Micronutrients: markers of status to understand function?

Susan Fairweather-Tait, Norwich Medical School
First, some definitions

**[Bio]marker**: a characteristic that is reliably and accurately measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to an intervention.

**Status**: “state of the body in relation to the consumption and utilisation of nutrients”

**Function**: physiological role [and health sequelae]

![Diagram showing the relationship between Status, Function, Biomarker that predicts function, and Biomarker that validates prediction.](image-url)
The challenges

- Biomarkers of status for many micronutrients are either inadequate or lacking

- We do not have a comprehensive understanding of all of the functions of micronutrients

- Even when functions are known, there may not be any *in vivo* biomarkers

- The health consequences of changes in status and / or function are not well documented
Questions that will be addressed

- What is a ‘good’ biomarker of status?
- How do biomarkers of status relate to function?
  - Iron
  - Selenium
  - Vitamin D
- What are the limitations of the present evidence?
What is a good biomarker of status?

Types of biomarkers
- Analytes in blood, tissues and other body fluids e.g. serum concentration
- Functional indices e.g. enzyme activity
- Physiological measurements e.g. growth
- Genetic / metabolic data
- ‘Signatures’ or patterns of sets of data (-omics technologies)

Attributes
- Sensitive
- Specific
- Reproducible
- Robust
- Resistant to other influences
- Changes under dietary conditions of deficiency and overload
- Dose-responsive within the normal dietary range

Heaven by Salvadore Dali

University of East Anglia
Biomarkers of status: iron, iodine, copper, zinc, magnesium, selenium, folate and related B vits, and polyphenols

Workshop of invited experts, Norwich UK, 2008
Reviews published in BJN supplement

R = Research only
F = Field (survey) use
Rating
--- ***Excellent
--- ** Good
--- * Limited use
--- Not useful
Systematic reviews of biomarkers of status (response to changes in intake): riboflavin, B12, vit D, copper, selenium, zinc, iodine and n-3 fatty acids
How do biomarkers of status relate to function?

Using iron as an example

Functions of iron
- Component of haemoglobin in red blood cells – transports oxygen around the body
- Component of myoglobin – storage and use of oxygen in muscles
- Component of haem and iron-sulphur complexes in enzymes responsible for electron transport and energy generation in mitochondrial respiration and the citric acid cycle etc.

Examples of biomarkers of status
- Hb concentration is used to measure the presence and severity of deficiency
  - Insensitive
- Transferrin receptor concentration measures the degree of iron deficiency
- Plasma/serum ferritin is used to measure the size of iron stores
  - Confounded by infection/inflammation
What is the relationship between iron status and health?

Iron deficiency affects

- Physical work capacity
- Pregnancy outcome
- Cognitive, motor and behavioural development in children
- Immune function
- HIV infection
• Of the **4415** articles identified through searching, **333 RCTs** were assessed as full-texts and the following included for the prioritised health outcomes:

  - **Tiredness**
    - 3 papers data extracted (3 studies)
    - 1 Piper fatigue scale
    - 1 Fatigue score
    - 1 Self-reported weakness

  - **Physical Performance**
    - 20 papers data extracted (20 studies)
    - Aerobic capacity/Endurance: Maximum capacity tests: VO₂max (8), VE max (1), time to exhaustion (6), max heart rate (2), etc.
    - Submax capacity test: VE (1), RER (6), HR (5), work rate (4), EE (5), %VO₂max (5), workload (3), etc.
    - Anaerobic capacity: Blood lactate (5), ventilatory threshold (3), etc.
    - Efficiency: Work efficiency (2), % efficiency during exercise (2), etc.

