Applications of Nutritional Biomarkers in Global Health Settings

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Abstract

In global health settings, there are three generic areas that require reliable biomarkers of nutritional status and function. Population surveillance needs to identify key nutrient deficiencies (or excesses) to monitor progress towards elimination of nutritional imbalances and to stratify populations into groups especially ‘at risk’ to whom public health resources can be focused. Clinical interventions need biomarkers to help identify disease pathways, to assist in targeting nutrient prescriptions, and to avoid potential harm (e.g. in the case of iron). Discovery science requires biomarkers in many domains, but especially in the study of nutrient-gene interactions and regarding the effects of nutritional status on the epigenome. Each of these applications imposes different constraints on the methodology though in all cases the optimum biomarker would have high sensitivity and specificity, would capture variation of functional significance, and would be cheap and easy to apply. These attributes are hard to achieve, and recent progress towards next-generation biomarkers, though holding much promise, has not yet delivered significant breakthroughs in the global health setting. Recent efforts to overcome these problems by two initiatives (BOND and INSPIRE) are highlighted as exemplars of a route map to progress.

Introduction

Following significant economic progress in many regions of the world, and consequent improvements in access to better quality diets, the residual global public health burden of nutrient (especially micronutrient) deficiencies is now concentrated on the world’s poorest populations that are largely confined to
sub-Saharan Africa and South Asia. In such regions, there are multiple overlapping applications for biomarkers of nutritional status ranging from individual clinical diagnosis, through group screening for targeted interventions, to population surveillance.

Screening for gross nutritional sufficiency of protein-energy supply tends to still focus on simple anthropometric indicators assessing stunting (height-for-age z score), underweight (weight-for-age z score), wasting (weight-for-height z score), and mid-upper arm circumference. Assessment of protein status still relies on crude indicators based on plasma protein concentrations (e.g. albumin) which are insensitive to all except gross protein deficiency. In these aspects, there has been little notable movement towards next-generation indices of nutritional status. One exception is the use of noninvasive measures of body composition, most importantly for assessing body fat, lean tissue, and muscle mass. In advanced research facilities, dual X-ray absorptiometry and air displacement plethysmography (e.g. BODPOD® and PEAPOD®) are being directed towards the further understanding of the etiology and later sequelae of early growth failure, but for most low-income clinics or field applications, bioimpedance assessment is the only really practical method.

Assessment of micronutrient status also remains a major challenge with few significant breakthroughs that have been adequately validated in recent years. The challenges are manifold. First, micronutrients can be divided into type-1 and type-2 micronutrients [1, 2]. For type-1 micronutrients, the category that includes all vitamins and most minerals, physical growth continues in the face of deficiency, and hence tissue levels are depleted, but characteristic clinical signs only become visible at extreme levels of deficiency. For type-2 micronutrients (e.g. zinc and protein), growth slows rapidly and hence tissue levels tend to be maintained making detection of deficiency very challenging. Second, for many nutrients, the levels in blood (the customary biopsy tissue) are homeostatically maintained by reserves in the liver or other tissues. Thus, measuring circulating levels of vitamin A, for instance, provides only a crude measure of vitamin A status and is more useful at the population level than at the individual level. Third, the circulating levels of many micronutrients are profoundly altered by inflammation raising challenges as to how to correct for these effects especially in low-income settings where infections are common. Such problems over the validity of existing biomarkers for nutrient status create a problem for developing new tests; namely, that there is no gold standard against which to reference new techniques.

Ideally, next-generation biomarkers would be based on functional tests. Some tests already exist. For instance the erythrocyte glutathione reductase activation coefficient test assesses the percent saturation of erythrocyte
glutathione reductase with its riboflavin-derived cofactor flavin adenine dinucleotide [3]. This has been shown to robustly correlate with the activities of other flavo-enzymes in other tissues thus providing a comprehensive status assessment [3]. There are a few other examples of such functional indicators, but others are still required.

It appears that the discovery of the iron-regulatory hormone hepcidin provides a step forward in assessing iron status, and consequently Drakesmith [this vol., pp. 59–69] allocated a full paper to this issue in this workshop. We believe that the opposing transcriptional regulators of hepcidin synthesis (iron deficiency and inflammation) reflect evolutionary pressures to optimize iron status in the face of possible infectious threats, and hence hepcidin can form the basis of a next-generation point-of-care diagnostic that could indicate ‘ready and safe to receive iron’ [4, 5].

