Nutrients, Endpoints, and the Problem of Proof

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Introduction

The early days of nutritional science were a heady time. Working out the metabolic roles and many benefits of specific nutrients was both exciting and clearly important. In more recent times, emphasis has shifted somewhat from metabolism to policy. In a sense, this represents a major victory for nutrition. Less than a century ago, E. V. McCollum had to struggle with the prevailing notion that food was primarily fuel. The idea that not eating something could make one sick was considered laughable. Now, it is taken for granted that the quality of what we eat is at least as important as the quantity, an understanding that leads naturally to the determining of nutrient intake requirements. It is not surprising, therefore, that 7 of the last 14 Atwater lectures dealt more or less explicitly with the issue of requirements. This one will be no exception to that pattern.

In my McCollum lecture of 5 y ago (1), I called attention to what I perceived as a broadening of the way deficiency disease might be conceptualized. The original nutrient deficiency diseases were all of short latency and involved discrete body systems and dysfunctions. Rickets, pellagra, and beriberi are good cases in point. And, while the working science has progressed far beyond these beginnings, these short latency diseases have remained the implicit model for much of our thinking about nutritional deficiency and, to a substantial extent, our determination of nutrient intake requirements. For example, in the first of the dietary reference intake books, that for bone-related nutrients (2), the intake requirement for vitamin D was explicitly pegged solely to the prevention of rickets/osteomalacia.

As is evident on a moment's reflection, short latency was a prerequisite for the discovery of a connection between nutrient intake and disease. Had the outcome of an inadequate intake not been discrete and not been manifested promptly, it is doubtful that we ever would have recognized the connection between cause and effect (i.e. nutrient intake and health or disease). But the science has gone far beyond that point, and it seems that there is no inherent reason why inadequate intake of the same nutrients involved in short-latency diseases could not be producing long latency deficiency disease as well. Moreover, as is now generally recognized, nutrients act through multiple mechanisms, and low intakes might be expected to lead to disorders quite distinct from the disease originally connected with the nutrient. Vitamin D is a good case in point. Total body inputs closer to those that must have prevailed during hominid evolution are associated with reduced risk of disorders as varied as Type I diabetes, hypertension, osteoporosis, various cancers, multiple sclerosis, and periodontal disease, to name only some (3–24). (For several of these disorders there is now what is re-

ferred to as "level I evidence," i.e. randomized controlled trials, confirming what had been found both in observational studies and in extensive bench and small animal studies.) This broadened scheme is illustrated graphically in **Figure 1**.

Endpoints and the nature of nutrition

Much discussion, and even controversy, today centers around the question of whether these newly recognized endpoints should be considered when determining nutrient intake requirements. This controversy arises out of what seems to me to be three distinct operative models of what nutrition is all about.

For the news media and governmental regulators, nutrition seems concerned mainly about killing oneself with a fork. It is about avoiding risks. It is about warnings and cautions. This approach is expressed formally in our nutrition facts label, now in force for nearly 15 y. At the top of the label, in bold-face type, are listed the nutrients whose intake should be limited, at least according to the conventional wisdom. Below, and without emphasis, there follows a list of nutrients that would generally be considered beneficial; their listing is, to a substantial extent, optional.

In much the same vein, news stories in the general media concerning research studies involving nutrients are almost always negative, i.e. their message is "you will be harmed if you eat too much of this," or, in some cases, if you eat it at all. For example, for purposes of advertising to children, cheese is labeled "junk food" in the U.K., presumably because of its salt and fat contents. In brief, both for the media and regulators, the message to the general public is one of potential harm rather than of actual benefit.

