Comparative nutrition and metabolism: Explication of open questions with emphasis on protein and amino acids

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The 20th century saw numerous important discoveries in the nutritional sciences. Nonetheless, many unresolved questions still remain. Fifteen questions dealing with amino acid nutrition and metabolism are posed in this review. The first six deal with the functionality of sulfur amino acids (methionine and cysteine) and related compounds. Other unresolved problems that are discussed include priorities of use for amino acids having multiple functions; interactions among lysine, niacin and tryptophan; amino acid contributions to requirements from gut biosynthesis; the potential for gluconeogenesis to divert amino acids away from protein synthesis; the unique nutritional and metabolic idiosyncrasies of feline species, with emphasis on arginine; controversies surrounding human amino acid requirements; and the potential for maternal diet to influence sex ratio of offspring.

amino acid deficiency | sulfur amino acids | arginine | lysine | niacin

Great progress was made in the nutritional sciences during the 20th century. By 1948, all of the essential vitamins had been discovered, and their synthesis was accomplished (1). The last of the amino acids found in food proteins (i.e., threonine) was isolated in 1935 (2). Defined functions for dietary essential mineral elements (Ca, P, K, Na, Cl, Mg, I, Zn, Fe, Cu, Mn, Cr, Se, F) and ultra trace elements (Si, Ni, Sn, V, B, As) also were discovered. Specific dietary requirements for essential nutrients were subsequently listed for animals by National Research Council (NRC) and for humans by Food and Nutrition Board committees of the National Academy of Sciences. Numerous Nobel Prizes were awarded for this body of work, particularly for the discovery of vitamins and their role in nutrition and disease.

World-wide, deficiencies of vitamin A, iron, and high-quality protein are generally considered the most serious problems in human nutrition. Prominent questions today are whether a role exists for nutrients beyond their role in preventing specific deficiencies. Thus, considerable research effort is being expended into the role of certain carotenoids in preventing macular degeneration and cataracts, specific vitamin E isomers as antioxidants, various hydroxylated vitamin D products for promotion of gut absorption of Ca and P as well as for treatment of osteoporosis and psoriasis, pharmacologic Se and conjugated linoleic acid for cancer prevention, and selected amino acids at pharmacologic dose levels for a host of clinical conditions. In addition, obesity and adult-onset diabetes have become serious problems in developed countries such as the U.S. Terms such as glycemic index, soluble vs. insoluble (and fermentable vs. nonfermentable) fiber, and indigestible starch have entered our vocabulary.

Bioavailability assessment of food nutrients has advanced to the point where we now have reasonable estimates of digestibility and absorbability of most nutrients in a food matrix. Vast differences exist in bioavailability both within and among nutrient groups (3, 4). Within dietary essential mineral elements, for example, some ingested elements in inorganic form are almost completely absorbed (e.g., K, Na, Cl, I, F), some have ≈70–90% absorption efficiency (P, Se, B, As), some have ≈30–40% (Ca, Mg, Cu), some have ≈15–20% (Fe, Zn, Ni), some have ≈5% (Mn, V), and some have <1% (Cr). Ratios of one element to another in a meal also are known to substantially affect absorption efficiency; this is illustrated by excess dietary Zn reducing Cu and Fe absorption and excess dietary P reducing Mn absorption (4, 5). Moreover, certain food ingredients in the vegetable category can bind mineral elements and reduce their bioavailability: phytic acid (inositol hexaphosphate) binds not only P, but also Ca, K, Zn, and Fe, and oxalates in foods like spinach can strongly bind Ca and Fe.

With vitamins, whether existing in foods as free vitamins or coenzymes, cooking, baking, and other food processing procedures can markedly reduce the bioavailability of vitamins like thiamin and folacin that contain a free amino group (3). Heat processing also reduces the bioavailability of certain protein-bound amino acids that contain a free amino group (lysine) via the same mechanism (Maillard reaction); this occurs prominently when cookies, cakes, or bread is baked and when bread is toasted (3).

