Can Metabolomics Be Used for Short and Long Term Dietary and Nutritional Assessment?

Preliminary Results From Proof of Concept Projects

John W. Newman

March 31, 2009
The Metabolome Is Derived From Both Endogenous and Exogenous Sources
Can Metabolomics Aid Dietary Assessment?

- A variety of studies are underway evaluating this question.
  - The MEDE Study – Aberystwyth Univ. / Newcastle Univ., United Kingdom
  - Plasma Biomarkers of Whole vs. Refined Grain – USDA, WHNRC, Davis CA.
  - Phytochemical Biomarkers of Plant Intake – INRA, Clermont-Ferrand, France
  - Circulating Markers of “Fish Oil” Consumption – USDA, WHNRC, Davis CA.
The MEDE Study
MEtabolomics to characterise Dietary Exposure

**GOAL**: Evaluate metabolomics as an alternative tool for dietary assessment

NuGO and SYSDIET Metabolomics workshop 24 February 2009
**Study design**: 2 test days per volunteer, designated A and B

**Pre-test day**
- No alcohol
- Minimal physical activity
- Standardised evening meal

**Test day**
- Blood - up to 30 mL
- Urine - total void(s)
- Saliva - alternative biofluid

Fasted state

Fed state:
- 2, 4, 6 and 8h later

**Standardised evening meal**
- Chicken breast with carrots, peas and potatoes
- Chocolate éclair
- Still mineral water

‘Metabolomically’ neutral, balanced, attractive

**Test breakfast**
- Orange juice
- Tea with sugar and milk
- Croissant
- Corn flakes with milk

NuGO and SYSDIET Metabolomics workshop 24 February 2009
Individual Metabolite Fingerprints Are Reproducible

- Plasma analysed by positive mode FIE-MS (m/z 100-220) very HIGH THROUGHPUT
- Discriminating individual by test day
- Satisfactory grouping of A & B visits

NuGO and SYSDIET Metabolomics workshop 24 February 2009
Metabolite Fingerprints are Dynamic and Respond to Diet

- Plasma analysed by positive mode FIE-MS (m/z 100-220)
- Discriminated by sample class
- Good state discrimination
  - fasting vs. fed
  - breakfast vs. lunch
Plasma Biomarkers of Grain Consumption

Changes in the Postprandial Plasma Metabolome with a High vs. Low Glycemic Diet Challenge

John Newman
Theresa Pedersen
Dmitry Grapov
Alison Keenan
William Keyes

Nancy Keim
William Horn
Sridevi Krishnan

Western Human Nutrition Research Center
430 West Health Sciences Dr., Davis, CA 95695
Subjects:

20 overweight women (BMI: 25-29.9).
3 day cross-over to diets rich in whole or refined grain

Sample Collection:

8hr fasting, 0.5, 2, 3.5, 6, 8 hrs postprandial post-test meal plasma.

Analyses:

Clinical lipids, insulin/glucose, leptin, calorimetry
LC/MS-based Metabolic Fingerprinting
Diet Glycemic Index Alters Plasma Biomarkers

- Short duration consumption of whole vs. refined grain diets shift postprandial metabolome profiles

Increased postprandial unknowns ([M+H]+ m/z 250, 283, 457) with low glycemic meal

Reduced postprandial phospholipids with low glycemic meal
Characterizing the Food metabolome to discover new biomarkers of food intake
a proof-of-concept study on Citrus


UMR 1019, Unité de Nutrition Humaine Centre Clermont-Ferrand-Theix, France
Markers of food / phytochemical intake in urine

- New markers of intake revealed by metabolomics

SOY
Isoflavones
13 potential biomarkers

CITRUS
Furanocoumarins & Terpenes
9 potential biomarkers

CRUCIFEROUS
Glucosinolates
2 potential biomarkers

Manach C. (INRA France) - NuGO and SYSDIET Metabolomics workshop 2009
**Markers of polyphenol intake in urine**

**Correlations between urinary polyphenols and intake of their major food sources**

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**Pearson correlations ($p < 0.05$)**

Ito et al. (2005) BJN
Mennen et al. (2006) BJN
Mennen et al. (2007) EJCN
Urine metabolome: Principle Components Analysis

- Volunteers are discriminated by type of beverage
- PCA trajectories show the temporal evolution of the urine metabolome

PCA Scores plot: all samples, ions p<0.05 ANOVA / beverage
Peak Annotation & Food Metabolome Databases Are Needed

Food composition tables
- Nutrients
- Phytochemicals

Phenol-Explorer
527 polyphenols in 591 food items

Phytochemical metabolite database

Isoflavones: 128 metabolites

Ph2-1
Ph2-2
Ph2-3
Ph2-4
Ph6-5
Ph7
Ph9-1
Ph9-2
Ph9-3
Ph9-4
Ph9-5
Ph9-6
Ph8-2

Endogenous metabolome

Manach C. (INRA France) - NuGO and SYSDIET Metabolomics workshop 2009
- We Need Databases of Metabolites With Searchable Meta Data Which Should Include
  - Food Sources
  - Spectral Data

6586 metabolites
1193 identified in blood
472 identified in urine

1932 food components
(first pass from FDA Food Additives List)

Oliver Fiehn UC Davis.
‘We need annotated and searchable spectral libraries for unknown identification.’

