Meat and Muscle Biology™

A Salute to PVT TIM HiLL: Indispensable Amino Acids and Global Human Health[1]

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Abstract: Students are taught the study mnemonic "PVT TIM HiLL" to memorize the 9 indispensable amino acids (IAA): phenylalanine, valine, threonine, tryptophan, isoleucine, methionine, histidine, leucine, and lysine. The Food and Agriculture Organization (FAO) recommended that IAA be treated as individual nutrients on food labels because "crude protein" alone does not indicate protein quality. Protein quality—determined by Digestible Indispensable Amino Acid Score (DIAAS)—is calculated for 3 life stages: birth to 6 months, 6 months to 3 years, and 3 years and older. Foods possessing DIAAS greater than 100 are "excellent" protein quality, and those with DIAAS between 75 and 99 are "good," whereas foods with DIAAS < 75 cannot make a protein claim. Processing, heating, and/or grinding can decrease or improve plant and animal IAA digestibility. For children 6 months to 3 years of age, ground pork, smoked-cooked bacon, cooked pork leg, cured ham, cooked pork loin, salami, beef/pork bologna, beef jerky, and medium and medium rare beef ribeye steaks can be described as "excellent." A range of research has reported that cooked ground beef can be classified as "good" or "excellent," whereas the Impossible® (Impossible Foods, Redwood City, CA) and Beyond® (Beyond Meat Inc., El Segundo, CA) meat-alternative burgers and well-done ribeye can be classified as "good" sources of IAA for young children. For persons aged >3 years, all meat categories but cooked ground beef can be classified "excellent" sources of IAA. For meat alternatives, Impossible Burger could be classified as "excellent," but Beyond Burger could only claim to be "good." Protein quality claims for individual food ingredients can be diminished when the food is consumed with a lower protein quality item such as a wheat flour bun. To provide meals that are adequate in all IAA, the protein quality in each food item must be determined. Mixed meals must be adjusted for protein quality by combining low-quality proteins (present in cereals and grains) with higher-quality proteins (present in foods of animal origin).

Key words: indispensable amino acid, protein, digestibility, meat, Digestible Indispensable Amino Acid Score
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Introduction

Undergraduate animal nutrition students often are taught the handy study mnemonic "PVT TIM HiLL" for memorization of the 9 indispensable amino acids (IAA): (1) phenylalanine, (2) valine, (3) threonine, (4) tryptophan, (5) isoleucine, (6) methionine, (7) histidine, (8) leucine, and (9) lysine. In 2013, the Food and Agriculture Organization (FAO) branch of the United Nations (UN) recommended that these 9 IAA be treated as individual nutrients and that information regarding their digestibility and bioavailability be listed on food labels (FAO, 2013). This recommendation is very important because the crude protein content currently listed on all food labels around the world are not indicative of the amino acid quality of the food, and therefore the consumer of that food item may believe that they are obtaining an adequate quantity of IAA, when in fact they are not.

The objective of this review is to scientifically justify the use of foods of animal origin as a complementary food in a balanced, mixed-food diet. Foods of animal origin provide highly digestible nutrients with fewer calories while providing the necessary IAA for the prevention and/or treatment of chronic disease and malnutrition associated with wasting, stunting, and underweight as well as overweight and obesity.

