Biomarkers of nutrition status: recent advances with metabolomics

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THE PURPOSE OF NUTRITIONAL ASSESSMENT

- Identify population groups who do not have adequate nutrition for preventing diseases or for optimal performance
- Develop nutrition programs for at risk populations and monitor their impact

Assess exposure to nutrients and non-nutrients

Characterize dietary patterns of individuals

Study health effects of dietary patterns, foods, nutrients
THE CHALLENGE OF NUTRITIONAL ASSESSMENT

• Still a huge challenge in the post-genomic era:
  - Heterogeneity and variability of food choices
  - Limited knowledge of the composition of foods beyond the ~60 essential nutrients & best-known food bioactives

• Methods must be accurate, sensitive and applicable to many populations

• Existing methods:

1- Anthropometric methods
2- Clinical examination
3- Questionnaires
4- Biomarkers
1-ANTHROPOMETRIC METHODS

- **BMI**, Hip/waist ratio...

![Anthropometric Methods Diagram]

**Indicator of adequacy of energy intakes**

**Correlation with diet quality?**
2-CLINICAL EXAMINATION

- Physical signs associated to severe vitamin or mineral deficiencies

Vit C deficiency (*Scurvy*)

Vit A deficiency (*Xerophthalmia*)

Iron deficiency (*Anemia*)

Vit D deficiency (*Rickets*)

Not for mild deficiencies
Today dietary assessment mainly relies on questionnaire instruments:
- Food Frequency Questionnaires (10-300 foods)
- 24hr recalls
- Food records

New tools (web-based; digital camera)

3- QUESTIONNAIRES: LIMITATIONS

• Recall errors, Misreporting, Difficulty to assess portion size (literacy problems, desire to reduce the inconvenience of reporting, desire to be perceived as compliant to a socially acceptable behaviour…)

• Inappropriate for some populations (children, obese people, elderly with cognitive impairment…)

Attenuation of associations with health outcomes

Could Exposure Assessment Problems Give Us Wrong Answers to Nutrition and Cancer Questions?
Arthur Schatzkin, Victor Kipnis

Is It Time to Abandon the Food Frequency Questionnaire?
Alan R. Kristal, Ulrike Peters, and John D. Potter

Are imprecise methods obscuring a relation between fat and breast cancer?
Sheila A. Bingham, Robert Luben, Ailsa Welch, Nicholas Wareham, Kay-Tee Khaw, Nicholas Day
# 4- Biomarkers

- Targeted analysis in biofluids (blood, serum, urine, saliva) or tissues (hair, nail, skin, erythrocytes, adipose tissue)

- Some biomarkers are nutrients or bioactives and reflect their status or exposure
- Some are used as surrogate biomarkers of food intake

### List of commonly used biomarkers

<table>
<thead>
<tr>
<th>Doubly labeled water</th>
<th>Total energy expenditure</th>
</tr>
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<tbody>
<tr>
<td>Urinary nitrogen</td>
<td>Protein intake</td>
</tr>
<tr>
<td>Urinary sodium</td>
<td>Sodium intake</td>
</tr>
<tr>
<td>Urinary sucrose and fructose</td>
<td>Sugar intake</td>
</tr>
<tr>
<td>Fatty acids (erythrocytes, adip tissue)</td>
<td>Dietary fats, fatty acids</td>
</tr>
<tr>
<td>Plasma Vitamin C</td>
<td>F&amp;V</td>
</tr>
<tr>
<td>Plasma carotenoids</td>
<td>F&amp;V</td>
</tr>
<tr>
<td>Plasma alkylresorcinols</td>
<td>Whole grain wheat and rye</td>
</tr>
<tr>
<td>Urine Methylhistidine</td>
<td>Meat</td>
</tr>
<tr>
<td>TMAO</td>
<td>Fish</td>
</tr>
<tr>
<td>Urine polyphenols</td>
<td>Red wine, citrus, tea, soy, olive oil…</td>
</tr>
</tbody>
</table>

*Jenab et al., Hum Gen 2009; Perez-Jimenez et al., AJCN 2010; Hedrick et al., Nutr J 2012*
HYPOTHESIS-DRIVEN APPROACH FOR BIOMARKER DISCOVERY

- Previous knowledge of a specific compound of the food
- Bioavailability and method of analysis of the specific compound

One compound ⟷ One food or food group

Specificity?
High dependence on factors affecting the biomarker concentration
(food matrix, interindividual variation in absorption and metabolism, smoking, condition of sampling and storage, analytical method…)

- New biomarkers needed to cover a larger spectrum of foods
**Metabolic profiling** of plasma/urine samples of consumers and non-consumers of a given diet/food/nutrient (cohort or intervention studies)

**Comparison with multivariate statistics**

Food metabolome = All metabolites that directly derive from digestion and metabolism of food chemicals
WORKFLOW FOR FOOD METABOLOME ANALYSES

1. High Resolution Mass spectrometry or NMR analysis

   Group 1
   Plasma, urine, saliva, etc...

