Biomarkers in nutritional epidemiology

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Traditional Epidemiology
Exposure --- Disease
Molecular Epidemiology
Markers of Exposures --- Markers of Disease
Exposure Internal Dose --- Biologically Effective Dose
Early Biologic Effect --- Altered Structure Function
Clinical Disease --- Prognostic Significance
Markers of Susceptibility

Biomarkers in Epidemiology

Figure 1-1. Sites of uptake, target tissue, and media for monitoring for xenobiotic compounds

Biomarkers of Dietary Intake
Specific Aims
1. To measure long-term dietary intake
2. To assess nutritional status*

Specific Applications
1. Indicators for intake of specific nutrients
2. Validating dietary assessment (e.g. FFQ)

Criteria for Biomarker Evaluation
1. Is it sensitive to intake?
2. Is it time-integrated?
3. What other factors (aside from intake) influence levels (e.g. genetic variants)?
4. What are the sources of measurement error?

1. SENSITIVITY TO INTAKE
- Sensitive to short or long-term intake

LIMITING FACTORS
HOMEOSTATS
- Saturation of absorption (e.g. Iron)
- Excretion of excess (e.g. Vitamin C)
- Complex hormonal pathways (e.g. Calcium, VitD)
Intake and levels rarely linear

BIOAVAILABILITY
Not all chemical forms of a nutrient are equally absorbed
Selenium

Serum Se
---
Sodium Selenite

1

1

1

1
2. **Time Integration**

Time scale of response

- Need months or years
- NOT days or weeks

Partially a function of biological samples

- plasma - up to several weeks
- RBC - 2-4 months
- adipose - 2-3 years

3. **Non-Dietary Determinants**

- Genetic
- Environmental
- Lifestyle

No association with disease

Misclassification

Associated with disease

Con founding

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**Misclassification**

- E.g. Vitamin E and Cholesterol

**Vitamin E: FFQ Estimate vs. Plasma**

- CRUDE: $r = 0.12$, $p = 0.19$
- ADJUSTED: $r = 0.28$, $p = 0.02$

Cholesterol

- Age
- Sex
- Caloric intake
- Triglycerides

**Other determinants inadequately characterized for most nutrient markers**

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**Diagram:**

- **Diet intake**
  - Folic acid
  - Methionine
  - 5,10-methylene THF
  - MTHFR

- **Folate metabolism**
  - DNA methylation
  - Methionine synthesis
  - DNA synthesis
  - Thymidylate dTMP
  - Deoxyuridylate dUMP

- **Pathways:**
  - Purine
  - Tetrahydrofolate
  - Dihydrofolate

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**Typical relation between nutrient intake and tissue nutrient levels**

**Selenium feeding trial**

High Se vs Mid Se vs Control

**Change in toenail Se (µmol/kg)**

- 4.9 µmol/d
- 2.6 µmol/d
- 0.4 µmol/d

Supplementation period
MTHFR - 2 COMMON POLYMORPHISMS

<table>
<thead>
<tr>
<th>C677T</th>
<th>Ala</th>
</tr>
</thead>
<tbody>
<tr>
<td>Function</td>
<td></td>
</tr>
<tr>
<td>Serum Folate</td>
<td></td>
</tr>
<tr>
<td>Serum Homocysteine</td>
<td></td>
</tr>
</tbody>
</table>

Val/Val homozygotes at lower risk of colorectal cancer

A 1298 C Glu Ala

Genetic basis of magnesium homeostasis

Absorption of magnesium in humans involves two transport mechanisms:
- A transcellular transport via TRPM6 and TRPM7
- A paracellular passive transport rising with elevated luminal Mg concentrations

2. Ion channel transient receptor potential melastatin (TRPM6 and TRPM7) play a central role in active Mg handling in intestine and kidney

