Metabolomics and the Molecular Phenotype of Obesity

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Endowment for the Biological Sciences
Clinical and Molecular Phenotyping

- Biological Data
- Transcriptomics
- Proteomics
- Metabolomics
- Predictive Model of the System
DNA

RNA

Proteins

Metabolites

The ultimate potential of a cell

The current direction of a cell

The functional capabilities of a cell

The limiting currency of a cell

Material

Information
The 'omic's

- **Genome**: ~30,000 genes
- **Transcriptome**: ~100,000 transcripts
- **Proteome**: ~1,000,000 protein forms?
- **Metabolome**: ~2000 to 5,000 metabolites
What is a Metabolite?

- Any organic molecule detectable in the body with a MW < ~2000 Da
- Includes peptides, oligonucleotides, sugars, nucleosides, organic acids, ketones, aldehydes, amines, amino acids, lipids, steroids, alkaloids and drugs (xenobiotics)
- Includes human & microbial products
- Concentration > 1nM*
Mass Distribution of Compounds in the Human Metabolome

- Metabolome
  - natively biosynthesized
  - monomeric
- Complex metabolites
- Xenobiome
Why Are Metabolites Relevant?

- Generate metabolic “signatures”
- Monitor/measure metabolite flux
- Monitor enzyme/pathway kinetics
- Assess/identify phenotypes
- Monitor gene/environment interactions
- Track effects from toxins/drugs/surgery
- Monitor consequences from gene KOs
- Identify functions of unknown genes

Metabolites are the Canaries of the Genome
Why Are Metabolites Relevant?

- Generate metabolic “signatures” for disease states or host responses
- Obtain a more “holistic” view of metabolism (and treatment)
- Accelerate assessment & diagnosis
- More rapidly and accurately (and cheaply) assess/identify disease phenotypes
- Monitor gene/environment interactions
- Rapidly track effects from drugs/surgery
2 Routes to Metabolomics

Quantitative Methods

Chemometric (Pattern) Methods

Condition 1
Control
Condition 2
The Technology of Metabolomics

- **Sensitivity**
- **Automated and High-throughput**
  - DIMS
  - IR and Raman Spectroscopy
  - GC/MS
  - HPLC/MS/MS
  - NMR

- Chromatogram
- Mass spectrum
## Separations Based Metabolomics Platforms

<table>
<thead>
<tr>
<th></th>
<th>CE-MS</th>
<th>GC-MS</th>
<th>LC-MS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>AD</strong></td>
<td>Small Injection Volumes</td>
<td>High resolution</td>
<td>Soft ionization</td>
</tr>
<tr>
<td></td>
<td>High Resolution</td>
<td>Library ID</td>
<td>Full metabolome coverage</td>
</tr>
<tr>
<td><strong>DA</strong></td>
<td>Low capacity</td>
<td>Chemical derivitization</td>
<td>Limited structural info</td>
</tr>
<tr>
<td></td>
<td>Difficult MS interface</td>
<td>Harsh ionization</td>
<td>Lower Resolution</td>
</tr>
<tr>
<td></td>
<td>Requires charged analytes</td>
<td>Limited metabolite applicability</td>
<td></td>
</tr>
</tbody>
</table>

- **ALL ESI-MS Methods Are Subject to Ion Suppression**
- **Response Factors of Analytes are Not Equal**

# Relative risk of health problems associated with obesity

<table>
<thead>
<tr>
<th>Greatly increased (relative risk &gt;&gt;3)</th>
<th>Moderately increased (relative risk 2-3)</th>
<th>Slightly increased (relative risk 1-2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetes</td>
<td>Coronary heart disease</td>
<td>Cancer (breast cancer in postmenopausal women, endometrial cancer, colon cancer)</td>
</tr>
<tr>
<td>Gall bladder disease</td>
<td>Osteoarthritis (knees)</td>
<td>Reproductive hormone abnormalities</td>
</tr>
<tr>
<td>Hypertension</td>
<td>Hyperuricemia and gout</td>
<td>Polycystic ovary syndrome</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td></td>
<td>Impaired fertility</td>
</tr>
<tr>
<td>Insulin resistance</td>
<td></td>
<td>Low back pain</td>
</tr>
<tr>
<td>Breathlessness</td>
<td></td>
<td>Increased anesthetic risk</td>
</tr>
<tr>
<td>Sleep apnea</td>
<td></td>
<td>Fetal defects arising from maternal obesity</td>
</tr>
</tbody>
</table>
Excess U.S. Medical Costs Related to Abnormal Body Weight
Causes of Obesity