Protein Malnutrition: A Statement of the Problem

According to multiple UN agencies, approximately 690 million people were impacted by undernutrition (8.9% of the global population) in 2019—an increase of 10 million in 1 year's time and a 60-million-person increase in the past 5 years (FAO et al., 2020). Low- and middle-income countries are simultaneously experiencing high rates of child undernutrition while seeing an increased diagnosis of obesity. The report summarized that "rising rates of overweight and obesity add to these (undernutrition) concerns. Childhood overweight and obesity are increasing in most regions, and in all regions for adults. In 2016, 41 million children under 5 years of age were overweight." Kulkarni (2018) described the "double burden of malnutrition" as a high prevalence of childhood undernutrition expressed coincidentally with an increase in the diagnosis of obesity-related noncommunicable diseases. For example, in Dr. Kulkarni's home country of India, 20% of adult men and women are classified as being in a state of "chronic energy deficiency" as indicated by a body mass index less than 18.2 kg/m^2. At the same time, those diagnosed as obese have increased from 10% of the Indian population in 2005–2006 to 20% in 2015–2016 (Kulkarni, 2018). Furthermore, if predictions of chronic noncommunicable disease remain unchanged, India will reach the highest number of diabetics in the world by the year 2040. The double burden of malnutrition is also illustrated by the "Three Faces of Malnutrition" (Figure 1) identified by the UN Children's Fund (UNICEF)/World Health Organization (WHO)/World Bank Joint Child Malnutrition Estimates (UNICEF et al., 2021): stunting, wasting, and overweight. Governments seeking to establish nutritional policy that addresses these divergent forms of malnutrition (underweight and overweight) are faced with a significant challenge. Once the physiological reasons for the contrasting phenotypic expression are established, a public education campaign must be disseminated. The nutritional physiology of this dichotomy is very complex, and public guidelines that are developed regarding nutritional recommendations must be easily understood by the public. That said, nutritional recommendations generated by governmental and nongovernmental agencies are often subject to incorrect or premature nutritional consensus, political ideology, corporate/industry interference, and/or imposition of the separate science of environmental sustainability.

Physiological development of indispensable amino acid malnutrition

Kwashiorkor is a very severe form of protein deficiency (characterized by general edema, swollen abdomen, muscle wasting, and flaky skin; Williams, 1933); however, the physiological conditions that lead to kwashiorkor are present in many children suffering from subclinical protein malnutrition (Semba, 2016). Kwashiorkor is the extreme result of dietary consumption

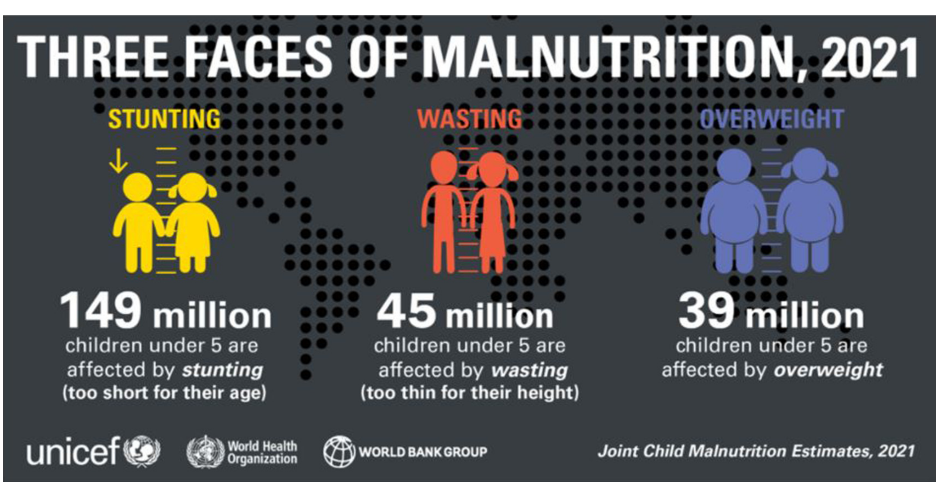

Figure 1. The "Three Faces of Malnutrition" as identified by the UNICEF/WHO/World Bank Joint Child Malnutrition Estimates (UNICEF et al., 2021). UNICEF = United Nations Children's Fund; WHO = World Health Organization.

trends that under-index high-quality protein foods and over-index less nutrient-dense, high-carbohydrate foods. Semba (2016) noted that the underlying physiological conditions that lead to the expression of kwashiorkor go unnoticed in many children who suffer from subclinical IAA malnutrition. This distinction is important to note, because even in developed countries, where the food options are abundant and accessible, an individual could be in a state of IAA malnutrition. Thus, the global epidemic of obesity that continues to increase in both developed and developing countries could be attributed to the double burden of malnutrition. It is presented as a high prevalence of childhood undernutrition expressed coincidentally with an increase diagnosis of obesity-related noncommunicable disease (Kulkarni, 2018). To understand the double burden of malnutrition, we must first acknowledge that undernutrition is not synonymous with a caloric deficit. In developed countries, the assumption is made that the average child is well fed because they are consuming a variety of food on a daily basis, yet they could be consuming diets deficient in one or more of the IAA. If these dietary patterns are maintained through the critical growth period and post puberty, the chronic nutrient deficiencies may manifest themselves as any number of obesity-related metabolic diseases (prediabetes, type 2 diabetes, metabolic syndrome, chronic fatigue syndrome). Berg et al. (2018) presented a macro-analysis of 10 research projects comparing swine diets adequate or deficient in the IAA lysine. These diets were fed for varying durations at different life stages of swine development. On average, the lysine-deficient swine averaged 19.3% more subcutaneous fat and had an 8.1% smaller cross-sectional area of the *longissimus dorsi* muscle. The most dramatic effect was seen in an 89.2% increase in intramuscular fat present in the *longissimus dorsi*. Central adiposity and accumulation of intramuscular triglyceride are medically documented indicators of the progression of chronic disease in humans. In these swine studies, a deficiency of lysine in a high-starch (high glycemic), corn-based diet resulted in wasting of muscle and an increase in adiposity that is indicative of human metabolic syndrome, prediabetes, and type II diabetes.