Molecular genetic approaches are being used today to engineer food crops to have increased concentrations of specific nutrients (6). β-Carotene is the direct vitamin A precursor in plant-derived food products. The entire biosynthetic pathway of β-carotene biosynthesis in plants is now known (7), and its first application was in the development of transgenic rice (i.e., golden rice) that contained a substantial increase in β-carotene (8). Biosynthetic pathways for α-tocopherol (vitamin E) and folacin have been established as well (6, 9, 10). Plant geneticists have also found ways to engineer food crops to have increased levels of lysine and iron, and decreased levels of phytic acid (6, 10, 11).

Everyone seems to be interested in their own nutrition and health, and this has led to a plethora of so-called "nutritional supplements" being made available (without prescription) to the general public. Those of us who consider ourselves nutritional professionals find this alarming. Indeed, many of these supplements have no demonstrated efficacy or safety data to justify their use. Also, several essential nutrients (and nonnutrients) available for sale to the general public are toxic at higher dose levels, e.g., Se, V, cysteine, and vitamin A.

My research program has focused on using animal models and chemically defined diets to study nutrition and disease problems that affect both animals and humans. In this review, I will describe and briefly discuss my own personal list of "15 vexatious

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Abbreviations: NRC, National Research Council; SAA, sulfur amino acid; DRI, Dietary Reference Intake.

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questions" that have intrigued me over the course of my career as an academic scientist.

The first six questions deal with sulfur compounds and sulfur amino acids (SAA, i.e., methionine and cysteine). The role of these compounds in protein synthesis, transmethylation, synthesis of glutathione, taurine, CoA, and phosphoadenosine-5′-phosphosulfate as well as in ameliorating various inflammatory conditions have had longstanding emphasis in my laboratory. Clearly, the elegant research contributions of the late Vincent du Vigneaud (12), an Academy member and Nobel Laureate, provided great inspiration for the nutrition work on SAA done in my laboratory. Sulfur amino acid work is of great practical relevance to animal nutrition in that well over 90% of SAA production is used to fortify diets for animals, particularly poultry. Poultry diets around the world are based on corn and soybean meal, and these diets for poultry, without fortification, are deficient in SAA.

Questions

Question 1: Why Does the Addition of Methionine, Alone, to a Protein-Free Diet Increase Nitrogen Retention, Protein Accretion, and Growth? Several investigators have reported that methionine supplementation of a protein-free diet reduces body weight loss and improves nitrogen balance in rats (13), chickens (14), pigs (15), and dogs (16). Our own work (17) has confirmed the earlier suggestion (18) that the methionine response is not due to methionine *per se* but instead to methionine furnishing sulfur for cysteine biosynthesis via transsulfuration. Indeed, cysteine supplementation elicits a response equal to or greater than methionine. Protein turnover (degradation and synthesis) is an ongoing body process, even when no protein is being consumed. A portion of the amino acids released from body protein catabolism is oxidized and therefore not available for resynthesis of new protein. The cysteine response observed when a protein-free diet is fed implies that this amino acid is substantially depleted from body pools, making it the first limiting amino acids for endogenous protein synthesis.

Question 2: Why Is Excess Dietary L-Cysteine So Much More Toxic than an Isosulfurous Excess of L-Cystine, *N*-Acetyl-L-Cysteine or L-Methionine? At isosulfurous levels, L-cysteine, L-cystine, *N*-acetyl-L-cysteine, and L-methionine are equally efficacious for growth of animals fed a cysteine-deficient diet (19). Nonetheless, at pharmacologic dose levels these SAA elicit far different results (20–22). Addition of 3% or 4% L-cysteine to a typical corn–soybean meal diet for chicks or rats causes heavy mortality within 5 days. Similar levels of L-cystine, *N*-acetyl-L-cysteine, or methionine result in no mortality after 10 days of feeding. Cysteine is absorbed from the gut faster than cystine (22), and it has potent reducing-agent activity as well as mineral-chelation activity (21). It can also bind plasma proteins (22). *N*-acetylcysteine is less toxic than cysteine, perhaps because the deacetylation process occurs slowly. This is fortunate in that *N*-acetylcysteine is being used increasingly in the clinical setting (23, 24). It, along with cysteine itself, is also available over-the-counter in both health-food stores and pharmacies. They shouldn't be!