3. Mutations in TRPM6 cause hypomagnesemia with secondary hypocalcemia (HSH)

Witte KK, E, Wenz, N, Nikitin

4. No population data on the common variations of TRPM6 and 7

Schlegelowsky KP et al. Hum Genet 2002;31:166-170

Mg preparation

<table>
<thead>
<tr>
<th>Inorganic</th>
<th>Strength</th>
<th>Shortage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Magnesium Oxide</td>
<td>2) extremely soluble in water</td>
<td>extremely low bioavailability</td>
</tr>
<tr>
<td>Magnesium Chloride</td>
<td>2) insolubility in water</td>
<td>side effect: diarrhea</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Organic</th>
<th>Strength</th>
<th>Shortage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Magnesium Citrate</td>
<td>1) has better bioavailability compared to inorganic form</td>
<td>expensive formula to achieve</td>
</tr>
<tr>
<td>Magnesium Gluconate</td>
<td>2) has better bioavailability than other organic forms in rats</td>
<td>less side effects</td>
</tr>
<tr>
<td>Mg-proline</td>
<td>3) has good compliance</td>
<td></td>
</tr>
</tbody>
</table>

Mg measurement

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Advantage</th>
<th>Disadvantage</th>
<th>Feasibility</th>
<th>Cost</th>
<th>Collect</th>
<th>Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td>the most frequently performed analysis</td>
<td>may not reflect an intracellular level</td>
<td>easy to get samples</td>
<td>economic</td>
<td>blood samples</td>
<td>sensitive in severe magnesium deficient patients, serum Mg ≤ 0.73 mmol/L</td>
</tr>
</tbody>
</table>

Erythrocytes:
- Contain high concentration of Mg ions, even more than serum
- More sensitive than serum in patients with magnesium deficiencies

Blood samples:
- A sensitive marker in diabetic patients

Mg status in other markers of blood samples


MTHFR C677T Genotype

Ma et al, 1996, PHS
### Specimen

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Advantage</th>
<th>Disadvantage</th>
<th>Feasibility</th>
<th>Cost</th>
<th>Collect</th>
<th>Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buccal cells</td>
<td>easy to get the samples</td>
<td>can not be used to evaluate intracellular Mg status</td>
<td>easy-to-get samples</td>
<td>economic</td>
<td>Sublingual</td>
<td>not sensitive</td>
</tr>
<tr>
<td>Bone and teeth</td>
<td>accurately reflected the level of dietary Mg</td>
<td>easy-to-get samples</td>
<td>Bone sampled</td>
<td>Teeth</td>
<td>sensitive to chronic hypertriglyceridemia</td>
<td></td>
</tr>
<tr>
<td>Muscle</td>
<td>a quarter of total body Mg is located in muscle</td>
<td>limited research</td>
<td>hard to get samples</td>
<td>muscle biopsy</td>
<td>sensitive measurements of Mg status and deficiency</td>
<td></td>
</tr>
<tr>
<td>Hair and nails</td>
<td>best inverse sampling procedure</td>
<td>samples can be taken over a long period of time</td>
<td>hairs are usually used in children studies</td>
<td>tooth can be used as biomarkers of long-term exposure</td>
<td>can be contaminated by the environment with food ( \text{or} ) water, ( \text{or} ) could affect concentration</td>
<td>hair or nails</td>
</tr>
<tr>
<td>Muscle Loading test</td>
<td>a simplified balance studies</td>
<td>normal kidney function is required</td>
<td>urine is collected for 24 hours</td>
<td>economic</td>
<td>urine and fecal collections</td>
<td>not reliable</td>
</tr>
</tbody>
</table>

### Measurement Issues

#### Time of Day
- Relation to meals

#### Contamination
- Especially trace elements
  - e.g. EDTA: ZINC, IRON

#### Stability
- Many nutrients degrade
  - \(-70^\circ \text{C} < -20^\circ \text{C}\)
- Freezer failures

#### Light-Sensitivity

### Laboratory Issues

- RANDOM MEASUREMENT ERROR
- LABORATORY DRIFT (BETWEEN-RUN)
  - SINGLE RUN
  - OR CASE/CONTROL PAIRS TOGETHER
- LABORATORY DRIFT (WITHIN-RUN)
  - RANDOMIZE WITHIN-RUN
- QUALITY CONTROL
  - TEST WITH BLINDED SPLIT SPECIMENS
  - CALCULATE: $CV(%) = \frac{S.d.}{\bar{x} \cdot 100\%}$
  - $\sigma_x^2 = \text{within-person variance}$
  - $\sigma_b^2 = \text{between-person variance}$

