ANIMAL MODELS OF HUMAN NUTRITION

Experimentation with animals has been fundamental to human nutrition knowledge. By using small animals, researchers can economically control experimental diets to determine the biological effects of dietary constituents. Studies with small animals fed controlled diets led to the discovery of most of the vitamins and essential trace minerals. Because of animal research, nutrient deficiency diseases of humans have been eradicated in many countries. Animals continue to contribute to our knowledge about diet and biological function. Animal research in the future should help us understand how diet affects disease, reproduction, brain function and longevity.

Advantages of using animals in nutrition research include: (1) large groups can be studied economically; (2) dietary consumption and living conditions can be carefully controlled; (3) short life-spans improve the feasibility and efficiency of developmental, longevity or
multiple generation studies; and (4) controlled breeding reduces biological variability. The use of animals in food and nutrition research protects human safety. If dietary deficiencies or imbalances are suspected of causing disease in humans, these hypotheses should be tested in controlled studies with animals. Likewise, new dietary additives should be tested in animals to protect human safety. Although animal research is indispensable for our expanding knowledge about diet and health, investigators must weigh the benefits of such knowledge against the discomfort of animals in research. New knowledge for the health of our own and possibly other species should be obtained with an ethical responsibility for the humane treatment of experimental animals.

History

Since earliest times, human have recognized that they share with animals a basic need for food and water. Accordingly, the history of nutrition is replete with examples of animal experimentation. Erasistratus (310–250 bc), tested his ideas that pneuma in the atmosphere became spirit in the body. He placed fowls in a jar and weighed them and their excreta before and after food. Galen (130–200 AD) studied digestion in hogs and concluded that in the stomach foods were reduced to smaller particles that could later be absorbed.

In 1774, Joseph Priestley (1733–1804) exposed mercuric oxide to focused sunlight to produce ‘pure dephlogisticated air’ (oxygen). He demonstrated that this air caused a candle flame to burn brighter and that mice could live in it.

Guinea pigs were employed by Crawford, Lavoisier and Laplace, and by Despretz in the late eighteenth and early nineteenth centuries to study respiration and body heat production. Lavoisier and Laplace invented an ice calorimeter and compared the amount of ice melted by a guinea pig with the amount of carbon dioxide produced. In addition to using guinea pigs, Lavoisier measured his own respiratory exchanges and those of his friends and assistants. These studies led to the conclusion that respiration was a combustion of carbon and hydrogen which transformed oxygen into carbon dioxide, similar to the burning of a candle or an oil lamp.

Spallanzani (1729–1799) first obtained gastric juice from the stomach of a hawk by using a sponge and string. He demonstrated that it dissolved flesh, bones and bread without putrefaction.

Boussingault performed the first balance study in 1839 on a cow, by measuring carbon, hydrogen, oxygen, nitrogen and salts in the feed, urine, faeces and milk. Later, he performed similar experiments with a horse and a turtle dove.

In 1849, Regnault and Reiset used rabbits, dogs and fowl to determine the effect of various foods on the ratio of carbon dioxide produced to oxygen inspired, later called the ‘respiratory quotient’. The animals were in a 45-l bell jar which was part of a sealed, closed-circuit apparatus.

Dogs were used extensively in the last half of the nineteenth century by German investigators studying protein and energy metabolism. Voit (1831–1908) demonstrated ‘nitrogen equilibrium’, and an increase from negative to positive nitrogen balance as dogs were fed greater amounts of meat. He also discovered that muscular work by the dog did not increase protein metabolism. Rubner (1854–1932), a student of Voit, employed dogs to show that similar measurements of energy utilization could be obtained by direct and indirect calorimetry, and he measured the ‘specific dynamic action’ (increased heat production) associated with protein consumption by studying a dog fed meat. From his measurements of metabolic rates of various species, including humans, Rubner developed his surface area law, which stated that the basal metabolism is proportional to the surface area of the animal.

