Use of Laboratory Studies for the Design, Explanation, and Validation of Human Micronutrient Intervention Studies1–3

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Abstract

Many micronutrient supplementation trials have led to important new findings relevant to public health, but some outcomes have been unclear or concerning. Can and should laboratory studies and animal models be used more extensively to pretest the proposed designs of human studies? This paper illustrates, as examples, the contributions that animal models have made to several major advances in understanding the biology of the micronutrients vitamin A and carotenoids, and it proposes that animal studies can play a more integrated role in public health nutrition by serving as a first line of interrogation for study designs and thereby as a means of refining the designs of human studies so that large, expensive, and logistically difficult human trials will yield the best possible information. J. Nutr. 142: 157S–160S, 2012.

Introduction

It is an accepted expectation that human studies of micronutrient supplementation will be based on the soundest of preliminary evidence. Yet several recent public health trials involving micronutrient supplementation have provided unexpected results that have raised concerns and suggest that additional precaution, and greater preliminary evidence, may be warranted. This article considers the role that animal models have played in micronutrient research, using vitamin A and β-carotene as case studies, and asks the question whether animal studies should play a stronger, more integrated role in public health research. For many decades, animal models have led the way in understanding the basic functions of micronutrients. Now, in an era of great interest in micronutrient supplementation for improving public health, and especially of high-dose supplementation in infants and young children at risk for nutritional deficiencies, what role should laboratory animal models play? Could such models be better utilized to guide the design of future human studies and help to explain unexpected results when they are found in human clinical trials? There are no ready answers to these questions, but their consideration is important in going forward to conduct the best possible public health nutrition research in the future.

Animal Models in Micronutrient Discovery: Vitamin A as an Example

Animal models have contributed greatly to the understanding of essentially all micronutrients. Vitamin A is briefly considered here as an example in which animal studies led the way in elucidating several critical mechanisms. In the early 1900s, the first signs of a nutritional deficiency of this previously unknown nutrient were failure to grow and survive, while ocular abnormalities were also noted (1,2); these were corrected by a lipid extract of “fat-soluble A.” Soon, a connection was made between this newly discovered dietary factor and the long-known human condition of xerophthalmia, seen in children and adults (3) [see (4,5) for reviews]. Unequivocal evidence that vitamin A is required for vision, growth, and survival was obtained through the elucidation of the chemical structure of vitamin A, all-trans-retinol, and its synthesis in the 1930s. By testing the repletion effects of the pure synthetic compound in animals and then in clinical applications, the connections between retinol, growth, and xerophthalmia were clearly

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established. This did not mean, however, that xerophthalmia was rapidly eradicated, because the educational and public health efforts to combat xerophthalmia took many additional years (4). Similarly, research now being conducted to understand the role of vitamin A and carotenoids in reducing the severity of infectious diseases also can be traced back to studies with animals first reported in the late 1920s (6,7). Today, both mechanistic animal studies and clinical trials are going on simultaneously.

More than a decade after the first synthesis of retinol, all-trans-retinoic acid (RA) was identified as the active carboxylic acid form of vitamin A and then synthesized (8), and studies in vitamin A-depleted rats treated with RA showed that RA could support essentially all of the monocular functions attributed to vitamin A (9). Other early discoveries focused on the specific role of retinal, the aldehyde form of vitamin A, in vision, and these investigations too made use of several animal models of visual function, including fish, frogs, and especially cattle, for which the size and availability of eyes was advantageous (10). Discoveries about β-carotene as a source of vitamin A followed a similar time line. More recently, animal models have played an important role in elucidating carotene cleavage mechanisms whereby β-carotene is converted to vitamin A, leading to an emerging understanding of the diversity that has been observed in human β-carotene utilization (11). Overall, the history of progress in vitamin A research provides ample evidence of the importance of animal models in micronutrient research, and the histories of other micronutrients offer other examples to illustrate similar points.