  - **Immune Function**
    - 3 White blood cell count
    - 1 Fibrinogen conc.
    - 1 Amyloid A conc
    - 1 sCRP
    - 1 Infections prevalence
    - 1 Infections intensity

  - **Secondary outcomes**
    - 3 papers data extracted (2 cognitive funct; 1 RLS)
    - 1 Maze test
    - 1 Card sort
    - 1 Attention test
    - 1 Memory test
    - 1 Learning test
    - 1 Serial 7
    - 1 Test
    - 1 IRLS

• The majority of analysis was based on iron intake (+/- supplement), although many trials also measured biomarker responses these were not generally analysed directly in relation to the health outcomes

• A small number of cohort studies were identified (3) which assessed the effect of training regimes on iron status markers; one assessed the relationship between iron status and physical ability in a frail elderly population
### Iron and physical performance (RCTs)

**Aerobic Capacity/Endurance**
- **Max capacity test**: VO$_2$max, max minute ventilation (VE), time to exhaustion, max work rate*, heart rate (HR), max workload*
- **Sub-max. capacity test**: VE, RER, HR, work rate*, energy expenditure (VO$_2$/RER), average oxygen pulse (VO$_2$/HR), % VO$_2$max, workload*

**Anaerobic capacity**
- Blood lactate threshold, ventilatory threshold

**Efficiency**
- Tea picked, voluntary physical activity, pieces of clothing sewn, % efficiency during exercise

*Can be measurement of maximal or sub-maximal capacity, depending on how assessed during the exercise trial.*
### Meta-analyses of aerobic capacity (effect of iron supplementation)

<table>
<thead>
<tr>
<th></th>
<th>Mean difference (95% CI)</th>
<th>p</th>
<th>No included studies</th>
<th>I² (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Max capacity test</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>VO₂max</strong></td>
<td>Athletes</td>
<td>1.99 (0.61, 3.37)</td>
<td>&gt;0.01</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Untrained</td>
<td>0.25 (-2.36, 2.87)</td>
<td>NS</td>
<td>2</td>
</tr>
<tr>
<td><strong>Time to exhaustion (min)</strong></td>
<td>Athletes</td>
<td>0.33 (-3.79, 4.44)</td>
<td>NS</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Untrained</td>
<td>1.70 (0.39, 3.01)</td>
<td>0.01</td>
<td>1</td>
</tr>
<tr>
<td><strong>Respiratory exchange ratio</strong></td>
<td>Athletes</td>
<td>-0.02 (-0.04, 0.00)</td>
<td>NS</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Untrained</td>
<td>0.02 (-0.02, 0.05)</td>
<td>NS</td>
<td>2</td>
</tr>
<tr>
<td><strong>Sub-Max capacity test</strong></td>
<td>Athletes</td>
<td>-6.45 (-14.11, 1.22)</td>
<td>NS</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Untrained</td>
<td>-5.00 (-11.18, 1.18)</td>
<td>NS</td>
<td>1</td>
</tr>
<tr>
<td><strong>Work rate</strong></td>
<td>Athletes</td>
<td>5.50 (-25.92, 36.92)</td>
<td>NS</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Untrained</td>
<td>-0.36 (-14.81, 14.09)</td>
<td>NS</td>
<td>3 (4 arms)</td>
</tr>
<tr>
<td>Study</td>
<td>Main performance test</td>
<td>Principal measures</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-------------------</td>
<td>------------------------------------------------------------</td>
<td>----------------------------------------------------------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fogelholm 1992</td>
<td>Incremental ergometer test</td>
<td>Blood lactate, VO$_{2\text{max}}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Powell 1991</td>
<td>Endurance run on treadmill</td>
<td>VO$_2$, lactate, RER (respiratory exchange ratio)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LaManca 1993</td>
<td>Cycle ergometer with increasing intensity</td>
<td>Heart rate (HR), lactate, RER, VO$_2$ max</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Friedmann 1999</td>
<td>Incremental treadmill test (+ training camp)</td>
<td>HR, lactate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Klingshirn 1999</td>
<td>Graded maximal exercise test on treadmill</td>
<td>RER, lactate concentration, time to exhaustion, VO$_{2\text{max}}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Schone 1983</td>
<td>Cycle ergometer with progressive work protocol</td>
<td>HR, lactate max, maximum workload, VO$_{2\text{max}}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yoshida 1990</td>
<td>3000m run + incremental treadmill test</td>
<td>RER, lactate threshold, time trial, VO$_{2\text{max}}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zhu 1998</td>
<td>15km bicycle ergometer + incremental test</td>
<td>Time trial, HR, energy expenditure, % VO$_{2\text{peak}}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Newhouse 1989</td>
<td>Incremental cycle ergometer + treadmill tests</td>
<td>Wingate power test, max &amp; mean power</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rajaram 1995</td>
<td>Treadmill with increasing elevation</td>
<td>Maximum time on treadmill, VO$_{2\text{max}}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lieden 1974</td>
<td>Increasing ergometer test</td>
<td>Work capacity at 170bpm HR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hinton 2000/Brownlie 2004</td>
<td>Incremental ergometer + 15km tests</td>
<td>Energy expenditure, HR, RER, % VO$_2$, Time trial, efficiency</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hinton 2007</td>
<td>Cycle ergometer tests</td>
<td>Total energy expenditure, max HR, RER, efficiency, ventilation threshold (VT), VO$_{2\text{peak}}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ericsson 1970</td>
<td>Bicycle ergometer tests</td>
<td>Physical work capacity (HR max)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cotes 1972</td>
<td>Cycle ergometer tests</td>
<td>HR, VO$_2$ @ 30W</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brutsaert 2003</td>
<td>Knee-extension exercise protocol</td>
<td>Muscle fatigue</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Edgerton 1979</td>
<td>Tea-picking (work productivity), daily activity</td>
<td>KG tea per person, right ankle movement</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Florencio 1981</td>
<td>Sewing clothes (work productivity)</td>
<td>Efficiency = ((no. pieces per day x minutes per piece) / minutes at work) x 100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Magazanik 1991</td>
<td>Treadmill with increasing gradient + training</td>
<td>VO$_{2\text{max}}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lyle 1992</td>
<td>Treadmill walking with increasing elevation</td>
<td>VO$_{2\text{max}}$</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Summary of the physical performance systematic review