Animals played an indispensable role in the discovery of the vitamins. In 1897, Eijkman, a Dutch physician in Java, reported that chickens fed polished rice developed a paralysis. This paralysis was similar to that of the disease beriberi in humans. Unmilled rice or a water extract of rice polishings cured the chickens. Hölst, a Norwegian, repeated Eijkman’s experiments, and obtained similar results with pigeons. However, he and Frölich reported in 1907 that guinea pigs fed a cereal diet developed scurvy, rather than the paralysis of beriberi. This fortunate discovery – of one of the few species in addition to humans that require ascorbic acid – soon led to a biological assay for the antiscorbutic potency of foodstuffs.

In 1906, Hopkins reported from the University of Cambridge, UK, that mice required the amino acid tryptophan. This initiated the concept of essential amino acids and differences in the biological value of proteins. Hopkins studied both mice and rats and reported in 1912 that animals could not live on purified protein, fat, carbohydrates, and minerals alone; other unknown nutrients were essential.

In 1907, at the University of Wisconsin, USA, McCollum set out to identify what was lacking in purified diets that did not nourish small animals. Frustrated by tedious studies with cows fed various grains, he recognized the advantages of studying animals which have a short lifespan and eat little. Smaller dietary volumes would make dietary control and analysis both practical and economical. McCollum decided to use rats because of their convenient size, omnivorous feeding habits, and lack of economic value. Within 5 years, McCollum and Davis reported a fat-soluble factor (later named vitamin A) in butterfat that was not present in lard or olive oil. Vitamin A deficiency was later found to be a major cause of human blindness.
In the UK, Mellanby used dogs, known to be susceptible to rickets, to show that the disease resulted from a dietary deficiency. Incidental to this research, Mellanby discovered that nitrogen trichloride bleaching of wheat flour caused canine hysteria, a nervous disorder that also occurred in ferrets, cats and rabbits. Rats, which had been used in safety testing, were less sensitive to the compound. Commercial use of nitrogen trichloride in flour for human consumption was discontinued after Mellanby’s discovery.

McCullum used rats to differentiate between the effects of vitamins A and D. He developed the ‘line test’, a measure of the width of a line of new calcification in the bone of a deficient rat given vitamin D. This became a widespread biological assay for vitamin D in foods.

Rats were the principal animal used to discover most of the vitamins, the essential trace elements, and the essential amino acids. As a result, more is known about the nutritional requirements of the rat than about any other species. Dogs, chickens, pigeons, mice and guinea pigs have also made valuable contributions to our knowledge of nutrition.

Animals as Models of Human Nutrition in Research

Factors considered when selecting an animal model include anatomical, physiological and biochemical similarities to humans, similarities of disease processes, susceptibility to aetiological agents, availability, cost, breeding lines available, behaviour, size, genetic characteristics, lifespan, resistance to infections, suitability for experimental manipulation, and accumulated pathobiological information. Considerations particularly pertinent to nutrition research are as follows.

Natural Diet in Habitat

The omnivorous feeding patterns of the rat usually make it a better model for human nutrition questions than a strict herbivore such as the rabbit. The natural diets of nonhuman primates vary considerably. Primates include insectivores, herbivores, and omnivores, which makes some primate species poor models for human nutrition research. Strict carnivores such as cats have associated differences in nutrient metabolism and requirements. For instance, the cat requires arachidonic acid and preformed vitamin A from animal foods, as it cannot synthesize those from the respective plant-food precursors, linoleic acid and β-carotene.

Nutrient Requirements

The requirement for vitamin C is a striking example of qualitative differences between species; few species require it in the diet. Humans share this uncommon requirement with other primates, the guinea pig, the red-vented bulbul, and some Indian fruit-eating bats.

Dietary fibre may provide a more subtle example of species differences in nutrient requirements. Two herbivores, the guinea pig and the rabbit, require dietary fibre, according to the US National Research Council (NRC). Other laboratory animals are apparently not as vulnerable to a fibre deficit. However, fibre is routinely added to research diets for rodents, and continued research may reveal reduced gastrointestinal diseases associated with fibre in the diets of primates.

Quantitative comparison of nutrient requirements across species is difficult: limited research on some species has yielded knowledge of adequate intakes without determination of minimum requirements. Differences in vitamin requirements between species are influenced by differences in production by intestinal flora. The mineral requirements of most species are quite similar, with the possible exception of a higher iron requirement of primates.

