Directions in Nutritional Assessment

Biomarkers and bio-indicators: providing clarity in the face of complexity

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Introduction

Among the greatest challenges facing the nutrition community is integrating nutrition into all aspects of global efforts in health promotion and disease prevention. Despite compelling evidence of its importance to human development,[1–3] a full appreciation of how nutrition affects health is still lacking throughout the continuum of health care providers, programmers and policymakers. In large part, this disconnect is the result of relying on evidence that is often not directly or specifically linked to the biology of nutrition in a meaningful manner.

Over the years, attempts have been made to put the differential responses due to inadequacy of individual nutrients into a classification scheme based on specificity of response. The classification of Type 1 and Type 2 nutrients offered by Golden et al is a widely accepted application of this approach.[4] Type 1 nutrients (e.g., vitamin A) are directly and inextricably linked to explicitly defined outcomes, e.g., xerophthalmia, by a well described biological relationship.

By contrast, Type 2 nutrients, such as zinc, are not directly linked to particular outcomes. In fact, the categorization of nutrients by this paradigm is dependent on context. For example, vitamin A is clearly a Type 1 nutrient with respect to its role in preventing xerophthalmia, but it appears to be a Type 2 nutrient with respect to its activity in reducing risk of infection. This is more than a semantic issue, as addressing such physiological impacts calls for an understanding of the actual root causes. That challenge requires tools capable of identifying relationships between nutrients and specific outcomes. It necessitates a clear appreciation of the functions of nutrients within relevant biological systems (e.g., immune, neurological). Further, it requires evidence that changes in nutrient status will, in fact, have functional consequences. These are the domains of nutritional assessment.

Objectives of nutritional assessment

Our ability to understand the role of nutrition in health is driven by our capacity to address three fundamental questions:

1. Where do normal nutrient requirements end and specific health /physiological condition-related needs begin?
2. What is the role of diet /nutrition in those conditions that would require special consideration above and beyond provision of a balanced diet providing all essential nutrients needed for growth, development and health?
3. What are the best types and amounts of evidence to support the establishment of standards of care and the development of programs to address the role of nutrition in health promotion and disease prevention?

These questions are based on the premise that nutrition is involved in all aspects of human biology. Nutrition status is achieved as a result of a series of behavioral, physiological and metabolic processes involved in the taking in, and utilization of, dietary substances /nutrients that must be present to support growth, repair and maintenance of the body as a whole or in any of its parts. This definition illustrates the complexity of nutrition, as well as the need to be integrative in assessing nutritional status. It shows the necessity for a variety of tools capable of probing this complexity at key points – not just in the ingestion/exposure of nutrients, but also in their metabolism/utilization and their function under conditions of both health and disease/stress. Accordingly, interpreting such results calls for an appreciation of the various direct and indirect interactions of nutritional status in affecting, and being affected by, these processes or conditions. These various factors must be considered in determining nutritional needs and standards of care or the roles of diet/nutrition in health and/or disease.

> "Nutrition requires a variety of tools capable of probing complexity at key points"

Historically, four approaches have been used to assess nutritional impacts on given health conditions. These are:

- measurement of dietary intake;
- inferences from anthropometry;
- assessment of biochemical indices / biomarkers of nutrient status; and
- responses to direct nutritional intervention.

Because context matters, it can be difficult to draw conclusions or generalize upon specific results based on only one of these approaches. **Textbox 1** contains some of the reasons why this is a challenge.

Textbox 1: Reasons for using an integrated approach to nutritional assessment

- In the absence of biochemical indices / biomarkers, intake data alone are insufficient to determine functional status or the effect of nutrients on an individual's health.
- In the current global context, the dual burden of overnutrition (overweight and obesity) and undernutrition (underweight and nutrient deficiency) co-exist at both population and individual levels. Thus we cannot rely on anthropometry alone to make a judgment about nutrition and health.
- With regard to nutrition and health relationships, it is difficult to make any inferences about biochemical indices without knowing an individual's intake.
- Physiology vs. Exposure: Abnormal circulating levels of a particular nutrient may be due to many factors, e.g., inadequate intake, inflammation, inherent biochemical abnormalities, pathologies, or problems associated directly or indirectly (via interactions with therapeutics / interventions) with a given condition.
- Without knowing the pre-intervention status of an individual, it is difficult to distinguish between the effects of correcting a primary nutrient deficiency and those of correcting a secondary nutritional anomaly associated with disease, medicines, etc.
- The ability to determine optimal nutrient doses for interventions is contingent on an appreciation of dietary intake, physiological need, nutritional status, and the impact of the condition on the processes of nutrition.