Dr. Davis Wishart, U. Alberta, Canada
Developing a database cataloging all of the small molecules within the human body, annotated with observed concentrations.

Agilent Technologies
Fiehn GC/MS
Metabolomics RTL Library

~1050 searchable spectra from 700 compounds
Red Blood Cell Phospholipids: 
Reporters of compliance and lipid intake

John Newman
Theresa Pedersen
Dmitry Grapov
Alison Keenan
William Keyes

Charles Stephensen
Gertrud Schuster
Patrice Armstrong
Alina Whetstone
Xiaowen Jiang
Controlled Feeding Shifts RBC Phospholipids in Mice

Dr. Gertrud Schuster, UC Davis, Dept Nutrition

- **Study:** Impact of DHA vs. EPA feeding on asthma symptom severity

- **Dietary Regimen:** 4wks ad lib. with 4 distinct diets.

- **Results:** RBC phospholipid fatty acids profiles reflected dietary lipids.

![Percent Composition of RBC Fatty Acids]

![PCA Scores Plot of RPB FAs]
Human Fatty Acids Profiles of Red Blood Cell Phospholipids

- **Study:** Impact of Fish Oil on Macrophage Stimulation in 5-LOX polymorphism

- **Dietary Regimen:** 6wks 0f 6g/day fish oil or placebo.

- **Results:**
  - RBC phospholipid are generally responsive.
  - Changes in EPA most dramatic.
Variability in Human RBC Membrane Fatty Acid

- Normalized RBC FAs to arachidonate, then greatest variance observed in:
  - Long Chain n3 PUFAs
  - trans-fatty acids

- Sub-populations observed based on LC n6/n3 ratios.
  - Diet vs. Metabolism?

Data courtesy of G. Shearer and W. Harris, Univ. South Dakota
With high trans-fat subjects removed, primary separation by:
- SAT/MUFA
- n6/n3 PUFAs
Interactions between Red Cell LC-PUFA & n6:n3

- As RBC PUFAs decrease
  - Elongation Index Increases
  - LCn6/n3 PUFAs increase (1:1 threshold)

- LCn6/n3 PUFAs increase, the Elongation Index increases.

Limited marker of “relative intake”
Plasma Oxygenated PUFA Metabolites: Novel reporters of compliance and dietary intake

Leukotrienes
Steroids ↔ Eicosanoids
PUFA
Cholesterol ↓ Vanilloids
Cannabinoids

John Newman
Theresa Pedersen
Dmitry Grapov
Alison Keenan
William Keyes

Gregory Shearer
William Harris
SANFORD SCHOOL OF MEDICINE
The University of South Dakota

Western Human Nutrition Research Center
430 West Health Sciences Dr., Davis, CA 95695
LC-ω3-PUFA Feeding Increases LC-ω3 Oxygenated Metabolites in Human Plasma

Hydroxy-, epoxy-, and dihydroxy-LCω3-PUFAs in the plasma of subjects (n=10) after 4wks of 4g/day EPA/DHA supplementation.
The Magnitude of Plasma Oxylipid Changes Depend on Baseline Concentrations

The fold-change in plasma hydroxy-PUFAs were inversely proportional to initial concentration. Reduction in arachidonate metabolites are only observed when basal levels are “high”.

Potential indication of an metabolic “Optimum”.

Human subjects (n=10),
Summary: Metabolomics for Dietary Intake Assessment

- Preliminary results from Proof-of-Principle studies suggest that Metabolomics can be applied to Diet Assessments.
  - An individual’s plasma metabolomic fingerprint is stable but can be altered by diet in the short term.
  - Urinary phytochemicals correlate with intake of their major food sources over a 24 hour period.
  - Expanded, searchable databases of food composition are needed.
    - Annotated with
      - Dietary source and Biological Significance (for the biologist)
      - Searchable spectral data (for the chemist)
  - Plasma and membrane lipids provide indications of dietary exposure, but homeostatic balances make these risky quantitative intake markers.
  - Standardized and validated test diets and nutritional challenges are needed.
  - The question is of global interest and international collaborations should be part of the solution.
Changes in Plasma LC-n3 Oxylipids are Tightly Correlated

Cross correlation matrix for plasma LC-\(\omega3\)-PUFAs (n=20, \(r>0.68, p<0.001\)).

EPA and DHA metabolites were tightly associated

Prostaglandins showed positive associations with epoxides of arachidonate, linoleate, and alpha-linolenate.