### SENSITIVITY TO INTAKE

#### TIME-TEGRATION

#### OTHER DETERMINANTS

#### MEASUREMENT ERROR

### SOURCES OF INFORMATION

1. Animal studies
2. Geographic correlation
3. Correlation with individual intake
4. Dietary manipulation
5. Repeated measures
Figure 9.2 Selenium concentration in toenails from four regions. Selenium exposure is known to be unusually low in New Zealand, intermediate in Boston and Georgia, and unusually high in South Dakota. Letters indicate members of the same family; dots indicate unrelated persons. (From Morris et al., 1983)

Reproducibility over 5 years:

<table>
<thead>
<tr>
<th>n</th>
<th>r</th>
</tr>
</thead>
<tbody>
<tr>
<td>128</td>
<td>0.50</td>
</tr>
</tbody>
</table>

First Specimens 1982-83
Second Specimens 1988

STUDY DESIGN OPTIONS

CASE CONTROL
- BUT DISEASE AFFECTS MARKER LEVEL?

COHORT → NESTED CASE-CONTROL
- WATCH OUT FOR EFFECT OF PRECLINICAL DISEASE
- e.g. MITF → CHOLESTEROL → CANCER RISK
- IN FIRST 2 YEARS OF FOLLOW-UP
*Introducing the concept of Mendelian Randomization—examining APOE genotypes
→ Cancer risk as a proxy for cholesterol phenotype → Cancer risk

PRINCIPAL CONSIDERATION
CASE AND CONTROL SPECIMENS SHOULD BE COLLECTED AND HANDLED IDENTICALLY

SOME NUTRIENTS OF INTEREST

FAT-SOLUBLE VITAMINS (A,E,D,B-CAROTENE)
- PLASMA OR SERUM

INTERVENTION TRIAL

<table>
<thead>
<tr>
<th>DAILY DOSE</th>
<th>BASELINE</th>
<th>8 WEEKS</th>
</tr>
</thead>
<tbody>
<tr>
<td>RETINAL ACETATE</td>
<td>25,000 I.U.</td>
<td>51</td>
</tr>
<tr>
<td>VITAMIN E</td>
<td>800 I.U.</td>
<td>1.1</td>
</tr>
<tr>
<td>B-CAROTENE</td>
<td>25 MG</td>
<td>170</td>
</tr>
</tbody>
</table>

i.e. VITAMIN E
B-CAROTENE

RETINOL - INSENSITIVE
VITAMIN D - SUNLIGHT A MAJOR

WATER SOLUBLE VITAMINS (C, B’s FOLATE)
- BLOOD REFLECTS RECENT INTAKE
- URINE REFLECTS RECENT INTAKE

C (PLASMA)
- REASONABLE CORRELATIONS WITH INTAKE
  ~ r = 0.3 – 0.4

B’s (A+B+C+D+M)
- SEVERAL ENZYME STIMULATION ASSAYS
  e.g. B-1 (THIAMIN)

MEASURES ERYTHROCYTE TRANSKETOLASE + THIAMIN

SPEARMAN CORRELATIONS BETWEEN FAT ASPIRATE AND RECORD ESTIMATES

<table>
<thead>
<tr>
<th>FAT</th>
<th>DIET</th>
<th>ASH</th>
<th>RECORD</th>
<th>% OF TOTAL FAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAT</td>
<td>SAT</td>
<td>0.14</td>
<td>MONO.</td>
<td>0.22</td>
</tr>
<tr>
<td>POLY</td>
<td>POLY</td>
<td>0.49</td>
<td>P/S</td>
<td>0.39</td>
</tr>
</tbody>
</table>

CORRECTED FOR ATTENUATION

<table>
<thead>
<tr>
<th>FAT</th>
<th>DIET</th>
<th>ASH</th>
<th>RECORD</th>
<th>% OF TOTAL FAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAT</td>
<td>SAT</td>
<td>0.16</td>
<td>MONO.</td>
<td>0.27</td>
</tr>
<tr>
<td>POLY</td>
<td>POLY</td>
<td>0.58</td>
<td>P/S</td>
<td>0.39</td>
</tr>
</tbody>
</table>

EPA | EPA | 0.49
FIGURE 1. Intercorrelations between estimates of polyunsaturated fatty acid intake from a food frequency questionnaire (FFQ), 2 weeks of diet records (DR), and subcutaneous fat aspirate (FA) among 118 Boston-area men. Modified from Hunter D Chapter 9 Nutritional Epi.