   Group 2

2. Multivariate statistics
   (PCA, PLS-DA...)

   Score Plot (Groups)

   Loading Plot (Markers)

   Visualization
   Discriminating ions

3. Identification of discriminating ions

   Spectral data (MS/MS,...)
   Databases, Libraries of spectra
   Analysis of standards

   Non-targeted method:
   Detection of as many compounds as possible

   In-house libraries
DISCOVERY OF BIOMARKERS OF FOOD INTAKE

- Controlled intervention study with orange juice

12 volunteers – 4 weeks
500 ml/d **Orange juice** / Control drink
Usual diet, Cross-over design
24h urine D30; LC-ESI⁺-Qtof

**Score plot PLSDA**

**HCA heatmap**

105 significant ions

**Pujos-Guillot et al., J Proteome Res, 2013**
BIOMARKER VALIDATION: PROLINE BETAINE AS AN EXAMPLE

- Associated with citrus intake in 3 acute studies, 3 medium-term interventions, 3 cohort studies
- Detected with NMR, LC-QTof, FIE-MS
- In morning spot urines, 24hr urine & post-prandial urine kinetics

Heinzmann et al., 2010, Lloyd et al., 2011 & 2013, Pujos-Guillot et al., 2013, May et al., 2013, Andersen et al., 2014

- Found almost exclusively in citrus fruits, with dominance in orange

Heinzmann et al., 2010; de Zwart et al., 2003; Slow et al., 2005

- Pharmacokinetics data

Heinzmann et al., AJCN 2010

- Validation in INTERMAP-UK cohort

ROC curve
Training set n=220
Validation set n=279

« Excellent biomarker »

250 ml orange juice challenge

Heinzmann et al., AJCN 2010
21 foods studied

Citrus, Apple, Raspberry, Aronia, Strawberry, Tomato, Soy, Cruciferous vegetables, Beetroot, Almonds, Nuts, Cocoa drink, Coffee, Red wine, Grape juice, Black tea, Green tea, Whole rye grain, Milk, Cheese, Salmon, Cod

- Acute or medium-term intervention studies (4 days-12 weeks; 4-61 subjects)
- >90% used urine samples (Spots, 24hr urines, or kinetics)
- NMR (8 studies), LC-MS (18 studies) or GC-MS (4 studies), multiplatform analyses (5 studies)

145 candidate biomarkers (75%= phytochemical metabolites)

Scalbert et al., AJCN 2014, 99(6):1286-1308

SU.VI.MAX2 sub-cohort (210 M & F; 55-70 y)

Selection of low and high consumers for 20 plant foods or food groups

Matched on:
- Sex
- Age class (10 ans)
- Sampling season
- BMI in 2 classes

Distribution of food consumption
Correlations between consumptions

Biobank (One morning spot urine)

UPLC-ESI-Qtof-MS (pos&neg)

Comparison of urine metabolomes of low and high consumers

PhenoMeNEp project
Coll. S. Hercberg, P. Galan, M. Touvier
UREN, Inserm/INRA/CNAM/Paris 13

METABOLOMICS FOR DISCOVERY OF PLANT FOOD INTAKE BIOMARKERS
METABOLOMICS FOR DISCOVERY OF PLANT FOOD INTAKE BIOMARKERS

Good discrimination for most foods, especially those consumed frequently & rich in phytochemicals
### Identification of Metabolites: The Bottleneck

- Databases are not complete
- Many food chemical metabolites are still unknown
- Standards are lacking

#### Spectral data

<table>
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<tr>
<th>m/z</th>
<th>%</th>
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<tr>
<td>100</td>
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</table>

#### List of discriminating ions

- CHZ OR 0-6 473 (14.063) 1: TOF MS ES+ 2.33e3
- 369.0930
- 347.1316
- 311.1183
- 153.1078
- 293.0958
- 715.2088
- 370.1058
- 693.2535
- 371.1097
- 716.2155

#### Databases and libraries of spectra

**Analysis of authentic standards**

- Names and chemical structures?

#### 75% of the detected ions are not easily identified

and require additional work

*(Analytical chemistry + bioinformatics)*
Coffee contains various bioactives implicated with human health and well-being.

Uncertainties on the effect of coffee on diabetes, cardiovascular diseases, neurodegenerative diseases, cancer…

…maybe due to inaccuracy of dietary assessment.

Different methods of preparation: Poor comparability across studies.

