Lipidomic Profiling: an Information Rich Tool to Explore the Impact of Dietary Lipids

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Topics to Cover

• Overview of targeted lipidomic concept, information and our approach

• Human and Animal n3PUFA Feeding Examples
  – RBC Response Phenotypes in African Americans
  – “Environmental” Influence on Plasma Variance
  – Impacts on lipoprotein particle chemical composition
  – Impact of complex dietary lipid manipulation

• Hot off the presses: Impact of Low vs. High MUFA ground beef consumption.
The “Omics” Cascade

GENOME

What can happen

TRANSCRIPTOME

What appears to be happening

PROTEOME

What makes it happen

PHENOTYPE

WHY WE CARE!

METABOLOME

What has happened and is happening

(i.e. Lipids, Amino Acids, Metabolic intermediates, etc.)
The Promise of Metabolomics

- **Knowledge** – Metabolic phenotyping can identify genetic differences

- **Application** – The potential to tailor nutrition & pharmacology by phenotype

- **Translation** – Individual phenotyping will enhance our ability to promote health

![Image of sub-populations with unique requirements]
Metabolomics Approaches

**METABOLITE FINGERPRINTING**
- Hypothesis Generating
- Unknowns Targets
- Includes both NMR and MS methods
- “Biomarker Development”

**TARGETED METABOLITE PROFILING**
- Hypothesis Driven & Generating
- Known Targets
- Platform independent
- “Metabolic Phenotyping”

Both Approaches Require Bioinformatics Analyses
Why focus on lipids? An Information Rich Profile

1) Important metabolic fuel and primary storage form.

2) Membrane composition effects cell and enzyme functions

3) Associated with health benefits and disease risk
   - vascular disease (e.g. atherosclerosis)
   - mental illness (e.g. schizophrenia)
   - inflammatory diseases (e.g. asthma)

4) Physiological regulators:
   - Adipokines
   - Endocrine regulators
   - Chemokines
Oxylipin Profiles: Regulatory “Markers” of Many Processes

Representative Eicosanoid Tree
Endocannabinoids: Regulators of Energy Balance
(e.g. Body Weight, Body Temperature and Satiety)

Increase Appetite (i.e. ↑Energy Intake)

Reduce Basal Body Temperature (i.e. ↓Energy Expended)

Increase Lipogenesis and Fat Mass (i.e. Net Orexigenic)

Promote a Positive Energy Balance

- Anandamide (↑)
- 2-Arachidonyl-Glycerol (↑)
- Sn-2-arachidonyl DAG (↑)
- 2-Arachidonoyl-Glycerol

- PPARγ (↓)
- PPARα (↑)

- Oleoyl-EA

- COX
- FAAH
- MAGL
- TRPV1

- CB1, 2

- Increase Appetite (i.e. ↑Energy Intake)
- Reduce Basal Body Temperature (i.e. ↓Energy Expended)
- Increase Lipogenesis and Fat Mass (i.e. Net Orexigenic)
- Promote a Positive Energy Balance
“Shotgun Lipidomics” has great utility but difficulty with isomeric resolution and sensitivity.

I. Lipid Extraction
   I. Oasis HLB SPE of Fluids
   II. Liquid/Liquid of Solids

II. Class Separation
   I. Aminopropyl SPE

• Ester Cleavage
  • Alkaline hydrolysis
  • Trans-esterification
Experimental Examples

• Phenotyping with Omega-3 Feeding Challenges
  – FL50 – Variable Response Phenotypes
  – ROAP – Changes in multiple compartments dependent on initial conditions
  – Changes in lipoprotein distributions (FONIA)

• Dietary PUFA Balance on Lipids and Lipid Metabolism (Ham1)

• Impact of beef consumption on HDL profiles in healthy men
Quantifying variable responses to dietary challenges can define phenotypes.
Fish oil feeding response phenotypes in African Americans

Study Design:
- 1° Goal - investigate n3 x ALOX5 gene interactions associated with elevated cardiovascular risk.
- African Americans (n=100) fed 3g/day of fish oil or soy/corn oil for 6wks.