- Genetics
- Behavior
- Environment
Environmental effects...
Investigational Weight Management Clinic
Nutrition Obesity Research Center Demonstration Unit

- Primary goal: Develop tools for multiscalar integration of clinical, behavioral and molecular phenotyping data in a clinical setting.
- Insurance-supported clinical care for 400 obese patient
- Undertaking a variety of studies related to nutrition and obesity
- Michigan Nutrition Obesity Center Demonstration Unit project: Broad phenotyping at baseline, 3 months, 24 months for 400 obese and baseline studies in 100 lean (BMI < 27).
Phenotypic response to diets

[Graph showing lipid levels (mg/dl) over time for Day 0 and Day 21 for PUFA and CHO diets for Total Cholesterol, Triglycerides, HDL, and LDL.]
Can macronutrient consumption be detected in fatty acid profiles?

**Lipomic assessment of plasma**

**Principal Component Analysis**

(% of total lipid in fraction)

<table>
<thead>
<tr>
<th>Day</th>
<th>Free Fatty Acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>14:0</td>
</tr>
<tr>
<td>2</td>
<td>14:2</td>
</tr>
<tr>
<td>7</td>
<td>16:0</td>
</tr>
<tr>
<td>21</td>
<td>18:2</td>
</tr>
</tbody>
</table>

- **B**
  - 18:2 and 14:0 predicts PUFA at day 2, 7, 21
  - 14:1 and 16:1 in TG and PL predicts day 2, 7, 21 CHO
  - 18:2 and 16:1 in CE predict day 21 CHO
Correlation between glucose and 16:1 levels in CE

- Baseline: $R^2 = 0.7241$
- Day 21 Pufa: $R^2 = 0.0142$
- Day 21 CHO: $R^2 = 0.4835$

Correlations between HOMA and 16:1 levels in CE

- Baseline: $R^2 = 0.2855$
- Day 21 Pufa: $R^2 = 0.0426$
- Day 21 CHO: $R^2 = 0.2295$

Identification of a Lipokine, a Lipid Hormone Linking Adipose Tissue to Systemic Metabolism

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Phenotyping of Patients

**Phenotyping and Analysis**

- **Investigational Weight Management Clinic (Rothberg)**
- **Laboratory for Physical Activity and Exercise Intervention Research (Gordon)**
- **MMOC Human Phenotyping Core (Horowitz)**
- **MMOC Molecular Phenotyping Core (Burant)**
- **NCIBI/CCMB (Athey, Cavalcoli)**

- **Anthropometric tests.** Height, weight, blood pressure, heart rate, temperature, skin fold thickness, waist-to-hip ratio, skin fold thickness. Dual Energy X-Ray Absorptiometry (DEXA, new).
- **Metabolic Assessment.** VO\textsubscript{2}peak, resting metabolic rate (RMR) and R/Q measurement. Oral glucose tolerance tests (for those without a diagnosis of diabetes), Total cholesterol, LDL, HDL, triglycerides, free fatty acid, insulin (at 0 and 30 and 120 minutes of oGTT), leptin, adiponectin, C-Reactive Protein.
- **Peripheral Blood Metabolomic Assessment (including lipomics).** The pattern of metabolite levels will be determined, including fatty acid profiles of lipid subclasses in EDTA collected plasma.
- **Peripheral Blood Transcriptomic Assessment.** Fasting blood collected for RNA expression will be collected in PaxGene tube.
- **Genomic Assessment.** DNA will be isolated from peripheral blood for assessment of DNA polymorphisms related to obesity and ability to lose weight (Boehnke, not funded).
- **Muscle and adipose tissue biopsy metabolite and transcript analysis.** Biopsies will be performed on the vastus lateralis muscle and anterior abdominal fat.
- **Behavioral assessment. 4-Day Food Intake Record.** A Depression inventory (Beck Depression (BD-II) 21 item questionnaire or Zung Self-Rating Questionnaire).
Gastric Bypass and Gastric Banding

Pre-operative Medications

Post-operative Medications
Weight Maintenance after Bariatric Surgery

![Graph showing weight change over years of follow-up after different bariatric surgeries.](image-url)
Gastric Bypass and Gastric Banding

