially in areas such as the outer ear.

KERATIN

Keratin: Insoluble in hot or cold water = hydrophobic

Keratin's amino acid composition varies depending upon the location in the body. (Strnad, Pavel et. al.).

"Keratins in different vertebrates have similar amino acid sequences as inferred from the observation that epithelial tissues of various species of teleost fishes, amphibians, reptiles, birds, and marsupial & placental mammals cross-react with anti-human keratin antibodies." (Bragulla, Hermann H. & Dominique G. Homberger).

"...the amino acid composition of a particular keratin is remarkably similar in different species. For example, bovine, rat, and human skin keratins are all rich in the amino acids glycine, serine, leucine, and glutamic acid."(Bragulla, Hermann H. & Dominique G. Homberger).

"dominated by glycine, serine, and alanine." (Khan, M.Y. & Sangeeta Saxena).

"Of the amino acids in keratin, cystine (dimer) may account for as much as 24 percent. The numerous disulfide bonds formed by cystine are responsible for the great stability [strength & rigidity] of keratin: it is completely insoluble in hot or cold water and is not attacked by

proteolytic enzymes (the enzymes that cleave protein molecules)." (Encyclopedia Britannica).

"feather keratins also characteristically contain large amounts of the amino acids serine, proline, valine, leucine, glutamate, and aspartate." (Bragulla, Hermann H. & Dominique G. Homberger).
"keratin is nutritionally useless to humans, since it is not hydrolyzed by digestive enzymes." (New World Encyclopedia contributors).

"Keratins contain a high proportion of the smallest of the 20 amino acids, glycine...and alanine." (New World Encyclopedia contributors).

Structural proteins may each vary in their amino acid compositions, but many of them have small amino acids that are able to pack together tightly. "A preponderance of amino acids with small, nonreactive side groups is characteristic of structural proteins, for which H-bonded close packing is more important than chemical specificity." (New World Encyclopedia).

The connective tissue protein keratin, does indeed have a high proportion of the smallest of the 20 amino acids glycine and alanine. Keratin's amino acid composition varies in location within the body, but is remarkably similar among species. Keratin has a fairly even distribution of amino acids, but is especially noted for having appreciable amounts of cysteine (sulfur) amino acids, in addition to fair amounts of glutamic acid, serine, glycine, alanine, and leucine. The high cysteine (sulfur) amino acid content contributes to the strength and rigidity of keratin.

Collagen's tensile strength is partly the result of abundant cross-linking between lysine residues (individual amino acids). The cross-linking continues throughout life, accumulating, making bones, skin, and tendons less and less elastic. Unlike keratin, collagen contains very little if any at all of the sulfur amino acids such as cysteine.

PHYSIOLOGICAL FUNCTION(S)

A key structural material making up the outer layer of human skin and a key structural component of hair and nails.

"For example, bovine, rat and human skin keratins are all rich in the amino acids glycine, serine, leucine and glutamic acid." (Bragulla, Hermann H. & Dominique G. Homberger.).

"Of the amino acids in keratin, cystine (sulfur amino acids; may account for as much as 24%. The numerous disulfide bonds formed by cystine are responsible for the great stability of keratin: it is completely insoluble in hot or cold water and is not attacked by proteolytic enzymes (the enzymes that cleave protein molecules)." (http://www.britannica.com/EBchecked/topic/315321/keratin).

DIETARY SOURCES OF CONNECTIVE TISSUE PROTEINS

TRADITIONAL BONE BROTH & GELATIN

A quintessential example of a substrate rich in connective tissue proteins is bone. While technically an organ, the composition of bone-protein is approximately 85 – 90% collagen-fibers. This distinct composition stresses the importance of consuming traditional bone broth, which is prepared by slowly boiling down collagenous tissues. However, due to the inherent nature of cross-linked collagen present in animal tissue, it dissolves very slowly – even when generously boiled. In general, scleroproteins do not denature as readily as other protein types, such as globular proteins (e.g., albumin in egg whites), and often require a two-step extraction process. A mild chemical treatment will facilitate extraction by cleaving these cross-links – thereby increasing solubility. Organic acids, such as acetic acid, are both commonly, and effectively, employed to carry out this function.

Literature values regarding the ideal concentration and extraction time suggests that an acetic acid concentration of 0.5 M at a temperature of 24.7 C for 32.1 hours results in the highest yield of solubilized collagen. This translates to 108.30 mL (~12 T.) of 5% acetic acid (vinegar is standardized to 5% acetic acid) in one gallon of water. While this concentration may adversely affect overall flavor, effective extractions have also been executed at 0.05 M acetic acid, which corresponds to 10.82 mL (2.20 tsp) per gallon. Ergo, manipulation of acid concentration permits a final product that is subjectively palatable for the consumer. The second step in this extraction process consists of boiling the acid-bone complex at a temperature of 80 °C for six hours, which results in complete denaturation of connective tissue proteins. By coupling both acid and thermal extraction techniques – the final product will resultantly provide an ample supply of collagen-constructing amino acids.

Another recent study found that "gelatin as a feed supplement protected against ethanol-induced mucosal damages in rats." This directly supports the traditional thought that broth is healing and coating to the gastrointestinal lining, and gives a scientific explanation for broth's ability to calm and soothe. Gelatin has also been found to improve body weight as well as bone mineral density in states of protein undernutrition. However, this is likely due to the overall increase in protein status with gelatin supplementation. Additionally, studies have shown that convalescing adults, who have lost weight because of cancer, fare better if gelatin is added to their diet. It is said to be tolerated when almost nothing else can be.

The term "gelatin" is loosely applied to the product obtained by the hydrolysis of collagen, which is the main organic constituent of bones, tendons, cartilage and skin.

The peptides of Pacidic cod skin showed potent non-competitive ACE inhibition (IC_{50} = 35.7 μM) and effectively protects cellular macromolecules from reactive oxygen

species (ROS) mediated damage. The peptide significantly reduced the oxidation levels of membrane lipids, proteins and DNA in RAW264.7 cells by effectively scavenging the intracellular ROS. Moreover, it was found that the peptide treatment upregulated the m-RNA expression of cellular antioxidative enzymes (superoxide dismutase, glutathione and catalase) and thereby enhanced the intracellular antioxidant mechanisms. These findings suggest that Pacific cod skin could be effectively converted to produce a bioactive peptide, which could be used as a functional food ingredient to control ACE activity and oxidative stress.

Other applications of gelatin are compositions consisting essentially of sugar, gelatin and citric or tartaric acid, with added flavoring and coloring; they contain only about 10% gelatin and about 87% sugar. As pointed out in the "Queries" section of the Journal of the American Medical Association, these desserts are frequently confused with pure unflavored gelatin and such confusion extends to medical literature.
In the years 1829-1838, one establishment alone served 2 3/4 million portions of gelatin bouillon. During this time, for reasons of economy, attempts were made to utilize gelatin at every meal and to make it replace all foods until the public became literally "fed up" on it.

When people tired of the monotony of a daily gelatin diet, the pendulum swung completely to the other extreme. Many factors aided this change of sentiment besides overuse and simple exaggeration of its virtues. In the first place each institution generally manufactured gelatin for its individual requirements and such production was by no means scientifically controlled. Carelessness in processing and in cleaning of equipment in many of the establishments resulted in a gelatin product which was badly decomposed and highly odorous so that the public soon began to object to the dishes prepared with the product and refused them. Likewise the particular virtues of the gelatin had been destroyed by the faulty processing.

"Gelatin chemically supplements the protein mixture of both the wheat kernel and oat kernel respectively."

"So small an addition as 3.5 percent of gelatin, because of its high lysine content, greatly increases the utilizability of the wheat proteins. Without this addition growth takes place at a rate approximating half the normal expectation. With the gelatin addition growth proceeds at the normal rate."

Glycosaminoglycans
There are other compounds in broth that gel besides collagen. The ground substance of cartilage is made of proteoglycans, huge sugar and protein molecules. Attached to a core protein are long strands of glycosaminoglycans (GAGs) also called mucopolysaccharides. These structures are naturally jellylike. As mentioned, the GAGs in cartilage are hyaluronic acid, chondroitin sulfate and to a lesser degree, keratin sulfate. Hyaluronic acid forms a central strand to which chondroitin and keratin sulfate bond.

Chondroitin sulfate

Chondroitin Sulfate is a jellylike substance, now famous as a supplement for joint pain associated with osteoarthritis. It functions to support and provide adhesiveness. It lines blood vessels and plays a role in lowering atherosclerosis, cholesterol and heart attacks.

Minerals are essential to life but they are not easy to digest. In the stomach, the presence of hydrochloric acid is necessary to physically break down our food, but also to extract elemental minerals from the food that we've eaten. A similar reaction takes place in the making of broth. An acid is necessary to remove the minerals from the bone. This is the purpose of using vinegar (acetic acid) when making broth. As stated in The Principles of Anatomy and Physiology, "If inorganic minerals are removed by soaking bone in a weak acid such as vinegar, it results in a rubbery, flexible structure." This rubbery flexible structure is the leftover collagen/gelatin. The chemical reaction that extracts the minerals is an acid base reaction, in which the vinegar is the acid, and the minerals are the base.

Bone contains calcium and phosphorus, and to a lesser degree, magnesium, sodium, potassium, sulfate and fluoride. Bone is an excellent source of minerals. All of the minerals present in bone, except fluoride, are macrominerals, which are essential for proper nutrition and are required in greater amounts than 100mg/day. The only macromineral not present in bone is chlorine. Minerals have numerous functions in the body beyond the composition of bone, which is why the body will rob the bones and tissues to maintain steady levels of minerals in the blood and other fluids.

Generally there are two ways this can happen, lack of intake in the diet, or lack of absorption in the intestines. Broth can be an excellent remedy for both of these causes of mineral deficiency because it provides easily absorbed extracted minerals, plus promotes healing of the intestinal tract. Unlike vitamins, minerals do not have defining deficiency diseases, but rather a collection of associated deficiency signs, symptoms and diseases. Interestingly, many of the deficiency symptoms of minerals are mood and behavior disturbances. This offers a scientific explanation for broth's ability to soothe and stabilize. It is reasonable to assume that previous to the development of pharmaceutical mineral supplements, bone broth was an important supply of minerals, especially in the winter when fresh fruit and vegetables are less available, and warm food is preferred.

The chemical ingredients extracted from broth are glycine and proline (collagen/ gelatin), calcium and phosphorus (minerals), hyaluronic acid and chondroitin sulfate (GAGs), and other minerals, amino acids and GAGs in smaller amounts.

Broth recipes stress the quality that can be obtained from using highly cartilaginous parts of animals. These parts will be joint areas, like chicken feet and beef knuckles, trachea and ribs, or anatomy with a concentration of glycosaminoglycans, like hooves and skin.

Its colloidal properties aid the digestion of any foods which cause the patient to suffer from 'sour stomach.' Even foods to which individuals may be definitely sensitive, as proven by

the leukopenic index and elimination diets, frequently may be tolerated with slight discomfort or none at all if gelatin is made part of the diet.

"Some of the medical communities in other parts of the world value gelatin too. In Chinese herbal medicine, gelatin is an important herbal remedy, in use for thousands of years. Its Chinese name is e jiao. It is classified as a tonic herb. Tonics strengthen or supplement insufficiency and weakness. They are considered nourishing and enhance the body's resistance to disease. They are used for states of deficiency. Gelatin is used to tonify the blood, in particular. This correlates to Western medical knowledge since, as we will see, glycine, a key ingredient in gelatin, plays a vital role in the blood. Also if gelatin is extracted from bone, then marrow, where blood cells are produced is also extracted. Chinese studies have shown gelatin to increase red blood cell and hemoglobin count, increase serum calcium level, increase the absorption and utilization of calcium, and prevent and treat myotonia atrophica (muscle wasting)." (Bensky, D, Chinese Herbal Medicine Materia Medica, Seattle, WA, Eastland Press Inc., 1993, p.332.)

A University of Michigan-led study shows that the fat tissue in bone marrow is a significant source of the hormone adiponectin, which helps maintain insulin sensitivity, break down fat, and has been linked to decreased risk of cardiovascular disease, diabetes, and obesity-associated cancers. The findings appear in today's online-ahead-of-print issue of *Cell Metabolism*. Bone marrow adipose tissue has primarily been associated with negative health effects, most notably because of a documented relationship to reduced bone mass and increased risks of fractures and osteoporosis. The new study however -- which included people with anorexia, patients undergoing chemotherapy, rabbits and mice -- suggests that this type of fat may also have benefits.

COLLAGEN HYDROLYSATE

Another potent source of these collagen-constructing amino acids is collagen hydrolysate (CH). Collagen hydrolysate possesses a glycine and proline concentration that is approximately 20X greater than other common high-protein food sources. Collagen hydrolysate and gelatin are fundamentally tantamount in primary amino acid sequence – with the exception being that the former is soluble in cold, hot, and brine water. A result of this solubility is reflected by the high degree of accretion; more than 90% of collagen hydrolysate is digested and absorbed within six hours of ingestion and preferentially accumulates in cartilage and bone. Research reveals a wide array of beneficial attributes associated with the supplementation of peptides present in hydrolyzed collagen. These benefits range from increasing bone mass density – consequently improving joint pain associated with osteoarthritis and osteoporosis, to exhibiting both anti-hypertensive and cardio-protective activities. More specifically, the CH-derived dipeptide, Pro-Hyp, has demonstrated effect on stimulating cell proliferation, increasing synthesis of hyaluronic acid, accelerating cell migration in the skin, and altering gene expression profiles in the skin. There is unanimity amongst this research that suggests these benefits are a result of hydrolysis, which incorporates physical, mechanical, and biochemical means of

denaturation. The result of this extensive process is a reduction in molecule size by up to 600-fold (i.e., 300,000 Da to 500 – 25,000 Da), which greatly aids in absorption. The recommended daily intake of collagen hydrolysate is between 8 – 12 grams, which can efficiently be added to bone broth to formulate a concentrated collagenous concoction.

Additional evidence suggests gelatin's ability to improve the digestion of milk. In the early 1900s gelatin was recommended as an ingredient in infant formula, to decrease allergic reactions, colic and respiratory ailments. Gelatin was also reported to increase the digestibility of beans and meat (which gives credence to the practice of serving meat with gravy). It was also found that gelatin increased the utilization of the protein in wheat, oats and barley, all gluten containing grains. Gluten is a notoriously difficult to digest protein for many people. Those that suffer from gluten allergy are diagnosed with Celiac disease, a debilitating condition.

"Furthermore, CTP (collagen tripeptide) was incorporated into tissues including skin, bone, and joint tissue. Thus, administering collagen as tripeptides enables efficient absorption of tripeptides and dipeptides."

Some of its benefits may not be associated with these three amino acids: "Hydroxyproline serves as a precursor for glycine synthesis, is an antioxidant in the body, synthesizes hyaluronic acid, functions as an ACE-inhibitor, etc. Thus, hydroxyproline possesses many benefits outside of directly synthesizing collagen." (Bruyere et al. 2012) This study by Bruyere et al. conducted a placebo controlled/double-blind study on 200 participants for a duration of six months. It found that there was an absence of adverse-effects in addition to elucidating the efficacy of the CH treatment ("We conclude that in this 6-month randomized placebo controlled study, CH is able to increase the proportion of clinical responders, as defined by an improvement of at least 20% in the VAS score, compared to patients receiving placebo"). Another interesting aspect of this study was that they found the benefits of CH supplementation began to be observed after three months of ingestion. Thus, strengthening the safety of long-term consumption in addition to the need to consume these peptides on a regular basis to garner their benefits.

BIOLOGICAL VALUE OF COLLAGEN HYDROLYSATE

CH is approved in Canada to improve joint pain:

- **1.2 – 10 g/day**
- **Recommended to use for a minimum of 5 months**
- **Source of the essential amino acids histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, valine for the maintenance of good health and involved in protein synthesis (CNF 2010; Eastoe 1955).**
- **Source of the non-essential amino acids alanine, arginine, aspartic acid, glutamic acid, glycine, proline, serine, tyrosine involved in protein synthesis (CNF 2010; Eastoe 1955).**

- **Source of the essential amino acid lysine to help in collagen formation (derMarderosian and Beutler 2011; Baziwane and He 2003; Garrison and Somer 1995; Jansen 1962).**
- **Helps to reduce joint pain associated with osteoarthritis (Bruyère et al. 2012; Benito-Ruiz et al. 2009; Clark et al. 2008).**

Other sources of connective tissues consist of offal (e.g., pig aorta and beef tripe), mollusks (e.g., squid and octopus), gastropods (e.g., abalone), and crustaceans (e.g., shrimp). The marine sources are unique in that they also supply high concentrations of essential amino acids. This is illustrated by the rich content of tryptophan contained in shrimp and squid meat, which is not observed in mammalian connective tissue proteins. While food that provides a direct source of connective tissue intuitively supplies the constituents necessary for their corresponding synthesis, both animal and plant sources absent in connective tissues contain scleroprotein-forming amino acids, their precursors, and co-factors, which directly participate in protein synthesis. In general, foods of both animal and plant origin containing the collagen-building amino acids will contribute to overall scleroprotein synthesis. Non-proteinaceous co-factors, for instance, vitamins, are of principal importance for protein synthesis in addition to the amino acids that directly participate in anabolism. Both vitamin(s) B- and C and required for proper cross-linking of collagen, specifically for the hydroxylation of proline to hydroxyproline, which signifies the importance of maintaining a balanced diet containing an array of both macro- and micronutrients. By coupling what are considered to be "high-quality proteins" by contemporary standards (i.e., PDCAAS), such as egg white, milk, meat, and soy with "low-quality proteins" such as traditionally made bone broth, hydrolyzed collagen, and organ meat – one can obtain the full spectrum of amino acids by accounting for any proteinaceous lapses to ensure maximum longevity.

PROTEIN TURNOVER

"The process by which body protein is continually degraded and resynthesizes is called protein turnover, a term that has been used collectively to include both protein synthesis and degradation. In addition to the exchange of amino acids into and out of protein, amino acids also are irreversibly lost through degradative pathways. For most adults who are in protein balance, the amount of amino acids degraded is equivalent to the amount in the diet… Degradation involves the removal of nitrogen, primarily as urea and ammonia, and the degradation of the carbon skeleton. The end result of the degradation of the carbon skeleton of amino acids is the provision of energy either directly or through the formation of compounds such as glucose and fatty acids, which can then be stored or metabolized to provide energy. The needs of the body regulate which of the possible pathways predominates; that is, when amino acids are used for the synthesis of protein, when they are oxidized for energy, and when they are used to form glucose." (Stipanuk, M.H. p. 213).

"…the rate of turnover of proteins in the body spans a broad range and that the rate of turnover of individual proteins tends to follow their function in the body; that is, those

proteins whose concentrations need to be regulated (e.g. enzymes) or that act as signals (e.g. peptide hormones) have relatively high rates of synthesis and degradation as a means of regulating concentrations. Conversely, structural proteins such as collagen and myofibril proteins or secreted plasma proteins have relatively long lifetimes." (Shils, Maurice E.; Shike, Moshe et. al. p. 35).

"While most proteins are degraded within 24 hours of being synthesized, some proteins are short-lived and exist for only minutes. While some proteins exist for months and even years, others are degraded within minutes. Gene ontology terms describing signaling functions are highly enriched among short-lived proteins, suggesting that rapid turnover is required for proper signal transduction. Indeed, defects in protein turnover are implicated in the pathogenesis of cancer and other types of human disease." (Loriaux, PM; Hoffmann, A.).

"Proteins are much larger and much more complex than carbohydrates and fats, with intricate, distinctive three-dimensional shapes. Protein makes up most of the weight of the human body that is not water. The body cannot store nitrogen or amino acids in the way it can store carbohydrates and fat." (Weil, Andrew p. 43).

What are the advantages and disadvantages of this highly inefficient protein turnover system that uses energy to cycle proteins and amino acids? Obviously, this system is inefficient with respect to energy, because energy is needed both for the degradation of protein and for the synthesis of new protein. Estimates of the energy cost of protein turnover suggest that 15% of basal energy expenditure is associated with the turnover of protein (Reeds and Garlick, 1984). However, substantial advantages also are conferred by the dynamism. Protein turnover provides benefits in the capacity to adapt to infection, at the expense of the loss of some body protein and the expenditure of energy.

Individual proteins vary in their rate of turnover, and the higher rates of turnover are found in regulatory proteins. The higher rates of turnover in these proteins allow for more rapid adaptation in the levels of regulatory proteins in response to changing conditions than is seen in proteins with much longer turnover times.

At the level of individual tissues, higher turnover rates are found in tissues that respond rapidly to changes in the environment (stomach, esophagus, liver etc.). The high rates of protein synthesis in tissues like stomach and esophagus reflect both the secretory function and the rapid replacement of cells of the gastric and esophageal mucosa. The liver also has a relatively high rate of turnover, which facilitates adaptation to changes such as alterations in nutrient intake. By contrast, the rate of protein synthesis is relatively slower in muscle tissue, and the response of this tissue to altered conditions (e.g. work induced hypertrophy) occurs more slowly.

"In response to altered demands or alterations in the environment, tissues can respond both by altering the overall rates of protein synthesis and degradation and by changing the spectrum of individual proteins being made. This adaptation allows the body to meet continually changing demands such as those associated with growth and development,

health, illness, pregnancy, and lactation. Protein turnover is, therefore, a 'substrate cycle' (or futile cycle); that is, there is continual synthesis and degradation, which requires energy but accomplishes no net change in the amount of protein. The benefit is that protein turnover provides the capacity for rapid adaptation when needed." (Stipanuk, M.H. p. 219).

Various forms of damage to proteins is one factor, but there are several other factors. The main reason for the high protein turnover is the continuous need for new proteins to perform new functions, and the destruction of proteins whose function is finished.