- Quality of included studies was low and risk of bias assessed as unclear or high in all studies
- Physical performance is mostly measured in athletes or training volunteers - not relevant for the general population
- Aerobic capacity was the most commonly assessed outcome: studies reported different tests that could not always be combined, making the meta-analysis difficult
- Lack of good quality studies prevented meta-analysis of other outcomes
- Future research should focus on standardising physical performance measures, using good quality and informative measures
How do biomarkers of status relate to function?

Deriving DRVs for selenium

**Input information**

- **Benefit**: Plasma GPx activity
  - (data from dose-response studies in China and NZ)

- **Risk of selenosis**: epidemiological data from China, and 200µg/d supplementation trial (Clark)

**EAR (50% response)**: 45µg/d

**UL (50% response)**: 3200µg/d

Adapted from Renwick et al 2008
Effect of selecting different functional endpoints

Input data
- Possible anti-cancer effect
  (Clark study and other trials)
- Prolonged prothrombin time (data from high Se areas in China)

\[
\text{EAR } 143 \mu\text{g/d}
\]
\[
\text{UL } 897 \mu\text{g/d}
\]

Adapted from Renwick et al 2008
Translating biomarkers of status into dietary recommendations

Dose-response relationship between selenium intake and plasma selenium and selenoprotein P concentrations

*Hurst et al. Am J Clin Nutr 2010*
Relationship between selenium intake, status (serum Se) and health outcome (prostate cancer)

**SELECT trial (2001-2004)**
- Double-blind randomised placebo-controlled trial of 35,533 men with raised serum prostate-specific antigen levels - USA, Canada and Puerto Rico
- Assigned to Se (200µg/d as SeMet), vit E, Se + Vit E, or placebo groups

![Graph showing serum Se levels over time with placebo and selenium groups](image)

