Biomarkers for Vitamins and Minerals

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Biomarkers for Vitamins and Minerals

- Current methods
- New methods for use now and in the very near future
- Innovations with potential for the longer-term
Current Biomarker Methods

• Multiple options for each nutrient, depending on purpose
  – Population-level prevalence vs individual-level status
    • supplement vs fortification
    • long-term markers of deficiency or markers of intervention efficacy
      (RBC folate vs serum folate)
  – Research to understand biological mechanisms, context

• Debate about appropriate cut-off values and difficulty in establishing standardized methods

• Practicality of biomarker methods given the data collection circumstances
  – Sample type, lab facilities, etc.
Biomarkers of Nutrition for Development (BOND) Program

NICHD and partners “... created the BOND Program to meet the growing need for discovery, development, and implementation of reliable and valid biomarkers to assess nutrient exposure, status, function, and effect.

A primary goal of the BOND project is to harmonize the processes for making decisions about what biomarkers are best for use in support of research, program development and evaluation, and generation of evidence-based policy.”


- Assembled expert panels to compile evidence for each nutrient
- Outcome will be publication of reviews for each nutrient
  - Iodine, Rohner et al, J. Nutr. August 1, 2014 vol. 144 no. 8 1322S-1342S
### Biomarkers for the 6 BOND nutrients

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Common Analytes</th>
<th>Sample Types</th>
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</thead>
<tbody>
<tr>
<td>Vitamin A</td>
<td>Retinol, retinol binding protein</td>
<td>Blood, breastmilk</td>
</tr>
<tr>
<td>Iron</td>
<td>Ferritin, transferrin receptor, iron, hepcidin, zinc protoporphyrin/heme ratio, hemoglobin/hematocrit</td>
<td>Blood</td>
</tr>
<tr>
<td>Zinc</td>
<td>Zinc</td>
<td>Blood, hair, (urine, nails)</td>
</tr>
<tr>
<td>Folate</td>
<td>Folate (serum or RBC), homocysteine, folic acid</td>
<td>Blood, (urine)</td>
</tr>
<tr>
<td>Vitamin B12</td>
<td>VitB12, homocysteine, methylmalonic acid (MMA), transcobalamin (holoTC)</td>
<td>Blood</td>
</tr>
<tr>
<td>Iodine</td>
<td>Iodine, thyroglobulin, TSH, T3/T4</td>
<td>Blood, urine</td>
</tr>
<tr>
<td>Inflammation</td>
<td>CRP, AGP</td>
<td>Blood</td>
</tr>
</tbody>
</table>
Vitamin A

- **Serum retinol**
  - widely used, de facto gold standard
  - difficulties with stability and measurement technology (HPLC in developing country settings)
  - under homeostatic control

- **Retinol binding protein**
  - 1:1 molar ratio with retinol (probably)
  - More stable and easier to assay (ELISA)
  - Also under homeostatic control

- **Relative dose response tests**
  - Collect blood sample, administer a vitamin A supplement, collect another sample in 4-5 hours
  - Change in retinol or RBP indicates depleted liver stores
**Iron**

- Anemia screening (HemoCue) is cheap, simple, and very widely used, but a non-specific test
- Serum ferritin has been the gold-standard
  - Not reliable in the presence of inflammation
- Soluble transferrin receptor (sTfR)
  - Increases in the case of deficiency
  - Not impacted by inflammation (probably)
  - In replete individuals it’s a measure of erythropoiesis
  - Standardization of assays has been a problem (establishing a cut-off value)
- Hepcidin: liver hormone regulating iron homeostasis
  - Newly emerging marker of the body’s iron management system
Inflammation

• A number of vitamin and mineral biomarkers are affected by inflammation
• Assess inflammation and use results to either exclude or adjust micronutrient results
• Two most widely used markers are acute phase proteins
  – C-reactive protein (CRP), fast rise and decline
  – α1 acid glycoprotein (AGP), slower rise, elevated longer
Fig. 2. Idealised behaviour of acute-phase proteins during the course of infection. Standardised changes in plasma retinol (●), C-reactive protein (CRP; ○), α1-antichymotrypsin (ACT; □) and α1-acid glycoprotein (AGP; ◀) in response to trauma or infection (●).
New methods for the near-term

• Practical approaches to measuring multiple markers
  – Multiplexing

• Data analysis beyond prevalence estimates
  – Much of the data we collect could be mined much more heavily to understand relationships between the nutrients, disease processes, etc.
  – Steps toward a systems approach (“systems thinking” Kaput et al 2014 Genes Nutr 9:378)
Multiple Micronutrient Assessment Tool (MMAT) for iron, vitamin A status

Measures AGP, CRP, ferritin, RBP and sTfR simultaneously in 8µL of serum or plasma

Simple technology makes this an appropriate method for use in low to middle income country labs.
Conclusions: The risk of ID in obese Mexican women and children was 2–4 times that of normal-weight individuals at similar dietary iron intakes. This increased risk of ID may be due to the effects of obesity-related inflammation on dietary iron absorption. Thus, ID control efforts in Mexico may be hampered by increasing rates of adiposity in women and children. Am J Clin Nutr 2011;93:975–83.
New approaches to data collection: Vitamin A across the postpartum period

Vitamin A dynamics in breastmilk and liver stores: A life history perspective  Fujita et al. 2011 American Journal of Human Biology
Long-term: biomarker discovery

- Omics methods facilitate the search for informative differences in chemical fingerprints between groups (like deficient vs replete)
  - Ultimately may identify novel markers of nutrient status

**Figure 2a:** Whereas genes and proteins are subject to regulatory epigenetic processes and post-translational modifications, respectively, metabolites represent downstream biochemical end products that are closer to the phenotype. Accordingly, it is easier to correlate metabolomic profiles with phenotype compared to genomic, transcriptomic and proteomic profiles.

Biomarker Discovery: Metabolomics

Compare gene expression, proteins and metabolites across phenotypes to understand networks (here wild type vs transgenic mice)

FIG. 6. Correlation network of select expressed genes, proteins and lipids. The shading inside the box indicates the relative amount in the transgenic animals compared to wild type controls (red=higher level; green=lower level) Clish et al 2004
Lab methods are expensive and generate extremely large data sets
– data analysis is time consuming and methods are still under development

Can be purely empirical (very slow progress)

Likely result will be new markers practical for field work, rather than full metabolomic profiling of large sample sets
Biomarker Discovery: Metabolomics

Slide by Ben van Ommen, INSPIRE Nov 2012

Cellular machinery maintaining homeostasis in metabolism, oxidation, inflammation

Healthy male adult
Insulin resistant male adult
Child with impaired GI-function

- How does this profile change with changing Riboflavin?
- When will it “crack”?
- How does this profile look like for a TT-er?
- What happens in case of low Selenium?
- What if I was a top athlete?
- What is normal for a child?

Can we build a “biological network” with these parameters related to phenotypic descriptions?
Thank you

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