Nutritional policymakers, on the other hand, seem to be operating out of a model which, in effect, focuses on determining the lowest intake one can get by on without developing explicit disease. Such recommendations used to be called "minimum daily requirements," a term that has been dropped but a concept that appears still to be dominant. Once again, vitamin D serves as an apt example. The recommended intake up to age 50 is 200 international units/d. That quantity is sufficient to prevent rickets in children and probably osteomalacia in adults. However, it is an input that has been shown to be inadequate even to sustain serum 25-hydroxyvitamin D during winter at most U.S. latitudes (25) and in many cases it is insufficient to produce the other benefits associated with vitamin D, alluded to briefly above [e.g. osteoporotic fracture reduction (4)]. It is difficult to understand the rationale for this approach, but it may partly be due to the fact that there is a bias toward intake recommendations close to intakes that prevail in the population currently and, for at least some nutrients, intakes that should be readily achievable with currently available foods, if only better choices among them were made.
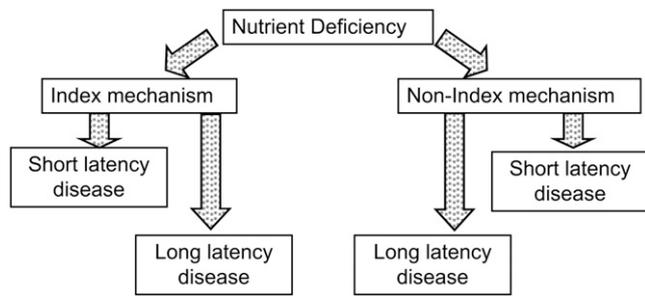
FIGURE 1 Schematic diagram depicting the various ways nutrient deficiency may lead to disease and dysfunction. The terms ''index disease'' and ''index mechanism'' refer to the original nutrient-disease connection for the nutrient concerned. For example, in the case of vitamin D, the index mechanism would be malabsorption of calcium and phosphorus, and the index disease, rickets (or osteomalacia). For the nonindex mechanism, once again for vitamin D, substitute genomic signaling, and for the nonindex diseases, substitute such dysfunctions as propensity to fall, periodontitis, hypertension, osteoporosis, type 1 diabetes, and cancer, among others. Reproduced from (45) with permission.

A model better than either of the foregoing, it seems to me, arises out of whole-organism physiology and rejects the simplistic, primary school approach to nutrition that can be caricatured as "one nutrient – one disease." It is based on the understanding that most nutrients act in all tissues, that all tissues need all nutrients, and that inadequate intake impairs all body systems (though obviously some more prominently than others). In brief, nutrition may be better conceptualized as the equivalent of preventive maintenance in a complex mechanical apparatus (such as an automobile). In addition to the obvious functions of fuel and bulk parts replacement, nutrition in this model is about keeping the various systems from wearing out or breaking down prematurely and its referent would be the intake that prevailed when human physiology evolved and to which that physiology is presumably adapted.

Vitamin D again illustrates this point nicely. The wide variety of vitamin D effects can be reduced to what is probably a single mechanism. 1,25-dihydroxyvitamin D3 serves as a key component of the signaling system by which many cells access their DNA libraries to produce cell-specific proteins important for functional response to a wide variety of stimuli. The circulating 25-hydroxyvitamin D concentration is rate limiting in this system and thus low vitamin D status results in blunted or inadequate tissue responses such as the regulation of epithelial proliferation or the production of antibacterial peptides by macrophages (26). As with mechanical maintenance, the apparatus will often run perfectly well in the face of inadequate intakes, without perceptible malfunction for considerable periods of time; however, ultimately systems fail, in ways unique to their own function, and in all cases, prematurely. Such a model reconciles the seeming paradox that an organism can have a nutrient deficiency and yet not be perceptibly sick, at least for some period of time.

Incidentally, it is sometimes argued that, while human physiology may, indeed, be tuned to primitive intakes, those same intakes may not be well adapted to contemporary conditions. And that is certainly plausible. However, lacking explicit evidence to that effect, it would seem to make better sense to presume the appropriateness of primitive inputs (where they can be known with some certainty) and to shift the burden of proof to those who say that such intakes are unsafe (rather than unnecessary) and that lower intakes would be more beneficial.

This approach to nutrition also plays into what has been termed the "squaring of the survival curve." Several years ago, Fries (27) noted that mean survival for humans was ~85 y, with a SD of ~4 y. Accordingly, excluding deaths from war, natural disasters, and the like, human survival ought to be close to 100% up to the mid 70s. The fact that it is not reflects the impact of the major chronic diseases, such as cancer and cardiovascular disease. Many behavioral changes can be made that work toward the desired squaring, ranging from seat belt use to avoidance of tobacco and excessive drinking, but clearly nutrition makes a contribution here as well. Grant (28), for example, has estimated that over one-half of the breast cancer mortality in Europe and North America has a nutritional basis. And certainly major public health campaigns such as those centered around cholesterol and salt (though they are an example of the negative approach to nutrition cited earlier) presume that bad diets contribute to premature mortality.

Nutrients and efficacy testing

How can this connection between nutrient intake and total body health be tested and, if correct, firmly established? Proof is plainly required today as the basis for nutrient intake recommendations, i.e. the current imperative is that such recommendations be "evidence-based," an objective with which it would be hard to disagree. But what constitutes evidence and by what means ought it be developed? These questions have been explored in greater depth elsewhere (29,30) and my purpose here will be to review some of the structural problems associated with evidence-based nutrition, as currently implemented, and to sketch out a partial solution to those problems.