The degradation of different types of proteins ranges anywhere from a few seconds (i.e. enzymes) to minutes, to days, months, and even years. Degradation occurs when proteins are made incorrectly due to a genetic mutation resulting in a faulty protein, when errors in translation from the genetic code to protein form, or when improper folding of a protein into its 3-D shape occurs. Over time, as proteins age, chemical damage is inevitable, such as the addition of carbohydrates or lipids onto proteins, and the loss of amide (the nitrogen containing portion) of the amino acid. Oxidation, which can alter the bonds that hold a protein together, can also result in degradation. Although, the rate of oxidative damage to proteins is generally very slow so that oxidative damage is probably a minor determinant of the overall rates of protein degradation and renewal. Proteins that are damaged are certainly degraded as quickly and efficiently as possible, as damaged proteins can cause all sorts of havoc to a cell that can be hard to repair. The improper disposal of faulty proteins is implicated in the development and progression of several diseases including cancer, multiple sclerosis, and Alzheimer's, among others.

More importantly, the rate of protein turnover is reflective of the ever-evolving need for new proteins to support cellular function that is in a state of constant flux. As you know, proteins can act as catalysts that speed up reactions, signal molecules, tiny pumps on the membranes of cells, and transporters that allow for the movement of substances in cells and between cells and tissues, as well as the migration of cells themselves. Proteins also function as regulators of cell functions, immune functions, and so much more. Thus, through rapid & continuous protein turnover, the cell is able to tightly regulate and adapt to changing metabolic and developmental needs. Protein turnover is so important that the body is willing to expend significant amounts of energy (~15% of basal energy expenditure) in the degradation and synthesis of new protein.

"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)." (Schutz, Y.).

"During any day an adult makes, and degrades, about 300 g of protein. In contrast, the normal intake of protein from the diet for an affluent, well-fed individual is about one third of that amount, or 100 g. This means that the body not only processes the protein that is taken in but also degrades about three times as much body protein. Thus, about 400 g of protein is broken down to amino acids by digestion and protein degradation." (Stipanuk, M.H.).

"…considerably more protein is mobilized in the body every day than is consumed…the overall turnover of protein in the body is several-fold greater than the input of new dietary amino acids…approximately 340 g of amino acids will enter the free pool daily, of which only 90g will come from dietary amino acids." (Shils, Maurice E.; Shike, Moshe et. al. p. 35).

EFFECT OF EXERCISE ON PROTEIN TURNOVER

A sedentary lifestyle has a profound negative effect on skeletal muscle. For example, a 7-day bed rest in young healthy males can decrease leg muscle mass by 3% and muscle O2 consumption by 4%. Much evidence shows that moderate exercise is beneficial for improving skeletal muscle mass as well as muscle and whole body health, while reducing the risk of metabolic syndrome

Even in the elderly, resistance exercise (e.g., weight-lifting) can enhance skeletal- muscle mass and strength. Indeed, dietary protein and moderate exercise have synergistic effects on skeletal-muscle protein synthesis. Thus, American College of Sports Medicine (ACSM) has recommended strength training for the elderly to sustain muscle mass and function.

Unless sufficient dietary protein is consumed during recovery for increased synthesis of muscle proteins, protein degradation will exceed protein synthesis, resulting in a loss of muscle mass and negative N balance. In support of this view, healthy adults who performed intensive exercise daily (9.9 kcal min^{-1} for 6 of 20 min periods) for 3 weeks and consumed 1 g protein per kg BW per day exhibited negative N balance during each day of the training program.

When the dietary intake of AA and energy is sufficient during a prolonged post-exercise period of recovery, a positive protein balance occurs in the whole body (including skeletal muscle). For example, in adequately fed subjects, the rates of muscle protein synthesis were increased by 112%, 65%, and 34% at 3, 24 and 48 h post exercise, respectively, whereas the rates of muscle protein breakdown were increased by 31% and 18% at 3 and 24 h post exercise, respectively, and returned to resting levels by 48 h.The effective time period for an anabolic response can last for up to 48 h after a single workout.

PROTEIN METABOLISM

The majority of protein digestion (≈ 80%) occurs in the upper small intestine and is caused by the action of the pancreatic and intestinal proteases, while only a small percentage (≈ 10 – 20%) of protein digestion occurs in the stomach due to the action of hydrochloric acid and pepsin. Once ingested, proteins are macerated by the grinding and crushing force(s) of chewing, which further increases the surface area for protease catabolism. This macerated food mixes with saliva to form a bolus. Once swallowed, this bolus enters the stomach, where it comes in contact with hydrochloric acid. The acidic environment of the stomach further denatures proteins in addition to activating the proteolytic enzyme – pepsin. Pepsin cleaves proteins into smaller polypeptide units, which then endeavor into the small intestine, where they are met with a host of pancreatic and intestinal proteases. Trypsin,

which cleaves peptide bonds adjacent to lysine and arginine residues, activates both carboxypeptidases and chymotrypsin (trypsin inhibitors interfere with this process, and thus, prevent proper protein digestion – e.g., soybeans). Carboxypeptidases further cleaves amino acids from the carboxyl terminus in a nonspecific manner, while chymotrypsin specifically cleaves peptide bonds next to phenylalanine, tyrosine, tryptophan, methionine, asparagine, and histidine residues. Elastase and collagenase cleave polypeptides into oligopeptides (i.e., 4 – 9 AA units) and tripeptides (i.e., 3 AA units), which are then further cleaved by intestinal tripeptidases into dipeptides (i.e., 2 AA units) and free amino acids. As a final step of protein catabolism, the remaining dipeptides are degraded by intestinal dipeptidases into free amino acids. Ultimately, polypeptides are broken down into oligopeptides, tripeptides, dipeptides, and free amino acids by pancreatic and intestinal proteases. Without this catabolism of polypeptides – dietary protein does not possess any nutritional value (Wu 2016).

While both di- and tripeptides are abundantly absorbed in the small intestine – there is virtually a complete absence in absorption of peptides longer than four amino acid units. As di- and tripeptides are absorbed by intestinal epithelial cells, they enter the *enterocytes* with the facilitation of sodium-dependent amino acid transporters. Once inside this region, intact di- and tripeptides are digested into free amino acids by cytoplastic peptidases and exported into circulation; only a minute fraction of these intact peptides enters the blood intact. The exception to this axiom being that neonates possess the ability to absorb intact peptides, which is a vital immune-establishing and nutrient-sequestering mechanism. However, this ability is quickly lost after birth in a process termed – *closure*. In this context, while few exceptions exist, dietary proteins are not absorbed in their innate form. Rather, they must first be digested into their basic *monomeric units* for absorption in the small intestine to subsequently transpire.

Once thoroughly catabolized, these ingested amino acids become available for protein turnover, which is the continual making (i.e., anabolism) and breaking down (i.e., catabolism) of protein(s). Because proteins cannot be stored like their macromolecular counterparts (i.e. carbohydrates and lipids; glycogen and adipose tissue, respectively), they must be constantly turned over; some proteins are constantly being synthesized while others are constantly being degraded. It is estimated that the daily rate of protein turnover is equivalent to approximately 300 – 400 grams per day, which accounts for nearly 15% of basal energy expenditure. This figure is largely in excess when compared to an average daily protein intake of 50 – 100 grams per day. Thus, a significantly higher portion of protein is mobilized within the body than is derived from the diet. To illustrate the magnitude of variability within protein turnover rates, enzymes and peptide hormones may possess a half-life of seconds to minutes while structural proteins, such as plasma proteins and collagen, may possess a half-life of months to years. Fundamentally, short-lived proteins are such due to their role in regulatory processes. They are typically located in tissues that respond rapidly to environmental deviations, such as the esophagus, stomach, and liver. In the instance of these examples, the esophagus and stomach are constantly replacing mucosal cells (i.e., esophageal and gastric

mucosa) while the liver must adapt to fluctuations in nutrient intake and detoxification mechanisms. Because these two metabolic pathways oppose one another (i.e., anabolism and catabolism), protein turnover is deemed a *futile cycle*. The metabolic payoff of protein turnover is therefore the capacity for acute and auspicious adaptation.

The process of protein turnover involves a delicate partitioning between catabolism and anabolism. Catabolism encompasses deamination of amino acids – the removal of the nitrogenous portion. Deamination releases ammonia into the bloodstream, which is toxic in high concentrations. Thus, ammonia is consequentially converted into more benign urea by the liver. The kidneys then filter urea, which is highly water-soluble, out of the blood for excretion. The remaining carbon skeleton becomes available for the provision of energy either directly (i.e., TCA and/or oxidative phosphorylation) or through the formation and/or storage of energy-laden compounds (i.e., fatty acids and glucose). The amino acids available in the cell from both dietary and degradative means are referred to as the amino acid pool. The net loss of urea via excretion represents a consistent drain from this endogenous pool. When urea excretion exceeds nitrogen intake – the body is in a state of a negative nitrogen balance, which is typically associated with wasting disease, hyperthyroidism, tissue damage, fasting, and injuries – such as burns. Inversely, when nitrogen intake exceeds urea excretion – the body is in a state of positive nitrogen balance, which is typically associated with periods of growth (e.g., maturation, pregnancy, and lactation), tissue repair, and hypothyroidism. Nitrogen loss is remarkably consistent amongst adults, which is estimated to be 36 and 10 mg/kg/day via urinary and fecal losses, respectively. Furthermore, nitrogen lost through routes including skin, sweat, hair, nails, and respiration is estimated to be 8 mg/kg/day in healthy adults (Wu, 2016).

Although nitrogen balance studies serve as the foundation for amino acid recommendations – this method possesses inherent flaws. These flaws continue to be elucidated and serve as a harbinger for updated methodology.

If the diet supplies more protein than the body is able to utilize, and does not require immediate energy expenditure, glucose is converted to glycogen and/or fat for storage for use at a later time. The process in which glucose is synthesized from proteins, or more specifically – amino acids – is referred to as *gluconeogenesis*. This process involves a biochemical pathway in which utilizes non-carbohydrate-based carbon skeletons, such as glucogenic amino acids (i.e., alanine, arginine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, histidine, methionine, proline, serine, and valine) and the glycerol backbone derived from triglyceride catabolism. Pending the anatomical location of glucose synthesis – specific carbon structures will be preferred. Moreover, the liver prefers alanine, glycerol, and lactate while the kidneys prefer glutamine, glycerol, and lactate.

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

1. Increased oxidation, with terminal end products such as CO_2 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)

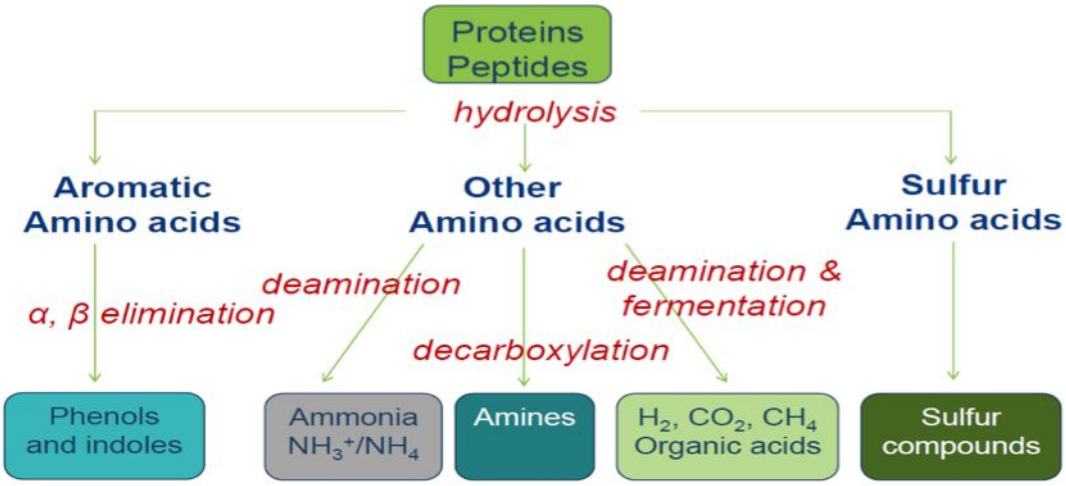
Most of the amino groups of the excess amino acids are converted into urea through the urea cycle, whereas their carbon skeletons are transformed into other intermediates, mostly glucose." (Schutz, Y.).

PROTEIN IMBALANCE

While protein is required for imperative metabolic functions – consuming this macronutrient in improper quantities and/or proportions can pose deleterious consequences. It has been established that the protein ceiling for humans is 35 – 40% of energy requirements. Quantities exceeding this amount may encumber the intestines, liver, and kidneys by providing overwhelming supplies of ammonia. Adverse health effects associated with excessive dietary protein intake may include increased calcium excretion (i.e., osteoporosis risk-factor), hyperaminoacidemia, hyperinsulinemia, dehydration, nausea, seizures, and elevated homocysteine levels (i.e., CVD risk-factor). However, most of these studies are done in patients with kidney disease and lowered glomerular filtration rates.

Essential amino acids, specifically when the ratio of essential to non-essential amino acids is too high, are uniquely prone to both oxidation and toxin formation. Extending this, when one essential amino acid is deficient – the body adapts by degrading all other amino acids present in excess. This incidence is a function of the limiting essential amino acid hindering the utilization of other amino acids implicated in protein synthesis. For example, when soybean meal is fed at levels high enough to satisfy the requirement for the limiting amino acid (i.e., methionine) – the consequential excess of corresponding essential amino acids cannot be used for body tissue synthesis. Thus, they must be delaminated and excreted from the body. This degradation process occurs in a tissue-specific manner, is energetically taxing (i.e., utilizes energy that may otherwise be productively implemented), and may result in toxic metabolite production. Formation of these toxins may be mediated by the gut microbiota (Figure 2). The majority of gut bacteria reside in the proximal colon, which precedes the distal colon (i.e., beginning of large intestine *vs.* end of large intestine), and preferentially metabolize carbon as the primary source of energy. When the diet lacks sufficient *prebiotics* that are resistant to digestion by bacteria in the proximal colon – microbiota in the distal colon will scavenge protein for energy in lieu of carbon – producing deleterious metabolic byproducts in the process, including p-cresol, skatole, and sulfide.

Figure 2. Microbial Metabolism of Proteins and Amino Acids



While this over-consumption can occur solely through dietary means, it is important to note that dietary supplementation of amino acids is ineffective and can also contribute to protein-derived toxin formation. These effects are particularly prominent with branched-chain amino acid (BCAAs) (i.e., leucine, isoleucine, and valine; essential amino acids) supplementation. Although approximately 35% of muscle proteins are comprised of BCAAs, supplementation of these amino acids provides little metabolic fuel, and more importantly, has been implicated in favoring the establishment of metabolic and neurodegenerative diseases, including uncontrolled diabetes and amyotrophic lateral sclerosis (Lou Gehrig's disease). Along with the inability for proteins to be stored, these factors necessitate the need for daily consumption of whole-food protein sources containing both essential and non-essential amino acids in proper proportions and quantities. Intake of protein exceeding metabolic demands is not used efficiently by the body, whether provided by dietary or supplemental means, and may ultimately result in toxin formation.

RECOMMENDED INTAKE OF DIETARY PROTEIN

With the expansive array of misinformation inundating mainstream media in tandem with a general lack of knowledge and proper research methods to ascertain accurate amino acid recommendations – the ambiguity of establishing an optimal diet can quickly become overwhelming. To assist in circumventing this onerous task, it is important to begin by modeling ancestral protein intakes. By doing so, the evolution of human foodways and the conserved aspects of these foodways can facilitate in elucidating protein requirements on a contemporary basis. With this, a comprehensive understanding for proper protein intake(s) may be achieved, and thus, utilized in advising accurate dietary recommendations.

PAST

We would not have progressed as a species without consuming a diet in which provided sufficient sustenance to continuously evolve our genetic makeup. This genetic makeup reflects the hunter-gatherer mode of life, which sustained our genus (i.e., *Homo*) for 99.6% of humanity. While this lifestyle is largely defunct in its pure form in today's epoch – our ability to extrapolate vital evolutionary information remains viable.

Many ethnographic reports of various hunter-gatherer societies showed that virtually all of the edible carcass was consumed. Except for the liver and possibly the kidney and tongue, there is virtually no carbohydrate available for consumption in the carcass of postmortem mammals. For worldwide hunter-gatherers, the most plausible (values not exceeding the mean MRUS) percentages of total energy from the macronutrients would be 19–35% for protein, 22–40% for carbohydrate, and 28–58% for fat. In the United States, the third National Health and Nutrition Survey showed that among adults aged ≥20 y, protein contributed 15.5%, carbohydrate 49.0%, fat 34.0%, and alcohol 3.1% of total energy intake. Dietary protein was estimated to have comprised between 19% and 50% of total energy intake, depending on the plant to animal subsistence ratio and the percentage body fat by weight in the prey animals. However, humans may not tolerate diets that contain >35–40% protein by energy. The avoidance of the physiologic effects of excess protein has been an important factor in shaping the subsistence strategies of hunter-gatherers. Many historical and ethnographic accounts have documented the deleterious health effects that have occurred when humans were forced to rely solely on the fat-depleted lean meat of wild animals. Excess consumption of dietary protein from the lean meats of wild animals leads to a condition referred to by early American explorers as "rabbit starvation," which initially results in nausea, then diarrhea, and then death. For a 12552-kJ (3000 cal) energy intake, the mean maximal dietary protein intake would be 35.1% of energy (range: 29.7–40.9% of energy). Therefore, dietary protein intakes greater than values in this range may result in hyperammonemia and hyperaminoacidemia, which in turn likely cause some of the clinical symptoms responsible for the rabbit starvation syndrome described by explorers. More recently, macronutrient estimates have been updated to 22% fat, 37% protein, and 41% carbohydrate.

One of the most vital factors in the course of this evolution was the consumption of animal proteins. Researchers have estimated the degree of animal protein consumption to be 45 – 65% of total calories energy by diverse hunter-gatherer populations. More specifically, only 14% of hunter-gatherer societies derived >50% of their calories from plant-based sources. Due to the scarcity of vegetation during winter months, early humans adapted by consuming nutrient-dense regions of animals, including organs, marrow, viscera, and fat to subsist during the winter months. These sources of animal proteins were not only essential to survival – they served as a catalyst for physiological advancement.

The consumption of meat, in specific, was – and continues to – serve as an invaluable factor in human growth and development. Early hominin brain expansion, social organization, and geographic movement is largely attributed to the consumption of meat. Moreover, the consumption of meat has been attributed with a height increase of 33% and 37% for females and
males, respectively, from the species transition of H. habilis to H. erectus. Because meat provides key essential micronutrients (e.g., iron, zinc, vitamins A, B1 (thiamine), B2, (riboflavin), B3 (niacin), B6 (pyridoxine), B7 (folate), B12 (cobalamin), K, calcium, etc.), essential fatty acids, and is a quality protein source – it is intuitive that humans would form an affinity with this substrate – given the tools necessary for its efficient acquisition and processing. Although 45 – 60% of calories were derived from animal proteins – hunter-gatherer diets were non-atherogenic (i.e., do not generate arterial plaque). This is discordant with the association between increased meat consumption in Western diets and increased risk of cardiovascular disease. This suggests other factors are responsible for this association as hunter-gather societies were relatively free of symptoms indicative of cardiovascular disease.

An analysis of the Paleolithic diet reveals a protein intake 2 – 3 times greater (19 – 35%) than current rates. Furthermore, ~32% of calories were derived from fat and the remaining ~38% from carbohydrates (Frassetto, et al., 2009). The type of fat and carbohydrates are important to specify as primarily unsaturated fat and no refined grains or sugars (with the exception of seasonal honey) were consumed (O'keefe, et al., 2004). However, with the ever-increasing growth in population, an abrupt agricultural shift ensued *ca* 10 – 12 thousand years ago. This shift has resulted in a narrowing of the diet overall and transition from lean meats to domesticated grains. The archeological findings associated with this foodway transition expounds an increase in morbidity – including increased infection rates, anemia and bone loss in addition to overall dental decline (Larsen and Spencer, 2003). Although the evidence insinuates humans once thrived off of high amounts of dietary protein – this does not imply that these genetic adaptations have been conserved in modern humans. It does, however, imply that humans are well adept at adapting to extreme and diverse diets. More importantly, while human foodways have drastically evolved, it illustrates an outline for modern day protein requirements that is a direct function of our species' ancestral roots.

In the past, those devoid of protein saw surrounding health deficiencies. Most of the initial data regarding vitamin B12 deficiency in infancy are from case studies of infants exclusively breastfed by mothers on vegan, vegetarian, or lacto-ovo vegetarian diets. Several authors have described developmental retardation and "infant tremor syndrome" in 4- to 11-month-old infants of vegetarian mothers from India. In both cases, the most common symptom was anemia, which was present in 56% of the infants of mothers with pernicious anemia and 100% of the infants of vegetarian mothers. Studies examining plasma vitamin B12 concentrations among preschool children and adolescents have suggested that vitamin B12 deficiency early in life compromises children's subsequent growth and development.

"For the Indians living inside the Rocky Mountain Range in the far North of Canada, the successful nutrition for nine months of the year was largely limited to wild game, chiefly

moose and caribou. During the summer months the Indians were able to use growing plants. During the winter some use was made of bark and buds of trees. I found the Indians putting great emphasis upon the eating of the organs of the animals, including the wall of parts of the digestive tract. Much of the muscle meat of the animals was fed to the dogs. It is important that skeletons are rarely found where large game animals have been slaughtered by the Indians of the North. The skeletal remains are found as piles of finely broken bone chips or splinters that have been cracked up to obtain as much as possible of the marrow and nutritive qualities of the bones. These Indians obtain their fat-soluble vitamins and also most of their minerals from the organs of the animals. An important part of the nutrition of the children consisted in various preparations of bone marrow, both as a substitute for milk and as a special dietary ration" (Nutrition and Physical Degeneration, 6th Edition, page 260).

PRESENT

Currently, it is evident that there is ubiquitous protein malnutrition in both undeveloped and developed geographical regions of the world. This is evidenced by the six million deaths per year due to protein inadequacies. This figure translates to approximately one billion people worldwide with protein intakes under biologically imperative quantities (Wu, et al.,2014.) This affliction affects all age groups and demographics. Currently, 165 million children under the age of five suffer from protein malnutrition worldwide. On the opposite end of the spectrum, individuals over the age of 50 begin to lose 0.5 – 1.0% of skeletal muscle mass per year due to sarcopenia. In the United States, 51% of home-bound elderly have a dietary protein intake level below the RDA of 0.8 g/kg/day, which is already insufficient to prevent skeletal-muscle mass (Campbell, et al., 2001). This necessitates the need to modify the existing RDA of dietary protein.