Animal models also have played important roles in both the discovery and validation of biomarkers that are useful for assessing micronutrient status in humans. Again taking vitamin A as an example, studies in rats first elucidated the relationship between hepatic retinol status and the production and section of plasma retinol-binding protein (RBP) by showing that RBP secretion is “blocked” when the concentration of vitamin A in the liver is very low, but, conversely, holo-RBP is readily secreted within a few hours after the administration of a small dose of vitamin A (12). This information gained in rats was then translated into the relative dose response test, first tested in a rat model (13) and subsequently adapted for use in clinical studies in children and adults [reviewed in (14)]. The relative dose response test has been used successfully in both field studies and hospital-based metabolic studies as an indirect assessment tool in humans, in whom direct measurements cannot be made, to provide insight into whether liver vitamin A reserves are adequate or depleted. Animal models have also led the way in kinetic studies of vitamin A transport and turnover [reviewed in (15)], providing a framework upon which human studies using stable isotopes as tracers in studies of retinol and carotene metabolism have been based (16–19). Today, the various “omics” tools for basic science discovery are ideal for initial use in animal models, because the background noise often present in human studies is reduced. Although any discoveries of potentially useful biomarkers still require validation in human studies, the efficiency of an “animal-first” study design is significantly advantageous.

General Advantage of Animal Models and a Few Caveats to Animal Research

In the present era, animal models have become essential for understanding the relationships of diet, genetic background, and disease prevention at several levels: physiological, metabolic, biochemical, molecular, and even behavioral. The genomes of human and animals are very similar at a global level (20). As our understanding of gene homology has improved, confidence has grown that what is learned in common animal models, and even in simple model organisms like Drosophila and worms, has a good likelihood of being relevant to human biology. Nonetheless, the utility of any proposed animal model needs to be closely assessed by evaluating how well its various characteristics fit the human situation it is meant to model. For any model, it must be appropriate for the specific hypothesis that it is being used to test.

Several of the advantages of animal models may be well known but still deserve mention. These include the smaller size and shorter reproductive cycle in small animal models compared to large animals and humans. Studies of nutrition and embryonic development can be exquisitely studied in the mouse and chick, for which precise stages from fertilization through various embryonic stages to birth have been defined. Examples of novel insights gleaned from such studies include important information on the linkage between RA exposure, gene expression, and body pattern formation (21). Similarly, in the case of another micronutrient that is also of interest in human studies of multimicronutrient supplementation, folate, animal models of supplementation in the preconceptual and gestational periods have defined interactions between genetic background and micronutrient intake (22). Animal models of postnatal development often provide insights into the entire perinatal period in humans, because rodents at birth resemble late-gestational or premature infants. Because the environment can be closely controlled in most animal studies, sources of infection and other “natural” events can be eliminated, or, conversely, specific commensal microbes or pathogenic organisms can be introduced and studied (23). Animal models of defined microbiota have been and will likely continue to be extremely important in understanding the interactions of gut microflora with the mammalian gastrointestinal and immune systems.

Animal models are also advantageous for micronutrient research concerning the mother-infant dyad. Mice, rats, pigs, cows, goats, sheep, and other species have all provided important information regarding nutrition as a determinant of prenatal and postnatal growth and maternal lactational performance. The ability to conduct multigenerational studies in a relatively short time is another strength of animal models. Such models have been and will continue to be essential for understanding the fetal and neonatal origins of adult disease (24–26). Moreover, many animal models relevant to medical nutrition have been established to test feeding and supplementation strategies, including micronutrient delivery by the parenteral route, and the appropriate amounts and forms of micronutrients for inclusion in infant formulas and medical foods. The complex nutritional and physiological interactions in the whole animal cannot be duplicated or substituted by the use of alternative in vitro models, such as cell cultures, and thus animal models are essential for furthering advances in biomedical research.

There are also caveats to the use of animal models. Although animal and human genomes are similar, there are also important dissimilarities. Before choosing an animal model for micronutrient research, it is necessary to understand the physiology of the animal, including its digestive system. Inborn habits, such as the practice of coprophagy, which may provide certain bacterially produced micronutrients, could compromise certain types of micronutrient research. However, with proper attention to selection, an animal model can be a good first representation for assessing the biological effects of micronutrients, both acutely and over the animal’s relatively short life span.
How Can Animal Studies Best Be Used as a Prologue to Human Studies?

Animal research as a prologue to human studies has an important role to play in optimizing the experimental design of human intervention trials. Several ways in which animal studies can contribute to stronger design of human trials are outlined in Fig. 1. Due to the shorter duration and lower costs of animal studies, they can be more comprehensive, with more doses, sampling times, etc. Animal research is ideal for efficiently conducting dose-response studies, which can help to define the most appropriate dose(s) for use in human studies. Studies to screen for interactions between multiple factors, such as micronutrient combinations, can also be efficiently conducted in animal models and may help to revise or eliminate nutrient combinations that are not promising. Only a few micronutrient studies in humans have used dose-ranging conditions, which may require sophisticated designs and research facilities (27). In general, as knowledge of comparative pharmacokinetics between animal models and humans has accumulated, the results obtained in animal models have become more trusted as “pilot data” to guide the design of human studies. Animal models are, of course, also ideal for testing protocols that involve potential risks, including testing nutrient doses outside the normal range of daily intake.