Results:
- Diet gene interactions showed increased oxylipin production in low risk genotype.
- Large variance in RBC membrane incorporation not associated with measured genotype.
Fish oil feeding response phenotypes in African Americans

- Changes in RBC n3 profiles correlate with stimulated immune cell n3 oxylipin production
- Two discrete response phenotypes are observed in this population.

Omega-3 Feeding in Humans (FL50)
Fish oil feeding response phenotypes in African Americans

A low meat dietary pattern may be associated with low fish oil response phenotype. No differences in lipid intake.
Simple Challenges have Broad Impacts

Dietary lipids do not effect all biological compartments in the same way
n3-FA Feeding Changes Circulating Lipid Profiles

Study Design:
- 1° Goal - investigate omega 3 impact plasma and blood cell membranes on lipid and lipid metabolites.
- Healthy men (n=9) and women (n=21) fed 4g/day of pharmaceutical grade omega 3 (P-OM3) for 4wks.

Results:
- Metabolites stratified subjects pre and post feeding.

Principal Components Analysis
A multivariate statistical analysis that uses all data to project variance into planes of separation.
n3-FA Feeding Changes Circulating Lipid Profiles

Circulating lipid pools are enriched in long chain n3 fatty acids and their metabolites, and depleted in long chain long chain n6 fatty acids.
The Magnitude of Change in Circulating Lipid Pools is Inversely Proportional to Baseline Concentration

P-OM3 feeding only affected 20 and 22 carbon fatty acids and their metabolites. Despite high compliance, some individuals showed minimal changes. Reduction in arachidonate metabolites are only observed when basal levels are "high".

18:2n6 and 18:3n3 change, but are unaffected by POM3 challenge.

While AA Decreased in Some but not all.

Increases in EPA & DHA Point of no change between Initial and Final Concentrations.

Changes in Plasma Fatty Acids vs. Baseline

Omega-3 Feeding in Humans (ROAP)
n3-FA Feeding Changes Lipoprotein Particle Profiles

**Study Design:**
- **1° Goal** - investigate the impact of omega 3 feeding on lipid and lipid metabolite distribution in lipoprotein particles
- Male and female subjects with metabolic syndrome were fed 4g/day of placebo (n=20) or pharmaceutical grade omega 3 (P-OM3; n=20) for 4wks.

**Data:**
- Lipid Distributions
- Particle size
- Clinical Lipids
- Flow Mediated Dilation
Population shows a range of phenotypes, but the directionality of change is constant.

Unresponsive individual

PC1 Score (12%)
PC2 Score (9.5%)

4 weeks
P-OM3

Placebo & Baseline

Pre Placebo
Post Placebo
Pre P-OM3
Post P-OM3
Pre POM-3
Post P-OM3

n3 Effect

Omega-3 Feeding in Humans (FONIA)
n3-FA Feeding Changes Lipoprotein Particle Profiles

- Increases in n3 and n3 metabolites
- Decreases in TGs but also all TG n6 Oxylipids
- Increase in LDL C20 Epoxides
- A lot of covariates with the Omega 3 index
n3-FA Feeding Changes Lipoprotein Particle Profiles

15-HEPE (EPA Alcohol)

- n3PUFA Alcohols increased in all fractions.

Unresponsive individual

15-HETE (AA Alcohol)
n3-FA Feeding Changes Lipoprotein Particle Profiles

- VLDL is preferentially enriched in ALA metabolites
- A lot of variance among the population
Investigating the dietary recommendation of lipid balance and composition on lipid metabolism and tissue lipid distributions
Dietary Fatty Acid Composition Changes Plasma Profiles and Hepatic Lipid Metabolism

Study Design:
- 1° Goal - investigate USDA Guidelines for lipid consumption on lipid distribution in peripheral tissues depots.
- Animals: Hamsters 5wks – 19wks.
- Diets: 1:1:1 SAT/MUFA/PUFA
  - Phase 1 - 2 cohorts
    - 20:1 Corn-based n6/n3
    - 40% vs. 7% caloric fat
  - Phase 2 - 40% Cohort split
    - 3:1 Corn/Flax-based n6/n3
    - 3:1 Corn/Fish-based n6/n3