Digestion and Absorption

The relative influence of gastrointestinal microorganisms on metabolism of digesta varies widely between species. Extensive fermentation in the pregastric chambers of ruminants and the hindgut of the horse makes these animals poor models for human nutrition. Dogs and pigs are similar to humans in many aspects of gastrointestinal morphology and physiology, including relative length of small and large bowels, ingesta transit times, influence of dietary factors on gastric emptying, glucose or xylose absorption, faecal fat excretion, activities of intestinal brush border and pancreatic enzymes at maturity, and colonic volatile fatty acid concentration. However, humans, dogs and pigs differ in the developmental patterns and primary structure of some digestive enzymes. The composition of bile and the structure of bile acids differs substantially between humans and pigs. Colonic volatile fatty acids may be an important energy source for the pig, but not for the dog; the importance of colonic fermentation for humans probably falls between these two species. Numerous strains of miniature pigs have been developed and used in experiments related to digestion and absorption.

Body Composition, Nutrient Metabolism, and Excretion

Species differences in chemical composition may influence the suitability of animals as models for human nutrition problems. As an example, differences in skeletal mass may reduce the usefulness of animal
models for bone health and related nutrients. The skeleton comprises about 16% of the adult human body, as compared to 11% of the rat, 10% of the rabbit, and 7.7% of a 28-week-old pig. This probably accounts for considerably higher amounts of calcium and phosphorus per kilogram of fat-free body tissue in humans than in pigs, cats, rabbits, rats, or mice. Human cortical bones have greater nitrogen and lower calcium: nitrogen ratios than those of pigs, cats, rabbits, rats, and fowl. Differences in nutrient excretion may complicate animal modelling further. For example, the rat excretes less than 1% of dietary calcium in the urine, which is an important route for calcium excretion (and its hormonal control) in humans. These examples may help explain why there has not been a satisfactory animal model for human osteoporosis.

Coprophagy (the ingestion of faeces) by rabbits and rodents can confound dietary intake measurements in nutrition studies. By increasing the total nutrient intake, coprophagy can lower apparent requirements and reduce signs of deficiency for many nutrients. Particularly affected are those nutrients synthesized or made more bioavailable through colonic microbial flora, including the B-complex vitamins and essential fatty acids. To study signs of deficiency for such nutrients, steps must be taken to prevent coprophagy.

Reproduction

Animal studies have demonstrated vital roles for nutrition from oestrus to parturition and lactation. For example, animal studies have shown the essentiality of zinc for normal oestrus cycling and fertility, development of preimplantation eggs, normal development of organ systems (especially the skeletal and central nervous systems), fetal growth, and normal labour and delivery. Zinc deficiency during specific stages of gestation causes postnatal abnormalities in behaviour and immune function.

Special considerations when evaluating animals as models of human reproduction include the number of fetuses, the type of placentation, the tendency to abort or reabsorb fetuses under teratological conditions, the rate of development, and the maturity of the fetus at delivery. Maturity at birth can determine the suitability of an animal model for studies of late gestational development. For example, epiphyseal calcification of the femur is much greater in newborn piglets than in newborn humans, and pigs walk soon after birth. This would limit the usefulness of the pig in some studies of prenatal bone development. Maturation at birth is also a concern when selecting animals to study early infant development. Another difficulty is that newborn mammals often require maternal lactation, limiting the investigator’s control of dietary variables in early life.

Animal Models of Nutrient Evaluation

Because living organisms are always used in research determining the essentiality of nutrients for life, they naturally provide the earliest means of evaluating foods as a source of nutrients. Biological assays for vitamins in foods were mentioned previously. Historically, animals were useful for food evaluation because chemical techniques were inadequate for direct analysis of foods. While chemical analysis difficulties have been largely overcome, difficulties predicting nutrient bioavailability have not.

