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Animal Models in Nutrition Research[1,2]

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Abstract

Current knowledge in nutrition is based largely on the use of appropriate animal models together with defined diets. Numerous examples are cited where animal models have been used to solve nutrient × nutrient interactions, to evaluate bioavailability of nutrients and nutrient precursors, and to test for nutrient tolerances and toxicities. Advantages, disadvantages, and idiosyncrasies of various animal species are discussed. J. Nutr. 138: 391–396, 2008.

Introduction

Combs (1) lists 3 key factors that were crucial in the discovery of essential nutrients: *1*) a recognition that certain diseases were associated with diet; *2*) development of suitable animal models that had a specific requirement for the nutrient in question, with subsequent use of bioassay procedures to first produce and then ameliorate 1 or more deficiency symptoms; *3*) development of defined purified diets that could be made singly deficient in the nutritional entity under investigation. Generally, the diet-disease association preceded both isolation of the nutrient per se as well as the establishment of nutrient function. Thus, the benefits of fruits for scurvy, seaweed or the ashes of sponges for goiter, and rice husks for beri-beri occurred long before defined diets were used in bioassays to fully explore the metabolic functions of vitamin C, iodine, and thiamin. Eijkman's classic work in the late 1800s with chicks fed a polished rice diet led to the discovery that beri-beri, or nutritional polyneuritis, was caused by a deficiency of what later was found to be thiamin, an essential nutrient that led to the term "vitamine" (later changed to "vitamin") being used to describe a category of (trace organic) nutrients necessary for life.

There were elements of great creativity and keen observation in the discovery of essential nutrients, but in many cases serendipity was involved as well. This was true for many of the hormone discoveries also. Research in the late 1800s that led to the discovery of insulin began with studies on the role of the pancreas in digestion (2). After removal of the pancreas of dogs, it was noticed that flies were attracted to their urine. On analysis, the urine was found to be loaded with sugar. This led to pancreatic secretions of animals being used to treat human diabetes and later (1953) to determination of the 51-amino acid sequence of bovine insulin.

The review that follows focuses on the contributions of experimental animal models to what is known today about nutrient-nutrient interactions, bioavailability of nutrients and nutrient precursors, and tolerance levels for excessive intakes of nutrients.

Nutrient-nutrient interactions

There are roughly 60 physiologically essential nutrients, and most of those that are present in foods and in the body do not exist as the nutrient per se but instead as components of proteins, fats, enzymes, coenzymes, nucleic acids, phytates, or bone components. Work with experimental animals has led to information on the structure of many of these nutrient-containing compounds. Clearly, digestive and (or) metabolic processes are required to transform these compounds into a form that the body can absorb from the gut, metabolize in the body, and use.

Animal models were used to characterize most of the classic mineral interactions. We now know that excess dietary P decreases Ca absorption, and likewise, that excess dietary Ca decreases P absorption. Also, vitamin D is required for efficient absorption of both Ca and P. The interactions between Zn and Cu; Mo and Cu; Zn and Fe; cysteine or ascorbate and Cu, Zn, or Fe; Mn and P; Se and vitamin E; I and Br; As and cysteine; phytates or oxylates and several macro- and micromineral elements have been identified and help explain the utilization efficiencies of mineral elements in various foods (3). Among the vitamins, animals were used to help us understand the tryptophan × niacin and lysine × niacin interactions (4,5) as well as the effect of Fe deficiency on the conversion efficiency of tryptophan to niacin (6). Also, progress in solving pellagra in humans was held back for many years because of lack of a suitable animal model. Ultimately, the dog (black tongue) became the best animal model for learning which foods were pellagragenic and which were ameliorative. The rat and pig were used to prove that excess dietary methionine (also *S*-methylmethionine) could spare, and even eliminate, the dietary need for preformed choline. The close functional relationship between folic acid and vitamin B-12 was also worked out using animal models, as was the establishment that both riboflavin and Zn were required to convert vitamin B-6 vitamers to pyridoxal phosphate. Before vitamin B-12 became available in crystalline form, clues as to its presence in animal or fermentation products but not plant products

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came from the "animal protein factor" work done with chickens and pigs in the period 1940 to 1950.