Results:
- Equivalent caloric intake
- 7% Caloric Fat diet
  - reduced feed efficiency
    - BW \( \downarrow \sim 10\% \)
    - no \( \Delta \) adiposity
  - increased hepatic de novo fatty acid synthesis.
- Corn/Fish increased D5D activity
- PUFA composition did not effect weight.
Dietary Fatty Acid Composition Changes Plasma Profiles and Hepatic Lipid Metabolism

Plasma oxylipins and endocannabinoids reflect n3 source.

Magnitude of change increased with time on diets.

Low fat diet increased de novo fatty acid synthesis and plasma 16:1/16:0.

Corn/Fish increased plasma 20:4n6/20:3n6

PCA Scores Plot

PCA Loadings Plot

Increased Long Chain n3s & Metabolites

Increased Liver FA Synthesis

Increased Liver LCFA Synthesis

Increased ALA Metabolites

Study Design:

• 30 normo-cholesterolemic men, 25-60 years old

• Randomized cross-over design for diet assignment

• Diet Treatments
  – 5 x 115g ground beef patties, 24% fat, per week for 5wks
    • 4 wk wash-out between diet treatments
    • Hi MUFA beef MUFA:SAT = 1.10, Low MUFA = 0.71

• Blood drawn before & at the end of each 5wk interval

• HDL isolated as the 1.063-1.200 g/ml fraction of plasma.
“Can Ground Beef Consumption Alter HDL Functionality?”

Results:

• Beef consumption ↑ relative abundance of DHA-derived oxylipins by ~15% w/o effecting the fractional abundance of DHA in HDL.

• Low MUFA & High MUFA beef decreased HDL 5-oxo-ETE (a potent eosinophil chemotactic factor) concentration by ~22% and ~14% respectively.
  – Overall, beef consumption reduced 5-oxo-ETE more in subjects in the lowest quintile of total plasma n3 concentrations, ~23% vs. ~8% in the highest quintile.

• High MUFA beef reduced total diol and ketone concentration in HDL by ~16% and ~15% respectively.
Oxylipin Profiles: Regulatory “Markers” of Many Processes

Representative Eicosanoid Tree

Pro-Inflammatory
Proliferative
Vasoconstrictive

Leukotrienes

LTA₄
Synthase
LТА₄
Hydrolase

5-HpETE
PGD₂
PGF₂

15-LOX

PGH₂

COX

PGE₂

TXA₂

TXB₂

PGI₂

CYP2C,2J

EETs

sEH

DHETs

12-LOX

15-LOX

12-HpETE

15-HpETE

5-oxo-ETE

GPX

GPX

GPX

16 min LC/MS/MS assay

Glucose Uptake
Insulin Secretion
Adipose Differentiation

Coagulant

Anti-Coagulant

15-oxo-ETE

Pro-Inflammatory
Anti-Proliferative
Vasodilatory

Anti-Inflammatory

Pro-Inflammatory

Proliferative

Vasoconstrictive

5-HpETE

GPX
eLOX3

Hepoxilin A₃

Trioxilin A₃

12-HETE

15-HETE

5-HETE

15-oxo-ETE

12-HETE

6-keto-PGF₁

15-LOX

PGF₂

PGI₂

PGF₂

TXA₂

TXA₂

Synthase

Synthase

5-oxo-ETE

5-HpETE

12-HpETE

HETE

HETE

HETE

HETE

HETE

HETE

HETE

HETE

HETE

HETE

HETE

HETE

HETE

HETE
In Summary

- Targeted analyses provide insight into metabolic responses and can segregate metabolic phenotypes.

- Variance in responses to dietary challenges need to be considered when designing dietary challenge studies.

- Results suggest value in screening plasma or RBC fatty acid profiles as a valuable cohort selection criteria to control experimental variance.
What is the pay off?

- Knowledge of individual metabolism & identification of unique metabolic responders, will revolutionize the ability of nutrition to deliver health benefits.
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