Bioavailability is the proportion of a nutrient in food which is absorbed and utilized. It is affected by chemical form, interactions with other food components, and, probably, physiological responses to food. For example, the zinc bioavailability of foods fed to rats depends on the amount of food fed, even though the foods are compared in amounts providing similar quantities of zinc. Pancreatic secretion responds to quantitative and qualitative differences in foods. Zinc is a cofactor of pancreatic carboxypeptidase, and perhaps the reabsorption of endogenous zinc from pancreatic secretions competes with absorption of dietary zinc. Zinc absorption mechanisms may also involve coabsorption with ligands such as histidine which have separate facilitated pathways of absorption. In situro measurements of zinc absorption are generally static, involving measurements of food composition or physicochemical properties in a gastrointestinal simulation. Until the dynamic nature of the gastrointestinal response to differing foods in varying quantities is understood, accurate in vitro simulation may not be possible. See Bioavailability of Nutrients

For investigating the bioavailability of some nutrients, even animal models may not be sufficient. For example, the haem form of iron present in foods of animal origin is much better absorbed by humans than ionic ferrous iron. This is not true for rats. Rats deficient in iron apparently absorb haem iron less efficiently than ferrous iron.

Food bioavailability is poorly understood for many nutrients, including the extensively researched macro-nutrient protein. The nutritional quality of dietary proteins has been evaluated since as early as 1917 by measures of animal growth or nitrogen retention. Despite attempts since the mid-1940s to replace the biological assays with a ‘chemical score’ of amino acid composition, the biological assays continue to be used extensively. Chemical scoring accounts for essential amino acid composition and requirements. However, scoring does not account for amino acid bioavailability, which can be reduced by chemical changes during heating, or by inhibitors of proteolytic digestion.

In vitro testing has progressed substantially and is often preferred by scientists for efficient use of resources. However, the complexity of living organisms remains
incompletely understood, and the nutritional value of foods or diets is frequently more accurately evaluated by direct in vivo testing than by modern, highly technological in vitro testing.

Animal Models of Disease States

With the help of animal models, a major cause of human disease – overt nutrient deficiency – was largely solved as a scientific problem (it unfortunately remains as a political and economic problem). Scientific emphasis has turned to diet and chronic disease. Perhaps because they are as yet unresolved, the problems of chronic disease seem more complex. Dietary variables are likely to be slight deficiencies, excesses, or imbalances in a lifelong interaction with other genetic and environmental factors. Animal models can provide the advantage of controlled environmental and dietary factors over a short lifetime. However, genetic differences, although confirming inheritability, limit application to humans. The use of animals to study nutrition and major chronic diseases is considered briefly below.

Atherosclerotic Cardiovascular Disease

The responsiveness of serum cholesterol and atherosclerosis to dietary cholesterol and saturated fatty acids varies among animal species. Rabbits, guinea pigs, swine, rhesus and cynomolgus monkeys are more susceptible than humans. Rats and dogs are more resistant. Baboons and vervet monkeys are in the same moderate range of susceptibility as humans. Genetic differences in lipid metabolism are confirmed by the variation observed within and between species. See Atherosclerosis.

Hypertension

Hypertension in response to dietary salt is not observed in all animal models but occurs in specific animal strains without sufficient renal capacity to excrete salt rapidly. The Dahl S and the Kyoto spontaneously hypertensive rat are susceptible to dietary salt, and the addition of potassium to high-salt diets reduces the incidence of stroke in these animals, and in the Sprague-Dawley rat. Research on animal models verifies an interaction between diet and genetic susceptibility to hypertension, and susceptible animals may be useful in identifying dietary variables that affect susceptible humans. See Hypertension. Physiology; Hypertension, Hypertension and Diet.

Obesity

Genetically obese strains of rodents – obese (ob/ob) and diabetic (db/db) mice and obese (fa/ fa) rats – confirm that inheritance can contribute to obesity. Obesity in such models has been associated with hyperinsulinemia and abnormal glucose tolerance. Hyperphagia also occurs, but pair-feeding studies indicate that genetically obese rodents have an unusually high body fat composition even with normal diets. Rats without genetic obesity increase their food intake and thermogenesis in response to a ‘cafeteria’ diet of mixed human food. The relationship between energy intake, physical activity, thermogenesis and bodyweight has been extensively studied in rodents. Results of these studies generally support the biological maintenance of a ‘set-point’ for bodyweight; this varies only slightly and controls energy balance. See Obesity, Aetiology and Assessment.