Question 3: Why Is Cystine the Least Digestible Amino Acid in Food and Feed Proteins? Most protein sources consumed by animals and humans have undergone some form of heat processing. This processing causes a significant portion of protein-bound cysteine to be oxidized to cystine, and protein-bound cystine is less digestible than protein-bound cysteine (25). The disulfide bridges created both within and between peptide chains when two cysteine residues condense to form cystine apparently restrict gut proteolytic enzyme attack. Whether the impaired digestibility results from presence of disulfide bonds within or between peptide chains is not known. Heat treatment together with alkaline food processing may also convert some of the dietary cystine to lanthionine (26), a crosslinked sulfur compound that has minimal SAA bioactivity (27). Thus, protein-bound cystine has a low bioavailability (28). This could be important clinically, because undigested cystine will pass to the colon where sulfate-reducing bacteria may degrade it to sulfides, and sulfides have been found noxious to colonic epithelial cells (29, 30). Still, the link between undigested SAA, particularly cystine, and colonic inflammation has not been firmly established.

Question 4: Are There Components of Foods and Feeds Other than Methionine, Choline, Betaine, Folacin, and Serine That Have Methyl Donating Capacity? Many foods and feedstuffs (e.g., soybean meal) contain significant and measurable quantities of *S*-methylmethionine (SMM), an analog of *S*-adenosylmethionine (SAM). As such, it may be capable of replacing (or sparing) SAM in biological methylation reactions such as choline biosynthesis from phosphatidylaminoethanol and creatine synthesis from guanidinoacetate. Our recent work using the chick as an animal model showed that SMM does indeed have choline-sparing activity (31). However, the methylation reaction in which homocysteine is converted to methionine prefers betaine as the methyl donor. Thus, methionine sparing by SMM was found to occur only when choline and betaine were deficient in the diet.

Dimethylsulfoniopropionate is another sulfur compound present in foods (32). Can consumption of this compound result in a choline-sparing effect similar to that observed with *S*-methylmethionine? Can ingestion of this compound reduce homocysteinemia via methylation of homocysteine to methionine? These two questions have not been answered.

Question 5: Why Are Sulfur Amino Acid Requirements for Adult Humans So Much Lower than Those for Adult Pigs? Amino acid requirements for maintenance have been determined based on attainment of zero nitrogen balance or on achievement of minimal oxidation of the test amino acid (direct oxidation method) or a target excess amino acid (indirect oxidation). With both humans and pigs, the maintenance SAA requirement ($mg \cdot kg^{-1} \cdot day^{-1}$) based on nitrogen balance has been found to be substantially higher than the maintenance lysine requirement (22). However, oxidation methodology has been used to set the official SAA and lysine requirements of humans (33), and this method has resulted in SAA requirement estimates that are less than one-half as great as the lysine requirement. Given that pigs and humans are similar physiologically and metabolically, how can the maintenance requirement ratio of SAA:lysine be so different between pigs and humans? Cysteine has an important precursor role (e.g., glutathione, taurine, CoA, and phosphoadenosine-5′-phosphosulfate biosynthesis) as well as an important role in synthesis of gut mucin and keratoid tissue that are ultimately sloughed from the body. Perhaps oxidation methodology underestimates the true requirement for an amino acid like cysteine. On the other hand, perhaps nitrogen balance methodology overestimates the requirement for an amino acid like cysteine. Clearly, these questions have not been resolved. Proper assessment of amino acid requirements is difficult and controversial (34–36).

Question 6: Why Do Some Dietary Copper Sources Provide Bioavailable Copper More Efficiently than Others, and How Does Cysteine Interact with Copper? Twenty years ago, cupric oxide (CuO) was the dominant source of Cu used in trace–mineral mixes for animals and in vitamin–mineral supplements for humans. However, research with pigs (37) and chickens (38, 39) has clearly shown that the Cu in CuO does not furnish any bioavailable Cu to the animal. However, copper oxide in the +1 state (i.e., Cu_2O, cuprous oxide) is used as well as the sulfate and chloride salts of Cu. Many mineral supplements for humans continue to rely on CuO as a source of Cu, probably because this salt of Cu

contributes to making a good (and smaller) pill (CuO is 80% Cu, whereas $CuSO_4 \cdot 5H_2O$ is only 25% Cu). Although definitive human data are not available on Cu utilization from CuO, the animal data make a convincing argument that CuO is probably poorly used by humans as well.