A response to the challenge: BOND

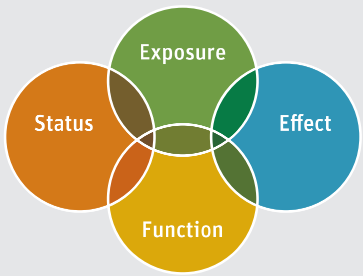
The Biomarkers of Nutrition for Development (BOND) program began in 2010 as a collaboration between the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD) of the US National Institutes of Health and the Bill & Melinda Gates Foundation (BMGF).[5] The goal is to provide information and services to support the entire food / nutrition research and global health community. Specifically, BOND is designed to develop consensus on accurate assessment methodologies that are applicable and relevant to users domestically and internationally in both the public and private sectors. BOND intends to serve the breadth of the food and nutrition user community, including: researchers (lab / clinical / surveillance), clinicians, program (planners / implementers / evaluators), and policy-makers (data consumers). Support to the global health community's efforts to address the increasingly complex food, nutrition and health context is provided through the discovery, development and implementation of new tools to evaluate these relationships.

In its first phase, the BOND project adopted a classification scheme to provide some clarity to these issues. The scheme was based on the assumption that the ability to answer the questions above is contingent on the tools needed to address: **1)** exposure (what has been consumed, including bioavailability); **2)** status (where an individual or population stands relative to an accepted cut-off, e.g., adequate, marginal, deficient); **3)** function (reflecting the role of a nutrient **within** a relevant biological system); and **4)** effect (the impact of a given status or intervention **on** nutrient status and function). **Figure 1** reflects the relationships of these categories.

Different perspectives: biomarkers vs indicators vs bio-indicators

From the outset, the focus of the BOND has been on tools that reflect the need for the discovery, development and deployment of biomarkers in these categories. However, there has always been a conundrum regarding the relevance of these categories to the needs of individual user groups, e.g., clinicians, researchers and program directors. More specifically, what are the roles

FIGURE 1: Relationships between exposure, status, function and effect



of: **a)** those outcomes that might be used to reflect some aspect of function or effect, but independently are not sensitive or specific measures of unique nutrient relationships; and **b)** those measures traditionally used for program development and evaluation, but again, not sensitive or specific to nutrition. **Textbox 2** provides definitions of three categories that again overlap, but clearly serve different roles depending on the user's needs.

> "BOND focuses on tools that reflect the need for the discovery, development and deployment of biomarkers in selected categories"

Textbox 2: Types of measures

Biomarkers: "A characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention." [6]

Bio-indicator: "[These] include biological processes, species, or communities and are used to assess the quality of the environment and how it changes over time."[7]

Public Health Indicators: "A measure used to express the behavior of a system or part of a system." [8]

The tension between types and utility of these measures has implications for the BOND user communities. For example, a user involved in public health program development or evaluation might rely on what are traditionally referred to as "indicators," reflecting a response of a system to a given intervention. In the field of nutrition, examples run the gamut from growth through changes in population status of a targeted nutrient and certain measures of neuro-development/capacity to universally accepted clinical parameters such as anemia. Indicators also include such broad, non-specific measures as mortality or disability-adjusted life year (DALY).

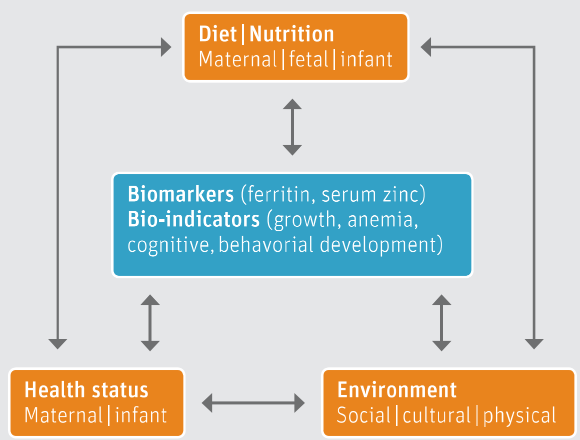
These are often used as triggers for implementing or stopping population-based programs. However, they tell us little about the specific nature of the relationships within diet, nutrition and health. The distinction between an *indicator* and a *biomarker* is not a simple matter of semantics; it reflects expectations about what that information means and how it should be used. For example, a DALY has meaning in the context of population trends and responses, but it lacks specificity with regard to causal factors and, particularly, the effects of nutrition. Moreover, the leap from a biomarker to an indicator is huge, and covers a large spectrum of potential biological relationships. That chasm requires an intermediary step to make a more logical connection.