Cancer

Approximately one third of human cancer mortality may be related to diet. Animal studies are useful in the investigation of specific dietary components and the mechanism of action. Animal studies, mostly using rats, have indicated that tumour development is enhanced in a variety of tissues by high-fat diets, especially diets rich in ω-6 (but not ω-3) polyunsaturated fatty acids. This effect of high-fat diets has not been completely differentiated from the effect of high energy consumption. Retinoids can prevent cancer at several sites in laboratory animals. However, research has focused on new synthetic retinoid compounds because the naturally occurring retinoids are commonly toxic at doses necessary to inhibit carcinogenesis. Animal models have shown that selenium inhibits both the initiation and proliferative phases of tumorigenesis. The effect of some dietary components may depend on the specific site, type and stage of carcinogenesis. The effects of specific nutrients may depend on the relative nutrient requirements of host and tumour. Zinc deficiency in animals increases the incidence of oesophageal carcinoma induced by methylbenzylaminoamine, but decreases the incidence of tumours induced by 3-methylcholanthrene and 4-nitroquinoline-N-oxide. See Cancer, Epidemiology; Cancer, Diet in Cancer Prevention; Cancer, Diet in Cancer Treatment.

Testing food additives for carcinogenicity requires animal models. Careful studies generally involve more than one species and exposure over an extended portion of the lifespan. However, the evaluation of research results must consider whether dietary characteristics have been experimentally exaggerated, and whether extrapolation to humans is realistic. A US food additive law, known as the Delancy Clause, prohibits the use of a food additive in any amount if it has been shown to produce cancer in animal studies or in other appropriate tests. This law has been controversial because it can be applied to prohibit any amount of a substance that may
have been tested in high amounts. Carcinogenicity of dietary constituents should be evaluated with consideration of average and peak human exposures to the substance, the potency of the substance, and the quality of experimental data.

Osteoporosis

Diets low in calcium or high in phosphorus increase bone reabsorption and decrease bone mass in rats, mice, cats, dogs and nonhuman primates. In contrast with experimental animals, calcium balance in humans is relatively insensitive to high dietary phosphorus. Animal research related to bone health has too often involved animals that were young and growing or animals that were very old. There are few animals that experience the spontaneous ovarian failure in middle age that occurs in humans. Researchers have not identified an appropriate animal model of postmenopausal or high-related osteoporosis (see Body Composition, Nutrient Metabolism, and Excretion, above). See Osteoporosis

Diabetes Mellitus

Experimental diabetes can be induced in animals by pancreatectomy or injection of β-cell toxins such as streptozotocin or alloxan. There is a genetically obese strain of diabetic (db/db) mice. Unlike most rodents, the sand rat remains lean and nondiabetic in the wild, but becomes obese and diabetic under laboratory feeding conditions. In normal strains of animals, greatly increased food intake for an extended time leads to adiposity and an increased incidence of insulin resistance, which is reversible with weight reduction. This insulin resistance is confounded by both adiposity and ageing. It has not been possible with animal research to show that high energy consumption causes diabetes.

Interpretation and Extrapolation of Data

Animal studies are most appropriately applied to humans when they complement and are consistent with human studies. Highly controlled experimental trials in animals can confirm epidemiological associations between diet and health in humans. Short-term experimental results in humans can be compared for similarity to short-term results in animals, and then continued to determine longitudinal effects in animals. As with all scientific research, reliable conclusions should be reproducible in different laboratories and supported by various methods. Relevance of animal research to humans is more likely if the results are confirmed in several animal species.

The highly uniform experimental conditions charac-

teristic of animal models can provide disadvantages as well as advantages. For example, energy restriction reproducibly increases longevity in rodents. However, such studies were usually conducted under laboratory conditions in which the rodents spent their entire lives in a small space; they formed no social relationships, did not reproduce, experienced low exposure to hazardous biological or chemical substances and sunlight, and had little need for physical activity or development of self-preservation skills. Scientists must evaluate whether the food-restricted animals would have an improved quality or even quantity of life if tested under more realistic living conditions.