The many amino acid interactions/interrelationships also profited from animal research. Thus, the methionine-cysteine, phenylalanine-tyrosine, arginine-proline and glycine-serine interactions are all examples of 1 amino acid sparing the dietary (or physiological) need for another amino acid (7). In other cases, negative effects of 1 or more amino acids were shown to affect utilization of other amino acids. This is illustrated with antagonisms among the branched-chain amino acids in rats and chicks (8), lysine antagonizing arginine in chicks and dogs but not in pigs or cats (9–13), and supplemental glycine being capable of partially reversing growth depressions in chicks and rats caused by excess methionine consumption (8,14). The negative interactions among amino acids have, for the most part, not been studied in humans.

Bioavailability

There are probably no better examples of how experimental animals have contributed basic nutritional information than studies conducted on bioavailability of nutrients and nutrient precursors (3,7,15). For the most part, energy values of foods, digestibility and protein quality of proteins, absorption efficiencies of mineral salts and the minerals in foods, absorption efficiencies of the vitamin components of foods and various vitamin precursors, and amino acid utilization from various amino acid precursors, analogs, and metabolites have been derived from studies employing animal models. Today, however, stable isotope techniques are allowing more and more nutrient bioavailability studies to be done using human subjects.

Minerals. There are vast differences in the absorption efficiency of mineral elements provided either as mineral salts or as components of food (16). The halogen elements Cl, I, and F are almost completely absorbed, and Na and K are generally absorbed with over 90% efficiency. In contrast, the essential mineral elements Cr and Mn are absorbed poorly, most estimates being in the range of 2% to 4%. Phosphorus from inorganic sources is absorbed with 70–90% efficiency, depending on P status, but much of the P in plant-based foods exists as a phytate complex, and in this form the efficiency of P absorption may be as low as 10%. The P in foods existing in either phospholipid or nucleic acid form, however, is absorbed as efficiently as inorganic P (3,15). Phytic acid in foods also has negative effects on the absorption of other mineral elements such as Ca, Zn, Fe, and Mn. Oxalates in foods such as spinach have similar effects. The negative effects of phytic acid on trace-mineral bioavailability are exacerbated by excessive Ca ingestion. Reducing agents such as cysteine and ascorbate enhance the absorption of Zn and Fe but decrease the absorption of Cu and Co.

Animal studies have led the way in providing answers to questions of bioavailability of mineral supplements ingested as mineral salts (3,15). Sulfates, chlorides, phosphates, acetates, citrates, and gluconates are generally the best sources of supplemental elements, but carbonates of Ca and Zn are well utilized also. Oxides and sulfides are generally poor sources of mineral elements, although food-grade ZnO and MnO are well utilized as bioavailable sources of Zn and Mn. Oxides of Fe and Cu have relative bioavailability values (i.e., efficacy value relative to a standard such as $FeSO_4 \cdot 7H_2O$ or $CuSO_4 \cdot 5H_2O$) of near zero (3,17).

Vitamins. Bioavailability of vitamins in foods and food ingredients has received little research attention, probably because it is easy and economical to supplement diets with crystalline forms of the vitamins. Nonetheless, in poor countries of the world, crystalline vitamins are often unavailable or unaffordable, such that multiple vitamin deficiencies are not uncommon. Animal models were instrumental in solving vitamin-deficiency diseases such as beri-beri (chick thiamin deficiency), scurvy (guinea pig ascorbic acid deficiency), pellagra (dog, rat, pig, and chick deficiency of niacin and tryptophan), rickets (dog, rat, and chick deficiency of Ca, P, and/or vitamin D), night blindness (rat deficiency of vitamin A), dermatitis (rat deficiency of vitamin B-6), low fertility and muscle dystrophy (rat deficiency of vitamin E), hemorrhagic disease (chick deficiency of vitamin K), and anemia (monkey, rat, and chick deficiency of folate and/or vitamin B-12). Many of these diseases were thought initially to be infectious and of bacteriologic origin. However, when an association with diet was noted, and when this was followed by development of animal-model bioassays with defined purified diets, progress was quickly made in defining the disease condition and in reversing or preventing it with the proper vitamin-containing food or (later) with the vitamin itself.