The RDA of protein was formulated via short-term nitrogen balance studies and represents only the minimum daily average dietary intake that meets the nutrient requirements of nearly all (97.5%) of healthy individuals in a particular stage of life (Institute of Medicine 2005). Ergo, because this figure manifested to simply meet minimum nitrogen balance requirements – it should not be used when constructing a diet to optimize or maintain physiology. The shortcomings of this enacted RDA have been substantiated on several accounts, particularly in terms of skeletal muscle loss in the elderly: (I) elderly men consuming a diet providing 0.8 g/kg/day of dietary protein for 14 weeks lost skeletal-muscle mass, (II) individuals (70 – 79 years old) lost the greatest amount of skeletal muscle when consuming <0.8 g/kg/day of protein for a 3 year duration, and (III) consuming a diet enriched with protein levels 25 – 35% above the RDA attenuates age-related muscle loss. These lines of evidence exemplify the importance of consuming dietary protein in levels above the RDA the ensure healthy aging (Campbell, et al., 2001) (Campbell, et al., 2002) (Houston, et al., 2008).

The total rate of protein deficiencies could increase substantially with the corresponding increase in population growth.

As noted previously, the quantity and quality of proteins are determinants of the adequacy of diets to meet human AA requirements. Consumption of animal-based foods (e.g., lean beef) is a simple and effective means to ameliorate the impairment of growth and development in millions of children worldwide. For example, Grillenberger et al. (2003) reported that, in 7-year-old children in Kenya who consumed basal diets (7300 kJ per day) consisting of almost exclusively staple crops (corn and beans) that met energy requirement, isocaloric supplementation (1050–1255 kJ per day) with meat improved growth and cognitive development. Of particular note, supplementation with animal protein increased upper arm muscle area by 80% in Kenyan children, compared with the control group. Similarly, in China, as consumption of animal-source foods increased by 115% between 1990 and 2010, the prevalence of growth stunting in children under 5 years of age decreased from 33% in 1990 to 9.5% in 2010 (Wu et al., 2014). Furthermore, in low-income countries, consumption of milk and other animal-source foods by undernourished children improved anthropometric indices, while reducing morbidity and mortality (Dror and Allen, 2011). Consumption of animal protein as ≥65% of total dietary protein can prevent protein deficiency in elderly subjects (Dasgupta et al., 2005). These findings indicate that plant proteins alone may not be adequate to support maximal growth and development in infants and children or optimize health in adults.

FUTURE

By corroborating the evidence outlined in literature-based models – one can substantiate the ideal contemporary diet to optimize human nutrition. (G. Wu et al., 2014).

An ideal human diet would consist of both animal- and plant- source foods in appropriate amounts and proportions to ensure intake of sufficient quantity and quality of proteins, while consuming adequate dietary fiber. Globally, plant- and animal-based foods contribute ~65% and 35% of protein, respectively, in human diets, and the the opposite is true in North America. While proper combinations of large amounts of legumes with cereals could provide sufficiently most AA, the global availability of legumes as a staple food is increasingly limited and in many parts of the world, these foods are not produced. At best, such combinations may meet protein requirements of adults with minimal physical activity but not for optimal growth or development in children. In home- bound elderly subjects, consuming <65% of total protein from animal-source foods results in the deficiency of at least one EAA, leading to protein undernutrition.Recent Proceedings of the Protein Summit 2, joining more than 60 nutrition scientists, health experts, and nutrition educators, suggest to increase plant but, in particular, animal protein intake because richer in leucine and consequently more effective to influence anabolic protein metabolism. The Panel conclusions are in apparent contradiction with the nutritional ecology statements, which strongly sustain the reduction of animal origin foods in the human diet and are currently concerned about the excessive, mainly animal protein intake in western and westernized Countries.

In view of large variations among people in any age population, caution must be exercised not to adopt "one shoe fits all" guidelines when establishing safe upper limits of dietary protein intake by humans. The results of dietary surveys indicate that protein intake by infants during the complementary feeding period in industri- alized countries is generally 2 to 3 times the RDA. Furthermore, based on the capacity of urea synthesis, Bilsborough and Mann estimated that healthy adults can tolerate a dietary intake of 3.5 g protein per kg BW per day without side effects. This is equivalent to 280 g protein per day for an 80 kg subject. Based on these studies, it appears that well- adapted healthy adults can tolerate a dietary intake of 3.5 g protein per kg BW per day for a prolonged period of time.

The dietary requirements of AA and protein are affected by: (a) dietary factors (e.g., AA and proportions, energy intake, presence or absence of other substances, and food processing); (b) physiological characteristics of subjects (e.g., age, sex, genetic backgrounds, circadian clock, hormones, pregnancy, lactation, and physical activity); (c) pathological states (e.g., infection, trauma, neoplasia, diabetes, obesity, cardiovascular disease, and fetal growth restrictions); and (d) environmental factors (e.g., temperatures, toxic agents, air pollution, dietary habits, sanitation, and personal hygiene). These factors should be taken into consideration in estimating the human requirements for dietary AA.

Our consensus opinion is that leucine, and possibly the other branched-chain amino acids, occupy a position of prominence in stimulating muscle protein synthesis; that protein intakes in the range of 1.3-1.8 g · kg(-1) · day(-1) consumed as 3-4 isonitrogenous meals will maximize muscle protein synthesis. These recommendations may also be dependent on training status: experienced athletes would require less, while more protein should be consumed during periods of high frequency/intensity training. Elevated protein consumption, as high as 1.8-2.0 g · kg(-1) · day(-1) depending on the caloric deficit, may be advantageous in preventing lean mass losses during periods of energy restriction to promote fat loss.

It is noteworthy that skeletal muscle is the major reservoir of AA in the body and undergoes decreases in both mass and physical strength with aging. Several lines of evidence show that the current RDA of protein is insufficient for adult humans with minimum physical activity. First, elderly adults who consumed diets providing 0.8 g protein per kg BW per day for 14 weeks lost skeletal- muscle mass. Second, men and women (70–79 years of age) lost the most amount of skeletal muscle during a 3-year period when they consumed the lowest amount of dietary protein(≤0.8 g protein per kg BW per day). Third, increasing dietary protein intake moderately above the RDA by 25–35% enhanced muscle protein anabolism and reduced the progressive loss of muscle weight in adults with advanced age. Thus, adequate protein intake is highly beneficial for healthy aging. To help older people (>65 years) maintain and regain lean body mass and function, the PROT-AGE study group recommends average daily intake at least in the range of 1.0 to 1.2 g protein per kilogram of body weight per day. Both endurance- and resistance-type exercises are recommended at individualized levels that are safe and tolerated, and higher protein intake (ie, ≥1.2 g/kg body weight/d) is advised for those who are exercising and otherwise active. Most older adults who have acute or chronic diseases need even more dietary protein (ie, 1.2–1.5 g/kg

body weight/d). Older people with severe kidney disease (ie, estimated GFR <30 mL/min/1.73m^2), but who are not on dialysis, are an exception to this rule; these individuals may need to limit protein intake. Protein quality, timing of ingestion, and intake of other nutritional supplements may be relevant, but evidence is not yet sufficient to support specific recommendations.

To meet the functional needs such as promoting skeletal-muscle protein accretion and physical strength, dietary intake of 1.0, 1.3, and 1.6 g protein per kg BW per day is recommended for individuals with minimal, moderate, and intense physical activity, respectively. Chronic high protein intake (>2 g per kg BW per day for adults) may result in digestive, renal, and vascular abnormalities and should be avoided.

Current evidence indicates intakes in the range of at least 1.2 to 1.6 g/(kg·day) of high-quality protein is a more ideal target for achieving optimal health outcomes in adults.

In addition, subjects who have consumed a low-protein diet for a prolonged period of time should not suddenly ingest large amounts of protein due to the reduced expression of hepatic urea-cycle enzymes for ammonia detoxification.

MEAL SPECIFICITY

Dietary protein is assumed to be of high quality (a typical mixture of animal- and plant-source
proteins) with a biological value of 75% (efficiency with which a truly digestible protein is utilized for maintenance and protein deposition in the body). There are reports that consumption of 25 to 30 g high-quality protein (0.333 to 0.40 g per kg BW) and adequate energy in a single meal maximally stimulates skeletal-muscle protein synthesis in the resting 75 kg young adult man. This translates to 75 to 90 g protein for a 25–30 g of protein per meal for 3 meals daily (1.0 to 1.2 g per kg BW per day). Of note, an increase in skeletal-muscle protein synthesis occurs within 1–2 h after consumption of dietary protein or AA and is sustained for 3 h thereafter. A convincing statement by the Panel, at least in our opinion, is the timing of protein intake to be equally distributed at a minimum quantity of 20–30g/meal during the three main courses.

The rate of skeletal-muscle protein synthesis in healthy adults is 25% higher when protein intake is evenly distributed across breakfast, lunch, and dinner, compared with a pattern where most protein is consumed at the evening meal despite the same daily intake of total protein. Timing of protein or AA consumption is important for muscle recovery after exercise. Skeletal muscle takes up nutrients (e.g., AA, glucose and fatty acids) from the blood circulation most efficiently within the first 30–60 min after an exercise program is completed, followed by great reductions several hours later.Thus, the response of muscle protein synthesis to exercise-induced anabolism is much greater when AA intake is initiated immediately after the end of exercise, as compared to 3 h after the end of exercise. There is evidence that the inclusion of high-quality animal protein or combinations of high-quality

plant-based proteins can stimulate muscle anabolism. Recent data also indicate that adequate intake of protein at each meal of the day has an advantage over a large amount of protein in a single meal to support skeletal-muscle mass and function.

AMINO ACIDS AND HEALTH

OVERVIEW

It has been well-established that when one essential amino acid required for protein synthesis is deficient, then all other amino acids are in excess and will be oxidized. If improper ratios of amino acids are ingested, a series of physiological responses ensue that are capable of altering eating behaviors in order to obtain sufficient amino acids. Overeating on low-protein diets or diets deficient in one or more amino acid have been repeatedly observed. Scientists have also concluded that there is the existence of an aversive response to diets deficient in or devoid of protein or deficient in at least one essential amino acid. In addition, the existence of a specific appetite for essential amino acids is strongly suggested based upon experiments with rats who were fed a diet devoid in one essential amino acid and were able to recognize its presence when offered a choice between different diets with or without this essential amino acid.

"The existence of a specific appetite for essential amino acids is strongly suggested, since rats fed a diet devoid in one essential amino acid are able to recognize its presence when offered a choice between different diets with or without this essential amino acid according to different paradigms (1999, 2002, 2001)...In addition, when rats were offered a low protein diet (5 – 8 % energy as protein), they tended to increase their food intake in comparison with a standard protein diet (14 % energy as protein) in order to increase their protein ingestion to a level allowing attainment of protein requirements..." (Tome, Daniel p. S27).

It has been observed that rats typically eat to obtain more than the minimum nitrogen and amino acid requirements. That being said, excesses of certain amino acids can produce toxins. The excess of a specific amino acids can produce a toxic effect associated with the amino acid or its metabolites, leading to an aversive anorexic response. Some results also suggest that a limit of protein intake may occur when these toxic protein wastes reach a level that could cause harm. In the same manner, a low concentration of amino acids in the plasma may signal an inadequate or unbalanced amino acid intake. The level of amino acids in the blood is, like glucose, under close regulation tolerating fluctuations no greater than 20%.

In the end, non-essential amino acids are vital in the maintenance of lung tissue, intestinal barrier function, and the integrity of vascular tissue. They also provide the body with the necessary ingredients it needs to make and maintain the prominent connective tissue proteins collagen and elastin. In addition, accumulating research points to the important role amino acids play in metabolic processes beyond protein synthesis such as with the

synthesis of the eminent antioxidant glutathione that plays a critical role in the body's ability to detoxify oxidative cellular waste products and keep a balanced oxidative state.

Since protein is so vital to the survival and overall health of the body, an organism will change its behavior in order to keep an amino acid balance in both the tissues and the blood. If excesses of essential amino acids have been ingested, particularly without nonessential amino acids, the body is forced into degrading these amino acids costing energy and producing many toxic metabolites along the way. On the other hand, if inadequate or imbalanced amino acid intake occurs, an organism will strive to obtain more protein. Regardless, protein levels in the blood are regulated in the same way as glucose, allowing for little in terms of fluctuations.

Since the body does not store extra amino acids like it stores glucose in the form of glycogen, the daily intake of adequate amounts of amino acids (both essential and non-essential) is critical. "The influence of protein and amino acid on the control of food intake and the specific control of protein and amino acid intakes remains incompletely understood. The most commonly accepted conclusions are: (1) the existence of an aversive response to diets deficient in or devoid of protein or deficient in at least one essential amino acid; (2) the existence of a mechanism that enables attainment of the minimum requirement for N and essential amino acids by increasing intake of a low-protein diet..." (Tome, Daniel).

"When one essential amino acid is deficient for protein synthesis, then all other amino acids are in excess and therefore will be oxidized." (R. Elango et. al. p. 20).

"if protein intake was to be restricted to a single plant source, such as wheat, rice, or legumes (other than soy), then the amount of protein required to meet essential amino acids needs may be increased." (Marsh, Kate A.; Elizabeth A. Munn et. al.).

Typically eat to obtain more than the minimum N & amino acid requirements: "Interestingly, it also seems that the spontaneous level of protein ingestion does not correspond to the minimal % protein as energy (10 – 12 % in the adult rat) required for N balance, but is usually higher and varies according to different factors, including age, the physiological state and the nature of the food." (Tome, Daniel p. S28).

"Some species, such as young cats, are known to survive well on food practically free of nonessential amino acids if the total amounts of amino acids are maintained. This may indicate that the extra essential amino acids simply act as amino donors for the nonessential amino acids through amino-transferase reactions; the carbon skeletons of the essentials being metabolized to oxaloacetate (glycogenic), acetylCoA (ketogenic), or both. However, the degradation pathways for essential amino acids are usually long and complicated; more than 50 extra enzymes would be required for these particular pathways.

Review of Normal Metabolism of Excess Amino Acids:

"...excess amino acids cannot be stored and therefore must be partitioned between incorporation into protein or oxidation." (R. Elango et. al. p. 20).

"...if the ratio of essential amino acids to total nitrogen in a food is too high, essential amino acids will be used as sources of Nitrogen for the nonessential amino acids." (DiPasquale, M.G. p. 149). **My Note: Thus, the body synthesizes the backbone of the nonessential amino acid, and then attaches a Nitrogen group obtained from the essential amino acid.

AA in plasma are regulated in the same way glucose is regulated:
"The level of blood amino acid is, like glucose, under regulation, and the homeostatic mechanism tolerates fluctuations in the blood concentrations of both glucose and amino acids of about 20 %. It is likely that the system responds precisely when protein and essential amino acid intake is inadequate, but allows a large range of adaptive capacities through amino acid degradation and substrate interconversion." (Tome, Daniel p. S29).

Excess Dietary Energy Improves Nitrogen Utilization & Protein Synthesis:
"Amino acids ingested without other energy sources (i.e. fats, carbs) are not efficiently incorporated into protein partly because of the energy lost during amino acid metabolism. Moreover, incorporation of each amino acid molecule into peptides requires three high-energy phosphate bonds. Consequently, excess of dietary energy over basal needs improves the efficiency of nitrogen utilization." (DiPasquale, M.G. p. 169).

Figure 3. Physiological Roles of Amino Acids

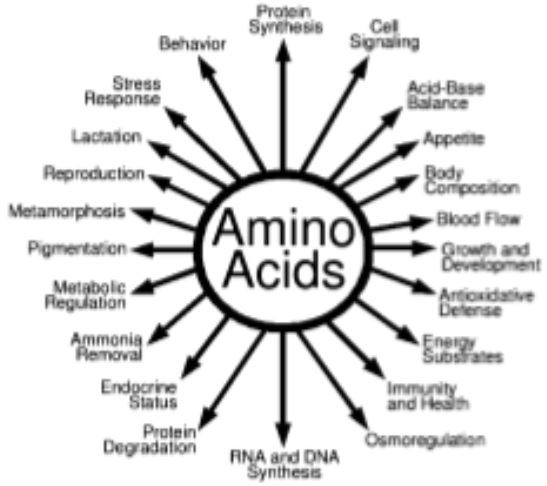


Figure 1 Roles of AA in nutrition and whole-body homeostasis. Besides serving as building blocks for proteins, AA have multiple regulatory functions in cells. These nutrients are crucial for growth, development, and health of animals and humans.

HEALTH EFFECTS SUMMARY

Dietary protein is also inversely related to blood homocysteine concentration , an independent risk factor for CVD. Meat-eating populations have been shown to maintain lower plasma homocysteine concentrations than nonmeat eaters. In numerous population studies, summarized by Obarzanek et al , higher blood pressure has been associated with lower intakes of protein. A 4-wk dietary intervention of hypertensive subjects showed that a high-protein diet (25% energy) was effective in significantly lowering blood pressure. Furthermore, many population studies have established that stroke mortality is inversely related to protein intake.

The thermic effect of food is the energy required for digestion, absorption, and disposal of ingested nutrients. Its magnitude depends on the composition of the food consumed:

- **Carbohydrates: 5 to 15% of the energy consumed**
- **Protein: 20 to 35%**
- **Fats: at most 5 to 15 %**

Because protein has >3 times the thermic effect of either fat or carbohydrate and because it has a greater satiety value than do fat or carbohydrate, increased dietary protein may represent an effective weight-loss strategy for the overweight or obese. Recent clinical trials have shown that calorie-restricted, high-protein diets are more effective than are calorie-restricted, high-carbohydrate diets in promoting and maintaining weight loss in overweight subjects while producing less hunger and more satisfaction.

Thus, protein undernutrition results in stunting, anemia, physical weakness, edema, vascular dysfunction, and impaired immunity.

High-protein intake may lead to increased intraglomerular pressure and glomerular hyperfiltration. This can cause damage to glomerular structure leading to or aggravating chronic kidney disease (CKD). Hence, a low-protein diet (LPD) of 0.6–0.8 g/kg/day is often recommended for the management of CKD. We reviewed the effect of protein intake on incidence and progression of CKD and the role of LPD in the CKD management. The LPD management in lieu of dialysis therapy can reduce costs, enhance psychological adaptation, and preserve residual renal function upon transition to dialysis. Adherence and adequate protein and energy intake should be ensured to avoid protein-energy wasting.

There is a concern that high protein intake may stimulate urinary excretion of calcium, which may contribute to bone loss and subsequent development of osteopenia and osteoporosis. However, in free-living individuals, high protein intake is likely associated with high calcium intake, and, therefore, may com- pensate for a moderate increase, if any, in urinary excretion of calcium

Based on an extensive and systematic review of the literature, Sahni et al. concluded that dietary protein provided a significant benefit on bone health in humans. Likewise, there is evidence that adequate protein intake increases peak bone mass in both young and older

adults. Thus, protein nutrition plays a key role in skeletal health to reduce risk for osteopenia and osteoporosis.

Recent epidemiological studies suggested that consumption of large quantities of protein (particularly animal protein) could be linked to an increase in risks of cancer and diabetes. Although some epidemiological research revealed a correlation between consumption of animal-based protein (e.g., red meats) and certain diseases (e.g., colon cancer and hypertension), it should be borne in mind that: (1) there is a clear difference between correlation and causation; and (2) the results of epidemiological studies do not establish a role for adequate consumption of animal-source protein (e.g., lean meat) in causing chronic diseases in humans. There are no rigorous long-term clinical trials involving meat-less diets for adults or children. Likewise, there is little evidence in human beings for the carcinogenicity of an adequate intake of animal protein. Lean meat is a major source of high- quality protein for human consumption. Recent studies from large cohorts such as the Nurse's Health Study, the Health Professional Follow-up Study, and the Multiethnic Cohort showed non-significant associations and even inverse associations between consumption of unprocessed red meat and colorectal cancer. Of note, findings from the intervention studies on diet and cancer, such as the Women's Health Initiative and the Polyp Prevention Trial, indicated that a decrease in dietary intake of animal protein (e.g., red meat and processed meat) did not reduce the risk of colo- rectal cancer and/or had no effect on adenoma recurrence in the large bowel.

When protein intake is ≤2 g per kg BW per day, there is little evidence of intestinal, hepatic, renal or cardiovascular dysfunction in healthy people. Furthermore, a diet providing protein intake as 25% of total energy (8.6–9.3 MJ per day) for 6 months does not affect renal function in over- weight and obese subjects with no pre-existing kidney disease.

The average protein intake (e.g., 1.07 g per kg BW per day for young adults) being 15% of dietary energy in the United States is well within the acceptable macronutrient distribution range but well below the recommended intake for most athletes.

Dietary intake of protein (up to 1.7 g protein per kg BW per day) recommended for athletes is well within the acceptable macronutrient distribution range. Based on an extensive review of the literature, Bilsborough and Mann suggested the maximum intake of 2 to 2.5 g protein per kg BW per day for healthy people, totaling 160 to 200 g protein per day for an 80 kg subject consuming 2900 kcal daily. This is equivalent to 25% of dietary energy from protein.

Consumption of animal-based foods (e.g., lean beef) is a simple and effective means to ameliorate the impair- ment of growth and development in millions of children worldwide. For example, Grillenberger et al.reported that, in 7-year-old children in Kenya who consumed basal diets (7300 kJ per day) consisting of almost exclusively staple crops (corn and beans) that met energy requirement, isocaloric supplementation (1050–1255 kJ per day) with meat improved growth and cognitive development. Of particular note, supplementation with animal protein increased upper arm muscle area by 80% in Kenyan children, compared with the control group.