Why are indispensable amino acids indispensable?

Nine amino acids are classified as indispensable because they cannot be created endogenously by the human body and must be obtained from food. Six amino acids are considered conditionally indispensable (arginine, cysteine, glutamine, glycine, proline, and tyrosine) when physiological conditions prevent endogenous synthesis (Fan et al., 2013). Basic biology has taught us that amino acids are the building blocks of all proteins; however, amino acid metabolomics are much more complex than that one, simple function. Table 1 is provided as an outline of many metabolic functions associated with amino acids. Many are precursors for biological compounds such as the heme in hemoglobin, creatine (which binds inorganic phosphorus to re-phosphorylate ADP to regenerate ATP during muscle contraction), glutathione

Table 1. The specific function and transitional products of amino acids (adapted from: van de Poll et al., 2005; Wu, 2009)

Amino Acid[1]	Transitional Products	Function
Arginine		Activates mTOR; antioxidant; regulation of hormone secretion; activates NAG synthase in mammals; ammonia detoxification; regulation of gene expression; immune function; N reservoir; protein methylation
	NO	Multifunctional signaling molecule; nutrient metabolism regulation, vasodilation, hemodynamics, angiogenesis, spermatogenesis, embryogenesis, fertility, immunomodulation, hormone secretion, wound healing, neurotransmission, tumor growth, mitochondrial biogenesis, and function
	Agmatine	Inhibition of NO systems, ornithine decarboxylase, and monoamine oxidase; ligand for α2-adrenergic and imidazoline receptors; ornithine precursor
	Ornithine	Ammonia detoxification; syntheses of proline, glutamate, and polyamines; mitochondrial integrity; wound healing
Cystine		Forms di-sulfide links in protein formation; sulfur transport
Glutamine		Regulates protein turnover through mTOR signaling, gene expression, and immunomodulation; fuels the rapid proliferation of certain cells; inhibits apoptosis; cofactor in synthesis of nucleotides (purine, pyrimidine), ornithine, citrulline, arginine, proline, and asparagine; nitrogen sink; NAD(P) synthesis
	Ammonia	Interorgan nitrogen transport; renal HCO_3^- production for regulation of acid-base balance
Histidine		Methylation of proteins; hemoglobin structure and function
	Histamine	Anti-inflammatory in allergic reactions; vasodilator; immunomodulation; gastric acid secretion
Isoleucine		Glutamine and alanine synthesis; maintain BCAA balance

Table 1. (*Continued*)