The liver and gall bladder are prominent storage sites for body Cu, but the bioavailability of Cu in pork liver (prominently used in pet foods) is near zero (40). The Cu in beef and chicken liver, on the other hand, is as bioavailable as that in $CuSO_4 \cdot 5H_2O$ (the accepted standard). What is the explanation for the poor Cu utilization in pork liver? A clear answer is problematic, although pork liver is known to be higher in cysteine than liver from other species, and cysteine is capable of binding Cu and therefore reducing its absorption from the gut (21).

Individuals with Wilson's disease (41) absorb too much and excrete too little Cu. Hepatologists treating these patients often use cysteine (or drug forms of cysteine such as D-penicillamine or dimercaptopropanol), *N*-acetylcysteine, or ascorbic acid as reducing agents and/or Cu-binding agents together with pharmacologic Zn supplementation to reduce dietary Cu absorption and enhance Cu excretion. Based on work with chicks fed high levels of Cu, cysteine compounds were found to be far more effective than either ascorbate or Zn in ameliorating the Cu-induced growth depression and reducing Cu deposition in the liver (42). Moreover, oral cysteine is over twice as effective as an isosulfurous level of either cystine or methionine (21). This finding, again, points to a marked difference between the pharmacologic effects of oral cysteine vs. cystine. The answer to this vexing difference between these two SAA probably lies in what is taking place in the gut, i.e., speed of absorption, amount taken up into mucosal protein, amount used for glutathione biosynthesis, and redox state and equilibrium.

Question 7: What Are the Priorities of Use When an Amino Acid with Multiple Functions Is Deficient in the Diet? Amino acids are used to synthesize a variety of different body proteins, e.g., myofibrillar, stromal, sarcoplasmic, keratoid, and acute-phase tissue proteins, hormones, enzymes, and specialized proteins such as metallothionein. Also, several amino acids have precursor roles. Concerning the synthesis priority of one type of protein over another when an amino acid is deficient, little is known about this intriguing question. Our work with chickens fed diets deficient in either histidine (43) or cysteine (44) suggested that protein synthesis is prioritized over either carnosine or glutathione synthesis. However, questions of priority remain for many amino acids that have important precursor roles: arginine for urea cycle function and synthesis of protein, creatine, polyamines, and nitric oxide; tyrosine for synthesis of protein, catecholamines, thyroxin, and melanin; tryptophan for synthesis of protein, serotonin, and niacin nucleotides; and glycine for synthesis of protein (contractile vs. collagen), heme, creatine, and uric acid. How gluconeognic amino acids are partitioned for gluconeogenesis vs. protein and precursor synthesis is another unresolved priority question. Other priority examples could be mentioned, but clearly, the priority for functional synthesis is an area of nutrition we do not fully comprehend.

Question 8: Why Does a Large Excess of Dietary Lysine Elicit a Growth Response in Niacin-Deficient Animals? Niacin activity comes not only from ingested niacin (or niacinamide) but also from ingested tryptophan. Most of the tryptophan flux during turnover goes to CO_2 (via α-ketoadipic acid), with only a small portion going to serotonin and nucleotides of niacin. α-ketoadipic acid is also an intermediate in lysine catabolism to CO_2. We demonstrated that addition of 1–1.5% excess lysine to a niacin-deficient diet elicits a growth response in chicks (45). The same lysine addition to a niacin-adequate diet caused a substantial growth depression. At the key branch point of tryptophan catabolism to either niacin nucleotides or CO_2 (i.e., at 2-amino-3-carboxymuconic acid semialdehyde), α-ketoadipate is projected to accumulate due to lysine catabolism; we suggest that this forces more of the 2-amino-3-carboxymuconic acid semialdehyde flux in the direction of niacin nucleotide synthesis, with less being directed to CO_2 via α-ketoadipic acid.