Similarly, in China, as consumption of animal- source foods increased by 115% between 1990 and 2010, the prevalence of growth stunting in children under 5 years of age decreased from 33% in 1990 to 9.5% in 2010. Furthermore, in low-income countries, consumption of milk and other animal- source foods by undernourished children improved anthropo- metric indices, while reducing morbidity and mortality. Con- sumption of animal protein as ≥65% of total dietary protein can prevent protein deficiency in elderly subjects. These findings indicate that plant proteins alone may not be adequate to support maximal growth and development in infants and chil- dren or optimize health in adults.

Dietary protein deficiency not only contributes to poor growth, cardiovascular dysfunction, and high risk of infectious disease, but also exacerbates the deficiency of other nutrients (including vitamin A and iron) and worsens metabolic profiles (e.g., dyslipidemia and hyperglycemia) in humans. This is because of the need for protein to: (a) digest and absorb dietary nutrients by the small intestine; (b) transport nutrients (including long-chain fatty acids, vitamin A, and iron) and other molecules (e.g., cholesterol and triacylglycerols) in blood; and (c) oxidize nutrients (including fatty acids and glucose) to water and CO2. Thus, the deficiency of protein and micro- nutrients (including vitamin A, iron, zinc, and folate) remains a major nutritional problem in poor regions of the world. A severe deficiency of an AA can cause death, as indicated by inadequate provision of arginine in infants.

On top of habitual protein intake (e.g., 1.07 g per kg BW per day in US adults), additional consumption of 0.31 g protein per kg BW per day to achieve an intake of 1.38 g protein per kg BW per day is beneficial for long-term management of BW to minimize white adipose tissue and maximize skeletal-muscle mass. In healthy adults, dietary protein is used to primarily support intracellular synthesis of polypeptides and low molecular weight bioactive substances. Thus, only 8% of dietary protein is used for gluconeogenesis, and the remainder for the maintenance of whole-body protein turnover and tissue-specific AA oxidation in humans.

Through metabolites and cell signaling, AA play an important role in regulating the oxidation of fatty acids and glucose in a cell- and tissue-specific manner. For example, enzymes of metabolic pathways are synthesized from AA. Second, the physiological levels of NO (a product of arginine catabolism) enhance the oxidation of fatty acids and glucose to CO_2 and water. Third, the physiological levels of glutathione (formed from cysteine, glycine and glutamate), taurine (a metabolite of methionine), glycine, proline, and hydroxyproline (derived from proline) protect cells and tissues from oxidative injury and inflammation. Fourth, thyroid hormones (derived from tyrosine) are required to maintain adequate rates of basal energy metabolism in humans. Fifth, creatine (formed from arginine, glycine, and methionine) is needed to store energy as phosphocreatine for muscular work and neurological func- tion. Six, carnitine (synthesized from lysine, methionine and serine) is required to transport long-chain fatty acids from the cytoplasm into the mitochondrion for β-oxidation to yield acetyl-CoA. Seventh, serotonin and melatonin (metabolites of tryptophan) inhibit the production of inflammatory cytokines to maintain the health of adipose tissue, while regulating food intake and behavior by humans. Finally, arginine, leucine, glycine, tryptophan and glutamine activate the mTOR signaling pathway to

stimulate skeletal-muscle protein synthesis,thereby partitioning dietary energy from the fat stores into muscle building.

Furthermore, in free-living subjects, adequate consumption of dietary protein can have a satiety effect and, therefore, reduce food or energy intake by inhibiting the release of ghrelin (an appetite-promoting polypeptide) and stimulating the release of peptide YY and glucagon- like peptide 1 (appetite-suppressing polypeptides).

In all the various versions of recommended AA requirements, only EAA are considered and represent only 8–27% of the RDA. This is clearly a limitation, as synthesizable AA are more abundant than EAA in tissues (e.g., skeletal muscle) and can limit protein synthesis in skeletal muscles.

As noted previously, the RDA of a protein was recommended to meet N balance and should not be considered as the optimal amount for maintenance, optimal health or specific functions of organs.

Protein at around 25-30% of calories has been shown to boost metabolism by up to 80 to 100 calories per day, compared to lower protein diets.

30 g total protein at each meal (15 – 18 g EAA and 2.5 g leucine)
Bone health > 1.2 g/kg/day
Satiety/weight control = 25% protein
CVD, blood pressure, etc. = 25% protein

AMINO ACID SPECIFIC EFFECTS ON HOST HEALTH

GLYCINE

OVERVIEW

Indeed, reduced growth has been shown in experimental animals on a low-glycine diet. Thus, while glycine is conventionally classified as a non-essential amino acid, studies in the last 20 years have indicated that glycine availability is insufficient in humans. The amount of glycine calculated to be available from its endogenous synthesis is insufficient to account for the glycine needed for its metabolism as a precursor of other metabolites and, in particular, for sustaining an adequate synthesis of collagen. It appears that glycine production by the body is not able to be increased according to demand. However, when extra amino acids are administered (including glycine), collagen synthesis increases. This response would not be expected if glycine was truly a non-essential amino acid that could be produced in whatever quantity needed for collagen synthesis and renewal, making dietary intake of collagen's amino acid precursors irrelevant to the rate of collagen synthesis. Coming full circle, the fact that a given amino acid can be synthesized during metabolism does not mean that there is enough to sustain all metabolic needs. The small amino acid glycine can be synthesized by the body, but there is accumulating evidence that this amount produced endogenously is insufficient for human needs.

SOURCES & INTAKE

Table 8. Glycine Balance Sheet

Process	Glycine flux	Source of data
Synthesis in metabolism	3 g/day	Table 1
Hydrolysis of dietary proteins	1.5 to 3 g/day	Section 2.1
Synthesis of metabolites	–1.5 g/day	Table 2
Synthesis of collagen	–12 g/day*	Table 3, section 4.3.1
Synthesis of other proteins	–1 g/day	Section 4.3.3
Balance	–8.5 to –10 g/day	

*The value of –12 g/day is derived from table 3, i.e. it assumes that 95% of the glycine released in the procollagen cycle is recycled. If the real recycling rate is lower than 95% the glycine flux needs to be increased accordingly.

(Meléndez-Hevia, Enrique p. 867).

"This first detailed calculation of the supply and consumption of glycine indicates that the capacity of human metabolism to supply glycine is insufficient to account for the glycine needed for metabolism as a precursor of other metabolites and, in particular, for sustaining an adequate synthesis of collagen. This result agrees with the suggestion from numerous nutritional studies over the past 20 years that glycine is a semi-essential amino acid in human nutrition, and that the stoichiometric problem discussed previously is not purely theoretical but has practical consequences for human nutrition, because glycine production cannot be regulated according to demand in the same way as most other biosynthetic routes. The observation of Babraj et al. (2005) that intravenous administration of extra amino acids results in a large and rapid increase in collagen synthesis strongly supports the conclusion [that glycine production by the body is insufficient]...[IF] glycine was truly a non-essential amino acid, the amount available from the diet would be irrelevant...As Christensen (1982) remarked, the fact that a given amino acid can be synthesized during metabolism does not mean that there is enough to sustain all metabolic needs. In fact, in normal adults on either a low-protein diet or a diet limited in the intake of non-essential nitrogen, de novo synthesis of glycine does not satisfy metabolic demand.. " (Meléndez-Hevia, Enrique p. 867-8).

"...the amount of glycine available from synthesis, about 3 grams/day, together with that available from the diet, in the range 1.5 - 3.0 grams/day, may fall significantly short of the amount needed for all metabolic uses, including collagen synthesis by about 10 grams per day for a 70 kg human." (Meléndez-Hevia, Enrique p. 853).

DEFICIENCY

"Collagen...appeared with the origin of animal life in the Precambrian explosion and its history thus spans the same time period as that of animals, around 580 million years. The high content of glycine in collagen (one-third of the amino acid residues) implies an important requirement for the availability of this amino acid to support a healthy turnover of collagen, high enough to avoid problems of accumulation of undesirable chemical modifications (glycation and others), as a protein-deficient diet induces decreased turnover of protein, especially collagen." (Meléndez-Hevia, Enrique p. 853).

"Note that a glycine deficit must affect all uses of glycine, not just collagen production, and an adverse effect on glutathione synthesis, for example, will have deleterious effects on the response to oxidative stress." (Meléndez-Hevia, Enrique p. 868).

"So, even though glycine cannot be regarded as essential for survival, because failure to maintain collagen in a healthy state is not lethal, it is required for adequate synthesis of collagen and for a healthy level of protein turnover. In fact, people with a protein-deficient diet adapt by decreasing protein turnover (Gibson et al. 2002). However, although this adaptation allows survival it has secondary effects, because the increased lifetimes of proteins increase the probability of their undergoing undesirable chemical modifications, such as oxidation, glycation and cross-linking, which can alter their activities. Even though the turnover of collagen may be slow, it is increased in elderly people, which may be explained by the increase in modified collagen which is more susceptible to collagenases. Thus, even though survival is not threatened by a shortage of glycine, the quality of life certainly is....with time the glycation of collagen promotes extra covalent cross-links between chains, reducing plasticity." (Meléndez-Hevia, Enrique p. 868-9).

A deficit in glycine may lead to decreased collagen synthesis and turnover. When protein is not renewed as it should, undesirable chemical modifications such as glycation or oxidation may occur compromising the health of the tissue. It appears that fructose has approximately ten times the glycation activity compared to glucose. Glycine is also a key precursor for one of the most important mammalian antioxidants, glutathione. Thus, a deficit in glycine may limit glutathione synthesis, decreasing the body's ability to respond to oxidative stress, a prominent stressor in our modern-day world (GSH imperative for PII detoxification). Long term shortages of glycine may contribute to the development of such connective tissue diseases as osteoarthritis and osteoporosis, degenerative diseases of old age that plague much of our society. In the end, a shortage of glycine may not directly threaten survival, but the quality of life may certainly be.

"The effects are in the long term, and shortage will contribute to the development of osteoarthritis and osteoporosis, typical degenerative diseases of old age..." (Meléndez-Hevia, Enrique p. 869).... "problems such as osteoarthritis that are related to connective tissues, where collagen and elastin are major components, may be due in part to glycine deficiency." ((Meléndez-Hevia, Enrique & Patricia de Paz-Lugo p. 778).

"In the short term, limited availability of glycine could be buffered by a reduction in its flow to high demand pathways, such as the formation of haem or creatine formation. Over longer

periods, limiting the flow to collagen which could translate to a constraint on linear growth, might be more efficient use of resources."

PHYSIOLOGICAL FUNCTION(S)

"utilization of glycine by the small intestinal mucosa to synthesize GSH (glutathione) is a physiologically important pathway, but the role of glycine as a powerful cytoprotectant (i.e., protector of cells) has only been recently recognized." (W.W. Wang et. al. p. 107-8).

"Glycine...is used extensively in metabolism as a chemical reagent for the synthesis of many compounds, such as glutathione, which plays a major role in combating redox stress. Glycine also participates in the detoxification of benzoic acid, and acts as a neurotransmitter. the food industry adds benzoic acid to many food products as a preservative, therefore causing depletion of the glycine metabolic pool." (Meléndez-Hevia, Enrique & Patricia de Paz-Lugo p. 776) & (Meléndez-Hevia, Enrique p. 868).

"Glycine is needed for glutathione production...Glutathione plays an important role as an antioxidant in many metabolic processes, especially in the red blood cells, red muscle, and heart for the detox of free radicals and toxic oxygen radicals...5-oxoproline, a product of glutathione metabolism, found in urine and supplies a way of measuring glycine deficiency. High amounts were found in the urine of vegetarians and subjects on a low protein diet." (Melendez-Hevia, Enrique et. al. p. 865-8).

"Glycine rectifies vascular dysfunction induced by dietary protein imbalance during pregnancy." (Brawley, L., C. Torrens et. al.).

L-Glutamine & Glycine: can restore nitrogen balance and increase nitric oxide production. Glutamine is a major vehicle for the transport of amino acids such as arginine. It is also needed in the synthesis of glutathione. The glutathione system is major for decreasing cellular oxidative stress and thus may play an important role in ischaemia/reperfusion injury (i.e. restriction of blood flow to tissues followed by tissue damage when blood returns)....Glutamine is a major fuel source for endothelial cells...and a regulator of nitric oxide (NO) biosynthesis in endothelium...may be used to repair cell damage." (Ming Pan, Craig P. Fischer et. al. p. 83).

One researcher addresses the semi-essentiality of glycine in infants, especially in preterm infants, and note that higher glycine intakes may benefit infants in general..."The semi-essentiality of dietary glycine for the growth of preterm infants who have low glycine intake was first demonstrated by Jackson & associates and was suggested to be due to the exceptionally high demands for growth, the relatively small amounts present in human milk, and the immaturity of the enzymes involved in glycine synthesis... In addition, based on studies using [radioactive] N15 glycine, in which almost no labeled urinary urea was identified, infants (especially those are small for gestational age) might benefit from higher dietary intakes."

"In the central nervous system, glycine plays a crucial role as neurotransmitter, thereby controlling intake of food, behavior, and complete body homeostasis. Glycine regulates the immune function, production of superoxide, and synthesis of cytokines by altering the intracellular Ca2+ levels. The conjugation of bile acids in humans and pigs is facilitated by glycine; thereby glycine indirectly plays a crucial role in absorption and digestion of lipid soluble vitamins and lipids. RNA, DNA, creatine, serine, and haem are generated by several pathways which utilize glycine. Collectively, glycine has crucial function in cytoprotection, immune response, growth, development, metabolism, and survival of humans and many other mammals...The biochemical studies on rats proved that glycine is synthesized from threonine (through threonine dehydrogenase pathway), choline (via formation of sarcosine), and serine (through serine hydroxymethyltransferase [SHMT]). Later on, in other investigations it was proved that the glycine synthesis in pigs, humans, and other mammals is through the above mentioned three pathways. From the recent studies it was stated that hydroxyproline and glyoxylate are substrates for glycine synthesis in humans and mammals" (Razak et al., 2017).

"One amino acid for which endogenous formation may be marginal is glycine and we have shown that in preterm infants glycine may be conditionally essential. Glycine may be the first limiting nutrient for growth in infants on low protein diets as weight gain is improved by dietary supplementation." (Jackson, Alan A. p. F152).

"During growth, the demands for glycine are high, both absolutely and relative to other amino acids. The accumulation of glycine as protein in the body of the developed fetus is twice to 10 times that for any other amino acid on a molar basis and glycine is one of two amino acids made in large amounts by the placenta for the fetus." (Jackson, Alan A. p. F152).
"The requirement of a newborn for glycine is high as its availability is linked to every aspect of growth, being consumed directly in the formation of purines and haem, and providing one third of the residues for the formation of collagen and elastin." (Jackson, Alan A. p. F155)

PROLINE

OVERVIEW

Another abundant amino acid in the body that is considered a non-essential amino acid is proline. Nearly all proteins contain proline, and overall, proline is the third most abundant amino acid in the body. Thus, proline has the highest requirement of all amino acids for whole-body protein synthesis. Half of this pool of proline is contained in collagen. "Proline is critical for the production of collagen and reducing the loss of collagen through the aging process. It also helps in the healing of wounds and cartilage; maintaining the integrity of joints, tendons, ligaments; and maintaining and strengthening heart muscle." (Braverman, Eric R. et. al. p. 183). Interestingly, proline also has antioxidant properties, and has an

important role in activating whole-body protein synthesis, outside of acting as a substrate for proteins.

Due to its essential role in so many metabolic and physiological processes in the body, one well-known amino acid researcher, Guoyao Wu states that considering proline as a non-essential amino acid, "... is very unfortunate and reflects a lack of knowledge about proline biochemistry and nutrition in mammals." Wu further explains, "We must recognize that the traditional view that proline is a nonessential amino acid for the adult mammal is solely based on older studies that used methods that are generally considered to be less accurate than other more newly developed methods."

Studies in animals (chickens & pigs) show that supplementing proline in a proline-free diet improved daily growth rate and feed efficiency, increasing overall protein utilization. This draws attention to the possible benefits of ingestion of proline, even though it is considered non-essential. There is more proline in dairy protein than muscle meat. Indeed, proline is the most abundant amino acid in milk proteins. Notably, proline is even more abundant in fish (especially salmon meal), poultry, meat, and bone meals that contain significant amounts of connective tissue proteins from connective tissues included in the meal.

"We must recognize that the traditional view that proline is a nonessential AA for the adult mammal is solely based on nitrogen balance studies. Whether proline is a nutritionally essential AA for animals should be reevaluated through careful design of experiments and use of meaningful criteria (including functional needs such as maximal growth performance, fertility, embryonic/fetal survival and growth, and immunity)... emerging evidence consistently points to proline as an important regulator of cell metabolism and physiology. Therefore, proline can be considered as a functional AA for humans, livestock species, poultry, and fish." (Wu, Guoyao p. 1060).

SOURCES & INTAKE

We must recognize that the traditional view that proline is a nonessential AA for the adult mammal is solely based on nitrogen balance studies. Whether proline is a nutritionally essential AA for animals should be reevaluated through careful design of experiments and use of meaningful criteria (including functional needs such as maximal growth performance, fertility, embryonic/fetal survival and growth, and immunity).

Studies in animals (chickens & pigs) show that supplementing proline in a proline-free diet dose depend on improved daily growth rate and feed efficiency while reducing concentrations of urea in plasma. **[Note: a decrease in urea indicates increased amino acid utilization]. This occurred despite the fact that proline's precursors arginine and glutamine had been supplied in what was deemed an adequate amount. This points to the essentiality of proline, and the possible benefits of ingestion of proline, even though it is considered non-essential.

"Nearly all proteins contain proline. It is a nonessential amino acid and the third most abundant amino acid in the body, exceeded in concentration only by glutamine and alanine [of which are also in significant concentrations in collagen]. Approximately half of the body's total proline content is contained in collagen, which makes up 30% of all protein in the body...collagen is essential for healthy skin, connective tissues, and bone. It [collagen] serves as the major reservoir for proline." (Braverman, Eric R. et. al. p. 183).

"Proline and its metabolite (hydroxyproline) are unique amino acids (AA) both chemically and biochemically. They constitute one-third of AA in the collagen proteins which comprise approximately 30% of body proteins." (Guoyao Wu et. al. p. 1054).

"Proline and hydroxyproline are major AA in the collagen proteins...and are major extracellular components in connective tissues (e.g., skin, tendon, cartilage, vessels of the vascular system, and bone)." (Guoyao Wu et. al. p. 1054).

"On a per gram basis, proline plus hydroxyproline are most abundant in collagen and milk proteins, and requirements of proline for whole-body protein synthesis are the greatest among all amino acids. Therefore, physiological needs for proline are particularly high during the life cycle." (Guoyao Wu et. al. p. 1053).

"There is more proline in dairy protein than in meat protein..." (Braverman, Eric R. et. al. p. 190).

"Proline represents 12% of proteins in the milk of mammals, including sows and cows. Indeed, proline is the most abundant AA in milk proteins....The abundance of proline in milk protein is consistent with a high requirement of proline for neonatal growth and development. Notably, the content of proline is even higher in meat AND bone MEAL, and POULTRY BY-PRODUCT MEAL than in milk. Indeed, proline and hydroxyproline are most abundant in meat and bone meal, poultry by-product meal, and salmon proteins. Therefore, these animal products are excellent sources of proline and hydroxyproline for post-weaning animals and post-hatching birds. In general, animal proteins contain 3 to 6 fold greater amounts of proline than plant proteins per gram of feedstuff. Among plant proteins, proline is the second most abundant AA in barley, wheat, and wheat middlings, and the third most abundant AA in corn (grain) and sorghum." (Wu, Guoyao p. 1058).**

****Note: The salmon proteins referred to in the above quote come from salmon heads, frames, and viscera forming what is known as salmon protein hydrolysate (SPH). In general, SPH was analyzed to be higher in glycine, glutamine, proline, alanine, aspartic acid, and arginine, thus reflecting the general amino acid pattern of tissue higher in connective tissue proteins. (Tucker, J.L. et. al. p. 1467 & 1468).**

Thus, summarizing the last two quotes, proline is higher in milk than in muscle meat proteins, and is even higher in meat & bone meals, poultry by product meals, and fish meals such as salmon...all of which contain tissues of the body high in the connective tissue proteins in which proline is typically found in high concentrations.

"Proline is most effective when adequate vitamin C is supplied at the same time." (Braverman, Eric R. et. al. p. 184). Proline requirements are high for infants who obtain their needs from their mothers' milk that is high in proline formed from arginine.

Concentrations of proline in tissue proteins increases markedly during pre- and postnatal periods, indicating that proline requirements for protein accretion increases substantially ... In the mammary glands of lactating mothers, arginine is actively used to form proline, resulting in an arginine deficiency and an abundance of proline in milk protein relative to the needs of the infants. (H. Vlaardingerbroek et. al.).

PHYSIOLOGICAL FUNCTION(S)

"Proline is critical for the production of collagen and reducing the loss of collagen through the aging process. It also helps in the healing of wounds and cartilage; maintaining the integrity of joints, tendons, ligaments; and maintaining and strengthening heart muscle." (Braverman, Eric R. et. al. p. 183).

"Interestingly, proline can scavenge free radicals, and this antioxidant property of proline may explain why its concentrations increase markedly in response to cellular oxidative stress." (Guoyao Wu. et. al. p. 1055). Proline acts in concert with arginine, glutamine, and leucine (i.e., activators of mTOR and regulators of polyamine production) to enhance protein synthesis in cells and tissues (e.g., the small intestine and skeletal muscle)..." (Guoyao Wu. et. al. p. 1055).

"Furthermore, proline is not considered a nutritionally essential or conditionally essential AA for humans without burns or injury. This is very unfortunate and reflects a lack of knowledge about proline biochemistry and nutrition in mammals." (Guoyao Wu et. al. p. 1054).

ARGININE

OVERVIEW

Arginine is a basic (charged amine side chain) amino acid in bodily fluids. By convention, arginine is considered a conditionally essential amino acid.