In exploring this conundrum, it is useful to look outside the *nutrition* field to see how other disciplines might be thinking about this common challenge. An illuminating example is to be found in the environmental sciences, where a quick search of the literature reveals that the term "bio-indicator" is an important part of that vernacular. In the context of an ecological system, the distinction between *biomarkers* and *bio-indicators* represents a hierarchy from the molecular level (toxin levels, sensitive and specific markers of an impacted biological system) to the macro-level (changes in population levels of sensitive species). An example of a bio-indicator in environmental science might be a sentinel species used to assess perturbations of a system. The population might be reduced but without specific biomarkers, and the reasons for that disappearance would be unknown. So biomarkers provide essential context to allow a more meaningful interpretation of bio-indicators and thereby inform interventions to remedy the problem. Thus bio-indicators seem to offer exactly the kind of transitional tool needed to fill that gap in nutrition between biomarkers and indicators.

> "Bio-indicators seem to offer exactly the kind of transitional tool needed to fill that gap in nutrition between biomarkers and indicators"

A recent example of this interpretive challenge can be seen in the controversy surrounding the utility of intermittent, high-dose vitamin A supplements.[9,10] In simple terms, the argument

FIGURE 2: Interpreting biomarkers/bio-indicators in a complex environment



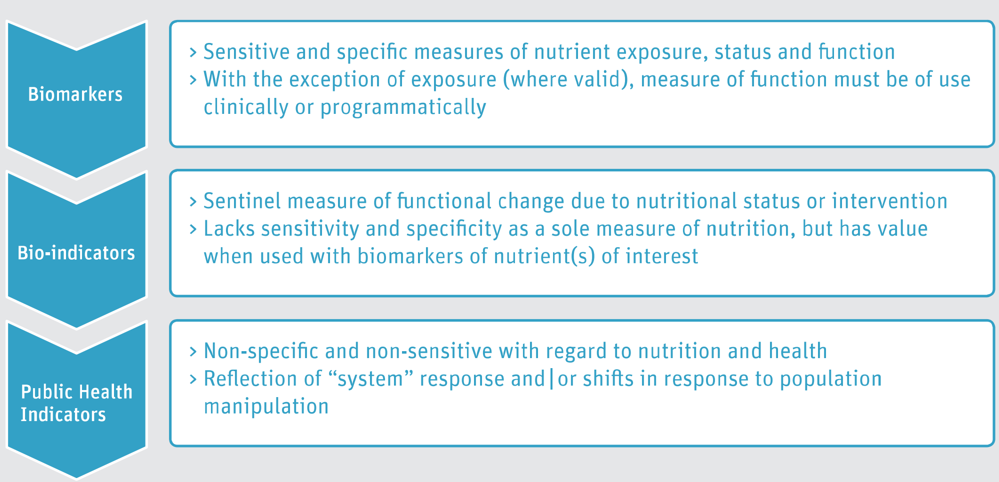
stems from trends in reduced mortality rates that suggest a diminished impact of vitamin A supplementation programs. Additional fuel for this controversy is offered from the paradoxical lack of sustained responsiveness of serum retinol to high-dose vitamin A supplements. Of course, there is no discourse about the essentiality of vitamin A or the need to be vigilant about ensuring its dietary adequacy. The biological conundrum is the link between the biomarker (serum retinol) and the public health indicator (mortality), without which it is difficult to make a case for either risk or benefit.

The decisions made by the *nutrition* community about such controversies are used by public health officials who need clear guidance as to why a change is needed and what are its implications. Thus the debate should be about risk and efficacy. Our ability to attribute trends in non-specific outcomes such as mortality to any specific factor depends on the extent of our knowledge of relevant causal pathways reflected by the relationship between nutrient biomarkers and bio-indicators reflecting functional effect(s). The difference among the three measures (biomarkers, bio-indicators and public health indicators) will be reflected in our ability to assess such pathways. The absence of the intermediary benefits of bio-indicators as indices of function highlights the dichotomy between such public health indicators as mortality and biomarkers. It also emphasizes the need to develop strategies that integrate the role of nutrition within biological systems in a manner that can be translated most effectively to standards of care and evidence-informed programs and policies.

The use of both biomarkers and bio-indicators would facilitate more discrete descriptions of the roles of nutrients in vulnerable individuals/populations, and enable more informative assessments of the responses to dietary/nutritional interventions. In many ways, this distinction speaks to expectation. A biomarker should yield information specific to a given physiological condition, whereas a bio-indicator should yield a relevant, albeit non-specific, reflection of the net effects of several factors. The impact of various factors on use and interpretation of biomarkers and bio-indictors is reflected in **Figure 2**.