Amino Acid[1]	Transitional Products	Function
Leucine		Regulate protein turnover through mTOR signaling and gene expression; activator of glutamate dehydrogenase; BCAA balance; important in regulation of energy and protein metabolism
Lysine		Regulates NO synthesis; antiviral; protein methylation, acetylation, ubiquitination, and O-linked glycosylation
Methionine	Homocysteine	Oxidant; independent risk factor for CVD; inhibits NO synthase
	Betaine	Methylation of homocysteine to methionine
	Choline	Precursor for synthesis of betaine, acetylcholine, and phosphatidylcholine
	Cystine	See above
	Taurine	Bile acid conjugation; neuronal cell development; osmo-regulation; antioxidant; anti-inflammatory; vascular, muscular, cardiac, and retinal functions
Phenylalanine		Tyrosine precursor; neurological development and function
Threonine		Abundant in mucins (necessary for intestinal constitution and function); glycine synthesis
Tryptophan	Serotonin	Neurotransmitter regulation of mood and sleep; inhibits inflammatory cytokines and superoxide; intestinal motility
	Melatonin	Regulation of circadian rhythms
	Niacin (B3)	Coenzyme and component of NAD and NADP
Tyrosine	l-dopa	Dopamine synthesis
	Dopamine	Neurotransmitter associated with the sensation of pleasure and motivation
	Noradrenaline, adrenaline	Neuro-hormones responsible for activation of sympathetic nervous system (fight-or-flight response)
	Tri-iodothyronine (T3), thyroxine (T4)	Regulation of basal metabolic rate
Valine		Glutamine and alanine synthesis; BCAA balance
Short-chain peptides containing IAA that form physiologically important molecules		
Arginine + **Methionine**	Polyamines	Gene expression; DNA and protein synthesis; ion channel function; apoptosis; signal transduction; antioxidants; cell function; cell proliferation and differentiation
Arginine + **Methionine** + Glycine	Creatine	Antioxidant; antiviral; antitumor; muscle and brain energy metabolism; neurological and muscular development and function
Cystine + Glutamic acid + Glycine	Glutathione	Endogenous antioxidant, free-radical scavenger, and immunomodulator
Lysine + **Methionine** + Serine	Carnitine	Transport of long-chain fatty acids into mitochondria for oxidation; energy stored as acetyl-carnitine; antioxidant

[1]Nonessential amino acids are standard font, **IAA are bold font**, and ***conditionally essential amino acids are bold-italics font***.

BCAA = branched chain amino acid; CVD = cardiovascular disease; IAA = indispensable amino acids; mTOR = mechanistic target of rapamycin; NAD = nicotinamide adenine dinucleotide; NADP = nicotinamide adenine dinucleotide phosphate; NAG = N-acetylglutamate; NO = nitric oxide.

(the body's endogenous antioxidant that defends against cellular damage caused by free radicals), and carnitine (facilitates the beta-oxidation of circulating fatty acids), as well as several of the conditional IAA. Furthermore, IAA alone are directly responsible for many biological functions. The physiological contribution of these nutrients becomes complex when one is attempting to discern the downstream disruption caused as a result of their absence or deficiency in the human diet.

Bioavailability of Indispensable Amino Acids

To provide meals that are adequate in all IAA, the quality of the protein in each food item must be determined, and mixed meals must be adjusted for protein quality by combining low-quality proteins (present in cereals and grains) with higher-quality proteins (present in foods of animal origin). The protein quality of a food item is primarily determined by the concentration of the first limiting IAA in that food item as determined from the true ileal digestibility of all IAA as fed or consumed. For these reasons, the FAO convened the "Expert Consultation on Protein Quality Evaluation in Human Nutrition" in Auckland, New Zealand in the spring of 2011. The group was formed to address the need to "define accurately the amount and quality of protein required to meet (the) human nutritional needs" of a growing world population (FAO, 2013). They further concluded that an accurate means of determining the amino acid nutrient bioavailability was the key to identifying foods and food combinations that could supply adequate nutrition for populations to thrive without over-taxing valuable land and water resources. The group recommended that protein quality be determined and defined through measurement of Digestible Indispensable Amino Acid Score (DIAAS). The key findings and recommendations from the Expert Consultation on Protein Quality Evaluation in Human Nutrition (FAO, 2013) were as follows:

- Quality of protein will be defined as a measure of DIAAS based on how well a food protein can meet the human body's requirements for IAA.
- DIAAS will be calculated for 3 life stages: (1) infants from birth to 6 months, (2) children from 6 months to 3 years, and (3) children >3 years, adolescents, and adults.
- DIAAS should be calculated from amino acid digestibility (nutrient absorption) at the terminal ileum of the small intestine.
    - "Digestibility should be based on the true ileal digestibility of each amino acid preferably determined in humans, but if this is not possible, in growing pigs."
- Dietary amino acids should be treated as individual nutrients, and information about digestibility/bioavailability of individual amino acids should be included on food labels.

The DIAAS will eventually replace the Protein Digestibility Corrected Amino Acid Score (PDCAAS) that has been used as a measure of protein quality by the FAO/WHO since 1991. It is conceivable that the PDCAAS will continue to be used until a sufficient database of ileal digestibility of DIAAS values are generated for commonly consumed human foods. Reviews by WHO (2007), Boye et al. (2012), Gilani (2012), Schaafsma (2012), and the FAO (2013) have outlined the limitations of the PDCAAS methodology. Bailey and Stein (2019) provided a summary of further reasoning for the use of DIAAS as the official metric for protein quality.