No specific biomarker available.
### BIOMARKERS OF COFFEE INTAKE

#### 25 very strong discriminating ions

<table>
<thead>
<tr>
<th>QTOF RT</th>
<th>ESI</th>
<th>m/z</th>
<th>Hypothesis</th>
<th>P_value</th>
<th>VIP</th>
<th>Identification</th>
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<tbody>
<tr>
<td>0.7</td>
<td>p</td>
<td>138.052</td>
<td>1-Methylurate [M-H]</td>
<td>8.68E-06</td>
<td>2.57</td>
<td>1-Methylurate*</td>
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<tr>
<td>3.6</td>
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<td>181.04</td>
<td>1-Methylurate [M-H] loss HCNO</td>
<td>6.48E-06</td>
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<td>6.75E-05</td>
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<td>4.4</td>
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<td>165.045</td>
<td>1-MethylurateXanthine [M-H]</td>
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<td>5.3</td>
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<td>357.104</td>
<td>Theobromine+AnhGlu [M+H]*</td>
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<tr>
<td>5.6</td>
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<td>200.108</td>
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<td>6.75E-05</td>
<td>2.42</td>
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<tr>
<td>5.8</td>
<td>p</td>
<td>197.068</td>
<td>1,7-Dimethylurate [M+H]*</td>
<td>8.51E-07</td>
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<td>2.42</td>
<td>1,7-Dimethylurate*</td>
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#### Many caffeine metabolites

- Not specific enough

* Validated with standard
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</table>

*Validated with standard

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* Rothwell et al., Plos One 2014, 9:e93474
Biomarkers performance (ROC curves)

**AUC**
- **Excellent**: 0.9-1
- **Good**: 0.8-0.9
- **Fair**: 0.7-0.8
- **Poor**: 0.6-0.7
- **Fail**: 0.5-0.6

**Biomarkers**
- Caffeine: AUC: 0.808
- Hippuric acid: AUC: 0.796
- Atractyligenine glucuronide: AUC: 0.979
- Cyclo-(Isoleucyl-prolyl): AUC: 0.968
- Trigonelline: AUC: 0.937

*Rothwell et al., Plos One 2014, 9:e93474*
**Biomarkers Performance (ROC Curves)**

**Figure 4.** ROC curve AUCs for single and combination biomarkers. Error bars represent 95% confidence intervals. clP, cyclo(isoleucyl-prolyl); MX, 1-methylxanthine; Tr, trigonelline; Atr, atractyligenin glucuronides; Caf, caffeine.

- Combination of markers increases performance
- Metabolomics provides multiple biomarkers
- Validation required (quantitative analysis in other studies)

Rothwell et al., Plos One 2014, 9:e93474
DATA SHARING FOR BIOMARKER VALIDATION

- Common data repository with metabolic profiles and food intake data
Define common objectives and priorities

• Consensus on most needed biomarkers

• Develop databases and bioinformatic tools for identification of unknowns

• Sharing data and resources (SOPs, chemical standards, samples, raw data…)

• Databasing biomarkers with a new validation scoring system

The food metabolome: a window over dietary exposure\textsuperscript{1–3}  
AJCN 2014, 99(6):1286-1308

Augustin Scalbert, Lorraine Bremner, Claudine Manach, Cristina Andres-Lacueva, Lars O Drugsted, John Draper, Stephen M Rappaport, Justin JJ van der Hooff, and David S Wishart
PHYTOHUB (www.phytohub.eu)

- An online database for dietary phytochemicals and their human metabolites

1,000 dietary phytochemicals

Dietary sources

Known metabolites

Predicted metabolites

Spectral data

Physico-chemical data

Links to other databases
TOWARDS THE USE OF METABOLIC PROFILES FOR NUTRITIONAL ASSESSMENT

Cohort studies

Biobank samples

Standardized Food Metabolome profiling (Biomarkers + known bioactives+ others)

Food metabololome profiles

Socio-economic data, Anthropometric data, Dietary habits (FFQ), Lifestyle, Genotype, Gut microbiota, Biological and Medical data, Other phenotypic data...

Diet-Health associations

Adequacy of dietary behaviour?

New food bioactives

Detailed information
Actual internal exposures of individuals
CONCLUSION

• Nutritional exposures are more complex than what has been covered so far: many non-nutrients to consider

• The Food metabolome contains a wealth of information that we are just starting to explore with MS-based metabolomics

• Profiling of urine or plasma metabolomes in interventions or cohorts studies is efficient to discover new candidate biomarkers of food intake (data-driven approach)

• International collaboration is essential to move forward (validation)

• Beyond biomarker discovery, food metabolome profiling in biofluids may become a new method for nutritional assessment
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