Goal: To detect differences in exposure (should they exist) between cases and non-cases

Two important issues:
(1) What is the relevant time period of exposure? Previous week, month, year, decade?
(2) Are exposure differences detectable?
  • a true range in exposure must exist ie, = between - person variation
  • you must be able to reasonably quantify an individual’s exposure ie, what is level of within-person variation?

Again, two components of variation to consider
(1) Between-person variation
(2) Within-person variation
  “noise” from biologic variation -
  e.g. circadian variation
  variation in dietary intake
  fasting
  laboratory error

ICCs can vary from study to study

Example: ICC for plasma estradiol over a 2-3 year period

Hankinson 0.68
Toniole 0.51
Cauley 0.36

What are some possible reasons for these Differences?

\[
\sigma^2_W = \text{between-person variance} = \text{total variance} - \text{within-person variance}
\]

\[
\text{Intraclass Correlation Coefficient (ICC)}
\]

• varies between 0 and 1 only
• 0=no reproducibility (ie, large within-person variability and 0 between-person var)
• 1=perfect reproducibility (ie, 0 within-person var and large between-person var)
Recall: always consider the magnitude of both components of variation…

• For example, if you are very interested in a specific exposure but there is limited between-person variation…..
  - if it is well-measured and there is little within-person variation over time you may be ok.
  - compare: \( \frac{5}{5+1} \) to \( \frac{5}{5+5} \)
    \[= 0.83 \text{ vs. } 0.5\]

• For example, if there is some degradation in your samples OR there is a fair amount of "random" within-person fluctuation in your marker….
  - if the "true" between-person variation is large, then you may still be ok.
  compare: \( \frac{5}{5+5} \) to \( \frac{10}{10+5} \)

How to get information on the ICC?

• Search literature for data on time-integration and reproducibility
• Conduct a pilot study - even repeated measures over a few weeks can be helpful (if ICC very low already, think before wasting money)

Calculating ICCs: Example of repeated hormone measures over time

• SAS Code:
  PROC MIXED;
  CLASS ID;
  MODEL LNASSAY = ;
  RANDOM ID;
  RUN;
• List each ID and time period as a separate record in data set with variables ID, TIME, and LNASSAY (note time is not in the model)

Example – data organization

<table>
<thead>
<tr>
<th>ID</th>
<th>TIME</th>
<th>LNASSAY</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>0.00</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>0.69</td>
</tr>
<tr>
<td>1</td>
<td>3</td>
<td>1.10</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>0.79</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>0.69</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>0.69</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>1.61</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>1.10</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>1.35</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>1.39</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>0.97</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>1.01</td>
</tr>
</tbody>
</table>

Example: Output

The Mixed Procedure

Model Information
Data Set WORK.ICC
Dependent Variable lnassay
Covariance Structure Variance Components
Estimation Method REML
Residual Variance Method Profile
Fixed Effects SE Method Model-Based
Degrees of Freedom Method Containment

Class Level Information
Class Levels Values
id 6 1 2 3 4 5 6

Dimensions
Covariance Parameters 2
Columns in X 1
Columns in Z 6
Subjects 1
Max Use Per Subject 12
Observations Used 12
Observations Not Used 0
Total Observations 12
### Example: Output, cont.

**Iteration History**

<table>
<thead>
<tr>
<th>Iteration</th>
<th>Evaluations</th>
<th>-2 Res Log Like</th>
<th>Criterion</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1</td>
<td>4.56015535</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>-1.98776029</td>
<td>0.00000000</td>
</tr>
</tbody>
</table>

Convergence criteria met.