- Individual amino acid absorption obtained from digesta collected from the ileum (for determination of DIAAS) can more accurately assess true protein digestibility than fecal samples (used in determination of PDCAAS) because proteins synthesized by microbes and other nondietary proteins are present in fecal matter, which will overestimate the digestibility of lower-quality protein.
- The PDCAAS method truncated values to 100%; DIAAS does not. Truncation at 100% fails to recognize the ability of high-quality proteins to complement low-quality proteins in mixed meals.

Animal models

It is intuitive that humans are the best means for determination of ileal amino acid digestibility values for humans; however, the FAO has recognized the limitations associated with obtaining human subjects for testing; therefore the growing pig, followed by the growing rat, should be considered in the absence of human test subjects (FAO, 2013). Gilani (2012) suggested that the pig is superior to the rat because pigs are meal-eating species in a manner similar to humans. Deglaire and Moughan (2012) note that rats are different from swine and humans because they possess a unique requirement for sulfur containing amino acids (SAA) owing to a high concentration of cysteine in their fur. Furthermore, pigs possess a gastrointestinal anatomy similar to humans, and the physiology and metabolism response to nutrients ingested by pigs is comparable to humans (Gilani, 2012). It is therefore recommended that food DIAAS values be determined from ileal digestibility values of amino acids obtained utilizing the growing pig when not determined in human subjects (FAO, 2013).

The procedures for the use of swine as a bioassay for the determination of DIAAS are provided in Stein et al. (2007). Briefly, values for the DIAAS of individual foods are calculated by first determining the standardized ileal digestibility of each amino acid in a food protein. This value is then multiplied by the concentration of that amino acid in the protein to calculate the amount (grams) of digestible amino acid per kilogram of that food. A digestible IAA reference value for each amino acid is then calculated by dividing the concentration of digestible amino acid by the reference value for a specific age group as classified by the FAO. The DIAAS values are then reported as the lowest value of the 9 digestible IAA.

Defining protein claims

As stated earlier, DIAAS values are calculated for 3 life stages: (1) infants from birth to 6 months, (2) children from 6 months to 3 years, and (3) children older than 3 years to adulthood (FAO, 2013). The FAO (2013) states that protein claims can be added to a food label based on the DIAAS for a specific food item and age group. Because this claim is based on the DIAAS, it considers the digestibility (bioavailability) of amino acids relative to the amino acid concentrations of the food item and the subsequent amino acid requirements for human health. If a food item has a DIAAS value greater than 100, it can be considered an "excellent" source of high-quality protein for the specific age group. A food item can be considered "good" protein quality if the DIAAS value is between 75 and 99. However, a food with a DIAAS less than 75 cannot make a protein claim (FAO, 2013). Given the recommendation by the FAO to replace PDCAAS with DIAAS as the means to label protein quality, there is great need to generate DIAAS values for food proteins commonly consumed around the world.

Meals consumed by humans most often consist of more than one food item. The FAO (2013) recognized that the classification of protein quality by DIAAS was superior to the former PDCAAS method because PDCAAS could not exceed (was truncated at) 100%. The reason for this was that previous FAO committees pointed out that excess amino acids cannot be stored in the body and amino acids present in food items with PDCAAS above 100%; therefore, they have no protein value for the body (Schaafsma, 2012). However, this logic fails to recognize that high-quality foods with PDCAAS values above 100% often are combined with low-quality foods with low PDCAAS values, and the PDCAAS of the combined meal may, therefore, be less than 100%. The DIAAS, which is not truncated, indicates that the test protein contains bioavailable IAA at a level greater than the human IAA requirements for their particular life stage. Foods that possess a DIAAS greater than 100% provide additional nutritional benefits because they can complement low-quality proteins in mixed meals. Bailey and Stein (2019) provide the following example. Wheat as a single meal ingredient has a DIAAS value of 45 (Mathai et al., 2017), yet when wheat is processed into a breakfast cereal, the DIAAS value is considerably reduced to a level of 1 (Rutherfurd et al., 2015). The other common ingredient for breakfast, milk possesses a DIAAS value over the requirements for IAA at 118 (Rutherfurd et al., 2015). The full-meal (combination of milk and cereal), calculated DIAAS value of a breakfast of 60% milk and 40% cereal is 107 (Rutherfurd et al., 2015). This example demonstrates how milk balances the requirement for all IAA in this common mixed meal. Furthermore (Shivakumar et al., 2019), recently reported that milk and eggs are an excellent complement added to a mixed meal of low-quality plant proteins.