The BOND and INSPIRE Initiatives

The Gates Foundation/National Institutes of Health/National Institute of Child Health and Human Development (NICHD)-sponsored programs BOND (Biomarkers of Nutrition for Development) [6] and INSPIRE (Inflammation and Nutritional Science for Programs/Policies and Interpretation of Research Evidence) [7], led by Dr. Daniel Raiten at NICHD, have pulled together experts to address the challenges highlighted above. Outputs, in both published and interactive web-based formats, from each initiative provide excellent resources describing the range of biomarkers currently available and in development.

The BOND program has started by addressing 6 key nutrients: vitamins A and B₁₂, iron, iodine, folate, and zinc. Table 1 (reproduced from BOND) shows the high-level summary of biomarkers available for vitamin A. Examination of this table reveals many of the challenges and roadblocks faced across the wider portfolio of biomarkers for micronutrient status. For instance, the most widely used measures (retinol-binding protein, serum retinol, and breast milk retinol) provide poor measures of status at the individual level, frequently fail to respond to intervention in predictable ways, and are affected by inflammation. Measures that provide a better index of the status of an individual require mass spectrometry, and hence are costly and have long lag times to the production of results, thus making them only applicable in research settings. Physiological function tests are either hard to implement (e.g. dark adaptation) or insensitive (e.g. self-reported night blindness). These limitations in assessing vitamin A status are replicated to a greater or lesser extent across the other 5 nutrients, but the BOND summaries provide investigators...
<table>
<thead>
<tr>
<th>Biomarkers</th>
<th>Type</th>
<th>Use</th>
<th>Utility</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum retinol-binding protein</td>
<td>Status (deficiency)</td>
<td>Population</td>
<td>Not released from the liver when retinol is limited Used as a proxy for serum retinol to identify vitamin A deficiency</td>
</tr>
<tr>
<td>Serum/plasma retinol</td>
<td>Status</td>
<td>Population</td>
<td>Most commonly used biomarker Correlates with the prevalence and severity of xerophthalmia and may change in response to interventions</td>
</tr>
<tr>
<td>Relative dose response</td>
<td>Status</td>
<td>Population/Individual</td>
<td>Based on hepatic accumulation of retinol-binding protein during vitamin A depletion Requires blood sample before and after an oral retinyl ester dose</td>
</tr>
<tr>
<td>Modified relative dose response</td>
<td>Status</td>
<td>Population/Individual</td>
<td>More responsive than serum retinol Qualitatively identifies low or adequate liver vitamin A reserves</td>
</tr>
<tr>
<td>Retinol isotope dilution</td>
<td>Status, marker of excess</td>
<td>Population</td>
<td>Most sensitive test to measure vitamin A status and intervention impact on vitamin A reserves Minimally invasive and accurate</td>
</tr>
<tr>
<td>Breast milk retinol</td>
<td>Status, exposure</td>
<td>Population</td>
<td>Good indicator of vitamin A status in areas where breastfeeding is common until at least 6 months of age Impacted by many factors</td>
</tr>
<tr>
<td>Retinyl esters</td>
<td>Status, marker of excess</td>
<td>Population/Individual</td>
<td>Validated qualitative measure of hypervitaminosis A May be confounded by liver disease at the individual level</td>
</tr>
<tr>
<td>Dark adaptation</td>
<td>Function (small scale)</td>
<td>Population/Individual</td>
<td>Dark-adapted final threshold is inversely and sensitively correlated with serum vitamin A levels in low to deficient ranges</td>
</tr>
<tr>
<td>Electro-retinography</td>
<td>Function</td>
<td>Population/Individual</td>
<td>Measures the bioelectrical response of the retina to a flash of light Invasive and nonsuitable for children</td>
</tr>
<tr>
<td>Pupillary threshold testing</td>
<td>Function</td>
<td>Population/Groups of individuals</td>
<td>Inversely correlates with serum vitamin A values in low to deficient ranges and the concentration of vitamin A in the retina Noninvasive, can be used in field conditions</td>
</tr>
<tr>
<td>Dietary assessment</td>
<td>Exposure (repeated testing)</td>
<td>Population/Individual</td>
<td>Qualitative measure of exposure Provides useful information to support biochemical biomarkers Seasonality of fruits and vegetables must be included</td>
</tr>
</tbody>
</table>

1 Reproduced with permission from BOND (https://www.nichd.nih.gov/global_nutrition/programs/bond/Pages/index.aspx).
at all levels with a roadmap for navigating towards optimally matching methods with applications.