*NPC trial effect cut-off 120µg/L

*Clark et al. JAMA 1996;276:1957-63

*Lippman, S. M. et al. JAMA 2009;301:39-51*
Relationship between nutrient intake, status and function

Intake

Morris & Tangney JAMA 2011;305:13-14
Scientific Opinion on the substantiation of health claims related to calcium and ... muscle function and neurotransmission... pursuant to Article 13(1) of Regulation (EC) No 1924/2006

**EFSA Journal 2009; 7(9):1210**

**Muscle function and neurotransmission**

The claimed effects are “muscle function”, “nerve transmission/function”, “nerves and muscle”. The target population is assumed to be the general population. Normal muscle function and neurotransmission are beneficial to human health.

A cause and effect relationship has been established between calcium and normal muscle function and neurotransmission in healthy subjects.

The evidence provided does not establish that inadequate intake of calcium leading to impaired muscle function and neurotransmission occurs in the general EU population.

The following wording reflects the scientific evidence: “Calcium contributes to normal muscle function and neurotransmission”.

Example of a well-accepted function that cannot be measured
How do biomarkers of status relate to intake?
Using Vitamin D as an example


What is the target 25(OH)D concentration for ‘optimal’ function?
Lack of consensus for target values for 25(OH)D

Examples from the literature

- Preventing rickets / osteomalacia: 25-30 nmol/L
  - US DRI committee: 40 nmol/L EAR: mid-point of the range (30-50 nmol/L) which includes an additional 30% to cover needs of 97.5% of the population
  - Optimal bone health: 75 nmol/L (e.g. Vieth 2011)

- Various non-skeletal effects, including CVD risk, colorectal cancer, prostate cancer, diabetes, immune function (e.g. respiratory function)...

- Multiple health outcomes: 75 nmol/L (consensus statement, Dawson-Hughes et al 2005)

- Need to define ‘optimal’ requirements in particular sub-groups of the population for a wide range of outcomes (Rank Forum, Lanham-New et al 2011)

- Effects of obesity: increases requirements? (Cheng et al 2010)

- Effects of inflammation: reduces 25(OH)D concentration but not ‘status’ (Reid et al 2011)
No effect of GPx1 polymorphism on mean plasma Se concentration or GPx activity, but the relationship between GPx activity and Se concentration was significantly affected by the SNP.

# Genotype-phenotype database

Functional SNPs and Intake/Status/Health data for selenium, folate, B12, zinc and iron

![Database Image]
Reflections on current evidence

- Intake-health relationships depend on the choice of biomarker(s) – can significantly influence dietary requirements and recommendations
- A dose-response relationship between status and some well-accepted functions cannot be demonstrated because life not sustained below the threshold at which an effect can be observed
- The baseline status of volunteers participating in an RCT may mask the functional effects of the nutrient and lead to an inappropriate interpretation of effects on health
  - this is sometimes the reason why there is disparity between results from different RCTs
- Genotype may have functional effects with some biomarkers
New work on biomarkers

Goal of the BOND Program
The BOND Program aims 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.

The Translational Track will develop processes to inform various user groups about appropriate biomarker selection and use. The development and use of the translational materials will then generate a research agenda that will support the next track.

The Research Track will support the discovery of biomarkers and development of their use.

What is the future for biomarkers?

Wise thoughts from the past

“*If your results don't make sense, think and think again! You may have made a mistake or you may have made a discovery. Above all, treasure your exceptions; you will learn most from them.*”

**Elsie Widdowson** (1906-2000)

“*Much needs to be learned about the quantitative extent to which genetic, epigenetic and dietary factors interact to determine the nutritional phenotype.*”


New / improved biomarkers of micronutrient status are needed – these will allow us to examine the relationships between intake, function and health to
- inform public health policies
- enhance personalised nutrition
- facilitate the development of healthier food products
Thank you for listening!

Acknowledgements

EURRECA colleagues, including the UEA team
  Rachel Collings
  Amelie Casgrain
  Lee Hooper
  Linda Harvey
  Rachel Hurst
  Maria King