Today we know that certain vitamins are extremely heat labile (thiamin, folate, pyridoxal forms of B-6) because of Maillard-type reactions. Also, the niacin in most cereal sources is poorly available, biotin is antagonized by avidin in raw egg white, and the biotin in wheat is not well absorbed; vitamin D bioactivity is enhanced by UV light, whereas UV light can inactivate riboflavin that is in solution; (excess) methionine can spare the requirement for preformed choline, raw fish may contain antithiamin (thiaminase) factors, and oilseed products (e.g., soybeans) may contain a substantial portion of their vitamin B-6 in glycosylated forms such as pyridoxine glucoside, a poorly bioavailable source of vitamin B-6 (18).

Among the fat-soluble vitamins, vitamin A and vitamin D have received the most attention. Although preformed vitamin A (esters of all-*trans* retinol) is found in foods of animal origin, carotenoids are the source of vitamin A activity in plant-based foods. At least 600 different carotenoids exist as yellow, orange, and red pigments in foods, but <10% of these have meaningful vitamin A bioactivity. β-Carotene is the carotenoid with the greatest vitamin A activity, but its cleavage efficiency in the gut by 15,15′-dioxygenase is very different among animal species: the chick and rat are as much as 4 times more efficient than the pig in converting β-carotene to retinol (18–20). There are 2 main factors that affect the bioavailability of β-carotene and other carotenoids as vitamin A precursors: *1*) release of carotenoids from the food matrix and *2*) efficiency of the dioxygenase enzyme in splitting all-*trans* β-carotene into 2 retinal molecules. These 2 factors, however, are affected by 3 other factors: *1*) species, *2*) nutrient status, and *3*) intake level of provitamin A carotenoids (21). In the human, it is assumed that 12 μg all-*trans* β-carotene in a mixed diet will yield 1 μg of all-*trans* retinol (22). Because various animal species absorb carotenoid compounds with greatly different efficiencies, selection of a suitable animal model for human vitamin A studies is problematic. Lee et al. (20) concluded that gerbils and preruminant calves were the best models for evaluating β-carotene conversion to vitamin A in humans. Other species, however, are more appropriate for studies of the effects of certain xanthophyll compounds (e.g., lutein, zeaxanthin) on macular degeneration and cataract formation.

Vitamin E deficiency exacerbates vitamin A deficiency in that vitamin E is necessary for cleavage of β-carotene to retinal. Vitamin E exists in foods in 8 different forms, 4 of which are tocopherols and 4 tocotrienols. D-α-Tocopherol has the highest

vitamin E bioactivity, but although β- and γ-tocopherols contain substantially lower vitamin E bioactivity values, these isomers of tocopherol nonetheless contain good antioxidant activity. The rat antisterility assay and beef cattle tissue tocopherol uptake assay have been compared with serum vitamin E uptake values in humans. The beef cattle estimates for the bioactivity of D-α-tocopherol, DL-α-tocopheryl, D-α-tocopherol acetate, and DL-α-tocopheryl acetate are more in line with the human estimates than are the rat bioactivity estimates.

There is little information on the bioavailability of vitamin D in food sources vs. that furnished via UV sunlight exposure. However, it is now known that the active form of vitamin D-3 (cholecalciferol) is actually a hormone, 1,25-dihydroxycholecalciferol. This compound is available for clinical use, and it is the most biologically active form of vitamin D. 1α-Hydroxycholecalciferol, however, is a precursor synthetic form of cholecalciferol that the liver can efficiently convert to the 1,25 hydroxylated form of cholecalciferol (23). 25-Hydroxycholecalciferol is the principal blood form of cholecalciferol, and this compound, available commercially, has more bioactivity than cholecalciferol itself but lower bioactivity than the 1α-hydroxylated forms of cholecalciferol.