The control of experimental diets in animal nutrition studies can present similar problems in extrapolation to humans. To enhance experimental control and comparability between studies, animal diets for nutrition research are often standardized, purified, and fed ad libitum. Purified diets are commonly composed of a small number of refined ingredients, such as commercially refined proteins, carbohydrates and fat, with added vitamin and mineral mixtures. While such diets allow a controlled supply of specific nutritional variables, they also limit the context of the experiment. Unlike many animal research diets, human diets contain thousands of compounds and are usually scheduled in meals. Metabolic adaptation occurs in response to eating schedules and to specific dietary components, but this is not well defined for most dietary constituents. As nutrition research moves from single nutrient deficiencies to more complex dietary interactions that affect health, laboratory diets and other conditions may need to become more like human conditions to facilitate comparability to humans. An example is the ‘cafeteria’ diet of mixed human food used to attain overeating in rodents.

Animal studies are often most valuable for initial nutritional studies preparatory to human studies and for studies of basic biological mechanisms. Animal models are often helpful in evaluating dose–response relationships. Some biological responses to dietary variables are more credible when an increased exposure results in an increased response. Essential nutrients can often be characterized by biological responses that are linear below requirements and possibly above safe amounts, but stable at intermediate intakes that do not overpower homeostatic adaptation. Quantitative relationships between dietary amounts and biological responses should also be tested in humans to determine realistic exposures and outcomes.

Limitations of Animal Models in Nutrition Research

Nutritional knowledge has rapidly expanded in the nineteenth and twentieth centuries. During this time, an
increasing share of biomedical advances depended on animal research. Some 67–75% of major biomedical advances in the 1900s required the use of animals. This trend was accompanied by an antivivisectionist movement in Victorian England in the late 1800s, which may be a precursor of the current ‘animal rights/liberation’ movement. This movement has raised ethical questions about animal research. The movement appropriately emphasizes the value of humane treatment of animals but inappropriately harms humanity when it prevents animal research and the resulting knowledge about health. Most people recognize the inconsistency of vandalism and terrorism in the name of animal rights. A more insidious threat to research is the reduction of resources through excessive regulation and bureaucracy. High-quality animal care helps both research results—and the researcher—by engendering public trust.

The US NRC's Committee on the Use of Laboratory Animals in Biomedical and Behavioral Research concluded in 1988 that animals should be used when research with animals is the best available method to improve the human condition. The committee also recognized the ethical obligation of scientists to ensure animal wellbeing and minimize pain and suffering through humane treatment. Scientists realize that they must be involved in public policy influencing legal regulations that benefit humans through science, while treating animals humanely and without inefficient bureaucracy.

One suggested means of minimizing animal pain and suffering has been for researchers to consider possible alternative methods. This may include differentiation among species; public opinion prefers the experimental use of rodents over the use of dogs, cats, and monkeys. Most nutrition research has been conducted with rodents, especially rats. However, as societal preferences influence researchers to use nonmammalian vertebrates, invertebrates, and microorganisms, the valid extrapolation of results to human nutrition becomes more difficult. Some nutrition knowledge may be gained by using cell and tissue cultures, human tissues removed at surgery or at autopsy, in vitro systems and mathematical models. Such methods can be more efficient, controlled and economical than research with living animals. However, researchers must be cautious not to extrapolate to human nutrition without adequate validation of the simpler model.

A straightforward alternative to using animals is to study humans directly. Although the technology for safe study of humans continues to improve, many nutrition research topics require animal models. Such topics include the effects of nutrition on reproduction, growth and development, longevity, and behaviour. Animal experiments can verify observations in humans that often cannot be controlled sufficiently to be conclusive. Use of animals for initial experiments in nutrition research improves efficiency and the rate of progress in knowledge. Animal studies facilitate the investigation of physiological and molecular mechanisms. Animal research has been instrumental in identifying required nutrients and developing the science of nutrition. For the foreseeable future animal research will be necessary to answer questions about nutrition and health that can be learned only from living organisms.

Bibliography


Janet R Hunt
US Department of Agriculture Human Nutrition Research Center, Grand Forks, USA