It is the very essence of science to propose hypotheses and to devise tests to see if they can be falsified. For example, it may be proposed that an inadequate intake of nutrient A contributes to the burden of disease B. Evidence-based nutrition seeks to define the rules for testing such a hypothesis. Given both the vagaries of random chance and the extreme variability of human populations in respect to their response to most interventions, it is currently recognized that the only investigational design that permits strong causal inference is the randomized controlled trial (RCT). Simply put, the inferential process is to evaluate the degree of difference produced by a particular intervention and, given the variability in population responses, assess the likelihood that a difference that large (or larger) might have occurred from random chance alone. Most other designs, lumped under the term "observational studies," are unable to distinguish whether the observed difference is due to the intervention or to some other unrecognized and often unmeasured factor.

So much is undisputed. RCT evidence is now required for registration of drugs and medical devices in most if not all of the developed nations. However, the success of the RCT in evaluating medical treatments has, perhaps, blinded nutritionists, regulators, and editors to the fact that it is a method ill-suited for the evaluation of nutrient effects. As a consequence, I submit that the field of nutrition needs to devise alternative designs to provide the required evidence base for its recommendations.

Here I review briefly some of the features of nutrients that do not fit the RCT context, using the contrasts between nutrients and drugs to illustrate the point.

Drugs are intended for, and evaluated in, sick people. Nutrients, and certainly nutrient intake recommendations, are first of all for well people. This distinction almost goes without saying. Nevertheless, it has important implications for study design. To begin with, drugs usually have only one, and at most only a few principal endpoints or outcome measures; the effect

of the drug on that outcome is usually measurably large; drug tests require the elimination or minimization of cotherapies with other agents that might affect the endpoint, and the response to the drug is typically evaluated relative to its absence. Furthermore, in most cases, drugs act promptly and their endpoints can be measured over relatively short periods of time.

Manifestly few of these features fit the nutrition context. Rather than having discrete outcomes, nutrients are polyvalent and, as noted above, exert their effects in many tissues. And those effects tend to manifest themselves in small differences over long periods of time. Furthermore, nutrients work together, rather than in isolation, and often their effects will not develop when the intakes of other nutrients are suboptimal. Hence, the nutritional equivalent of drug cotherapy, rather than being eliminated, must be optimized. And, whereas with new drugs one can summon a measure of equipoise to the effect that the drug may be no more beneficial than no treatment at all, that is never possible with nutrients. Moreover, there is, effectively, never a nutrient-free state against which the nutrient effects can be compared. Rather, one must contrast low intakes with high intakes. But nutrient response is seldom monotonic and because of its threshold characteristic (e.g. the hemoglobin response to iron or the calcium absorptive response to vitamin D), the control group must be receiving an intake below the threshold, i.e. an intake that is not only nutritionally inadequate but designedly so.

Failure to pay attention to this feature results in null-effect trials, as was the case for calcium supplementation in the Women's Health Initiative (31) and in the Calcium and Preeclampsia Prevention trial (32). Both were RCT, but both used control group calcium intakes at or above the currently recommended levels. Both reported lower overall incidence rates than had been expected but showed little additional effect of the added calcium on the design outcome measure. The study designers were effectively caught in a catch-22 situation. They could not ethically expose women to an inadequate calcium intake with its demonstrated increase in risk not only of preeclampsia (for Calcium and Preeclampsia Prevention) but of the other outcomes for which an adequate calcium intake is important. Hence, they were forced to ensure that the control group was receiving a nutritionally adequate calcium intake. In the final analysis, all that both trials showed was that getting more calcium than was needed to support the many demands of pregnancy and aging did not further reduce the risk of preeclampsia or fracture, at least when averaged over a large number of individuals. While that may be useful information, it does not address the primary question of whether preeclampsia and hip fracture are preventable consequences of inadequate calcium intake. That cannot be done if the study design does not include a group receiving an inadequate intake.

This dilemma illustrates the principal problem with the randomized trial when applied to the development of nutrient evidence. Whatever the disease outcome may be in a particular study, the low intake group must develop, depending upon statistical design, anywhere from roughly 13 to 26 excess incident disease cases or untoward events (e.g. preeclampsia, hip fracture, myocardial infarction, cancer, etc.). That is, the investigators must design the study so that the unsupplemented group experiences this excess of often serious disease. That is ethically unacceptable on its face (33) and it is doubtful that any Institutional Review Board, if it understood the issues concerned, would approve such a study. This ethical problem is not helped by starting with a low-risk group (as is recently proposed for osteoporotic fracture trials). One cannot escape the inexo-

rable fact that the untreated group has to experience that same excess of presumably preventable disease. What using a low-risk population does is increase the size and cost of the study, not its ethical acceptability.