Plant sources of protein such as pulses (i.e., beans, lentils, peas) are included in mixed meals around the world either as a voluntary choice (choosing a vegetarian diet) or because of the absence or expense of consuming foods of animal origin. Pulses generally have a greater DIAAS than cereal grains, yet are limiting in SAA such as methionine. Furthermore, they contain antinutritional factors such as protease inhibitors that reduce the digestibility and subsequent absorption of amino acids and other micronutrients (Rutherfurd et al., 2015; Shivakumar et al., 2019). For example, in growing male rats, Rutherfurd et al. (2015) determined that cooked rice—which had a DIAAS of approximately 60—was limiting in lysine but had a high concentration of digestible SAA. They concluded that when rice is consumed with peas (with a DIAAS of approximately 58), the greater concentration of SAA in rice complements the lower concentration in peas, whereas the higher concentration of lysine in peas complements rice to balance the amino acid pattern of this mixed meal.

Impact of processing on DIAAS

The FAO report (2013) recognized several factors, such as processing, heating, and antinutritional factors in foods, can decrease the bioavailability or digestibility of different amino acids. That said, processing and heating can also improve the bioavailability and digestibility of IAA. For example, Fernandes et al (2010) reported that soaking and cooking common beans (*Phaseolus vulgaris* L.) improved digestibility by reducing the impact of antinutritional protease inhibitors, phytase, lectins, and tannins commonly found in pulses. We have established that swine are an excellent model for evaluating the impact of food and food combinations on the progression of obesity-related metabolic disease. Therefore, we can also look to swine as a model for how further processing impacts the digestibility of food. According to Kiarie and Mills (2019), grinding, pelleting, and extrusion of swine feed increased feed intake and enhanced nutrient efficiency. They note, "Finer feed particle size enables optimal nutrient utilization and enhances animal performance due to increased surface area allowing better contact with digestive enzymes." The grinding process, similar to chewing, destabilizes plant fiber that restricts the digestibility of many plant nutrients. The benefits of further processing are well established in agricultural swine science for formulation of a feed ration (a human equivalent of a meal), complete in all the necessary nutrients, that is more efficiently digested. More efficient digestion of food requires less quantity of food to be fed to meet the physiological nutrient needs of the animal.

Further processing of foods improves the bioavailability of plant sources of IAA; however, there has been limited information regarding the impact of processing on protein digestibility of muscle foods. Table 2 displays the DIAAS of many different food items. For children from 6 months to 3 years of age, ground pork burger, smoked bacon, smoked-cooked bacon, fresh pork leg (74°C), alternatively cured ham, conventionally cured ham, cooked pork loin (heated to 63°C, 68°C, or 72°C), salami, beef/pork bologna, beef jerky, raw ground beef, and beef ribeye steaks cooked to medium rare (56°C) and medium (64°C) degree of doneness can be described (FAO, 2013) as "excellent" quality proteins. Cooked ground beef was inconsistently classified as either "good" or "excellent," whereas both meat alternative burgers (Impossible Burger [Redwood City, CA] and Beyond Burger [El Segundo, CA]) and well-done ribeye would be classified as "good" sources of IAA for young children. For the reference demographic representing persons 3 years or older, all meat categories but cooked ground beef can be classified as "excellent" sources of IAA. For the meat alternatives, the Impossible Burger could be classified as "excellent," but Beyond Burger could only claim to be a "good" IAA source for humans 3 years and older.