Golberger in 1922 (46) is generally given credit for discovering a cure for black tongue in dogs and pellagra in humans (47), but it was not until 1937 that Elvehjem *et al.* (48) isolated nicotinamide from liver extracts and showed that this compound would cure black tongue in dogs. Seventy years earlier, German chemists had actually synthesized nicotinic acid, but because this compound did not cure beri-beri in humans (now known to be caused by thiamin deficiency), it remained an unappreciated chemical entity for several decades (1). Today we know that diets poor in niacin and tryptophan cause pellagra, but we also know that iron deficiency anemia and poor protein quality (i.e., lysine deficiency) exacerbate the condition. Iron is required in two of the several enzymatic reactions leading to niacin biosynthesis from tryptophan (49). We also know that coffee consumption is a factor to be considered in pellagra, because coffee is rich in niacin (50). We suspect that diets very low in lysine result in minimal α-ketoadipic acid production from lysine, such that more of the tryptophan-derived 2-amino-3-carboxymuconate semialdehyde flux will be directed toward α-ketoadipic acid and, therefore, less will be directed toward niacin nucleotide biosynthesis.

Question 9: Why Is It That Growth on a Diet That Is Equally Deficient in an Amino Acid and Two Different B Vitamins Will Respond Markedly to Dietary Addition of Any One of the Three Deficient Nutrients? In underdeveloped countries, poor nutrition is characterized by multiple nutrient deficiencies. We developed a soy–protein isolate basal diet that could be made markedly deficient in several essential nutrients, e.g., methionine, choline, riboflavin, vitamin B_6, and Zn (51). Surprisingly, when diets were made approximately equally limiting in any pairs or trios of these nutrients, marked growth responses were found to occur from any one of the deficient nutrients. Thus, the order of limiting amino acid concept in which responses will not occur to a 2nd or 3rd limiting amino acid unless the 1st (or 1st and 2nd) limiting amino acid is supplemented does not apply when multiple deficiencies of amino acids, vitamins, and trace minerals coexist in a diet. Logical explanations for this phenomenon are not obvious.

Question 10: Does a Single Deficiency of One Amino Acid Cause the Same Degree of Growth Depression as an Equal Deficiency of Another Amino Acid? All single amino acid deficiencies also involve a profile of excess amino acids over and above the single deficiency, and each single deficiency results in a unique and different profile of excess amino acids. The excess amino acids can have very different effects on voluntary food intake, depending on which specific amino acid is deficient. Using a chemically defined amino acid diet, Sugahara *et al.* (52) evaluated single deficiencies (60% of required level) and compared them to a deficiency of all essential amino acids (i.e., all at 60% of required level). Single deficiencies of phenylalanine plus tyrosine, tryptophan, or isoleucine resulted in poorer growth (due to lower food intake) than that which occurred from a deficiency of all amino acids together. The excess amino acids over and above each single deficiency, although having variable effects on voluntary food intake, did not have negative effects on food efficiency, i.e., relative to the deficiency of all amino acids. Why certain dietary excess amino acid profiles cause food intake reductions while other profiles do not remains a mystery.

Question 11: To What Extent Does Gut Synthesis of Indispensable Amino Acids Contribute to the Amino Acid Requirements of Pigs? Torrallardona *et al.* (53) used ^{15}N and ^{14}C labeling experiments to evaluate gut amino acid biosynthesis and subsequent ileal absorption in 20-kg pigs. Amino acid absorption of microbial origin was estimated at 1.1 g/day for lysine, 2.0 g/day for leucine, 1.8 g/day for valine, and 0.8 g/day for isoleucine. These quantities are not insignificant. In fact, they exceed the estimated maintenance dietary needs for these amino acids. Thus, the true maintenance requirements for amino acids must be the sum of true ileal digestible dietary needs plus the amount provided by gut microbial synthesis. For a 20-kg pig, this would make the total Lys maintenance requirement 1.4 g/day rather than the dietary Lys maintenance requirement of 304 mg/day (54). Because the gastrointestinal tract of pigs is similar to that of humans, microbial synthesis and subsequent absorption of amino acids probably also contribute importantly to the maintenance amino acid requirement of humans (55, 56).