Early Clinical Effects
Differentiating Roux-en-Y and Gastric Banding-30 min of MMTT

Administer 250 cc Ensure + 650 mg acetaminophen

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>RnY/GB Before Sx</th>
<th>RnY/GB After Sx</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asparagine</td>
<td>1.176728</td>
<td>14.33873</td>
</tr>
<tr>
<td>Phenyl sulfate</td>
<td>2.852039</td>
<td>6.798669</td>
</tr>
<tr>
<td>5-(hydroxymethyl)-5-methylphenoxy)-2,2-dimethyl-Pentanoic acid (Gemfibrozil M4)</td>
<td>1.31608</td>
<td>2.222061</td>
</tr>
<tr>
<td>2-Hydroxyethinylestradiol</td>
<td>1.966002</td>
<td>2.107524</td>
</tr>
<tr>
<td>docusate</td>
<td>1.079641</td>
<td>2.097368</td>
</tr>
<tr>
<td>beta-D-Fucose</td>
<td>0.932653</td>
<td>1.984876</td>
</tr>
<tr>
<td>Lactate</td>
<td>0.786977</td>
<td>1.876784</td>
</tr>
<tr>
<td>D-Glucose</td>
<td>0.782593</td>
<td>1.874516</td>
</tr>
<tr>
<td>Citric acid</td>
<td>1.323149</td>
<td>1.744332</td>
</tr>
<tr>
<td>trihydroxyoctadecenoic acid</td>
<td>4.808725</td>
<td>1.620464</td>
</tr>
<tr>
<td>1D-Myo-inositol 1,3,4,5-tetrasik phosphate</td>
<td>1.34063</td>
<td>1.536745</td>
</tr>
<tr>
<td>2-Aminopropiophenone</td>
<td>1.703495</td>
<td>1.519994</td>
</tr>
<tr>
<td>hydroxy capric acid</td>
<td>0.91429</td>
<td>1.479224</td>
</tr>
<tr>
<td>2-Hydroxymestranol</td>
<td>1.844499</td>
<td>1.468781</td>
</tr>
<tr>
<td>Pro Lys Pro</td>
<td>1.77825</td>
<td>1.444315</td>
</tr>
<tr>
<td>Glu His</td>
<td>1.656207</td>
<td>1.142015</td>
</tr>
<tr>
<td>Creatine</td>
<td>0.461833</td>
<td>1.083051</td>
</tr>
<tr>
<td>Mono-N-depropylprobenecid</td>
<td>1.271909</td>
<td>1.054801</td>
</tr>
<tr>
<td>GPEtn(16:0/22:4(7Z,10Z,13Z,16Z))</td>
<td>1.152962</td>
<td>1.050375</td>
</tr>
<tr>
<td>Ribitol</td>
<td>1.479468</td>
<td>1.041754</td>
</tr>
<tr>
<td>1-eicosanoyl-2-(11Z,14Z-eicosadienoyl)-sn-glycerol</td>
<td>1.221211</td>
<td>1.035339</td>
</tr>
<tr>
<td>GPEtn(18:0/18:3(9Z,12Z,15Z))[U]</td>
<td>0.829258</td>
<td>0.947206</td>
</tr>
<tr>
<td>D-Glucose</td>
<td>1.020315</td>
<td>0.943904</td>
</tr>
<tr>
<td>6,9-hexadecadienoic acid</td>
<td>2.105966</td>
<td>0.764784</td>
</tr>
<tr>
<td>N-(2-phenoxy-ethyl) arachidonoyl amine</td>
<td>1.014266</td>
<td>0.763771</td>
</tr>
<tr>
<td>Allopregnanalne sulfate</td>
<td>0.886469</td>
<td>0.75287</td>
</tr>
<tr>
<td>GPEtn(18:1(11Z)/18:1(9Z))[U]</td>
<td>0.872243</td>
<td>0.722102</td>
</tr>
<tr>
<td>Dihydriodicolic acid</td>
<td>0.668707</td>
<td>0.67994</td>
</tr>
<tr>
<td>Amiloride</td>
<td>0.672242</td>
<td>0.637524</td>
</tr>
<tr>
<td>undecenoic acid</td>
<td>0.706845</td>
<td>0.633441</td>
</tr>
<tr>
<td>Glutamic Acid</td>
<td>0.660295</td>
<td>0.600171</td>
</tr>
<tr>
<td>Arginine</td>
<td>1.681267</td>
<td>0.596823</td>
</tr>
<tr>
<td>Lauric acid</td>
<td>1.637326</td>
<td>0.583346</td>
</tr>
<tr>
<td>2-Hydroxy-3-(4-methoxyethylphenoxy)-propanoic acid</td>
<td>0.286136</td>
<td>0.569132</td>
</tr>
<tr>
<td>GPIns(18:1(9Z)/18:1(9Z))</td>
<td>0.93842</td>
<td>0.533196</td>
</tr>
<tr>
<td>GPCho(O-12:0/O-12:0[U])</td>
<td>1.992268</td>
<td>0.510135</td>
</tr>
<tr>
<td>1-(9Z-heptadecenoic)-2-(9Z,12Z-heptadecadienoyl)-sn-glycerol</td>
<td>0.204326</td>
<td>0.487977</td>
</tr>
<tr>
<td>Methylprednisolone succinate</td>
<td>0.602441</td>
<td>0.428638</td>
</tr>
<tr>
<td>2,4-Dihydroxybutyric acid</td>
<td>0.191259</td>
<td>0.410316</td>
</tr>
</tbody>
</table>
Potential effects of increased dietary protein to enhance weight loss