In contrast with how drugs are usually evaluated, nutrients typically act in concert and depend upon one another. For example, it has been shown, and can easily be demonstrated, that the absorption of calcium is dependent both upon the quantity of calcium ingested and the efficiency of vitamin D-mediated absorption (34). In brief, full vitamin D repletion will not result in appreciable calcium absorption if there is not much calcium in the diet and, conversely, high-calcium diets will not result in appreciable quantities of calcium being absorbed in the absence of adequate vitamin D. Similarly, the beneficial effects of calcium on bone status, demonstrated over and over again (35), turn out to be dependent upon an adequate protein intake. In a secondary analysis of the results of their randomized trial of calcium and vitamin D supplementation in older adults, Dawson-Hughes et al. (36) showed that the effect of calcium supplementation on bone mineral density (BMD) was confined to those individuals in the highest tertile of protein intake, with calcium having no effect whatsoever at lower protein intakes. My colleagues and I have confirmed this result in the balance data generated in the long-running Omaha Nuns Study (37) and Hannan et al. (38) showed the same constructive interaction of calcium and protein for the Framingham cohort.

Failure to attend to these and similar interdependencies among nutrients may well be a part of the explanation for the heterogeneity of results from different research centers and investigators. It certainly is a factor in the strange meta-analyses published for calcium and for vitamin D several years ago (39,40). In the calcium meta-analysis, all studies that attempted to normalize vitamin D status were excluded, with the analysis confined to studies that used calcium alone. Conversely, in an analysis of vitamin D effects, all studies using calcium as well were excluded. Not surprisingly, the conclusions were that both calcium and vitamin D were without significant effect on bone, despite the fact that essentially all studies using a combination of calcium and vitamin D had exhibited strong positive effects on BMD and/or fracture. It will not do to argue that the goal of the analysis was to see what the effects of calcium or vitamin D by themselves might have been. Even if that were the right question, and I am not sure that it is, it could only be answered by testing the effect of withdrawing or supplementing the nutrient concerned in individuals whose nutritional status with respect to all other nutrients was entirely adequate.

In brief, the field of nutrition must, I believe, apply the brakes to its mad, downhill rush to embrace a drug-based standard of proof and, instead, pause long enough to develop its own standards – standards that would involve both different designs and a differing approach to endpoints.

Alternative designs

Difficult as it might seem, the problem of an alternative design is not, by any means, intractable. It is a technical issue, largely beyond the scope of this presentation and one that I have developed at somewhat greater length elsewhere (29). It may be sufficient here simply to remind ourselves of 3 points. First, RCT have severely limited generalizability (41,42). Second, nonrandom loss of sampling units from an RCT and/or noncompliance with the intervention destroys the inferential power conferred by randomization, effectively reducing an RCT to the status of a nonrandomized, concurrent cohort study. Third, evidence of

harm, and the consequent withdrawal of drugs that produce unacceptable side effects, is seldom based on evidence derived from RCT but, instead, on designs considered inferentially weaker. Clearly, therefore, we have been able to make policy decisions and to act on evidence less persuasive than that provided by RCT. Obviously, it would be unethical to design and conduct a trial precisely to show harm for a specific medical intervention. Still, if we can impute drug harm without an RCT, so too ought we be able to impute nutrient benefit without an RCT. In brief, the RCT, as implemented, is often unable to carry the load we optimistically place upon it.

Global indices

What I want to focus on in more depth is the choice of an endpoint or outcome measure for studies evaluating nutrient effects. I have already indicated why single system or single disease outcomes, while originally useful in discerning the classical nutritional deficiency diseases, are not the sort of endpoint the field needs today. What is required is an endpoint that captures the multiplicity of nutrient effects, i.e. a global index of some sort. Global indices are not new to clinical research but they have rarely if ever been used as the design endpoint. The Data Safety and Monitoring Board of Women's Health Initiative used a global index to evaluate the response to estrogen, but it had not been the design endpoint and was not the basis for the Data Safety and Monitoring Board's recommendations to the project management (43). What I propose here is specifically that, where possible, a global index be confected as the primary design endpoint for most studies of nutrient effects.

Developing such an index is clearly feasible, although it is not a trivial affair, as the relative weights given to various body systems and outcomes can easily become controversial and should probably require some sort of consensus if the results of such studies are to command acceptance by the nutrition science community.