Virtually nothing is known about vitamin K bioavailability in foods (18). All 3 forms of vitamin K—phylloquinones (K-1) in plants, menaquinones (K-2) in fermentation products, and menadiones (K-3), i.e., water-soluble synthetic forms of vitamin K—are bioactive. Bioavailability comparisons among the several menadione sources have been done exclusively in animal models, primarily chicks, using prothrombin time as a response criterion.

Upper tolerance levels for nutrients

There is a paucity of human information on excess ingestion of both nutrients and nonnutrients (23). Studies in animals have shown that most nutrient and drug toxicities are exacerbated when purified diets based on casein or amino acids (e.g., enteral and parenteral formulas) are consumed. This is illustrated by work in our laboratory on Cu toxicity in the young chick (24) and methotrexate toxicity in adult rats (25,26). The National Research Council has compiled information on both mineral (27) and vitamin (28) tolerances of animals, and these publications are very helpful in summarizing upper tolerance limits for these nutrients. For amino acids, FASEB issued a report in 1992 (29) that dealt with safety issues surrounding excess amino acid intakes, but it was obvious from this report as well as the 2002 Food and Nutrition Board report (30) that most of what is known about amino acid tolerance levels has come from animal research.

The safety margin for excess consumption of vitamins is large, even for vitamin A, which is considered perhaps the most dangerous of the vitamin excesses. Animal studies have predominated over human studies on vitamin excesses, with 2 notable exceptions: niacin and vitamin C. Because pharmacologic niacin doses of 1 to 3 g/d, 67 to 200 times the adult requirement, are used clinically to treat hypercholesterolemia and schizophrenia, the human literature is extensive on side effects (e.g., flushing) of excess niacin ingestion (23,28,31). In terms of growth depression in chicks, dietary levels of niacinamide above 5000 mg/kg cause a linear decrease in weight gain, but niacin levels as high as 20,000 mg/kg (600 mg/d; 3 $g \cdot kgBW^{-1} \cdot d^{-1}$) are without effect (32). Regarding vitamin C, with the exception of guinea pigs, apes, and a few birds and fish, animals do not require a dietary source of ascorbic acid. Thus, human studies of vitamin C excess are as prevalent as animal studies (28).

With mineral excesses, there is concern about excess Se and F among the essential mineral elements. Also, there is extensive animal literature on excess consumption of nonnutrient elements such as Cd, Hg, Pb, and As (27). The chick has been particularly helpful as an animal model for the Cu toxicity problems associated with Wilson's Disease and Indian Childhood Cirrhosis. Supplements of L-cysteine, L-ascorbate, and Zn are used by hepatologists to treat these Cu toxicity diseases. Chick work has shown that L-cysteine is more effective than either Zn or L-ascorbate in ameliorating Cu toxicity; also, cysteine is more effective than either cystine or methionine (33). The chick was also used to define I toxicity and its reversal by administration of supplemental Br (34,35). Both rats and humans have been used to evaluate pharmacologic doses of Se for cancer prevention (36).

There is keen interest in the potential benefits of consuming amino acid supplements at levels beyond nutritional requirements (37). Unfortunately, little evidence exists for tolerable upper amino acid limits in humans (29,30). Most of what is known about pharmacologic amino acid dosing comes from studies in animals. It is clear from these studies that individual amino acid excesses are more anorexigenic when provided in low-protein than in high-protein diets (8,38,39). Most of this early work was done with rats fed casein-based purified diets. Use of diets containing mixed ingredients and with normal protein levels is probably more relevant in terms of extrapolation to humans. Our work with chicks and pigs, therefore, involved individual amino acid excesses in diets containing 20% to 23% protein from corn and soybean meal (11,12,14,40–43). This work showed that a large dietary excess (4 g/100 g) of methionine, cysteine, or tryptophan was more growth depressing than the same excess of other amino acids. Also, branched-chain amino acids were better tolerated than excesses of other amino acids.