Further processed meat products such as bacon, ham, salami, bologna, and jerky resulted in some of the highest DIAAS scores for both young children and those 3 years and older. Many of these products are derived from less expensive lean trimmings, making them an inexpensive food source of IAA that appeal to a wide range of consumers (even kids). A partial reason for the increased digestibility may be attributed to grinding (as we noted in the grinding of plant-based foods). Grinding of whole muscle foods may have an impact similar to chewing, which increases the surface area of proteins during digestion. Addition of ingredients that prevent oxidation during heat processing may also have an effect on maintaining the integrity of proteins prior to digestion in the small intestine. The decrease in DIAAS (Table 2) of cooked ground beef from raw ground beef reported by Bailey et al. (2020a) supports this observation. Grinding the raw beef trimmings increased the DIAAS, yet the application of the high cooking temperature in a relatively short period of time in the absence of added ingredients that prevent oxidation resulted in a lower DIAAS, especially in the reference demographic for young children.

Table 2. Digestible Indispensable Amino Acid Scores (DIAAS) and first-limiting amino acids (AA) determined for human foods using the pig or rat model[1,2,3]

		Reference Protein Pattern		
	Animal Model	Young Children (6 Months to 3 Years)	Older Children, Adolescents, and Adults	Reference
Cereal Grains				
Corn, yellow dent, raw	Pig	–	48 (lysine)	Cervantes-Pahm et al., 2014
Millet, cooked	Rat	10 (lysine)	–	Han et al., 2019
Oats, rolled, cooked	Rat	54 (lysine)	–	Rutherfurd et al., 2015
Rice, polished, cooked	Rat	37 (lysine)	–	Han et al., 2019
Sorghum, raw	Pig	–	29 (lysine)	Cervantes-Pahm et al., 2014
Wheat, raw	Pig	45 (lysine)	54 (lysine)	Mathai et al., 2017
Wheat, whole, cooked	Rat	20 (lysine)	–	Han et al., 2019
Burger bun, bleached wheat flour	Pig	26 (lysine)	31 (lysine)	Fanelli et al., 2021
Plant Proteins				
Kidney beans, cooked	Rat	59 (SAA)	–	Rutherfurd et al., 2015
Peas, cooked	Rat	58 (SAA)	–	Rutherfurd et al., 2015
Soy protein isolate	Pig	84 (SAA)	98 (SAA)	Mathai et al., 2017
Dairy Proteins				
Milk protein concentrate	Pig	120 (SAA)	141 (SAA)	Mathai et al., 2017
Skimmed milk powder	Pig	105 (SAA)	123 (SAA)	Mathai et al., 2017
Whey protein concentrate	Pig	107 (histidine)	133 (histidine)	Mathai et al., 2017
Meat Proteins				
Beef, "top side steak," raw	Pig	97 (valine)	–	Hodgkinson et al., 2018
Beef, ground, raw	Pig	111 (tryptophan)	121 (leucine)	Bailey et al., 2020a
Beef, ground, fully browned	Pig	92 (leucine)	99 (leucine)	Bailey et al., 2020a
Beef, ground, 80% lean, 71°C	Pig	102 (valine)	110 (valine)	Fanelli et al., 2021
Pork, ground, 80% lean, 71°C	Pig	111 (valine/leucine)	119 (valine)	Fanelli et al., 2021
Beef, ground, 93% lean, 71°C	Pig	111 (valine)	119 (valine)	Fanelli et al., 2021
Beef, "top side steak" boiled, 71°C	Pig	99 (valine)	–	Hodgkinson et al., 2018
Beef, "top side steak" roasted, 71°C	Pig	91 (valine)	–	Hodgkinson et al., 2018

Table 2. (*Continued*)