If one takes vitamin D as an example, one notes at the outset that there are credible scientific data suggesting that vitamin D has an effect on blood pressure, insulin sensitivity, bone density, fall frequency, osteoporotic fracture risk, calcium absorption efficiency, resistance to infection, periodontal disease, and the development of various epithelial cancers, to mention only some (3–24). A global index combining some or all of these effects would operate in 2 distinct but additive ways. First, by summing within individuals, a global index improves the power to detect differences that, system by system, may be relatively small. And by summing across individuals, a global index specifically captures the fact that some people may have an improvement in, for example, blood pressure (but not insulin sensitivity nor infection resistance), while others may have appreciable changes in insulin sensitivity (without much in the way of the other effects, etc.). It is plausible, for example, that perhaps only one-fourth of individuals would respond appreciably in terms of blood pressure and maybe one-third with respect to insulin sensitivity, and so forth. Because these individuals cannot be identified in advance, any study looking for such an endpoint as an isolated outcome will be hampered by the fact that two-thirds to three-fourths of the individuals are inherent nonresponders. However, assuming stochastic independence of the 2 outcomes, a global index combining just these 2 endpoints reduces the nonresponders to about one-half of a given cohort. Adding other endpoints would decrease the proportion of nonresponders still further.

The construction of a global index will obviously have to be specific to the nutrient or nutrients concerned and the selection
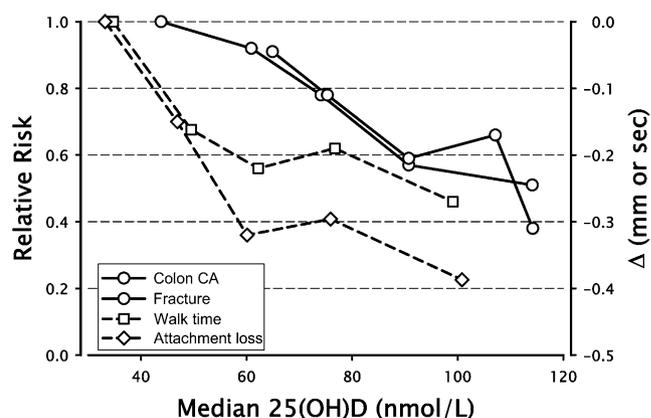

FIGURE 2 Relationship of disease risk (left axis) or quantitative dysfunction (right axis) to vitamin D status (horizontal axis). For all 4 endpoints, disease risk or dysfunction decreases as vitamin D status improves. CA, Cancer. Walk time is the time for an 8-foot walk and the right axis plots decrease in walk time; attachment loss refers to tooth attachment, and, as for walk time, the right axis plots decrease in attachment loss. [Redrawn from Bischoff-Ferrari, et al. (44).]

of endpoints making up the global index must have a basis in biology (e.g. in animal studies or cell biology). Bischoff-Ferrari et al. (44) recently paved the way for just such an index for vitamin D effects in a review article in which she and her colleagues aggregated effects of vitamin D on BMD, bone fracture rate, colon cancer risk, tooth attachment loss in periodontal disease, and lower extremity neuromuscular function. These authors did not attempt the construction of a formal index but stopped just short of doing so. **Figure 2** is adapted from their publication and illustrates graphically the similar trends of 4 distinct endpoints as a function of vitamin D status.

Another useful feature of a global index is the ease with which it is able to incorporate negative as well as positive effects. Weighing negative and positive effects involves value judgments, a fact that makes confection of an index difficult, as trade-offs will not be the same for every one. Nevertheless, it is entirely feasible, if not always easy.

As should be clear, the advantage of a global index is that it better corresponds with the actual function of the nutrients concerned than could any single-system outcome measure. Also, because it offsets the diluting effect of nonresponders, it would be predicted to greatly increase the power of a study to find clinically significant effects.

To sum up, I think that there would be general agreement to the effect that nutrition is important, despite the fact that the still growing number of failed trials of individual nutrients might suggest that no nutrient actually made much of a difference, a conclusion that is absurd on its face and ought to have alerted us to the possibility that there was something wrong with how we were investigating the matter. To provide the proof needed to sustain revised intake recommendations, we shall have to find a design better suited to nutrients than the randomized controlled trial as currently implemented, and we need to develop a series of global indices, nutrient by nutrient, which better capture the polyvalent nature of most nutrients. Perhaps it would be useful for the ASN, in collaboration with concerned governmental entities such as the USDA, to convene a workshop to address these structural issues. Such deliberation may well be arduous and frustrating, but it is terribly important and, in my view, well worth the effort.

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