Species comparisons

Animal research has contributed heavily to what we know today about nutrition and metabolism. In recent years, the young pig has come into particular prominence as an animal model, i.e., for studies of amino acid metabolism (9,44), total parenteral nutrition (45–48), rotavirus infection (49), and bacterial and viral pneumonia (50). Other than monkeys, pigs are thought to be the most nearly like humans of any animal model (51,52). Among the animal species that have contributed useful nutrition information, many exhibit well-documented differences in how they use, metabolize, and excrete nutrients. Some species-specific features are listed below:

1. Doubling of starting body weight is generally considered a minimum requirement for growth bioassays with young animals. The time required to accomplish this doubling varies greatly among species: 3 d in chicks, 7 d in rats, 14 d in mice, 20 d in pigs and puppies, 50 d in kittens, and 5 y in 6-y-old children.
2. Protein accretion per se dominates the amino acid requirements of growing mammals and avians, but the slower growth rate of children results in maintenance dominating the amino acid requirements of children.
3. Humans generally give birth to a single offspring, whereas rodents, pigs, dogs, cats, and rabbits are multiparious.
4. Rodents and chicks are "nibblers," whereas pigs, dogs, and humans are "meal eaters."
5. Nutrient losses in sweat (Na) and menstruation (Fe) occur in humans, but these losses are of little consequence in rats, pigs, chicks, and dogs.