There are traps to be avoided in using biomarkers and bio-indicators in nutrition. Without the contextual information pro-

FIGURE 3: Interrelationships among biomarkers, bio-indicators and public health indicators



The nature of the intersections between biomarkers, bio-indicators and public health indicators in making nutrition/health links.

vided by biomarkers, including information about nutrient exposure, a bio-indicator can be misinterpreted and therefore trigger actions that will not achieve the desired goal. For example, using anemia as a public health trigger without the necessary contextual information from biomarkers of inflammation, iron status, hemoglobinopathies etc. could result in an intervention of no benefit, and indeed potential harm, to at least half of a targeted population.[11,12] At the clinical level, this issue can affect the ability to make a differential diagnosis; at the population level, it can affect the ability to address a large-scale problem without increasing concerns of safety or inefficient use of limited resources. **Figure 3** shows the relationships of biomarkers and bio-indicators used in making nutrition / health decisions.

Future directions and conclusions

The value of biomarkers and bio-indicators to clinical and population-based nutritional assessment is clear in terms of both diet / nutrition and health links. It also provides clarity with regard to the effect of either nutritional status or interventions. A need exists, however, to understand the relative value of these terms as well as the relevant tools and operative contexts of each. Both terms are essential in the vernacular of nutritional assessment. As both terms emerged to facilitate communications about multifunctional outcomes of complex systems, their use should assist *nutrition* researchers in such emerging areas as the microbiome and its role in human health and development. That area calls for bio-indicators reflecting function implications of changes in the gut ecology and biomarkers reflecting specificity of these effects as well as precipitating factors (e.g., dietary changes).[12]

> "The value of biomarkers and bio-indicators to nutritional assessment is clear in terms of both diet | nutrition and health links"

The goal of the BOND project is to add value to efforts to address nutrition in health through an open and inclusive deliberative process. The first phase of BOND focused on the traditional approach to nutritional assessment with emphases on single nutrients (iron, zinc, iodine, folate, vitamin A, vitamin B_{12}) and biomarkers of exposure, status, and function. The project's second phase will take a systems approach that will address the roles of multiple nutrients in various health and developmental contexts. The targets will include the neurological system (central and peripheral), growth (linear and body composition),[8] and specific nutrient clusters within each. In each case, the focus will be on the linkage between specific biomarkers and bio-indicators that reflect the roles of nutrients within these systems. For example, for neuro-development, nutrient clusters might include aromatic amino acid precursors of neuro-transmitters, vitamins (pyridoxine, riboflavin, thiamin) that serve as cofactors in those metabolic pathways, and bio-indicators of function (e.g., evoked potentials, measures of cognitive function, measures of behavioral development).

In addition, an ongoing focus must be on the implementation of available biomarkers and bio-indicators to meet the clinical and programmatic needs of low- / middle-resource settings. As was evident during Phase I of BOND, there are numerous platforms available for deployment of biomarker methodologies (e.g., dried blood spot / paper-based,[10] multiplex platforms,[11] "lab-on-a-chip"[12]). The real challenges will be in matching particular biomarkers to particular purposes, and in determining which biomarkers can be deployed in cost-effective ways to provide the necessary contextual information for the interpretation of useful bio-indicators.

Finally, while the goal might be to have a battery of sensitive and specific tests to allow for a meaningful diagnosis or assessment of need at scale, vigilance will be needed to ensure the recognition that an integrated approach to effective implementation must include input from the continuum of expertise and delivery systems (health, food, agriculture, etc.) that will enable a coordinated and effective solution to what will be a complex situation.

Useful nutritional assessment is a complex undertaking that must take into consideration a myriad of factors related to diets, food habits, nutrient utilization, physiological status and health status. As we learn more about human biology, we must look for tools capable of probing this complexity at various points, and for ways of integrating the information those tools can provide. While such tools have traditionally been referred to as biomarkers, in nutrition that term has become elastic through use both in situations of specific relevance to certain nutrients and in non-specific evaluation of context. Therefore, a new paradigm is proposed to facilitate communication about, and implementation of, nutritional assessment. This paradigm distinguishes the term "biomarker" as a specific measure of the amount, activity or function of a given nutrient, and adds the term "bio-indicator" as a measure of the net effects of contextual factors, including nutrition and non-specific outcomes.

The evidence generated from the study of these relationships must be translatable to a broad community of potential users with varying degrees of technical expertise. The continuum represented by biomarkers, bio-indicators and public health indicators reflects the nature of the needs across the food, nutrition, and health enterprise. The appreciation of the relative strengths and weaknesses of these measures can help to ensure that these tools are used most effectively and that they reflect the goals of full integration of food and nutrition in all aspects of the efforts to improve the health of individuals and populations.

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