Question 12: Why Are 10–50% of Absorbed Amino Acids Wasted (Catabolized) When Fed to Growing Animals Well Below Required Levels for Maximal Protein Accretion? Numerous studies have now verified what might be referred to as the inefficiencies of amino acids used for protein accretion (54, 57–62). Thus, at well below required levels, amino acids recovered in whole-body protein represent only 50–90% of the amino acids fed (i.e., absorbed, because the amino acids fed are crystalline amino acids or derived from highly digestible casein). The amino acid that stands out as being the most inefficiently used is tryptophan. Over 50% of absorbed tryptophan is apparently not used for protein synthesis (i.e., it cannot be recovered in whole-body protein). Work in this area also suggests that the efficiencies of utilization for each essential amino acid are constant at all levels of intake between maintenance and ≈90% of the requirement for maximal protein accretion. The loss of (limiting) amino acids for functional protein synthesis may be related to the demands of amino acids for gluconeogenesis (63).

Question 13: Why Do Feline Species Often Die Within 24 h When Fed an Arginine-Free Diet? Felids evolved as true carnivores, and as such they have numerous nutritional idiosyncrasies (64, 65). In contrast to omnivorous mammals like dogs and pigs, cats either totally lack or have low levels of key enzymes for synthesis of vitamin A from β-carotene, arachidonic acid from linoleic acid, taurine from cysteine, niacin from tryptophan, and ornithine from glutamic acid. Unique among nutrient deficiencies (in any species), ingestion by cats of a single meal of an arginine-free diet causes severe pernicious effects, including anorexia, hyperammonemia, emesis, ataxia, and even death (66, 67). Cats have a low capacity for gut mucosal ornithine biosynthesis from glutamic acid, because of low activities of pyrroline-5-carboxylate synthase and ornithine aminotransferase. Thus, with arginine deprivation, ornithine becomes critical for the liver to take up ammonia as carbamoyl phosphate, and intestinal mucosa is the primary site of *de novo* ornithine biosynthesis.

Arginine biosynthetic capacity is very different among species. Avian species lack a mitochondrial source of carbamoyl phosphate synthase, and therefore synthesize no arginine. Other than felids, however, mammalian species synthesize enough arginine (in kidney) to meet about one-half of the requirement for maximal growth. For maintenance in adult mammalian species (other than cats), enough arginine is made from citrulline in the kidney to meet the entire requirement. Thus, adult pigs (68) and adult humans (69) do not have a dietary requirement for arginine. For growth, ornithine cannot replace arginine, but citrulline can. Oral ornithine is absorbed and taken up by the liver where arginine is indeed synthesized, but the activity of hepatic arginase is so high that virtually all of the liver arginine is catabolized to ornithine and urea. Even if ornithine of gut or liver origin could be transported to the kidney where arginase activity is low (70), kidney tissue cannot convert ornithine to citrulline due to lack of the enzyme ornithine transcarbamoylase. In contrast, oral citrulline is absorbed but not taken up by liver tissue, instead going to the kidney where net arginine synthesis takes place (70). Because enough citrulline is synthesized in gut mucosal tissue to meet the minimal maintenance need for arginine in pigs and humans, nitrogen balance is maintained, and neither hyperammonemia nor orotic aciduria occur when an arginine-free diet is fed (68–70). Whether extrahepatic tissues other than gut mucosa can produce citrulline is not known with certainty, although the small intestine likely accounts for the vast majority of circulating citrulline (71).

What about arginine for adult pregnancy? Thirty years ago, we tested this hypothesis in gravid swine, completing studies (which would not be approved by today's animal care committees) in which an arginine-free purified diet was fed throughout the entire 114 days of pregnancy (72, 73). Pregnancy outcome was not affected in terms of litter size and birth weight, nitrogen retention was normal, and no hyperammonemia or orotic aciduria occurred. Lactation performance also was normal. Because the animals used were young first-litter females that were still experiencing some maternal growth, the conclusion was that swine pregnancy, regardless of parity, does not require a dietary source of arginine (74). Human pregnancy also may require no dietary arginine, but it is unlikely this experiment will ever be done!