Attempts to design next-generation methodologies are very challenging for a number of reasons. First, the endeavors to develop more precise, reliable, and cost-effective assays and to multiplex these cannot overcome the basic physiological reasons that limit interpretation. Second is the question of a lack of a gold standard reference method against which to calibrate any new methodologies under development. For instance, innovative studies of the plasma proteome have elegantly demonstrated both expected and novel proteins correlated with micronutrient status in Nepalese children [8] but in this, and similar, endeavors it is difficult to escape from the circular logic inherent in comparing one technique to another in the absence of a single gold standard. Perhaps the only way around this will be to use multiple methods each with individual uncertainties and to triangulate between them. Such a process is complex to perform and would need to be implemented across various sex and age groups, and account for physiological variables such as inflammation; this explains why it has rarely, if ever, been achieved.

**Biomarkers Employed in Discovery Science Applications in Global Health**

There is still much to be learnt about the fundamental relationships between diet, health, and disease in both first- and third-world settings, and by virtue of the greater variations in nutrient intakes in poor populations there is a strong imperative to study such populations to seek new solutions. Sometimes studies conducted in nutritionally marginalized populations can provide insights that could also be applied across better-nourished populations. We provide one such example here to illustrate both the power and the limitations of complex biomarker studies.

Epigenetic changes induced in the very early phases of the life cycle are believed to provide at least a partial explanation for the known linkage between the nutritional and environmental circumstances of a mother’s pregnancy and the lifelong health of her children or her children’s children [9]. One class of epigenetic changes, namely DNA methylation, requires an adequate supply of methyl groups that in turn is dependent on a series of interlocking metabolic pathways in which nutrients provide both substrates (choline, betaine, methionine, and folic acid) and enzyme cofactors (vitamins B<sub>2</sub>, B<sub>6</sub>, and B<sub>12</sub>; fig. 1). Knowledge of intermediary metabolites (homocysteine, cysteine, dimethyl glycine (DMG), S-adenosylmethionine (SAM), and S-adenosylhomocysteine (SAH)) may also help inform how nutrient balances could affect DNA methylation [10].
We used a set of biomarkers measured during early pregnancy to capture the methyl donor 'minimetabolome' in order to test the hypothesis that deficiencies in these pathways could affect how DNA is remethylated in the very early embryo [11]. We had previously shown that the methylation of metastable epialleles (regions of the genome where methylation patterns are known to be established very soon after conception) is influenced in rural Gambia by whether babies are conceived in the hungry or the harvest season [12]. Surprisingly, the babies born in the harvest season, i.e. when foods are most abundant, showed lower levels of methylation suggesting that the effects were mediated by more complex aspects of nutrient supply than simply the abundance of the diet. We therefore prospectively studied a new cohort of women conceiving in the two seasons [13]. We also studied a group of nonpregnant women with monthly blood samples in order to gain a complete picture of how their diet and methyl donor biomarkers varied throughout the year. Figure 2a shows the annual variations in these nonpregnant women for some of the key intermediary metabolites and shows the large swing in the SAM/SAH ratio, a key determinant of the

**Fig. 2.** Seasonal differences in biomarkers of the methylation potential in rural Gambian mothers. **a** Seasonal variation in some key methyl donor pathway biomarkers assessed monthly in 30 nonpregnant, nonlactating Gambian women. **b** Heat maps of methyl donor biomarkers assessed in early gestation in 165 women. Each vertical bar represents a subject whose biomarker concentration is expressed as a z score relative to the overall mean. ActB\(_{12}\) = Active B\(_{12}\). * p < 0.05, ** p < 0.01, *** p < 0.001. (For figure see next page.)
methylation potential. The heat map in figure 2b presents data from 165 women equally divided into those who conceived in the hungry and harvest seasons. It shows that many of the substrates, cofactors, and intermediary metabolites involved in methylation pathways differed significantly between the two seasons, and, as observed previously, there were also significant differences in the methylation of metastable epialleles in the offspring which were significantly correlated with their mothers’ biomarker levels. These results provided first-in-human proof that a mother’s diet at conception could epigenetically alter her baby’s DNA in ways that might have lifelong effects on health.

These are important results but they reveal some significant challenges with respect to the use of biomarkers. First, the assays were costly and time consuming being performed by traditional wet laboratory methodologies. Performing them on a next-generation metabolomics platform would have been preferable if a platform existed that could yet compete with wet laboratory precisions and accuracy, but this goal has yet to be achieved. Second, although the levels of several of the metabolites significantly predicted DNA methylation and all of these were in the correct direction based upon first principles of the biochemical pathways involved, it has remained hard to decipher which nutrient interventions would be most beneficial due to the complexities of the modeling required [14]. Additional analytes and a much larger dataset will be required in the hope of directing the next steps towards developing a cocktail of nutrients to optimize methylation and infant outcomes.

In summary, there remain very significant challenges in designing next-generation biomarker-based methods for global health diagnostics and research. These challenges will be surmountable if sufficient efforts and resources are allocated to finding solutions.

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