Biomarkers: examples from cancer epidemiology

In memory of Sheila Bingham

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Nuffield Department of Clinical Medicine
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Sheila Bingham (Rodwell) 1947-2009

Outstanding contributions to nutrition include:

- nutritional biomarkers
- health effects of dietary fibre
- effects of meat on endogenous nitrosation
- application of detailed nutritional methods to large-scale epidemiology

Established the MRC Centre for Nutrition and Cancer in Cambridge in 2005

Long standing contributions to:
- Committee on Medical Aspects of Food Policy
- Scientific Advisory Committee on Nutrition

Honours:
- Fellow of the Academy of Medical Sciences
- Professorships in Cambridge and Coleraine
- Appointed OBE in June 2009
• Biomarkers for validation
  – Example of choice of food diaries in British arm of EPIC

• Biomarkers for large-scale epidemiology – examples from EPIC
  – Long-term reliability
  – Confounding
  – Reverse causation

• Biomonitoring
  – Standardized methods
  – Stable assay: chemical standards for accuracy, negligible assay drift

• Conclusions
<table>
<thead>
<tr>
<th>Reference measure</th>
<th>Dietary measure</th>
<th>Timing</th>
<th>Spearman correlations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urinary nitrogen from up to 8 x 24-h collections, g/24 h</td>
<td>Dietary nitrogen, g/d</td>
<td>During 1 year</td>
<td>0.67* 0.49*</td>
</tr>
<tr>
<td>Urinary potassium from up to 8 x 24-h collections, g/24 h</td>
<td>Dietary potassium, g/d</td>
<td>During 1 year</td>
<td>0.64* 0.41*</td>
</tr>
<tr>
<td>Energy from 16 x 1-d weighed records, MJ/d</td>
<td>Dietary energy, MJ/d</td>
<td>During 1 year</td>
<td>0.59 0.52</td>
</tr>
<tr>
<td>Dietary fat from 16 x 1-d weighed records, % energy</td>
<td>Dietary fat % energy</td>
<td>During 1 year</td>
<td>0.77 0.64</td>
</tr>
<tr>
<td>Serum cholesteryl ester saturated fatty acids, %</td>
<td>Saturated fatty acids, % of fat</td>
<td>One blood sample</td>
<td>0.16 0.26</td>
</tr>
<tr>
<td>Serum cholesteryl ester linoleic acid, %</td>
<td>Linoleic acid, % of fat</td>
<td>One blood sample</td>
<td>0.62 0.53</td>
</tr>
</tbody>
</table>

* Energy-adjusted. Data from 156 women in Bingham et al 1997 and 403 women in Brunner et al 2001
Application of detailed nutritional methods to large-scale epidemiology


- MRC Centre for Nutrition and Cancer: UK Dietary Cohort Consortium of 8 prospective studies with 4-7 day food diaries, total cohort about 100,000 participants
  - EPIC-Norfolk
  - EPIC-Oxford
  - UK Women’s Cohort Study
  - Whitehall II
  - MRC NSHD
  - ProtecT
  - Oxford Vegetarian Study
  - Guernsey Study
Dietary fibre and colorectal cancer: UK Dietary Cohort Consortium

Quintiles of fibre, food diaries

Quintiles of fibre, FFQs

579 cases/1996 controls in 7 cohorts. Dahm et al JNCI 2010
Dietary fat and breast cancer
Dietary total fat as % energy and breast cancer: EPIC-Norfolk 2003 and CNC 2011

Bingham et al Lancet 2003; Key et al AJCN 2011
## Trials of low fat diet and breast cancer risk

<table>
<thead>
<tr>
<th>Trial</th>
<th>Number of cases</th>
<th>RR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Women’s Health Initiative (Prentice et al JAMA 2006)</td>
<td>1727</td>
<td>0.91 (0.83-1.01)</td>
</tr>
<tr>
<td>Canadian trial (Martin et al Cancer Res 2011)</td>
<td>220</td>
<td>1.19 (0.91-1.55)</td>
</tr>
<tr>
<td>Pooled</td>
<td>1947</td>
<td>0.94 (0.86-1.03)</td>
</tr>
</tbody>
</table>
Food diaries in large-scale epidemiology

• Biomarkers show food diaries are more precise than FFQs for some nutrients, at least over a short period
• But extended analyses in Britain have not confirmed that fat estimated by food diaries is positively associated with breast cancer
• Diaries have limitations
  – Slow and very expensive to code
  – Generally nested case-control design – versus full cohort analysis with FFQs
  – Diary coding is subject to errors, rather like laboratory assays. Therefore case-control sets should be batched, and coding variations should be measured, as is done for laboratory assays
• The future for large-scale epidemiology is electronic methods
Biomarkers for large-scale epidemiology

- Long-term reliability
- Confounding
- Reverse causation
Reproducibility of some biomarkers over 1-3 years, Nurses’ Health Studies

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>ICC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma beta-carotene</td>
<td>0.73</td>
</tr>
<tr>
<td>Plasma alpha-tocopherol</td>
<td>0.83</td>
</tr>
<tr>
<td>Red blood cell n-3 PUFAs</td>
<td>0.68</td>
</tr>
<tr>
<td>Red blood cell n-6 PUFAs</td>
<td>0.39</td>
</tr>
<tr>
<td>Plasma 25-OH vitamin D</td>
<td>0.72</td>
</tr>
<tr>
<td>Plasma genistein</td>
<td>0.02</td>
</tr>
<tr>
<td>Blood pressure</td>
<td>0.62</td>
</tr>
<tr>
<td>Serum cholesterol</td>
<td>0.65</td>
</tr>
</tbody>
</table>

Authors: ICC of 0.4 and above is sufficient to use single measure.