Question 14: Are the Estimated Protein Requirements for Humans Optimal in All Circumstances? The Dietary Reference Intake (DRI) Committee of the Food and Nutrition Board (33) has suggested a minimal protein requirement for adults of 0.66 $g \cdot kg^{-1} \cdot day^{-1}$ and a recommended intake level of 0.80 $g \cdot kg^{-1} \cdot day^{-1}$, the latter amounting to 56 g/day for a 70-kg person. Obviously, any listing of a protein requirement depends on both protein digestibility and protein quality, i.e., the ability of a protein to supply indispensable amino acids. Because obesity is a serious problem in the U.S., some have suggested that a protein intake almost double the 0.8 $g \cdot kg^{-1} \cdot day^{-1}$ intake suggested by the DRI Committee may be beneficial to weight control (75, 76). Thus, diets with reduced carbohydrates and higher protein (1.5 $g \cdot kg^{-1} \cdot day^{-1}$) may stabilize blood glucose and increase the body lean/fat ratio. The mechanism proposed is that extra protein is needed to provide branched-chain amino acids, especially leucine, for regulation of muscle protein synthesis, insulin signaling, and glucose recycling via alanine. The estimated average requirement for branched-chain amino acids (i.e., leucine, isoleucine, and valine) by the DRI Committee is 68 $mg \cdot kg^{-1} \cdot day^{-1}$ for adults, but Riazi *et al.* (77, 78) suggest that more than double this intake may be required to achieve minimal oxidation of their indicator excess amino acid, phenylalanine. The branched-chain amino acid requirement for school-age children was also found to be considerably higher than the DRI Committee estimate (79).

The requirement for protein and individual amino acids has been estimated for pregnancy of both humans (33) and swine (74). Pregnancy includes amino acid needs for maternal maintenance (and maternal growth if dams are young), growth of the products of conception (placenta and fetal tissue), and growth of the mammary gland (80). With swine, amino acid requirements have actually been estimated for each of these components at various stages of gestation (80, 81). The lysine requirement (g/day) was found to be more than twice as great during the last one-third of gestation as during the first two thirds. The NRC Committee on Swine Nutrition (74) lists both protein and amino acid requirements as being the same at all stages of gestation; this can't be correct. With swine, it is easy to calculate that feeding a single gestation diet at 2 kg/day throughout gestation results in overfeeding protein and amino acids during the first 70 days

of gestation, but underfeeding protein and amino acids during the last 44 days (80). Perhaps taking account of the greater need for protein and amino acids during the last one-third of gestation in both pigs and humans would result in better lactation performance. This has not been tested empirically, although the DRI Committee (33) has acknowledged this higher requirement for protein and amino acids in late gestation by recommending that the protein intake during the last trimester of human pregnancy be increased by 6 g/day.

Question 15: Can Maternal Diet Affect the Sex Ratio of Offspring? Rosenfeld *et al.* (82) and Rosenfeld and Roberts (83) fed (ad libitum) female mice a diet either high in saturated fat (lard) or a diet low in saturated fat but high in carbohydrates from 4 to 45 weeks of age. A total of 1,048 offspring were born from 108 pregnancies. Sex ratio of offspring was close to 1:1 for dams bred at 10 weeks of age, regardless of maternal diet. However, sex ratio of offspring for dams bred at 20, 28, or 40 weeks of age was 0.67:0.33 (male/female) for dams fed the high-fat diet. Conversely, in mature dams fed the low fat-high carbohydrate diet, the sex ratio of offspring was skewed toward females (0.39:0.61 male/female). Explanations for these fascinating observations have been proposed but not empirically tested. However, Krüger *et al.* (84), in their 30-year evaluation of sex ratio in springbok (an African antelope), suggest that sex ratio determination most likely occurs at or near the time of embryo implantation. One wonders whether sex-ratio skewing due to diet could occur in dairy cows, and if so, how long the feeding period would need to be to effect the change. Clearly, any technique, nutritional or otherwise, that would yield more female calves from gravid dairy cows would be of great benefit to the dairy industry.

Conclusion

The years ahead will see continued advances in the nutritional sciences, particularly in areas involving how nutrient levels ranging from deficient to surfeit may affect overall health, longevity, mental capacity, and gene expression. Many of the big questions in nutrition have been largely answered, but there are many problems still remaining that deserve our attention.

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