**Covariance Parameter Estimates**

<table>
<thead>
<tr>
<th>Cov Parm</th>
<th>Estimate</th>
</tr>
</thead>
<tbody>
<tr>
<td>id</td>
<td>0.06294</td>
</tr>
<tr>
<td>Residual</td>
<td>0.01349</td>
</tr>
</tbody>
</table>

**Fit Statistics**

- -2 Res Log Likelihood: -2.0
- AIC (smaller is better): 2.0
- AICC (smaller is better): 3.5
- BIC (smaller is better): 1.6

ICC = 0.06294 / (0.06294+0.01349) = 0.82

### What to do if your ICC is low?

- Collect repeated samples from participants
- Collect repeated samples from a subset of subjects and use measurement error correction techniques
- Change the matrix used (e.g., a more time-integrated measure)
- Control for factors (or match on or restrict) that may be reducing ICC
  - e.g. day of menstrual cycle
  - fasting status
  - time of day of collection
  - stable weights

### Calculating CVs

- Coefficient of variation assesses the variability of a sample that has been measured multiple times
- In simplest form, the %CV is (standard deviation / mean) * 100
- This is done on the original scale
- Can calculate, then average
  - Within pairs (e.g. in a batch)
  - Within a batch
  - Between batches

### Example

- Assays performed in 7 batches over the course of 2 months
- Two QC samples (pooled from several individuals in underlying population) placed in each batch
- Lab had good CVs when pilot testing this assay

### Example – results

- Mean: 98
- SD: 6
- Min: 83
- Max: 108

![Graph of example results]

### Example – QC data to get inter-assay CV

<table>
<thead>
<tr>
<th>ID</th>
<th>Batch</th>
<th>Assay</th>
<th>CV (100*SD/mean) for ID=1 is</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>1.00</td>
<td>- 100 * 2.22 / 2.75 = 80.6%</td>
</tr>
<tr>
<td>1</td>
<td>4</td>
<td>2.00</td>
<td>- 100 * 1.00 / 0.50 = 200.0%</td>
</tr>
<tr>
<td>1</td>
<td>10</td>
<td>2.00</td>
<td>- 100 * 2.52 / 5.33 = 47.2%</td>
</tr>
<tr>
<td>1</td>
<td>11</td>
<td>6.00</td>
<td>- Average CV over all IDs = 109.3%</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>0.00</td>
<td>- Can use proc univariate</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>0.00</td>
<td>PROC SORT; BY ID;</td>
</tr>
<tr>
<td>2</td>
<td>7</td>
<td>0.00</td>
<td>PROC UNIVARIATE;</td>
</tr>
<tr>
<td>2</td>
<td>9</td>
<td>2.00</td>
<td>VAR Assay;</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>3.00</td>
<td>BY ID;</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>5.00</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>8</td>
<td>8.00</td>
<td></td>
</tr>
</tbody>
</table>
Other methods of calculating CVs

- A number of methods possible
- Use PROC ANOVA in the original scale, the CV is part of the output (or if on ln-scale then CV is root mean square error)
- Usually the hormone data are skewed, so often calculate CV on ln scale.
  - Use PROC MIXED
  - This procedure is very flexible for different QC schemes

Example 1: One QC pool

- Replicates of one QC pool in each batch
- SAS Code:
  ```sas
  PROC MIXED;
  CLASS BATCH;
  MODEL LNASSAY = ;
  RANDOM BATCH;
  RUN;
  ```
- List each QC sample as one record in data set with variables BATCH and LNASSAY