Returning to the Animal for Explanations: Bench to Bedside to Bench Again

Animal models can potentially play a very important role in explaining perplexing results from human studies. The purpose of such post hoc research is, first, to elucidate what has already happened and, second, to guide better human studies in the future. The role that animal research has played in carotenoid research provides a strong case in point. When randomized human trials designed to determine if $\beta$-carotene supplementation would reduce the risk of lung cancer were conducted in the 1990s, the research community was taken aback by the unexpected and perplexing findings of no benefit, or increased risk, in smokers and former smokers in large-scale intervention studies [reviewed in (28,29)]. The scientific question then became, could a high dose of $\beta$-carotene, such as has been used in these human trials, promote smoke-induced lung carcinogenesis, and if so, how? To address these concerns, Russell et al. (30) developed a new animal model, the smoke-exposed ferret (a species that absorbs a portion of dietary $\beta$-carotene intact and therefore is suitable as a model for $\beta$-carotene supplementation in humans), to examine the metabolism of $\beta$-carotene, lung pathology, and potential molecular changes that might explain the mechanisms of the unexpected carcinogenesis observed in the human trials. The results provided several potential explanations, including unanticipated oxidative metabolites of carotenoids and altered levels of vitamin A metabolites in lung tissue of the smoke-exposed, $\beta$-carotene–treated animals [reviewed in (31)]. Had such studies been done first, could the human intervention studies have been redesigned and been successful? In writing about the enigma of $\beta$-carotene in carcinogenesis, Russell (31) directly addressed the importance of animal models and drew the conclusion, with which I concur, that “the metabolism and breakdown of products should be thoroughly investigated in animal models before embarking on large scale intervention trials, particularly when using unusually high dose that greatly exceed normal dietary levels.”

Should Public Health Nutrition Be Investing More Up Front in Animal Studies?

Researchers in genetics, metabolic diseases, pharmacology, and drug discovery have embraced animal models as a fundamental part of advancing the understanding of clinical disease and treatment. Has public health nutrition lagged behind? Should

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**FIGURE 1** Proposed schema for optimal use of animal models in preparation for human studies.

1. Initial hypothesis for human micronutrient supplementation study

2. Conduct animal studies to assess:
   - Dose-ranging
   - Single vs. multi-nutrient
   - Variability
   - Biomarker testing
   - Direct testing on tissues that are not available in human studies
   - Long-term follow up
   - Better dose selection if only a single dose can be tested
   - Better understanding of interacting effects; better selection of treatments to be included/not needed
   - Better power analysis to assure adequate sample size
   - Better biomarker selection
   - Better understanding of physiological effects underpinning observed outcomes
   - Better understanding of potential safety & efficacy in vivo

3. Refinement of design for human study

4. Greater confidence that human study will be safe, and results will be decisive
nutrition research follow steps akin to toxicological research in which animal models are routinely used to generate safety data prior to clinical studies, and should more extensive data on safety and efficacy be required before embarking on micronutrient supplementation studies in infants and young children? Reviews conducted after the fact of the intervention study known as the Pemba trial, which administered high-dose oral iron supplementation with folic acid to children in an area of endemic malaria, suggested that adverse outcomes could have been predicted (32). This in itself is disturbing, while additionally the design of the study did not allow the effects of iron and folate to be understood independently (33). Could animal studies to pretest and refine this intervention have led to a better outcome? Possibly yes. Recently, human studies of vitamin A supplementation in the neonatal period, when numerous vaccines are administered, have also yielded unexpected results, including unanticipated differences in the responses of boys and girls (34,35). Will animal models, including comparisons of males and females, help to elucidate unanticipated interactions between vitamin A administration, gender, and vaccination? The need to understand these issues is great, because vitamin A supplementation campaigns have become a part of public health policy in many developing countries. An integrated approach to public health nutrition research, linking preliminary animal studies to the design of clinical trials, could result in more successful outcomes and engender greater public confidence in nutrition as a science.

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Literature Cited