SOURCES & INTAKE

Sources of arginine include gelatin, seafood, watermelon juice, nuts, seeds, algae, meats, rice protein concentrate, and soy protein isolate, but is low in the milk of most mammals (including cows, humans, and pigs)." (Guoyao, Wu et. al. "Arginine Metabolism..." p. 153-154).

"L-Arginine (Arg) is a basic amino acid (AA) in physiological fluids. Its content is relatively high in seafood, watermelon juice, nuts, seeds, algae, meats, rice protein concentrate, and soy protein isolate, but low in the milk of most mammals (including cows, humans, and pigs)." (Guoyao, Wu et. al. "Arginine Metabolism..." p. 153-154).

"It - {PDCAAS} - does not, however, provide an indication of other potential attributes of the protein. For example, like other nut proteins, hemp proteins contain a high amount of arginine (94-128 mg/g protein), relative to other food proteins, including whole wheat (48 mg/g protein)." (House, James D. p. 11806).

DEFICIENCY

Holt and Albanese (1944) reported that feeding an Arg-deficient diet to adult men for 9 days decreased both the number and motility of sperm cells by 90%. This striking observation underlines a critical role for Arg in spermatogenesis and argues that functional needs beyond tissue protein synthesis should be important criteria for the classification of AA as nutritionally essential or nonessential. Thus, enhancing Arg provision may improve fertility in males." (Guoyao Wu et. al. p. 157).

"An NO deficiency is a major factor contributing to endothelial dysfunction, which occurs in a variety of metabolic disorders, including diabetes, hypercholesterolemia, hypertension, tobacco smoking, and malaria (Wu and Meininger 2000). Several lines of evidence demonstrate that the Arg administration is effective in reversing endothelial dysfunction under these conditions.... The mechanisms by which Arg administration may prevent cardiovascular dysfunction include: (1) restoring endothelial NO synthesis and decreasing superoxide production; (2) reducing vascular oxidative damage; and (3) inhibiting platelet adherence and aggregation, leukocyte adherence to the endothelium, and the proliferation of vascular smooth muscle cells." (Guoyao Wu et. al. p. 160-161).

"Arg regulates NO synthesis by NOS2, production of antibodies by B-cells, as well as T-cell receptor expression and B-cell development. Thus, Arg plays an important role in both innate and acquired immunity. Inadequate intake of dietary Arg impairs NO synthesis by both constitutive and inducible NOS in mammals, indicating a role for adequate Arg nutrition in immune function." (Guoyao Wu et. al. p. 162).

"Dietary supplements of 0.2 and 0.4% arginine to 7 to 21 day old pigs dose dependently enhances the plasma arginine concentrations, reduces the plasma ammonia level (20 and 35%), and increases weight gain (28 and 66%). These compelling metabolic and growth data demonstrate unequivocally that arginine is insufficient for supporting the maximal growth in milk-fed young pigs and that this arginine deficiency represents a major obstacle to realizing the growth potential in piglets. Arginine is an essential amino acid for the maximal growth of young mammals." (Wu, G. "Arginine Nutrition in Neonatal Pigs.").

PHYSIOLOGICAL FUNCTION(S)

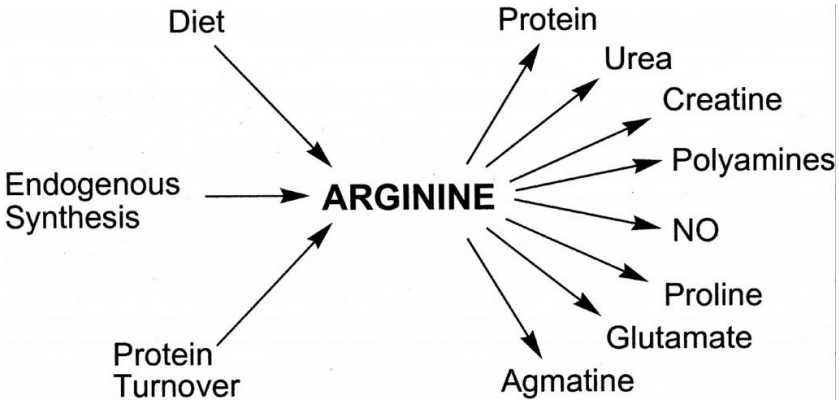
The results of both experimental and clinical studies indicate that arginine is a nutritionally essential amino acid for sperm production, survival of the embryo, fetal and neonatal growth, as well as maintenance of vascular tissue, and the healthy flow of blood. Moreover, a growing body of evidence clearly indicates that dietary supplementation or direct administration of arginine to the blood is beneficial in improving reproductive, cardiovascular, pulmonary, renal, gastrointestinal, liver and immune functions, as well as facilitating wound healing, maintaining tissue integrity, and enhancing insulin sensitivity. Additionally, arginine or L-citrulline may provide novel and effective therapies for obesity, diabetes, and the metabolic syndrome." (Guoyao Wu et. al. "Arginine metabolism...").

A multitude of research has been compiled that points to some specific amino acids critical to vascular health. The first and most prominent amino acid in terms of vascular health is arginine. Arginine promotes a cardio-protective vascular profile via the production of nitric oxide, which induces a variety of physiological processes that ultimately aid in vascular health. Another amino acid, glutamine, acts as a major fuel source for endothelial cells, also helping to regulate the formation of nitric oxide in the endothelium, and may even be used to repair cell damage within the vasculature. Promotes a cardio-protective vascular profile via the production of nitric oxide, which induces vasodilation and decreased platelet aggregation & adhesion, prevention of atherosclerosis, thrombosis & inflammation. The release of nitric oxide contributes to the non adhesive properties of vascular endothelium (Wu, Guoyao) & (Ming Pan, Craig P. Fischer et. al. p. 85). L-arginine is the exclusive precursor of nitric oxide (NO) biosynthesis. (Ming Pan, Craig P. Fischer et. al. p. 81).

Arginine also plays an important role in the intestines where it improves intestinal barrier function. Glutamine has been shown to maintain the intestinal barrier by optimizing gut mucosal repair. Similarly, threonine helps with maintenance of gut barrier integrity by aiding in mucin synthesis, critical for the gut's defense system. In addition, the sulfur amino acids (methionine and cysteine) produce metabolites which play important roles in

the intestinal immune response. Also note, the small intestinal mucosa actually uses amino acids, rather than glucose, as its major fuel.

Figure 4. Arginine's Role in Physiological Processes



Additionally, arginine aides in reproduction (via spermatogenesis & increased embryonic survival):
"On the basis of nitrogen balance, Arg was traditionally not considered as a nutritionally essential AA for healthy adult humans... However, this notion is not supported by studies on fertility in both males and females. Seminal fluid is particularly abundant in polyamines (putrescine, spermidine and spermine), polycationic products of Arg degradation, that are essential for cell growth and differentiation.."

Growing evidence indicates that Arg supplementation may be a novel therapy for obesity and metabolic syndrome. First, dietary supplementation with Arg decreased plasma levels of glucose, homocysteine, fatty acids, and triglycerides, and improved insulin sensitivity in chemically induced diabetic rats, genetically obese Zucker diabetic fatty rats, and diet-induced obese rats. Similar results have been reported for obese humans with type-II diabetes receiving oral or intravenous administration of Arg. Second, L-citrulline or Arg supplementation retarded the progression of high fat diet-induced atherosclerosis in obese rabbits. Third, supplementing conventional diets for growing-finishing pigs with Arg reduced body-fat accretion, enhanced muscle gain, and improved the metabolic profile. A distinct advantage of Arg over drugs (e.g., metformin and thiazolidinediones) is that dietary Arg supplementation reduces adiposity, while improving insulin sensitivity. The possible underlying mechanisms for the effect of Arg may involve multiple NO (nitric oxide) - dependent pathways that favor the whole-body oxidation of fatty acids and glucose." (Guoyao Wu et. al. p. 160). "high concentrations of arginine in plasma enhance nitric oxide availability and improve vascular sensitivity. Thus, dietary supplements with arginine may provide a novel means to treat obesity and the metabolic syndrome in mammals...Arginine &/or its metabolites (nitric oxide & polyamines) may enhance the proliferation, differentiation, and function of brown adipocytes...[possibly leading to a decrease in fat mass]." (Wu, Guoyao).

"Arg depletion and putative reductions in the synthesis of NO, polyamines, and collagen may predispose an individual to delayed gastrointestinal recovery from injury, multi-organ system failure, and endotoxemia. Most studies have demonstrated beneficial effects of Arg on improving gastrointestinal function and gastric ulcer healing, accelerating intestinal mucosal regeneration, enhancing bacterial clearance, and reducing histological bowel necrosis" (Guoyao Wu et. al. p. 161). "Up to 40% of dietary arginine is oxidized in intestinal cells..." (Gropper, Sareen S. p. 196)... "Arginine supplementation is effective in improving intestinal barrier function and vascular development." (W.W. Wang et. al. p. 106).

"Another unique effect of arginine in protein metabolism concerns its marked effect on wound collagen synthesis. Arginine significantly enhances reparative collagen synthesis (as assessed by hydroxyproline content) in rodents and in healthy human volunteers... The exact mechanisms that mediate this response have not been elucidated." (Kirk, Stephen J. et. al.).

"The cardioprotective effects of L-arginine and NO were ascribed to endothelial cell preservation, decreased neutrophil activation, improved coronary blood flow, and a reduction in free-radical-mediated injury...L-arginine conferred a direct and dose-dependent protective effect in isolated heart cells." (Shiono, Noritsugu et. al. p. H805-H815).

"An intact endothelium & adequate nitric oxide production are essential in preventing atherogenesis. In animals and human models, hypercholesterolemia has been shown to inhibit nitric oxide-dependent vasodilation. These effects can be reversed acutely by administering larger doses of the nitric oxide precursor L-arginine and might decrease the development of presclerotic plaques...the long term supplementation of L-arginine reduces intimal thickening and enhances endothelium-dependent acetylcholine-induced relaxation after arterial injury..." (Ming Pan, Craig P. Fischer et. al. p. 85).

Feeding an arginine-deficient diet to adult men for 9 days decreased both the number and motility of sperm cells by 90% despite N balance at equilibrium. This striking observation underlines a critical role for arginine in spermatogenesis. In addition, extensive studies with pregnant dams have shown that dietary arginine is required for the optimal survival and growth of embryos and fetuses.These findings argue strongly that functional needs beyond protein synthesis and N balance should be important criteria for the dietary requirements of AA and proteins.

GLUTATHIONE

OVERVIEW

Glutathione is a powerful antioxidant that exists as a tripeptide consisting of cysteine, glutamate, and glycine.

SOURCES AND INTAKE

The adequate provision of glycine, glutamate, and cysteine (i.e., tripeptide) is critical for the maximization of glutathione synthesis. Both glycine and cysteine have been labeled as possible limiting amino acids.

DEFICIENCY

Inadequate protein or inadequate amino acid precursors may lead to the suppression of glutathione turnover and synthesis possibly leading to a deficiency of glutathione. "A deficiency of glutathione contributes to oxidative stress, which plays a key role in both aging and the pathogenesis of many diseases, including kwashiorkor, seizure, Alzheimer's, Parkinson's, liver disease, cancer, heart attack, stroke, and diabetes. Animal and human studies demonstrate that adequate protein nutrition is crucial for the maintenance of glutathione homeostasis. The supply of dietary protein and cysteine can measurably alter the ability of the organism to maintain glutathione synthesis. There are convincing data to support the view that cysteine is generally the limiting amino acid for glutathione synthesis in humans. However, there are other aspects of dietary amino acid balance that have an important effect on glutathione homeostasis. The adequate provision of glutamate (glutamine or BCAAs leucine, isoleucine, and valine) and glycine (or serine) is also critical for the maximization of glutathione synthesis...glycine can become limiting in the synthesis of glutathione." (Castellanos, V.H. et. al. p. 497).

"...the supply of protein and cysteine can measurably alter the ability of the organism to maintain glutathione synthesis." (Reeds, Peter J. p. 1839S).

"Cellular free cysteine is maintained at low concentrations [due to the incorporation of cysteine] into glutathione, and cysteine is generally considered to be the rate-limiting substrate for the formation of glutathione. However, glycine has been postulated as the limiting amino acid for glutathione synthesis under some circumstances." (Jackson, Alan A.).

"Deficiency of sulfur amino acids or protein content in the diets of healthy humans has been previously shown to result in suppression of GSH turnover in vivo. Further, animals fed diets specifically lacking GSH precursor amino acids, especially cysteine, develop GSH deficiency." (Sekhar, R.V., M.D.)

"The deficiency in cysteine and glycine is intriguing because both of these amino acids are considered to be nonessential, meaning that they can be synthesized endogenously...indeed diabetic patients have reported abnormal overall protein balance." (Sekhar, R.V., M.D.)

PHYSIOLOGICAL FUNCTION(S)

Glutathione is an important antioxidant that protects epithelial cells in the vascular system and in the intestines. In the vascular system, the endothelium relies heavily on glutathione for the detox of the toxic metabolite H_2O_2 (a strong oxidant that when released into the cytoplasm of the cell - fluid part - may cause the cell to die). Glutathione synthesis is particularly disturbed in patients with uncontrolled diabetes that commonly suffer from protein imbalances causing them to be lacking in the appropriate amino acid precursors necessary for glutathione synthesis. Of course, they may also be consuming glutathione at higher rates due to the higher oxidation levels that coincide uncontrolled glucose levels. Nevertheless, adding more of the amino acid precursors of glutathione to the diet of diabetic individuals can restore glutathione synthesis, decreasing oxidative stress and damage. Cysteine, glutamic acid and glycine are the three essential precursors for endogenous glutathione production. Supplementation with NAC, cysteine, and glycine has been shown to increase GSH levels. Vitamin C also raises plasma GSH concentrations.

"There also may be accelerated consumption [i.e. utilization] of GSH in diabetic patients with uncontrolled hyperglycemia." (Sekhar, R. V., M.D.). "It is estimated that up to 55% of diabetic patients in the U.S. do not attain the American Diabetes Association's recommended glycemic goals, and 67% do not attain the more stringent glycemic targets of the American Association of Clinical Endocrinologists...increasing GSH levels with oral precursor supple. may be a viable intervention to target diabetic oxidative stress." (Sekhar, R.V. M.D.).

OVERALL AMINO ACID HEALTH EFFECTS

VASCULAR HEALTH

"Endothelial cell amino acid uptake and metabolism play an important role in regulating and maintaining vascular function. Amino acids are the principal energy sources for endothelial cells and their metabolic products influence many physiologic and pathologic processes. These include vascular tone, coagulation and fibrinolysis, cell growth and differentiation, and immuno-inflammatory responses. The maintenance of normal plasma amino acid levels helps to assure efficient metabolism, which is critical for normal endothelial function. A change in extra-cellular amino acid concentrations or impairment in endothelial amino acid metabolism have been shown to be associated with a variety of diseases -- including hypertension, atherosclerosis, and coagulation disorders. Furthermore, specific amino acid supplements have been shown to prevent atherogenesis, stroke, and to play a role in treating hypertension." (Ming Pan, Craig P. Fischer et. al. p. 79). **Note: vascular endothelium uses glucose, glycogen, and fatty acids as energy sources in addition to amino acids. Glucose can only be used to a certain extent. In excess, glucose will cause damage to the vasculature. (Ming Pan, Craig P. Fischer et. al. p. 80).

Amino acid uptake and metabolism by endothelial cells play a vital role in regulating and maintaining vascular function. Amino acids are the principal energy sources for endothelial

cells and their metabolic products influence many physiological and pathological processes. A change in extra-cellular amino acid concentrations or impairment in endothelial amino acid metabolism have been shown to be associated with a variety of diseases including hypertension, atherosclerosis, and coagulation disorders.

CARDIAC HEALTH

"Diabetes mellitus is associated with an increased rate of cardiac amino acid catabolism that could interfere with cardiac function." (Scognamiglio, Roldano M.D. et. al. p. 1106). It has been shown that hyperglycemia is associated with a significant arterial-venous difference of branched-chain amino acids across myocardium....branched chain amino acids are the principal amino acids taken up by the heart" (Scognamiglio, Roldano M.D. et. al. p. 1106).

INTESTINAL HEALTH

"...amino acids, rather than glucose, are the major fuel for the small intestinal mucosa." (Wu, G. p. 1249).

"The cells of the small intestine become important sites of conditionally indispensable amino acid synthesis, with some amino acids (e.g. glutamine and arginine) becoming nutritionally indispensable (essential) under circumstances of intestinal metabolic dysfunction. However, the quantitative requirement levels for conditionally indispensable amino acids have not been determined and these, presumably, vary greatly according to the specific condition." (Institute of Medicine-IOM p. 594).

"At present [2009],...based on our current knowledge, glutamine, glutamate, arginine, glycine, lysine, threonine, and sulfur-containing amino acids are expected to hold great promise in the management of a wide array of gut-related disorders in both humans and animals." (W. W. Wang et. al. p. 108).

"...dietary glutamine, glutamate, aspartate, and arterial blood glutamine are major fuels for SI mucosa and are responsible for providing energy required for intestinal ATP dependent metabolic processes such as active nutrient transport and high rates of intracellular protein turnover. Second, ornithine (a product of arginine, glutamine, and proline) is the immediate precursor for polyamine synthesis, which is essential to proliferation, differentiation, and repair of intestinal epithelial cells. Third, arginine is the physiological precursor of nitric oxide, which plays an important role in regulating intestinal blood flow, integrity, secretion, and epithelial cell migration. Fourth, glutamate, glycine, and cysteine are precursors for the synthesis of glutathione, a tripeptide critical for defending the intestinal mucosa against toxic and peroxidative damage." (Wu, G 1998).

"Methionine and cysteine also appear to be metabolized by intestinal cells. Studies suggest that 52% of methionine intake is metabolized in the gut. Cysteine is obtained directly from

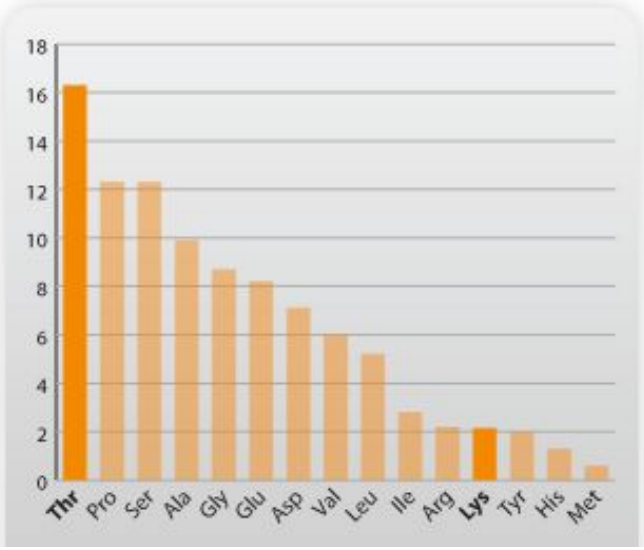
the diet or from methionine." (Gropper, Sareen S. p. 196).... The major end products of Met and Cys metabolism are glutathione (GSH), homocysteine (Hcy), and taurine (Tau), which play important roles in the intestinal immune response." (W.W. Wang et. al. p. 107).

Threonine- (high amounts in cooked brain and egg - See Values in Protein Analysis Calculations): "particularly important for mucin synthesis and maintenance of gut barrier integrity. The retention of dietary threonine by the intestine (up to 60%) is high. In intestinal mucosa, a major fate of threonine is incorporation into mucins, which are major glycoproteins protecting the epithelium from injury." (W.W. Wang et. al. p. 108).

A major fraction of the dietary threonine is absorbed in the upper part of the small intestine - the ileum. The remaining fraction is recovered at the end of the ileum (indigestible threonine). The fraction that is absorbed by the ileum is not entirely delivered in the portal blood which collects the nutrients from the digestion process. Only 40% of the luminal threonine reaches the portal blood. A significant part of the digestible threonine is indeed uptaken by the digestive tract itself; the intestinal cells (enterocytes) use 60% of the threonine intake, which is twice more than what was found for lysine.

This important threonine uptake by the gut is consistent with the high threonine content of the digestive secretions, among which is mucus. The mucus gel layer, secreted by goblet cells scattered along the gut villi, covers the wall of the digestive tract. It is an important component of the non-immune gut barrier that acts to protect the gut against digestive enzymes and physical damage by digesta. Mucus is mostly made of water (95%) and mucins (5%) which are large molecular weight glycoproteins very rich in threonine.

Figure 5. Mucin Amino Acid Composition



Glutamine - (high amounts in milk & wheat - See Protein Analysis Calculations): "is a source of energy for immunocytes, thereby optimizing gut mucosal repair... serves as a primary fuel source for lymphocytes, macrophages, neutrophils, and natural killer cells. [all immune cells]" (Stuart, Paula S. et. al.).

Glutathione is the most important cellular antioxidant in mammals and has a critical function in responding to reactive oxygen species and maintaining cellular redox status. ...Mediating oxidant stress and maintaining normal redox status is especially important in intestinal epithelial cells, which function as an innate defense barrier against luminal toxins and oxidants derived from the diet. In this regard, glutathione is essential for normal intestinal function and is related to an increased susceptibility to carcinogenesis, oxidative injury, metal intoxication, and common intestinal pathologies. (Shoveller, Anna K. p. 1611). "Glutathione synthesis represents a physiologically important pathway for glycine utilization by small intestine mucosa... dietary serine and glycine may be substantially catabolized by SI mucosa." (Wu, G.).

"Glutamate is also used with glycine and cysteine to make the tripeptide glutathione in the enterocyte (and other body cells). Glutathione functions as an antioxidant; it reduces many ROS and lipid and ROOH in the intestinal cell. Unless these reactive species are destroyed, they can damage cellular DNA, proteins, and PUFAs in intestinal cell membranes to cause membrane peroxidation and cell necrosis (death)." (Gropper, Sareen S. p. 195).... "GSH in the gut lumen and enterocytes is of critical importance in maintaining normal intestinal function, in part, by protecting epithelial cells from damage by electrophiles and fatty acid hydroperoxides."(W.W. Wang et. al. p. 107).