Example 1 – data organization

<table>
<thead>
<tr>
<th>Batch</th>
<th>Assay</th>
<th>lnAssay</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.00</td>
<td>0.00</td>
</tr>
<tr>
<td>1</td>
<td>2.00</td>
<td>0.69</td>
</tr>
<tr>
<td>2</td>
<td>3.00</td>
<td>1.10</td>
</tr>
<tr>
<td>2</td>
<td>6.00</td>
<td>1.79</td>
</tr>
<tr>
<td>3</td>
<td>2.00</td>
<td>0.69</td>
</tr>
<tr>
<td>3</td>
<td>2.00</td>
<td>0.69</td>
</tr>
<tr>
<td>4</td>
<td>5.00</td>
<td>1.61</td>
</tr>
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<td>4</td>
<td>3.00</td>
<td>1.10</td>
</tr>
<tr>
<td>5</td>
<td>1.00</td>
<td>0.00</td>
</tr>
<tr>
<td>5</td>
<td>4.00</td>
<td>1.39</td>
</tr>
<tr>
<td>6</td>
<td>1.00</td>
<td>0.00</td>
</tr>
<tr>
<td>6</td>
<td>1.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>

Example 1: Output

<table>
<thead>
<tr>
<th>Iteration History</th>
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<th>-2 Res Log Like</th>
<th>Criterion</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1</td>
<td>-77.1004239</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>-80.66539233</td>
<td>0.00000000</td>
<td></td>
</tr>
</tbody>
</table>

Convergence criteria met.

- Covariance Parameter Estimates
  - Batch 0.001189
  - Residual 0.001211

The Mixed Procedure

Fit Statistics
- -2 Res Log Likelihood: -80.7
- AIC (smaller is better): -76.7
- AICC (smaller is better): -76.1

Intra-batch CV = 100*Sqrt(0.001211) = 3.5%
Inter-batch CV = 100*Sqrt(0.001211+0.001189) = 4.9%

Strange properties of CVs

- The square root of the variance on the ln-scale is the CV on the original scale
  - Sqrt(var on ln scale) ~ SD/Mean on original scale
- In the literature, the “inter-batch” or “inter-assay” CV is usually calculated as the “total” CV
- CVs are always expressed as percents

What types of biologic materials are available to assess what “true exposures”

- Blood
  - RBC (fatty acids, folate, adducts, glycoslation)
  - WBC (genetic markers)
  - Plasma (antibody, protein, cytokines, hormone chemistry)
- Urine (hormones, mutagenicity assays)
- Saliva (hormones, cotinine, antibodies)
- Buccal cells (genetic markers)
- Nails/Hair (Trace elements: selenium, fluoride)
- Adipose tissue (fat soluble vitamins, fatty acids, pesticide)
- Other organs/tissues (genetic markers: germline and/or somatic)
What assays from what biologic materials

1. Hypothesized exposure/disease relationship
e.g., long-term vs. short-term exposure of interest
2. Study design e.g., long term measure more likely to represent exposure before disease
3. Ease of collection and processing/storage
e.g., acceptance by study participants
4. Ease of assay
e.g., is one matrix technically more difficult to work with
5. Versatility of matrix
e.g., more parameters can be measured in blood than in adipose

ADVANTAGES
- "OBJECTIVE"
- "INEXPENSIVE"
- MAY REPRESENT INFORMATION NOT AVAILABLE FROM FOOD INTAKE DATA
- ESPECIALLY FOR NUTRIENTS WHICH ARE HIGHLY VARIABLE IN INDIVIDUAL FOODS
- MAY BE AVAILABLE IN RETROSPECT (ANALYSIS OF STORED SPECIMENS)

DISADVANTAGES
- MAY NOT BE SENSITIVE TO INTAKE
- MAY NOT BE TIME-INTEGRATED
- EXPENSIVE
- MARKERS NOT AVAILABLE FOR MANY NUTRIENTS
- SPECIMENS MAY BE DIFFICULT TO OBTAIN

NO MEASURE OR POOR MEASURE
- CARBOHYDRATES, SUGAR
- FIBER
- PROTEIN
- CALORIES

CONCLUSION
EACH NUTRIENT HAS A DIFFERENT OPTIMAL METHOD OF ASSESSMENT

<table>
<thead>
<tr>
<th>QUESTIONNAIRE</th>
<th>BIOCHEMICAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>TOTAL FAT</td>
<td>CAROTENE</td>
</tr>
<tr>
<td>RETINOL</td>
<td>VITAMIN E</td>
</tr>
<tr>
<td>CHOLESTEROL</td>
<td>MG</td>
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