6. Consumption of gestation diets very low in protein concentration, or of poor protein quality, has little effect on pregnancy outcome in swine, but it results in either abortion or very poor pregnancy outcome in rats (53–56).
7. Animals have the advantage of allowing invasive tissue sampling to assess nutrient status, but humans have the advantage of reporting how they "feel" when nutrient deficiencies or toxicities are imposed. However, monitoring compliance with dietary protocols is easy with animals but difficult with humans.
8. Adipose tissue is the main site of fatty acid biosynthesis in pigs and ruminant animals, but adipose tissue and liver are equally important as sites in rats and rabbits. Lipogenesis in chicks occurs mainly in the liver; the site in humans is controversial, although the liver is known to be a significant site (57).
9. The rate of gluconeogenesis in nonruminant animals is lowest after feeding and highest during an energy deficit; in ruminant animals the rate of gluconeogenesis is highest after feeding (57).
10. Because of rumen fermentation, ruminant animals derive far more energy from fiber and volatile fatty acids than nonruminant animals; likewise, they obtain far more of their amino acid needs from nonprotein nitrogen and ammonia (microbial protein synthesis) than nonruminant animals.
11. Rodents and rabbits practice coprophagy, whereas pigs, chicks, and humans do not.
12. Pigs and humans obtain usable energy from fermentable fiber, but chicks do not.
13. Most mammals use sucrose (or fructose) poorly during the neonatal period, but young chicks and neonatal humans use sucrose efficiently (58).
14. Mammalian neonates use lactose efficiently, but avians use both lactose and galactose poorly (59).
15. Vast differences exist among species in their ability to absorb β-carotene and other carotenoids intact (19,20).
16. Chicks and rats convert β-carotene to vitamin A more efficiently than pigs (18,19).
17. Avian species excrete urine and feces together, whereas mammalian species excrete them separately; avians also excrete uric acid as an end product of nitrogen metabolism, whereas mammals excrete urea. Most fish species excrete ammonia via the gills as an end product of nitrogen metabolism.
18. Avian species do not have a mitochondrial source of carbamoyl phosphate synthetase and therefore, unlike mammals, have no net arginine biosynthesis. Several fish species are similar to avians in this regard (9).
19. Pigs and rats use D-tryptophan as effectively as L-tryptophan; chicks, dogs, mice, and humans use D-tryptophan poorly (7).
20. Virtually all animal species use D-methionine almost as effectively as L-methionine, but apes and humans cannot invert D-methionine to L-methionine efficiently (7).
21. Excess dietary methionine can eliminate the need for dietary preformed choline in mammals but not in avians (15,60).
22. Regular cornstarch causes diarrhea when included in diets for puppies (it must be pregelatinized or extruded), but this does not occur when cornstarch is fed to rats, chicks, pigs, or kittens.
23. Chicks, rats, and mice respond more rapidly to vitamin and mineral deficiencies than pigs or humans.
24. Placental transfer of Fe from dam to fetus during late pregnancy in humans is much more efficient than that occurring in late pregnancy of swine (61).
25. Both glycine (or serine) and proline are considered dietary essential amino acids for avians but not for mammals (62,63).
26. Modest excesses of dietary L-cysteine (but not L-cystine) are lethal when included in diets for chicks but not for rats or pigs (43).
27. Hydroxy and keto analogs of amino acids are utilized more efficiently by chicks than by rats; the efficiency with which these amino acid precursors are utilized by humans is not known (7).
28. Excess dietary lysine antagonizes arginine in chicks, rats, and dogs but not in pigs or cats (8–13).
29. Chicks receive (some) endogenous nutrition (from the yolk sac) during the first week of life; mammals receive early nutrition from suckling the dam.
30. Chicks do not have a swallowing reflex, and they have a much shorter intestinal tract than rats, pigs, or humans; chicks also do not have a pyloric sphincter. Thus, rate of food passage through the gut is much faster in chicks than in rats, pigs, or humans.
31. Sea water consumption results in negative water balance in virtually all mammalian and avian species but not in feline species (64).
32. Feline species, including the domestic cat, evolved as strict carnivores. As such, their nutritional idiosyncrasies are legend: inability to effectively convert tryptophan to niacin, β-carotene to vitamin A, linoleic acid to arachidonic acid, cysteine to taurine, and glutamate in the gut to either ornithine or citrulline (65–67).
33. Cats develop severe hyperammonemia and often die within 24 h of consuming 1 or more meals of an arginine-free diet; no other nutrient void in any species causes death this quickly (9,65–67).
34. "Chemical" diabetes occurs in mammals, but not birds, treated with either alloxan or streptozotocin (68).
35. Recent estimates of the human adult requirements for methionine plus cyst(e)ine and threonine based on oxidation methodology are roughly 50% of the estimated lysine requirement (30), whereas adult pig requirements for methionine plus cyst(e)ine and threonine exceed the requirement for lysine (69). Some have questioned whether either the human or pig requirements may be wrong (9,69).
36. Phytate phosphorus utilization is improved markedly by addition of 1α-hydroxylated cholecalciferol compounds to chick diets containing surfeit vitamin cholecalciferol (70,71), but neither pigs nor laying hens respond in a similar fashion (72,73).
37. In terms of amino acid limitations, a casein diet for rats, mice, and pigs is first limiting and singly deficient in cyst(e)ine; a casein diet for chicks is first limiting in arginine and second limiting in cyst(e)ine (74).
38. The Ca requirement is 5 times higher for egg-laying hens than for growing avians and mammals.

It is obvious from this (incomplete) list of species differences that it is important to choose the right animal model for predictions of what might happen in humans. Other considerations include availability of facilities and cost of the experiments to be performed. Clearly, research with animal models has been valuable in advancing our knowledge of nutrition. The first 50 y of the 20th century might be thought of as the qualitative era of nutrition wherein most of the essential nutrients and their functions were discovered. The last 50 y could be thought of as the quantitative era, a time when nutrient requirements,

nutrient-nutrient interactions, and pharmacologic aspects of nutrients were the focus. A 2006 Experimental Biology (History of Nutrition) symposium provided excellent reviews of how research with food animals has contributed to our knowledge of nutrition concepts and principles in energetics (75), carbohydrates and lipids (57), proteins (76), and body composition and growth (77).

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