"Glutathione synthesis is sensitive to protein intake and pathological conditions. Hepatic GSH and mucosal and systemic GSH concentrations decline with poor protein intake as well as during inflammation and disease..." (Gropper, Sareen S. p. 199). "...blood levels of glutathione from patients with active Chron's disease were reported to be lower than in controls while normal levels were observed in patients with inactive disease...[glutathione] has been shown to play an important role in the protection of intestinal mucosa against exogenous injury...As glutathione is synthesized from glutamate, cysteine, and glycine, diet content in these amino acids could be critical." (O. Miralles-Barrachina; G. Savoye et. al. (1999).)...chronic inflammatory disorders of the colon are associated with glutathione depletion...glutathione levels were lower in patients with active inflammation compared to those with inactive disease." (Ruan, Eduardo A, MD. et. al.).

Mediating oxidant stress and maintaining normal redox status (i.e. oxidative balance) is especially important in intestinal epithelial cells, which function as an innate defense barrier against toxins and oxidants present in the gut that are derived from the diet. In this regard, glutathione is essential for normal intestinal function. A low concentration of glutathione in the gut is related to an increased susceptibility to carcinogenesis, oxidative injury, metal intoxication, and common intestinal pathologies. (Shoveller, Anna K. p. 1611). In addition, chronic inflammatory disorders of the colon are associated with glutathione depletion. Thus, for intestinal health, dietary intake of glutamate, cysteine, and glycine could be critical. One researcher has gone as far to say, "...administration of specific dietary substrates and precursors for GSH [glutathione] synthesis is an effective strategy to improve gut mucosal functions and may prevent or treat intestinal diseases." (W.W. Wang et. al. p. 107).

"...administration of specific dietary substrates and precursors for GSH synthesis is an effective strategy to improve gut mucosal functions and may prevent or treat intestinal diseases." (W.W. Wang et. al. p. 107)

RESPIRATORY HEALTH

"The structure of the lung is altered by severe calorie-protein restriction in rodents producing an emphysema-like lesion after several weeks of severe calorie restriction. Biochemical and morphological evidence suggests destruction of collagen & elastin in nutritional emphysema. The matrix of the interstitium, mainly collagen and elastin fibers, provide tensile strength and elasticity to the lungs, and it is the breakdown of these fibers that leads to emphysema... Clearly, the lungs of starved animals contain less connective tissue than normal, but whether this is due to breakdown of existing connective tissue or lack of accumulation of new connective tissue is unsettled...." (Riley, David J. p. 1657S-8S).
****Note: the dietary regimen required to produce these lesions in animals is a reduction of calorie-protein intake to 1/3 of normal for 4 - 6 weeks. "Short term fasting [in animals] decreased the rate of lung protein and collagen synthesis and increased proteolysis." (Riley, David J. p. 1658S).**
Even short-term fasting (in animals) decreased the rate of lung protein and collagen synthesis. Rats that are protein deficient have lesser amounts of collagen and elastin per lung. This is alarming since an alteration in lung elastin metabolism may lead to a variety of pathological changes in the lung. Even if relatively normal lung function does subsist despite an altered level of functional elastin, it is suggested from research on animals that the lungs will become increasingly susceptible to smoke-induced lung injury.

"In animals, severe protein-calorie restriction produces a lesion resembling human emphysema. Malnutrition may play a role in accelerating the progression of emphysema in humans; however, there are no clinical studies conclusively supporting the possibility...[although] a high incidence of emphysema was found in lungs of victims of starvation in the Warsaw ghetto." (Riley, David J.).
"protein-deficient rats had lesser amounts of collagen & elastin per lung." (Kalenga, M.).

Amino acid intake also influences the health of lung tissue. With its high connective protein content, the integrity of lung structure is highly contingent upon adequate protein intake. It is clear that the lungs of starved animals contain less connective tissue than normal and often have lesions resembling those found with human emphysema. Furthermore, it has been postulated that malnutrition may play a role in accelerating the progression of emphysema in humans, but more research is needed.

PROCESSING EFFECTS

Protein in general is fairly well digested. High quality proteins are easily digested by the body. In general, animal proteins are digested more efficiently than plant proteins. This is due partly to the presence of limiting amino acids, anti-nutritional factors, and fiber that may bind some amino acids. Animal proteins have been found to be about 90-99% digestible, whereas plant proteins are about 70 - 90% digestible.

DENATURATION

Cooking will also affect digestibility. Cooking renders many foods such as meat and eggs more digestible by inducing the protein to unfold due to the presence of heat, thereby exposing more of the protein and enabling the digestive enzymes to gain broader access to the peptide bonds. In addition, anti-nutritional factors that may bind dietary amino acids and digestive enzymes may be deactivated with cooking. However, it has also been suggested that the most significant effect of cooking on meat-eating is tenderizing, because this allows a high rate of intake. " The meat of wild tropical and temperate mammals is generally low in fat and rich in collagen, making it tough to chew. Meat with more connective tissue is tougher. Cooking above 80 degrees Celsius essentially denatures these connective tissue proteins to form gelatin. This allows muscle fibers to be easily separated, and gives them a short, brittle texture allowing for easy mastication. Cooked meat is therefore much easier to eat than raw meat." (R. Wrangham, N. Conklin - Brittain p. 40).

High temperature has two major effects on the physical properties of meat (Bouton and Harris, 1972; Purslow, 2005; Tornberg, 2005). At around 40 °C, muscle fiber proteins start to denature, leading to the contraction, drying and toughening of the meat. Between 50 and 60 °C, collagen denatures, causing gelatinization and solubilization of the connective tissues sheaths surrounding the muscle fibers. As cooking proceeds the meat becomes increasingly more tender and hence can be more easily chewed. Thus, cooking should decrease the cost and time of gastric and intestinal performance, thereby increasing net energy gain." (Boback, Scott M. Christian L. Cox et. al. p. 651-2).

Such effects of cooking may be applied to plant foods as well. A major effect of cooking, accordingly, may be that by tenderizing or softening food, it shortens the digestive process and therefore reduces the overall energetic costs. However, extreme processing of food that includes very high temperatures and/or alkaline processing may cause the formation of compounds that render the amino acids unavailable, thus decreasing their digestibility. Fiber and anti-nutritional factors such as trypsin inhibitors may also lower digestibility.

Meat toughness is predictable from the connective tissue content, and accounts for much of the variation in preference among Western consumers. Cooking above 80 degrees Celsius coagulates the connective tissue collagen and hydrolyzes it to a soluble protein (gelatin).

Cooking and grinding separately, and in combination, decrease the amount of energy required to digest and assimilate a meal...the cooking of meat has as much impact on facilitating digestion as grinding or chewing...Compared to the raw meals, the cooked meals

took less time (for intact meals) and energy (for intact and ground meals) to digest and assimilate. Cooking softens and solubilizes the collagen-rich connective tissues that surround the muscle fibers thereby increasing access of the tissue to gastric acids and proteolytic enzymes. Therefore, like grinding, cooking reduces the structural integrity of the meat, thereby speeding up gastric digestion.

These combined benefits to oral and gastric digestion support the hypothesis that by cooking meat, humans experience an energy savings (Wrangham, 2006). The magnitude of such a savings has not yet been experimentally quantified. " (Boback, Scott M, Christian L. Cox et. al. p. 655).

However, cooking also has its limitations, and there is a threshold in which these benefits are garnered. Specific cooking methods (i.e., steaming, boiling, microwaving, stewing, frying, broiling, etc.) will variably influence final nutrient quality – as is particularly observed with water-soluble and heat-labile constituents, including vitamins, minerals, phytonutrients, and enzymes. It is generally recommended that when heating foods containing high concentrations of these compounds, such as vegetables (e.g., broccoli and root vegetables), steamed should be used to avoid the significant nutrient loss that is commonly observed when microwaving, boiling, and/or frying these same commodities. Thus, it is not only the application of heat that alters protein digestibility, but more importantly, the form of heat that will dictate whether the protein will elicit a more or less digestible form compared to its antecedent, native state.

Moreover, formation of compounds associated with the exacerbation of aging, inflammation, and degenerative disease states, referred to as *advanced glycosylation end-products* (AGEs), are significantly abated when moist heating methods (e.g., steaming) are employed in lieu of dry heating methods (e.g., frying). The high temperatures applied while frying and broiling of foods provides the proper environment for AGE formation, which seldom occurs in steamed or raw plant foods. Once ingested, AGEs enter systemic circulation and undergo two fates: approximately two-thirds are covalently integrated into tissues and remain within the body indefinitely while the remaining 1/3 is excreted by the kidneys. This high accretion rate, coupled with the well-established literature concerning the deleterious nature of AGEs, necessitates the need to avoid a diet high in food products containing these pre-formed compounds whilst maintaining a suitable intake of minimally processed fruits and vegetables.

"High-temperature cooking produces AGEs/ALEs which, if ingested, would contribute to the body's AGE burden. The greatest quantity are created by frying or broiling foods containing fats or meats. Few are found in boiled or raw vegetarian foods….Inflammatory markers in the blood of diabetic humans and animals increased substantially after a few weeks on a high-AGE diet. This indicates that AGEs/ALEs do enter the systemic circulation from food digestion and increase inflammation. Similarly, although CR often improves the health and extends the lifespan of laboratory mice, when nondiabetic mice are maintained on a CR diet that is cooked to increase dietary AGEs, they have higher serum AGEs, oxidative stress, inflammatory markers, organ damage, and shorter lifespans than

matched CR controls that received the same total calories, but not cooked food. A cautious person with an interest in optimizing health and lifespan might choose diets that minimize ingested AGEs and ALEs." (Fahy, Gregory M.; Vest, Michael D.; Coles, L. Steven; Harris, Stephen, B. p. 602).

It was estimated that ≈10% of ingested immunoreactive AGEs are transported into circulation, two-thirds of which remain in the body, and are incorporated covalently in tissues. Only one third is excreted via the kidneys." (Luevano-Contreras, Claudia & Chapman-Novakofski, Karen).

DIGESTIBILITY

"In general, the amino acid score has the largest impact on the PDCAAS value, due to the high values observed for protein digestibility." (House, James D. p. 11806). High quality proteins are easily digested by the body." (Stuart, Paula S.). "High quality proteins are both complete and highly digestible, meaning the smaller quantities need be ingested than would be the case for proteins of lower quality." (DiPasquale, M.G. p. 149).

"Animal proteins have been found to be about 90 to 99% digestible (the true digestibility values for various mixtures of beef and pig skin were 95-100% Zarkadas, et. al.), whereas plant proteins are about 70 to 90% digestible. Meat and cheese, for example, have a digestibility of 95%, and eggs are 97% digestible. Cooked split peas are about 70% digestible, and tofu is about 90% digestible." (Gropper, Sareen S. et. al. p. 237). Digestibility of protein in raw quinoa was 78%, significantly lower than that of casein, 91%, and also somewhat lower than that of the raw, washed quinoa sample, 83%...the process used to remove the outer layers of the seeds containing saponins increased the protein digestibility significantly, by 7%. Heat treatments increased the protein digestibility over that of raw quinoa samples." (Ruales, Jenny & Baboo M. Nair).

"Egg white protein is generally considered to be less digestible than heat-pretreated egg white protein. However, no data are available concerning the magnitude of this impairment in vivo. In this study, it was shown that after ingestion of 25 g of raw egg protein, almost 50% is malabsorbed over 24 H. The higher digestibility of cooked egg protein presumably results from structural changes in the protein molecule induced by heating, thereby enabling the digestive enzymes to gain broader access to the peptide bonds. It has been suggested that the reduced digestibility of raw egg white is at least partially related to the presence of trypsin inhibitors in raw egg white." (Evenepoel, Pieter p. 1721). The true ileal (small intestine) digestibility of cooked and raw egg protein amounted to 90.9 +/-0.8 and 51.3 +/- 9.8%, respectively." (Evenepoel, Pieter p. 1716).

"It has been shown that meat protein aggregation during cooking is linked to the increase in protein surface hydrophobicity (i.e. protein insolubility), which can reduce the rate of protein digestion by digestive tract enzymes." (Bax, Marie-Laure p. 2569).

"Heat treatments had different temperature-dependent effects on the meat protein digestion rate and degradation potential. At 70 °C, the proteins underwent denaturation that enhanced the speed of pepsin digestion by increasing enzyme accessibility to protein cleavage sites. Above 100 °C, oxidation-related protein aggregation slowed pepsin digestion but [still] improved meat protein overall digestibility." (Bax, Marie-Laure p. 2569).

The digestibility of meat (up to 100%) is not much affected by cooking. For these reasons, little attention has been paid to the possible importance of cooking in facilitating meat-eating for humans. Instead, the relevance of cooking for the evolutionary significance of meat-eating has been discussed largely with respect to the special context of its value in de-frosting large kills. However, it has also been suggested that the most significant effect of cooking on meat-eating is tenderizing, because this allows a high rate of intake. The meat of wild tropical and temperate mammals is generally low in fat and rich in collagen, making it tough to chew. Meat toughness is predictable from the connective tissue content, and accounts for much of the variation in preference among Western consumers. Cooking above 80 degrees celsius coagulates the connective tissue collagen and hydrolyzes it to a soluble protein (gelatin). This allows muscle fibers to be easily separated, and gives them a short, brittle texture allowing easy mastication. Cooked meat is therefore much easier to eat than raw meat." (R. Wrangham, N. Conklin - Brittain p. 40).

Even if the digestibility of meat is not affected by cooking, we can expect its nutritional value to be increased as a result of cooking's effect in making it tender. Tenderizing should lead to a shorter time for a given weight of meat to be chewed and/or for it to be subsequently digested. Such effects of cooking should affect both meat and plant foods. A major effect of cooking, accordingly, may be that by tenderizing or softening food, it shortens the digestive process and therefore reduces the energetic costs of digestion." (Wrangham, R. 2006 p. 317). Note: Digestibility is being defined here as "the proportion (by dry weight) of a food item that can be digested," which is the standard usage, according to Wrangham, R....It is NOT directly referring to the bioavailability of amino acids within the meat...but if you are able to eat more cooked meat than raw meat this may be a null point.

SIDE NOTE: "Chimpanzees ...prefer meat that is relatively tender, such as younger prey, and blood, feces, brains and guts (Goodall, 1986). (Tenderness is greater in younger animals, Shorthose and Harris, 1990.) Prey items are sometimes abandoned after the softer parts have been eaten (personal observation of R. Wrangham). (R. Wrangham, N. Conklin-Brittain p. 40).

Table 9. Protein Digestibility Percentages by Food

Table 8. Some values (%) for digestibility of proteins in man.

Protein source	True Digestibility Mean	Reference
Egg	97	4
Milk, Cheese	95	4
Meat, Fish	94	4
Maize	85	4
Rice, polished	88	4
Cottonseed	90	115
Sunflower seed, flour	90	115
Wheat, whole	86	4
Wheat, refined	96	4
Wheat flour, white	96	115
Wheat gluten	99	115
Oatmeal	86	4
Millet	79	4
Peas, mature	88	4
Peanuts	94	115
Peanut butter	95	4
Soyflour	86	4
Soy protein isolate	95	115
Beans	78	4
Corn, whole	87	115
Farina	99	115
Triticale	90	115
Corn, cereal	70	115
Wheat, cereal	77	115
Rice, cereal	75	115
Oats, cereal	72	115
Maize + beans	78	4
Maize + beans +milk	84	4
India rice diet	77	4
Indian diet + milk	87	4
Chinese mixed diet	96	4
Brazilian mixed diet	78	4
Filipino mixed diet	88	4
American mixed diet	96	4
Indian rice + beans diet	78	4

Table 10. Digestibility of Protein and Selected AA in Various Foods

Mixture	Protein	Lys	Met	Cys	Thr	Trp
Casein	99	100	99	100	100	100
Skim milk	95	96	92	94	95	98
Beef (roast)	100	100	100	100	100	100
Beef salami	99	99	99	100	100	100
Sausage	94	94	91	95	92	93
Egg white solids	98	97	98	97	96	97
Tuna fish	97	97	95	96	98	97
Chicken franks	96	97	97	100	95	96
Pea flour	88	92	77	84	87	82
Pea, Century (autoclaved)	83	85	62	85	78	72
Pinto bean (canned)	79	78	45	56	72	70
Lentil (autoclaved)	85	86	59	75	76	63
Fababean (autoclaved)	86	85	59	75	76	63
Soybean	90	87	82	82	84	89
Soybean protein isolate	98	98	94	94	96	98
Rapeseed protein concentrate	95	91	92	93	91	93
Peanut	96	90	85	89	89	94
Peanut meal	91	88	89	89	87	—
Peanut butter	98	96	94	100	97	99
Sunflower meal	90	87	92	91	90	—
Wheat	93	83	94	97	91	96
Rolled Oats	94	90	92	98	90	97
Rice-wheat-gluten	93	85	81	95	88	92
Wheat flour-casein	95	91	91	89	90	90
Macaroni-cheese	95	95	93	98	92	98
Potatoes-beef	86	89	83	89	83	86
Rice-soybean	90	89	77	82	84	87
Corn-pea	83	85	84	86	82	80
Corn-soybean	93	93	87	94	93	98

*Source, Sarwar (99).

(Source for Digestibility Charts: Food & Agriculture Organization of the United Nations. (copyright 1991). "Protein Quality Evaluation." Report of Joint FAO/WHO Expert Consultation: FAO Food & Nutrition Paper #51. Bethesda, Md. USA, 4-8 Dec. 1989.).

ANTI-NUTRIENTS

The presence of fiber will have a depressant effect on protein digestibility. (House, James D. p. 11806).

Trypsin inhibitors in soy protein, tannins in legumes and cereals, and phytates in cereals decrease the bioavailability of amino acids from the food sources..." (R. Elango et. al. p. 23).

ANIMAL PROTEINS

Pending the source, the time-temperature relationship will vary in order to result in optimal protein bioavailability while simultaneously avoiding toxin formation. The current established recommendation for the ideal denaturation point, and corresponding pepsin digestion of meat, is 70 °C (158 °F).[6] Temperatures above 100 °C (212 °F) abate pepsin digestion by causing proteins to tangle together into a dense mass in a process known as protein aggregation. By causing the proteins to aggregate, the amenability of cleavage sites is greatly reduced – thereby lowering digestibility efficiency. The recommended temperature of 70 °C (158 °F) also complements and falls below the lower-limit temperature of Maillard browning, which is 140 °C (285 °F). During Maillard browning, amino acids react with reducing sugars to form Maillard reaction products (MRPs), which may form AGEs. While these MRPs contribute organoleptic properties to foods (e.g., pigments, aromas, and taste) – they are accompanied by the loss of essential amino acids, particularly lysine, and a reduction in protein digestibility overall (Seiquer, 2006; Jenkins 1988). When digestibility falls below 80% - it is typically associated with proteins in which have been subjected to heat-induced damage. This figure contrasts the 90 – 99% digestibility that typically represents animal proteins. By adhering to a cooking temperature of 70 °C for meat products, pepsin accessibility to protein cleavage sites is enhanced while simultaneously avoiding the formation of amino acid-degrading byproducts.

PLANT PROTEINS

Similar to animal proteins, plant proteins are also susceptible to AGE formation when exposed to high temperatures. A common example of this phenomenon is acrylamide formation when frying potatoes and roasting coffee beans. This carcinogen is a product of the amino acid, asparagine, which reacts with reducing sugars (e.g., glucose or fructose) present in the food in the above mentioned mechanism – Maillard browning. This intensifies the necessity to properly manipulate plant-based proteins as they are inherently less digestible than animal proteins at an approximate range of 70 – 90%.This observed reduction in digestibility is in part due to anti-nutritive factors, which interfere with proper digestion, and therefore, assimilation of nutrients: tannins precipitate proteins; phytic acid

functions as a *chelator*; lectins bind carbohydrates; *protease* inhibitors, such as the trypsin inhibitors found in soybean, compromise digestive enzyme function; and saponins form complexes with proteins, lipids, and minerals. Fortunately, these anti-nutritive compounds can often be destroyed or largely abated with heat and/or germination methods.

Studies have demonstrated the greatest reduction in anti-nutrients accompanied by a corresponding increase in both protein and starch bioavailability in legumes to be 121°C (250 °F) for 10 minutes. To augment this, washing and/or germinating preceding cooking will further increase protein digestibility; simply washing quinoa free from the saponins residing on the outer layer can increase protein digestibility by up to 5%. Further removal of this outer layer can increase digestibility by an additional 2%. Germination of nuts, seeds, cereals (i.e., grains), and psuedocereals significantly reduces anti-nutrient content while concomitantly increasing the provision of supplementary nutrients, including unsaturated fatty acids and phytonutrients. In buckwheat, for example, linoleic- (C18:2) and linolenic acid (C18:3) concentrations have been shown to increase from ~39 and 1% to ~51 and 19%, respectively, while rutin and quercetin concentrations concurrently increase from ~0.63 mg/g and 0.35 mg/g to ~22.4 mg/g and 23.1 mg/g, respectively. For perspective, rutin possesses approximately 2.4X the antioxidant capacity of vitamin C and E while quercetin possesses approximately 4.7X the antioxidant capacity of vitamin C and E. By optimizing both the cooking method and conditions, the energetic cost of digestion is greatly reduced while conferring maximum nutritional benefit to the host.