Kotsopoulos et al CEBP 2012
Vitamin C and stomach cancer
Plasma and dietary vitamin C and risk of stomach cancer in EPIC

Quartiles of dietary vitamin C

Quartiles of plasma vitamin C

215 cases and 416 controls. Jenab et al Carcinogenesis 2006
### Vitamin C, smoking and stomach cancer in EPIC

<table>
<thead>
<tr>
<th>Smoking status</th>
<th>Plasma vitamin C µmol/l</th>
<th>RR for plasma vitamin C increase of 19.4 µmol/l</th>
<th>Extended follow-up, RR for dietary citrus fruit increase of 50 g/d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Never</td>
<td>43.5</td>
<td>1.13 (0.86-1.47)</td>
<td>1.00 (0.90-1.11)</td>
</tr>
<tr>
<td>Former</td>
<td>40.6</td>
<td>0.91 (0.67-1.24)</td>
<td>1.01 (0.90-1.14)</td>
</tr>
<tr>
<td>Current</td>
<td>38.9</td>
<td>0.67 (0.43-1.06)</td>
<td>0.86 (0.74-1.01)</td>
</tr>
<tr>
<td>$P$ for difference</td>
<td>NS</td>
<td>-</td>
<td>0.07</td>
</tr>
</tbody>
</table>

Jenab et al Carcinogenesis 2006; Gonzalez et al Int J Cancer 2012
Vitamin C and stomach cancer: comments

• Stomach cancer risk appears to be more related to plasma vitamin C than to dietary vitamin C, or dietary citrus fruit

• But we can’t yet exclude:
  – residual confounding - e.g. by smoking
  – reverse causality – growing tumour present before diagnosis may affect absorption and metabolism

• More data needed, with longer follow-up
Selenium and prostate cancer

• Generally agreed that dietary measures are not useful, therefore biomarkers are needed

• Reproducibility seems good:
  – e.g. Taylor 1987, serum over 1 y, ICC = 0.76
Plasma and toenail selenium and risk of prostate cancer in EPIC and Netherlands Cohort Study

**EPIC:** quintiles of plasma selenium

**NCS:** quintiles of toenail selenium

NCS 540 cases/1211 subcohort, van den Brandt CEBP 2003
Smoking and selenium

Concentration as % of never smokers

Selenium and prostate cancer: comments

• Different biomarkers - such as plasma and toenails - may give different results and may be differentially affected by confounding factors

• A large randomized trial has shown no benefit of supplementation - in a high selenium area (SELECT in the USA)
Vitamin D and cancer

• Generally agreed that dietary measures are not useful, therefore biomarkers are needed

• Reproducibility seems fair to good:
  – Al-Delaimy et al Biomarkers 1987, EPIC-Netherlands, serum over 2-5 y, ICC = 0.65
  – Meng et al CEBP 2012, WHI, plasma over 5 y, ICC = 0.59 (0.54-0.64)

• Confounding may be a big problem?
Serum 25-OHD, BMI and physical activity

Vitamin D, prostate and colorectal cancer in EPIC

Prostate 652 cases/752 controls, Travis AJE 2009.
Reverse causality? – example of plasma 25-OH vitamin D after elective knee surgery

33 patients, knee arthroplasty. Reid et al AJCN 2011
Reverse causality: example of cholesterol and colon cancer

- Rose 1974
  - Prospective study, 90 deaths from colon cancer
  - Cases had lower than expected blood cholesterol

- Kritchevsky 1991
  - Cholesterol measured every 2 months in prospective study, 103 incident cancer cases
  - Cholesterol started to decrease 2 years before cancer diagnosis
Serum lipids and colorectal cancer in EPIC

1238 cases/1238 controls. Mean follow-up 3.8 y. Van Duijnhoven et al Gut 2011
## Cholesterol and cancer, Mendelian randomization study

<table>
<thead>
<tr>
<th>Analysis</th>
<th>Change in LDL cholesterol</th>
<th>RR</th>
<th>$P$ for difference in RRs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Measured cholesterol</td>
<td>50% increase</td>
<td>0.91 (0.83-0.99)</td>
<td></td>
</tr>
<tr>
<td>Genetic predictors of cholesterol</td>
<td>50% increase</td>
<td>1.04 (0.95-1.15)</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Copenhagen City Heart Study and Copenhagen General Population Study. Benn et al, JNCI 2011
Requirements for future studies

Long-term reliability

Available data are sparse - and non-existent for some biomarkers

Need data over 10 years - or more

Repeat samples in whole cohort would help

Confounding

A big problem: more emphasis needed on factors which affect biomarkers, e.g. smoking, to quantify and understand mechanisms

Reverse causation

Needs much more investigation

Large studies with long follow-up

Repeat samples would help