Amino Acid Effects

- Postprandial meal-induced visceral signals
- Release of PYY and other enteric hormones
- Vagal nerve stimulation
- Direct action of amino acids in the brain

Mixed Meal Tolerance Test: Pre and Post Weight Loss

Each represents the time course of the indicated metabolite/hormone following administration of 250 ml of Ensure as a mixed meal tolerance test (0,30,60,90,150 minutes)
Mixed Meal Tolerance Test: Pre and Post Weight Loss
Mixed Meal Tolerance Test: Amino Acid Dynamics

Graphs showing BCAA, Glycine, and Alanine dynamics in Lean, GABP, and LAGB groups with Weight Loss and Baseline comparisons. Scatter plots of HOMA vs. Baseline [BCAA], Glucose excursion, Baseline [Glycine], and Glycine excursion with correlation coefficients R² = 0.4195, 0.4547, 0.4367, 0.8737, and 0.5805.
Change in Amino Acid dynamics following Roux-en-Y gastric bypass
Cerebral Spinal Fluid Amino Acids

Aspartic Acid

Leucine

Glycine

Clock Time

MEAL SNACK

Can the CSF protect its amino acid levels?

- Assess Plasma and CSF Amino Acid and Lipid Profiles at baseline and following 10% weight loss.
- Defined Diet for 72 hrs. prior to sampling.
Obese individuals have elevated plasma levels of amino acids (and other nutrients)

<table>
<thead>
<tr>
<th>Amino acids ((\mu\text{M}))</th>
<th>Obese</th>
<th>Lean</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Valine</td>
<td>281.4 (249.2, 332.9)</td>
<td>235.3 (204.1, 257.0)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Leucine/Isoleucine</td>
<td>170.0 (150.2, 200.8)</td>
<td>149.0 (132.5, 176.6)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Glutamate/Glutamine</td>
<td>118.4 (91.4, 143.7)</td>
<td>81.2 (66.7, 95.2)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Glycine</td>
<td>282.6 (245.6, 319.6)</td>
<td>328.4 (265.6, 403.0)</td>
<td>0.0007</td>
</tr>
<tr>
<td>Alanine</td>
<td>433.4 (394.5, 492.3)</td>
<td>367.3 (297.1, 420.0)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>72.6 (66.3, 78.9)</td>
<td>61.6 (55.1, 68.8)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>79.5 (68.5, 90.0)</td>
<td>67.1 (56.7, 73.5)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Aspartate/Asparagine</td>
<td>20.1 (17.3, 23.8)</td>
<td>16.5 (13.5, 19.7)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Arginine</td>
<td>135.2 (116.5, 148.5)</td>
<td>115.3 (101.6, 137.0)</td>
<td>0.0007</td>
</tr>
<tr>
<td>Citrulline</td>
<td>32.0 (27.9, 40.3)</td>
<td>36.3 (30.5, 40.7)</td>
<td>0.04</td>
</tr>
</tbody>
</table>
Metabolomic measurements can provide clues to the dynamic relationship between genes and environment in people

The metabolome is complex and changes appear coordinated

Statistical and visualization methods can provide otherwise hidden relationships between phenotypic characteristics