	Animal Model	Reference Protein Pattern: Young Children (6 Months to 3 Years)	Older Children, Adolescents, and Adults	Reference
Beef, ribeye roast, 56°C	Pig	104 (valine)	111 (valine)	Bailey et al., 2020a
Beef, ribeye roast, 64°C	Pig	121 (valine)	130 (valine)	Bailey et al., 2020a
Beef, ribeye roast, 72°C	Pig	99 (leucine)	107 (valine)	Bailey et al., 2020a
Beef jerky	Pig	102 (SAA)	120 (SAA)	Bailey et al., 2020a
Bologna, pork and beef	Pig	118 (leucine)	128 (leucine)	Bailey et al., 2020a
Salami, pork	Pig	107 (SAA)	120 (valine)	Bailey et al., 2020a
Pork, belly, raw	Pig	111 (valine	117 (valine)	Bailey et al., 2020b
Pork, belly, smoked bacon	Pig	109 (valine)	117 (valine)	Bailey et al., 2020b
Pork, belly, smoked, cooked ready-to-eat	Pig	126 (tryptophan)	142 (valine)	Bailey et al., 2020b
Pork, leg, fresh, 74°C	Pig	115 (valine)	124 (valine)	Bailey et al., 2020b
Pork, leg, alternatively cured, smoked, 74°C	Pig	123 (valine)	133 (valine)	Bailey et al., 2020b
Pork, leg, conventionally cured, smoked, 74°C	Pig	117 (valine)	126 (valine)	Bailey et al., 2020b
Pork, loin, 63°C	Pig	129 (valine)	139 (valine)	Bailey et al., 2020b
Pork, loin, 68°C	Pig	109 (valine)	118 (valine)	Bailey et al., 2020b
Pork, loin, 72°C	Pig	109 (valine)	117 (valine)	Bailey et al., 2020b
Meat Alternatives[4]				
Impossible Burger, 71°C	Pig	91 (SAA)	107 (SAA)	Fanelli et al., 2021
Beyond Burger, 71°C	Pig	71 (SAA)	83 (SAA)	Fanelli et al., 2021
Meal Combination of Burger and Bun				
80% lean beef burger + bun	Pig	98 (valine)	105 (valine)	Fanelli et al., 2021
80% lean pork burger + bun	Pig	100 (lysine)	107 (valine)	Fanelli et al., 2021
Impossible Burger + bun	Pig	73 (lysine)	86 (lysine)	Fanelli et al., 2021

[1]First-limiting amino acid (AA) is in parentheses.

[2]Unreported DIAAS values for certain reference patterns are noted by "–."

[3]AAA = aromatic amino acid; SAA = sulfur amino acid.

[4]Impossible Foods (Redwood City, CA) and Beyond Meat Inc. (El Segundo, CA).

Putting it all together: The mixed meal

Mixed meals are the norm for most humans, and a sandwich is a very convenient meal combination. Therefore, Fanelli et al. (2021) determined the final DIAAS for the sandwich combination of a ground beef, ground pork, and Impossible Burger patty on a bun. It is important to remember that the DIAAS value of a food protein is determined by the ileal digestibility of the first limiting IAA of that food item. In this case, the bun had a DIAAS value of 26 based on the requirement for young children and 31 for humans older than 3 years as determined by the low digestibility of lysine. Therefore, any complementary source of protein must compensate for the lysine deficiency in the bun. Individually, pork and beef had DIAAS values of 102 and 111, respectively, for young children, whereas the Impossible Burger was slightly lower at 91. The pork/bun combination was able to maintain a DIAAS of 100, but the beef/bun (DIAAS = 90) and Impossible/bun (DIAAS = 73) could not. The IAA requirements for humans older than 3 years of age were able to be met by the pork/bun (DIAAS = 107) and beef/bun (DIAAS = 105) sandwich; however, the Impossible Burger did not possess a sufficient quantity of the amino acid lysine to overcome the deficiency of lysine in the bun. This demonstrates that the Impossible Burger cannot function as a protein complement to the bun, and this resulted in a decrease in protein classification of the mixed meal from "excellent" to "good."

These meal combinations demonstrate the complexity of complementary proteins in a mixed meal. For example, let us examine the Impossible Burger and bun relative to the recommended amino acid scoring pattern for humans 3 years and older. The first limiting IAA in the Impossible Burger by itself are the SAA, but the second limiting amino acid is lysine, with a DIAA reference ratio of 1.13. This level of lysine will not complement the lysine deficiency of the bun because lysine is deficient in the meal combination. In contrast, the DIAA reference ratio for lysine in the 80% lean cooked ground beef is 1.49 and in pork is

1.62, which is sufficient to maintain an "excellent" protein quality score of these sandwiches.

The complexity of IAA mixed meal complements are a lot for the average consumer to mentally digest (pun intended). Even if the IAA contents of these foods were listed on the ingredient label as the FAO (2013) recommended, this would not necessarily be helpful to consumers attempting to create a mixed meal balanced in IAA requirements because food labels do not account for the digestibility of the protein. Furthermore, plant proteins offer an even greater level of complexity owing to the antinutritional factors that are naturally present. Consequently, obtaining a protein balance to meet human amino acid requirements in mixed meals is made much simpler by utilizing muscle foods compared with plant-based sources of protein.

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