AMINO ACID PROFILES OF VARIOUS FOODS

SHEEP

Table 11. Amino Acid Composition (g/100 g dry, defatted matter) of Sheep Organs

Amino acid	Breed	Brain	Heart	Kidney	Liver	Lung	Spleen	Stomach	Testicle	Tongue
Alanine	Dorper	3.2 ± 0.21	2.7 ± 0.20	3.6 ± 0.17	3.5 ± 0.12	4.6 ± 0.11	4.1 ± 0.28	4.1 ± 0.17	4.3 ± 0.32	3.5 ± 0.20
	Merino	3.5 ± 0.21	3.0 ± 0.22	3.9 ± 0.17	3.4 ± 0.13	5.0 ± 0.13	3.8 ± 0.28	4.1 ± 0.17	4.1 ± 0.35	3.1 ± 0.20
Threonine	Dorper	2.8 ± 0.17	2.2^{b} ± 0.08	2.1^{b} ± 0.28	3.3 ± 0.11	3.0 ± 0.19	2.8 ± 0.11	2.8 ± 0.13	2.6 ± 0.15	2.3 ± 0.09
	Merino	3.0 ± 0.17	2.5^{a} ± 0.08	3.1^{a} ± 0.28	3.0 ± 0.12	3.5 ± 0.23	2.8 ± 0.11	2.6 ± 0.13	2.9 ± 0.17	2.3 ± 0.09
Serine	Dorper	3.2 ± 0.22	2.4 ± 0.08	3.4 ± 0.16	4.0 ± 0.19	4.0 ± 0.21	3.2 ± 0.16	3.8 ± 0.18	3.4 ± 0.35	2.7 ± 0.14
	Merino	3.4 ± 0.22	2.5 ± 0.09	3.9 ± 0.16	3.9 ± 0.21	4.4 ± 0.25	3.4 ± 0.16	3.5 ± 0.18	3.7 ± 0.39	2.9 ± 0.14
Arginine	Dorper	4.9 ± 0.43	3.7 ± 0.47	2.9 ± 0.81	4.5 ± 0.46	5.3 ± 0.38	3.8 ± 0.40	6.1 ± 0.75	5.2 ± 0.52	4.6 ± 0.48
	Merino	5.2 ± 0.43	3.4 ± 0.51	4.7 ± 0.81	4.4 ± 0.50	5.2 ± 0.46	4.0 ± 0.40	4.5 ± 0.75	5.6 ± 0.57	4.4 ± 0.48
Glutamic acid	Dorper	6.1 ± 0.57	7.2^{b} ± 0.44	10.0 ± 0.49	12.6 ± 0.86	10.8 ± 0.73	9.7 ± 0.82	7.7 ± 0.85	10.5 ± 1.03	6.0 ± 0.67
	Merino	5.9 ± 0.57	9.0^{a} ± 0.48	11.0 ± 0.49	10.0 ± 0.94	11.1 ± 0.89	11.1 ± 0.82	8.1 ± 0.85	12.2 ± 1.13	6.5 ± 0.67
Valine	Dorper	2.7 ± 0.15	2.1 ± 0.11	3.3 ± 0.12	3.7^{a} ± 0.09	3.6^{a} ± 0.09	3.5 ± 0.19	2.8 ± 0.09	2.5 ± 0.15	2.5 ± 0.12
	Merino	3.0 ± 0.15	2.4 ± 0.12	3.6 ± 0.12	3.4^{b} ± 0.10	3.9^{a} ± 0.11	3.4 ± 0.19	2.6 ± 0.09	2.7 ± 0.16	2.3 ± 0.12
Histidine	Dorper	1.6 ± 0.12	1.3 ± 0.07	1.6^{b} ± 0.08	2.2 ± 0.08	2.1^{b} ± 0.11	2.3 ± 0.16	1.3 ± 0.07	1.2^{b} ± 0.05	1.2 ± 0.08
	Merino	1.2 ± 0.12	1.4 ± 0.07	2.0^{a} ± 0.08	2.1 ± 0.09	2.5^{a} ± 0.13	2.1 ± 0.16	1.2 ± 0.07	1.4^{a} ± 0.05	1.2 ± 0.08
Aspartic acid	Dorper	5.7 ± 0.46	4.8^{b} ± 0.18	6.7^{b} ± 0.27	8.2 ± 0.34	7.4 ± 0.41	6.6 ± 0.22	6.1 ± 0.42	7.3 ± 0.45	4.9 ± 0.28
	Merino	5.9 ± 0.46	5.7^{a} ± 0.19	8.0^{a} ± 0.27	7.3 ± 0.38	8.7 ± 0.50	7.2 ± 0.22	5.9 ± 0.42	7.9 ± 0.49	5.4 ± 0.28
Lysine	Dorper	4.7 ± 0.31	3.5 ± 0.20	4.3 ± 0.30	5.6 ± 0.21	5.6 ± 0.26	5.3 ± 0.26	5.1 ± 0.21	4.8 ± 0.23	4.9 ± 0.23
	Merino	4.7 ± 0.31	4.0 ± 0.22	5.0 ± 0.30	5.3 ± 0.23	5.9 ± 0.32	5.4 ± 0.26	5.0 ± 0.21	5.4 ± 0.26	5.1 ± 0.23
Proline	Dorper	2.7 ± 0.18	2.6 ± 0.13	3.5^{b} ± 0.10	4.0 ± 0.10	4.5 ± 0.09	4.0 ± 0.16	4.9 ± 0.16	5.5 ± 0.26	3.3 ± 0.13
	Merino	2.9 ± 0.18	2.8 ± 0.14	4.1^{a} ± 0.10	3.8 ± 0.11	4.6 ± 0.12	4.3 ± 0.16	4.8 ± 0.16	5.1 ± 0.28	3.1 ± 0.13
Methionine	Dorper	1.2 ± 0.06	1.1 ± 0.04	1.4^{b} ± 0.03	1.4 ± 0.04	1.2 ± 0.03	1.3 ± 0.03	1.3 ± 0.06	1.1^{b} ± 0.03	1.2 ± 0.03
	Merino	1.3 ± 0.06	1.2 ± 0.05	1.5^{a} ± 0.03	1.3 ± 0.04	1.3 ± 0.03	1.3 ± 0.03	1.2 ± 0.06	1.4^{a} ± 0.04	1.1 ± 0.03
Tyrosine	Dorper	2.5 ± 0.21	1.7 ± 0.10	3.2 ± 0.11	3.8^{a} ± 0.10	2.8 ± 0.08	2.9 ± 0.19	2.4 ± 0.17	2.2^{b} ± 0.09	2.0 ± 0.12
	Merino	2.7 ± 0.21	1.9 ± 0.10	3.5 ± 0.11	3.4$_{b}$ ± 0.11	2.8 ± 0.10	3.2 ± 0.19	2.4 ± 0.17	2.9^{a} ± 0.10	2.1 ± 0.12
Cysteine	Dorper	0.6 ± 0.08	0.4 ± 0.04	1.0^{b} ± 0.05	1.2 ± 0.05	0.9 ± 0.04	0.7^{b} ± 0.06	0.4 ± 0.06	0.7^{b} ± 0.04	0.3 ± 0.04
	Merino	0.7 ± 0.08	0.5 ± 0.04	1.2^{a} ± 0.05	1.2 ± 0.06	1.0 ± 0.05	1.0^{a} ± 0.06	0.5 ± 0.06	0.9^{a} ± 0.04	0.3 ± 0.04
Isoleucine	Dorper	2.1 ± 0.13	1.8^{b} ± 0.07	2.6^{b} ± 0.09	3.2^{a} ± 0.08	2.2 ± 0.11	2.2 ± 0.13	2.2 ± 0.10	2.1^{b} ± 0.12	1.9 ± 0.09
	Merino	2.3 ± 0.13	2.1^{a} ± 0.08	2.9^{a} ± 0.09	2.8^{b} ± 0.09	2.0 ± 0.13	2.5 ± 0.13	2.0 ± 0.10	2.6^{a} ± 0.13	1.9 ± 0.09
Phenylalanine	Dorper	2.8 ± 0.22	1.9 ± 0.09	3.1^{b} ± 0.07	4.1^{a} ± 0.10	3.1^{b} ± 0.07	3.3 ± 0.16	2.4 ± 0.12	2.4^{b} ± 0.07	2.1 ± 0.10
	Merino	3.0 ± 0.22	2.1 ± 0.09	3.5^{a} ± 0.07	3.7^{b} ± 0.11	3.4^{a} ± 0.09	3.4 ± 0.16	2.4 ± 0.12	2.9^{a} ± 0.08	2.3 ± 0.10
Leucine	Dorper	5.8 ± 0.36	4.7^{b} ± 0.17	6.9^{b} ± 0.15	8.2^{a} ± 0.15	7.4^{b} ± 0.15	7.3 ± 0.31	5.8 ± 0.21	5.6^{b} ± 0.16	5.2 ± 0.20
	Merino	6.2 ± 0.36	5.3^{a} ± 0.19	7.7^{a} ± 0.15	7.4^{b} ± 0.17	8.2^{a} ± 0.19	7.3 ± 0.31	5.8 ± 0.21	6.4^{a} ± 0.18	5.2 ± 0.20
Glycine	Dorper	2.7 ± 0.15	2.7 ± 0.23	2.6^{b} ± 0.25	3.3 ± 0.09	4.7 ± 0.14	4.1 ± 0.27	6.1 ± 0.34	6.1 ± 0.29	4.2 ± 0.30
	Merino	3.1 ± 0.15	2.8 ± 0.25	3.8^{a} ± 0.25	3.2 ± 0.09	4.7 ± 0.17	4.4 ± 0.27	6.1 ± 0.34	5.3 ± 0.32	3.8 ± 0.30
Hydroxyproline	Dorper	0.1^{b} ± 0.02	0.4 ± 0.07	0.5^{b} ± 0.04	0.2 ± 0.03	1.2 ± 0.07	0.7^{b} ± 0.09	1.9 ± 0.18	2.0 ± 0.18	0.9 ± 0.06
	Merino	0.2^{a} ± 0.02	0.4 ± 0.07	0.8^{a} ± 0.04	0.2 ± 0.03	1.1 ± 0.09	1.0^{a} ± 0.09	2.0 ± 0.18	1.7 ± 0.20	0.9 ± 0.06

^{ab} means between breeds, within organs, with the same superscript do not differ ($P<0.05$).

BEEF TRIPE

"Glutamic acid was the most abundant amino acid in the tripe and accounts for almost 1.5 residues in 10 and aspartic acid approximately 1 in 12...The high glycine and arginine contents and the lower levels of lysine reflect the amount of connective tissue proteins present." (Zarkadas, George C. et. al.).

Table 12. Amino Acid Composition of Beef Tripe

AA	untreated	
	mean ± SEM[a]	CV
aspartic acid	83.76 ± 1.11	3.11
threonine	38.33 ± 0.93	5.68
serine	39.33 ± 1.45	8.67
glutamic acid	138.11 ± 2.62	4.47
proline	65.09 ± 3.76	13.56
glycine	80.38 ± 3.19	9.32
alanine	60.75 ± 0.41	1.63
cysteine	6.76 ± 0.10	3.65
valine	51.97 ± 2.28	10.32
methionine	25.46 ± 0.39	3.64
isoleucine	42.41 ± 0.51	2.80
leucine	73.50 ± 1.38	4.41
tyrosine	35.18 ± 0.73	4.90
phenylalanine	36.69 ± 1.17	7.53
histidine	26.10 ± 1.61	14.46
lysine	70.74 ± 3.19	9.69
arginine	76.78 ± 1.21	3.72
tryptophan	5.99 ± 0.36	14.15
4-hydroxyproline	37.59 ± 0.77	4.78
5-hydroxylysine	3.43 ± 0.21	14.28
isodesmosine	0.204 ± 0.022	18.81
desmosine	0.159 ± 0.024	34.12
N^{α}-methyllysine	0.078 ± 0.01	27.77
N^{ε}-dimethyllysine	0.555 ± 0.069	26.23
N^{ε}-trimethyllysine	0.340 ± 0.008	43.97
N^{τ}-methylhistidine	0.416 ± 0.045	22.50
ammonia	12.27 ± 1.28	24.52
total AA nitrogen (N)		
g of AAN/16 g of N[b]	90.19	
g of AAN/kg of protein	177.39	
g of AAN/kg dry weight	158.39	
total protein[c]		
g/kg of dry weight	901.95	
WE, mg/nmol	0.105696	
CF, mg/nmol	0.106753	
CF', mg/nmol	0.119552	

[a] Mean values and standard error of measurements (S
of variation; ns, not significant. [b] Total amino acid nitroge
mass and WE, CF, and CF' constants were determined

Table 13. Essential AA Scores of Beef Tripe in Comparison to Other Animal Proteins

Table 3. Essential Amino Acid (EAA) Scores of Beef Tripe and Other Animal Proteins and EAA Requirements of Preschool Child

	EAA				
		bovine tripe		other animal products	
EAA	EAA[a] requirements for preschool child (2−5 years old)	untreated	extracted	egg[a]	beef[b]
	Milligrams of Amino Acid per Gram of Total Protein[c]				
histidine	19	26	22	22	34
isoleucine	28	42	43	54	48
leucine	66	73	75	86	81
lysine	58	71	64	70	89
methionine + cysteine	25	32	39	57	40
phenylalanine + tyrosine	63	63	76	93	80
threonine	34	38	45	47	46
tryptophan	11	6	6	17	12
valine	35	52	49	66	50
% total protein					
EAA_E[e]	33.9	40.3	41.9	51.2	50.4
EAA_{10}[d] including arginine		47.9	49.7		
total EAA,[e] mg/g of N		2615	2755		
PER_{10}[e] predicted by eq 8		2.52	2.49		
	Percent Protein Digestibility in Man				
		90	90	95	98
	Percent Amino Acid Score				
		54.5	54.5	100	100
	Protein Digestibility Corrected Amino Acid Score				
		50	50	95	98
limiting EAA		Trp	Trp		

The essential amino acid profiles and protein ratings of the beef tripe before and after extraction are compared with those of the reference pattern (FAO/WHO/ UNU, 1985) for a 2-5-year-old child and with two high-quality animal proteins such as hen's whole egg and bovine skeletal muscle tissue, and the results are shown in Table 3. The egg and skeletal muscle have true protein digestibilities of 95 and 98%, respectively. Mean values for corrected amino acid scores (PDCAAS) ranged from 95% in hen's whole egg to 50% in beef tripe."

Beef tripe provides adequate amounts of all of the essential amino acids ranging from 40.3 to 41.9%, which is considerably higher than the 33.9% reference protein value given by FAO/WHO, and it is limiting only in tryptophan, reflecting the amount of connective tissue proteins present. (Zarkadas, George C. et. al.).

Beef tripe (stomach) is also given a low PDCAAS of 0.50 (on scale of 0 to 1), limited in tryptophan due to its high connective tissue content. The most abundant amino acids in beef tripe are glutamic acid, followed by aspartic acid. It is also high in glycine and arginine, with lower levels of lysine, once again reflective of the connective tissue proteins present. Following the same amino acid pattern, the offal of rams is very high in glutamic acid, and is also high in aspartic acid, glycine, and proline. Glycine and proline are particularly high in the stomach and the testicle of the rams.

Beef tripe contained high levels of collagen (20.1%) and elastin (1.0%) limited only in tryptophan. (Zarkadas, George C. p. 2563).

SQUID

Connective tissue makes up a larger portion of the overall protein in squid muscle compared to fish muscle. Collagen, in particular, is somewhat higher in squid muscle than in fish muscle. Collagen may form 2-11% and 2-16% of true proteins in skinned mantle (body) and skinned arms of the squid.

The four major squid consuming countries of the world are Japan, Spain, Korea, and Italy. Japan consumes by far the most, at least half of the total world catch. (Bemtsen, Steven Eldon p. 1). "Among them, Japanese common squid, arrow squid, and long-finned squid are a special choice. Sashimi and sushi prep. require the removal of skin. The skin is treated as waste at home, in fish shops, and fish processing and refrigerating factories." (T. Nagai p. 271).

"...it can be assumed that on the average the meat of squid mantles contains about 3 times more collagen than fish meat (Kolakowski & Gajowiecki, 1973)... The essential amino acid content of the edible portion of squid is similar to that of fish with the exception that squid contains a higher level of tryptophan and a lower level of valine." (Bemtsen, Steven Eldon.).

"Fractions of protein from the squid mantle muscle differ from those of marine vertebrates. The stromal fraction, composed mainly of connective tissue, represents ~11% of all proteins, whereas fish muscle reaches ~2%." (G. de la Fuente-Betancourt et. al.).

"Collagen, which is present in considerably large amounts up to about 11% of total protein in the muscle of Illex argentinus..." (A. Thanonkaew).

"The muscle tissues of some edible marine invertebrates contain somewhat larger amounts of collagen. In the skinned mantle (body) and skinned arms of squid Illex argentinus, collagen has been found by Sadowska to constitute 2-11% and 2-16% of true proteins, respectively." (Sikorski, Zdzislaw E. p. 39).

"Abalone, octopus, and squid meats are mainly muscle tissue with a lot of connective-tissue collagen and a complex fiber arrangement." (McGee, Harold p. 225).

"The muscle fibers of squid and octopus are extremely thin - less than a tenth the diameter of a typical fiber in a fish or steer, which makes the flesh dense and fine-textured. They're arrayed in multiple layers, and greatly reinforced with strengthening and toughening connective-tissue collagen, some three to five times more than fish muscle has." (McGee, Harold p. 230).

"Unlike the fragile collagen of fish, squid and octopus collagen is extensively cross-linked and behaves more like the collagen of meat animals." (McGee, Harold p. 230).

"...squid and octopus must be cooked either barely and briefly to prevent the muscle fibers from toughening, or for a long time to break down the collagen...Continued gentle simmering for an hour or more will dissolve the tough, contracted collagen into gelatin and give the flesh a silken succulence." (McGee, Harold p. 230).

Instead of storing energy in the form of fat, molluscs accumulate other amino acids - proline, arginine, alanine, and some combined forms (?) - as well as glycogen, the animal version of starch..." (McGee, Harold p. 230).
Another Side Note:

"Fish muscle and skin collagens differ from bovine meat and hide collagens in having significantly higher contents of 7 essential amino acids and a considerably lower concentration of hydroxyproline residues. A characteristic property of shrimp collagen is a high content of tryptophan [essential amino acid] residue." (Sikorski, Zdzislaw E. p. 39).

EARTHWORMS

"The earthworm proteins were of high quality, comparable with those of cows' milk and eggs." (Paoletti, M.G. et. al.).

INSECTS

Blasquez, Julieta Ramos-Elorduy et. al. (2012). "Could Grasshoppers be a Nutritive Meal?" Food and Nutrition Sciences. Vol 3. pp. 164-175.

SNAILS

Adeyeye, E. I. & E.O. Afolabi. (2004). "Amino Acid Composition of Three Different Types of Land Snails Consumed in Nigeria." ***Food Chemistry.*** **Vol 85. pp. 535 - 539.**

FISH MEAL

FAO - Torry Research Station. "Fish Meal." FAO Corporate Document Repository. No. 49, ID:26385. <http://www.fao.org/wairdocs/tan/x5926e/x5926e01.htm>.

FISH COLLAGEN

Warm water fish have more proline and hydroxyproline in their collagen. Note that proline and hydroxyproline make collagen more rigid. This extra rigidity may not be as necessary in cold water.... but may be necessary for fish in warm water in addition to warm blooded animals... "Tilapia is a warm water fish and its amino acid profile was different from cod. The greatest difference was in the ratio of glycine to glutamic acid, which was higher for

cod gelatin, and in proline and hydroxyproline which was higher tilapia. The imino acids (proline & hydroxyproline) impart considerable rigidity to the collagen structure. The cod gelatin had markedly higher glycine to imino acid mole ratio (2.3 compared to 1.5 calf skin gelatin)." (Gudmundsson, Magnus et. al. p. 39).

"Fish collagens in general have lower amino acid contents than mammalian collagens..." (Jamilah, B. et. al.).

"considerably lower number of Pro-rich regions of the collagen or gelatin molecules in cold water fish, than in warm blooded animals." (M. C. Gomez-Guillen et. al. p. 26)....[thus, marine gelatins tend to be less stable with a decreased thermostability than mammals].

BONE PROTEIN

"raw bone meal contains 24.9% crude protein of which 80.8% is connective tissue proteins." (Zarkadas, C.G. et. al.).

"Mean values for the PDCAAS ranged from 14.4 to 16.4%, with tryptophan as the first limiting amino acid in bone proteins." (Zarkadas, C.G. et. al.).

"The least common protein residues in processed bones are cysteine and tryptophan...these two amino acids (tryptophan and cysteine) are absent from collagen isolated from a variety of connective tissues....collagen from bone contained significantly lower amounts of all essential amino acids required for human nutrition than either whole egg or cow's milk protein...with deficits in tryptophan, S containing amino acids, and aromatic amino acids....when compared with most food proteins, collagen is deficient to different degrees in all essential amino acids except arginine." (Zarkadas, C.G. et. al.).

KEEP ALL OF THIS

LIZARD MEAT

Abu-Tarboush, Hamza M. **(12/01/1996). "Nutritional quality of Dhub (lizard) meat (uromastys aegyptius Blanford 1874) and characterization of its protein using electrophoretic techniques". *Ecology of food and nutrition (0367-0244)*, 35 (4), p. 275.**

EGGS

Complete protein
PDCAAS: 1.0 (actually greater than 1.0, but this system does not go above 1.0) otherwise egg would score about a 1.2.

“Soy, whey casein, and egg whites have perfect scores of 1.0, while beef has a score of 0.92. Generally, any food product with a score above 90% is considered a good protein source.” (Collins, Nancy PhD, RD, LD/N).

MILK

"Milk has traditionally been thought to provide adequate amounts of all amino acids to neonates. However, results of recent studies with lactating sows indicate that milk provides at most only 40% of arginine for protein accretion in 7- to 21-d-old suckling pigs and that arginine deficiency is a major factor limiting their maximum growth. Besides arginine, the amount of milk-borne proline that enters the portal vein is inadequate to support proline requirements for protein synthesis in the piglet. Thus, a 7-d-old pig must synthesize daily at least 0.68 g arginine/kg body weight. Based on a degradation rate [0.93 g/(kg body weight * d)] of i.v. infused proline in young pigs, the de novo synthesis of proline must occur at a rate of at least 1.11 g/(kg body weight * d) or at least 60% of the proline need for protein accretion. Additionally, based on glycine and alanine content in sow milk, milk meets at most 23% and 66%, respectively, of the needs for protein synthesis in piglets, which must synthesize daily at least 0.71 g glycine and 0.18 g alanine/kg body weight. Interestingly, although aspartate plus asparagine and glutamate plus glutamine represent 23 and 42%, respectively, of the total NEAA in sow milk, this food provides at most only 8 and 9% of aspartate and glutamate for protein deposition in suckling pigs, respectively."(Wu, G. "Functional A.A..." p. 32).

"Because the composition of AA in the body is similar among species, requirements of intracellular AA (e.g. relative proportions of AA) for protein synthesis are not expected to vary substantially. Thus, new knowledge gained from animal models has important implications for human nutrition. First, as reported for sow-reared piglets, provision of arginine from human or primate milk is inadequate for optimal protein accretion in breast-fed infants. This necessitates endogenous synthesis of arginine from glutamate, glutamine, and proline in infants." (Wu, G. "Functional A.A..." p. 35).

"Additional work has also identified that young and gestating mammals cannot synthesize sufficient amounts of all NEAA to support maximum embryonic/fetal survival, neonatal growth, as well as vascular and intestinal health." (Wu, G. "Functional A.A..." p. 31).

"A growing body of literature has led to the development of the concept of functional AA (FAA), which are defined as those AA that regulate key metabolic pathways to improve health, survival, growth, development, lactation, and reproduction of organisms. A deficiency of a FAA (either EAA or NEAA) impairs not only protein synthesis but also whole-body homeostasis. Notably, supplementing a specific FAA (e.g. glutamine or arginine) to a conventional diet that was traditionally thought to provide adequate AA can maximize growth potential in young animals and prevent diseases (e.g. obesity, diabetes, necrotizing enterocolitis, and intrauterine growth retardation) in both animals and humans." (Wu, G. "Functional A.A..." p. 31).

"Dairy products, meat, fish, eggs, and poultry are examples of foods that contain complete proteins." (DiPasquale, M.G. p. 148).

PLANT ORIGIN

SOY

Soy protein can be added to the list of complete proteins for humans, along with milk and egg proteins..." (DiPasquale, M.G. p. 161).

PDCAAS of soy protein: 1.0

CONSIDER DELETING SOY?
KEEP

GRAINS

"...grain protein has a low PDCAAS because of the low lysine, but it has a high methionine content...most pulses have a low PDCAAS (of 0.6-0.7 on a scale of 0 to 1) because of the low methionine, but they have a high lysine content. Because they complement each other, the two proteins together make up a high-quality protein source. When both are eaten in roughly equal quantities in a diet, the PDCAAS of the combined constituent is 1.0, because each constituent's protein is complemented by the other." (DiPasquale, M.G. p. 161).

OSBORNE CLASSIFICATION

1. **Albumins = water soluble**
2. **Globulins = soluble in dilute saline**
3. **Prolamins = soluble in 70% Et-OH**
4. **Glutelins = soluble in dilute H+/OH-**

LEGUMES

"The nutritive value of legume proteins has been known to be low in comparison to animal proteins. This has been attributed to poor digestibility, deficiency of sulfur amino acids, and the presence of anti-nutritional factors." (El-Hady, E.A. Abd, R.A. Habiba.).

HEMP

Table 14. Comparison of Hemp Protein to Other Protein Sources

protein source	PDCAAS (%)
casein	100
egg white	100
beef	92
soy protein isolate	92
chickpeas (canned)	71
pea flour	69
kidney beans (canned)	68
dehulled hemp seed	61
pinto beans (canned)	57
rolled oats	57
lentils (canned)	52
hemp seed	51
hemp seed meal	48
whole wheat	40
almond	23

[a]Data for all non-hemp protein sources derived from the Joint FAP/WHO expert consultation on protein quality evaluation,(*5*) with the exception of the data for almonds.(*15*)

(House, James D. 2010).

"In comparison to other protein foods, the PDCAAS value for hemp protein sources is positioned in the same range as the major pulse protein sources (lentils, pinto beans), and above cereal grain products, such as whole wheat." (House, James D. p. 11806).

"hemp meal has a high protein content, typically 30-50%."... "hemp seeds and its derived products contain all essential amino acids required by humans... However, limited in lysine, with leucine and tryptophan as the 2nd or 3rd limiting amino acids. All other amino acids yielded scores greater than 1.0." (House, James D. p. 11804-5).

"relative to other dietary protein sources, the limitation in the lysine content of hemp protein positions it in the same range as the main cereal grains (whole wheat=0.44; corn=0.54). Oil seed meals, due to their proportion of lysine, yield higher relative amino acid scores (soybean meal= 1.05; canola meal= 1.01)." (House, James D. p. 11804-5).
****the values above are amino acid scores (without digestibility factored in).**

AMINO ACIDS (HIGH/LOW) COMPOSITION IN FOODS

Table 15. Amino Acid Rankings by High and Low Food Sources

AMINO ACID	HIGHEST FOOD SOURCE(S)	LOWEST FOOD SOURCE(S)	NOTES
Tryptophan	Milk, spirulina, pigeon, Alaska king crab, quail	Gelatin, snails, fish collagens	Connective tissue proteins lack tryptophan, which gives them the low amino acid score they have
Threonine	Spirulina, pigeon, veal brain	Quinoa, pickled pork hocks, cured and pickled pork feet, gelatin powder, gelatin desserts, snails	Threonine is low in collagen and foods with high connective tissue proteins.
Isoleucine	Pigeon, quail, grasshopper, spirulina	Fish collagen, gelatin, pork feet, pork ears, pork skins	Isoleucine is high in egg, meats, spirulina, grasshoppers, and chicken heart; low in foods high in connective tissue proteins such fish collagen, pork feet, ears, skins, and gelatin.
Leucine	egg, pork brain, chicken heart, chicken liver, beef liver, spirulina, kidney beans, and cottage cheese	cabbage, pickled pork hocks, pickled pork feet, gelatin, gelatin desserts, fish collagen	low in foods high in connective tissue proteins & the plant cabbage

Lysine	**Beef loin, frankfurter, sardines, anchovies**	**fish collagen (skin), sprouted wheat, cereals**	**Low in fish collagen (skin) and cereal grains (i.e., Gramineae)**
Methionine	**egg, cottage cheese, sardine, anchovy, cockroach, quail, and pigeon**	**cricket, gelatin, pork skins, feet, ears, hocks, cabbage, leeks, chives, beans-kidney and soy**	**high in cockroach; low in many foods with high connective tissue proteins; low in some plants - cabbage, leeks, chives, beans (typical limiting amino acid in beans)**
Cysteine	**egg, Allium family (e.g., leeks and garlic), beef and chicken liver, pork brain, and cockroach**	**milk, cabbage, kidney beans, pork hocks, pork ears, pork skins, feet, whale, seal, gelatin, bamboo shoots, cottage cheese, white fish meal, fish collagen (skin), earthworms, chives**	**Low in many foods high in connective tissue proteins.**
Phenylalanine	**egg, veal brain, kidney beans, fish meal, cottage cheese, beef liver**	**gelatin powder & desserts, fish collagen (skin), grasshoppers, earthworms, adult cricket**	**Low in many foods high in connective tissue proteins.**
Tyrosine	**cricket, cockroach, grasshopper**	**gelatin, pork feet, pork ears, pork skins, and pork hocks**	**High in Orthoptera - order of insects that includes grasshoppers, cricket, and**

			cockroaches; low in many foods high in connective tissue proteins. Can be decarboxylated to form tyramine during fermentation
Valine	spirulina, lizard meat, cockroach, chicken heart, meat, liver, kidney beans, cottage cheese, egg, milk, ground lamb	pork feet, pork hocks, gelatin, fish collagens, snails, earthworms, seal, whale, pork skins, pork ears, sausage, Popeyes Fried Chicken, KFC Fried Chicken, leeks, cabbage	Low in many foods high in connective tissue proteins. Note fried chicken from fast food is low in valine, reflecting higher connective tissue content which will be confirmed with the presence of connective tissue amino acids in its composition as well (i.e. hydroxyproline).
Histidine	Whale, seal, pigeon	pork hocks, skins, ears, feet, bamboo shoots, fish collagen, earthworms, grasshoppers, leeks, cabbage, spirulina, chives, squid-mollusks	Low in many foods; high in connective tissue proteins. Can be decarboxylated to form histamine during fermentation (i.e., biogenic amine)
Aspartic Acid	egg, pork brain, soybeans, chicken heart,	beef lungs, wheat, gelatin, fried chicken -	High in seafood items; Low in foods high in

	black beans and kidney beans, beef loin, frankfurter, King Crab, bamboo shoots, sardines, and anchovies	**Popeyes & KFC, fish collagens (skin), cricket (adult), snails**	**connective tissue proteins.**
Glutamic Acid	**Fried Chicken (KFC & Popeyes), cottage cheese, cabbage, chives, wheat, milk**	**lamb lungs, pork hocks, pork feet, gelatin, bamboo shoots, fish collagen, earthworms, grasshoppers, crickets**	**Low in many foods high in connective tissue proteins. Functions as a neurotransmitter and accounts for >90% of synaptic connections**
Alanine	**spirulina, pickled pork hocks, pork ears, pork skins, pork feet, gelatins (powder and dessert), fish collagen, grasshoppers**	**earthworms, wheat, snails, cabbage, milk**	**High in gelatins, fish collagens, pork hocks, ears, skins, feet, and spirulina.**
Arginine	**gelatin powder, gelatin desserts, fish collagens (skin), beef tripe, Alaska King Crab, pork skins, pork ears, pork feet, pork hocks, spirulina, chives, mollusks, quinoa, soybeans, Frankfurter (beef), pork bologna, lamb lungs, ostrich leg,**	**Adult cricket, milk**	**Highest in foods with highest concentrations of connective tissue proteins.**

	beef tenderloin, beef loin		
Glycine	Lamb lungs, pickled pork hocks, pork ears, pork skins, pork feet, gelatin (powder & desserts), fish collagens	cabbage, cottage cheese, milk, egg, chicken meat, earthworms, lizard muscle meat	High in foods high in connective tissue proteins; Low in meat, egg, milk, cheese, earthworms.
Proline	milk, beef lungs, wheat flour, cabbage, pork hocks, pork ears, pork skin, pork feet, gelatin, cottage cheese, fish collagens	salmon, alaska king crab, sardines, anchovies, snails, lizard meat	Seafood is low in proline - gives rigidity to collagen! which is not ideal in the water; High in foods high in collagen and dairy.
Hydroxyproline	chicken and beef liver, ground beef, beef loin, beef tenderloin, KFC & Popeyes Fried Chicken, fish collagens (skin)		Surprisingly high HP content in Popeyes & KFC fried chicken and very high in fish collagens! Also some (much less in comparison to fish collagens etc.) in beef loin and beef tenderloins, possibly reflecting higher connective tissue proteins
Serine	egg, fish skin - cold water cod,	: milk, chicken thigh meat,	Serine conc. differ greatly in

	raw leeks, kidney beans	gelatin, fish collagen, warm water tilapia, lizard meat, snails	the skin of warm water and cold water fish.

COMPLEMENTARY PROTEINS

"Fortunately, the amino acid deficiencies in a protein can usually be improved by combining it with another so that the mixture of the two proteins will often have a higher food value than either one alone. For example, many cereals are low in lysine, but high in methionine and cysteine. On the other hand, soybeans, lima beans, and kidney beans are high in lysine but low in methionine and cysteine. When eaten together these types of proteins give a more favorable amino acid profile...The general consensus at present (2008) is that the various complementary proteins do not have to be consumed in the same meal in order to supply the necessary amino acids but can in fact be consumed over the course of the day...However, in athletes trying to maximize protein synthesis and muscular hypertrophy it is necessary to have a full complement of amino acids present for every meal..."(DiPasquale, M.G. p. 155-157).

Table 16. Complementary Protein Examples

Food Group	Limiting Amino Acids	Combine with...
Legumes	Tryptophan, Methionine	Grains, Nuts/Seeds
Grains	Lysine, Isoleucine, Threonine	Legumes, Dairy
Nuts/Seeds	Lysine, Isoleucine	Legumes

WOULD YOU LIKE TO PUBLISH THE CALCULATIONS OR SHOULD WE DELETE THIS SECTION?

THIS WAS ALREADY IN A SPREADSHEET FROM REBECCA--SHOULD BE EASY TO FIND. JUST ATTACH THE DATA HERE OR AT THE END OF THE DOCUMENT FOR REFERENCE. I MAY USE THAT DOC IN THE FUTURE FOR THE ULTIMATE MEALS.

See "Protein Analysis Calculations" Document for an amino acid breakdown and total amino acid score (without digestibility factored in & based only on essential amino acids as non-essentials don't have recommended intakes yet) of a wide array of foods. This data was compiled and calculated from NutritionData.com. Note the color coding that brings to attention high and low values, along with unexpected values that stand out. **Also, the amino acid scores are calculated using the amino acid requirements used in calculating the PDCAAS score, which are the requirements for a 1 to 3 year old child.

Extra details for use when viewing the chart...
Specific Information on Food Sources Found in the Chart in "Protein Analysis Calculations" doc described above (Again, Data from NutritionData.com):
[A] - Milk, whole, 3.25% milkfat
[B] - Hard-boiled egg
[C] - Beef, variety meats and by-products, brain, cooked, and pan-fried
[D] - Lamb, variety meats and by-products, brain, cooked, and pan-fried
[M] - Lamb, ground, cooked, broiled
[E] - Pork, fresh, variety meats and by-products, brain, cooked, braised
[F] - Pork, fresh, variety meats and by-products, brain, raw
[G-] Veal, variety meats and by-products, brain, cooked, braised
***[A]* - Soybeans, mature seeds, dry roasted [soy nuts]**
***[B]* - Chicken, heart, all classes, cooked, simmered**
***[C]* - Chicken, broilers or fryers, thigh, meat only, cooked, stewed**

[D] **- Chicken, liver, all classes, cooked, pan-fried**
[E] **- Beef, variety meat & by-products, liver cooked, pan-fried**
[F] **- Beef, variety meat & by-products, lungs, cooked, braised **Note: Beef lungs are a source of Vitamin C (15% D.V.) & Vit B12 (12% D.V.).**
[L] **- Beef, ground, cooked, broiled**
[H] **- Fish, salmon, Atlantic, wild, cooked, dry heat**
[I] **- Squid, Mollusks, mixed species, cooked, fried**
[J] **- Beans, black, mature seeds, cooked, boiled, without salt**
[K] **- Beans, kidney, all types, mature seeds, cooked, boiled, without salt**

Amino Acid Scores of Other Various Foods (obtained from NutritionData.com):

- **Chicken, broilers or fryers, back, meat and skin, cooked, stewed = 130**
- **Tempeh = 79 (lacking sulfur a.a.'s Mt & Cys)**
- **Tofu, firm, prepared with calcium sulfate and magnesium chloride (nigari) = 68 (lacking sulfur a.a.'s Mt & Cys).**
- **Tofu, salted & fermented (fuyu) = 107**
- **Soy protein isolate = 108**

Grain proteins, pulses, and seeds/nuts all have limiting (essential) amino acids that are required for protein synthesis and are not present in adequate enough concentrations. Grains are low in lysine, but high in methionine. Legumes are low in methionine, and are high in lysine. Thus, legumes and grains may be paired together to make a complete protein. Seeds and nuts are typically deficient in lysine or isoleucine, the exception being oily seeds such as soybeans or canola that have higher (essential) amino acid scores with adequate amounts of lysine.

The general consensus is that complementary proteins don't have to be consumed at the same time, but may be consumed over the course of a day, although consumption of complementary proteins at the same meal is thought to be ideal for optimum protein synthesis. The most common combinations include legumes and grains or seeds/nuts, grains with legumes or dairy, and nuts/seeds with legumes.

ULTIMATE MEAL

Both animal and plant products are excellent sources of select vitamins (e.g., vitamin B12 from meat and folate from green leafy vegetables), whereas animal products generally provide more biologically available minerals than plant products.

Animal-source foods (e.g., meat, dairy products, egg, poultry, seafood, and other products) contain higher quantities and more balanced proportions of AA relative to human tissues, than plant-source foods (e.g., rice, wheat, corn, potato, vegetables, cereals, beans, peas, processed soy products, nuts, and seeds).

For example, beef meat contains 63–68% protein on the dry matter basis, but most staple foods of plant origin (except for legumes) have a protein content <12% (dry matter basis) and are deficient in most AA, including lysine, methionine, cysteine, tryptophan, threonine, and glycine.

To meet the Institute of Medicine-recommended dietary allowance of meth-onine plus cysteine by the 70 kg adult human, daily intake of meat, wheat flour, or rice would be 45, 285, or 493 g dry matter, respectively. The excessive amount of carbohydrates that would be consumed in the wheat flour or rice can be converted into fat in the body, thereby contributing to development of obesity, dyslipidemia, and other metabolic disorders.

Based on this calculation, consumption of meat can substantially reduce the need for plant-based foods to meet adequate AA requirements of humans. Importantly, meat is a rich source of both taurine (a sulfur-containing AA essential for protecting the eyes, heart, skeletal muscle, and other tissues of humans from oxidative damage and degeneration) and carno- sine (an antioxidant dipeptide that maintains neurological and muscular functions). Of note, plants do not contain taurine or carnosine. Additionally, protein in animal products has a higher digestibility (~95%) than proteins isolated from plants (~85–92%) or proteins in whole plant foods (~80–85%) which generally contain anti-nutritional factors.[3]

Several lines of evidence show that animal-source protein has a greater nutritional value than plant-source protein to sustain skeletal-muscle mass. First, dietary supplementation of 17.5 g milk protein per day during a 12-week resistance exer- cise program increased lean body mass (3.9 vs. 2.8 kg) than an isonitrogenous amount of soy protein. Second, compared with soy protein, dietary supplementation with 24 g whey per day to young men enhanced their lean tissue gains (3.3 vs. 1.8 kg) after 36 weeks of resistance exercise training.Third, ingestion of animal-source protein by healthy adults ranging from 17.5 to 40 g from whey, skimmed milk, or beef stimu- lated skeletal-muscle protein synthesis to a greater extent than the same amount of soy protein under resting and post-exercise conditions. Fourth, long-term vegetarianism resulted in reduced skeletal-muscle mass in older women, compared with consumption of an omnivorous diet (18.2 vs. 22.6 kg lean body mass). Thus, as a nutritional strategy, adequate consumption of animal protein (e.g., nutrient-dense lean meat) can reverse the decline in protein intake by adults in the age groups of ≥51 years. This

simple means is vitally important for sustaining skel- etal-muscle mass and improving health in aging adults.

Appropriate proportions of animal- vs. plant-source proteins in diet should provide sufficient EAA and synthesizable AA, as well as their optimal ratios relative to lysine

Increase consumption of lean protein, such as skinless poultry, fish, and game meats and lean cuts of red meat. Cuts with the words round or loin in the name usually are lean.

Dietary protein is assumed to be of high quality (a typical mixture of animal- and plant-source proteins) with a biological value of 75% (efficiency with which a truly digestible protein is utilized for maintenance and protein deposition in the body).

SUMMARY

In summary, adequate consumption of high-quality protein is essential for optimal growth, development, and health in humans. An appropriate mixture of animal- and plant-based foods is a practical way to ensure balanced provision of dietary AA for the young and the adult. There is not a fixed amount of protein intake that suits all the people in all age groups. Rather, individuals should adjust their intake of protein and other nutrients according to metabolic rates, physiological needs, and health status. A sufficient supply of both EAA and synthesizable AA (so-called nutritionally nonessential AA) plays a key role in sustaining skeletal-muscle protein synthesis, mass, and function (including physical strength), while improving insulin sensitivity, ameliorating ageing-associated sarcopenia, and reducing white-fat accretion. To date, there are myths about AA and protein nutrition in humans due to inadequate understanding of the science. Sufficient intake of high-quality protein from animal products (*e.g.*, lean meat and milk) is essential for optimal growth, development, and health of children, as well as for optimal maintenance, function and health of tissues (including skeletal muscle, brain, heart, kidneys, liver and gut) in adults. However, consumption of protein above safe upper limits should be avoided to prevent any adverse health problems.

At present, the specific dietary requirements of non-essential amino acids remain esoteric and obfuscated due to the strict emphasis that has conventionally been placed on essential amino acid growth studies. Although this area of research is still in its nascent stage, unequivocal evidence reflects the imperative nature of non-essential amino acids when constructing a diet designed to optimize growth, development, reproduction, and homeostasis. Although exact figures are currently unavailable, the information contained in this review may be overlapped to effectively conjecture the dietary aspects that will satisfy overall physiological requirements. Correspondingly, (I) the upper limit of energy in which humans can effectively derive from proteins is considered to be 35 – 40% of the diet; consumption exceeding this amount can result in death, (II) assuming an individual is healthy, the protein is of mixed quality, and that the body will efficiently assimilate the protein – the recommended total intake is 10 – 30% (1.0 – 1.6 g/kg/day) of the diet, pending daily energy expenditure, (III) when the ratio of essential to non-essential amino acids is

high, toxin formation is accelerated, (IV) when one essential amino acid is limited – all other amino acids will be oxidized, and (V) amino acid intake should encompass a complementary balance of both essential and non-essential amino acids. Taking this information into account with the method of cooking to ensure that (I) optimal denaturation is achieved without forming protein aggregates, (II) there is minimal destruction of synergistic nutrients, and (III) high temperatures that promote the formation of AGEs are not utilized – many of the pathophysiological perturbations that plague our society today may be prevented.

Coming full circle, the biological significance of consuming customarily-considered non- and essential amino acids in proper proportions is irrefutable. This contests the traditional classification scheme for amino acid essentiality, which as discussed in this review, possesses significant limitations that are just now coming to light as a result of contemporary technology and refined research methods. As such, the concept of "ideal proteins" should be modified to include adequate provision of the entirety of amino acids while eliminating the dated "essential" and "non-essential" nomenclature that has erroneously been associated with human nutrition. With this paradigm shift in nutritional biochemistry, the recommended intake of proteinogenic amino acids will need to be revised as experimental data continues to surface. While accurate amino acid recommendations are currently convoluted, by coinciding the culmination of substantiated suggestions offered throughout this review with the complementary protein sources outlined on the ultimate amino acid map – the ingredients and instructions necessary to construct the cookbook of vitality are presented.

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