Metabolomics and the Molecular Phenotype of Obesity

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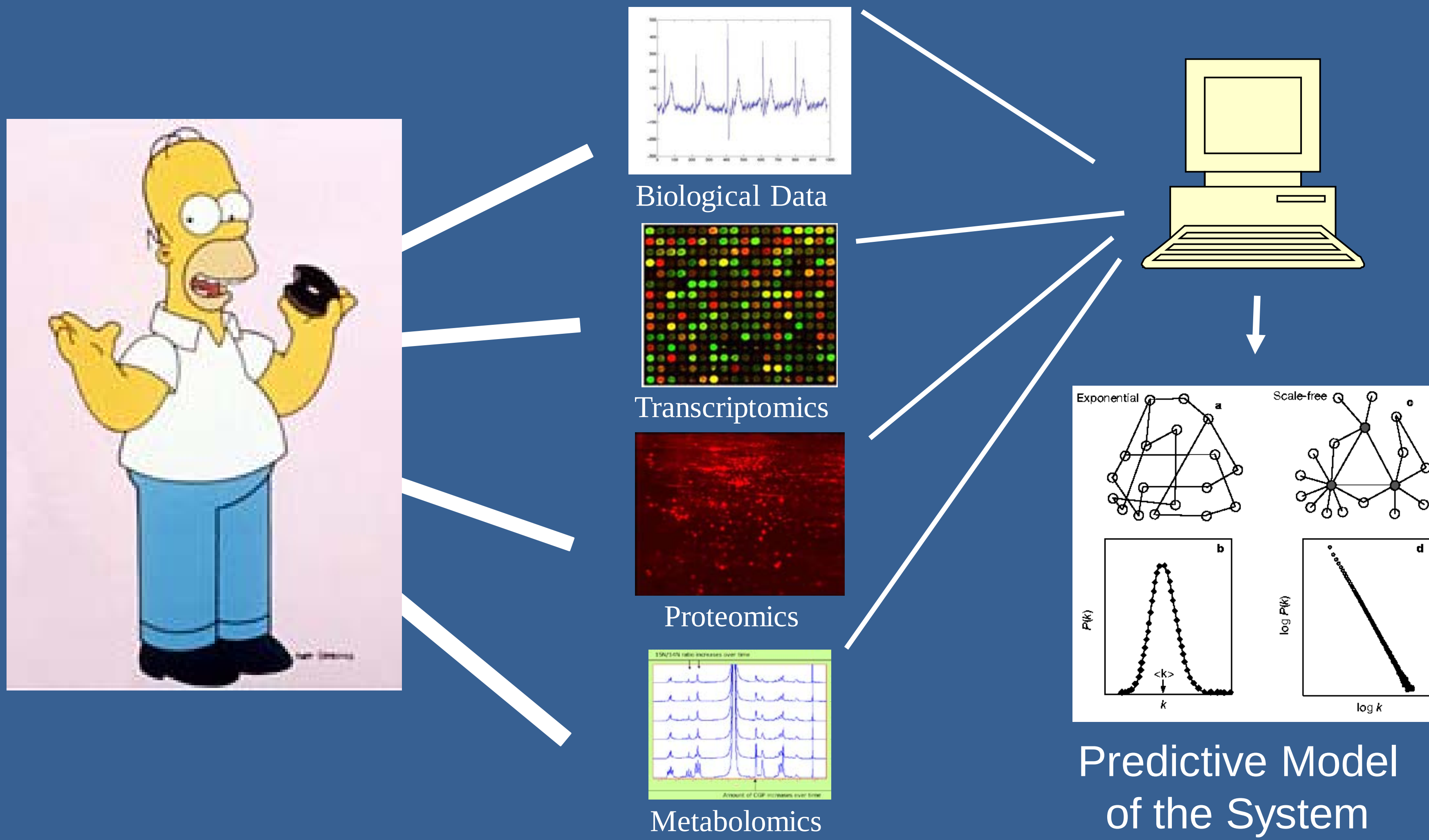
Endowment for the
Biological Sciences

Clinical and Molecular Phenotyping



Systems Roles

Material

DNA — The ultimate potential of a cell

RNA — The current direction of a cell

Proteins — The functional capabilities of a cell

Metabolites — The limiting currency of a cell

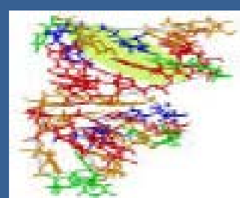
Information

The 'omic's

Genome

~30,000 genes

Transcriptome

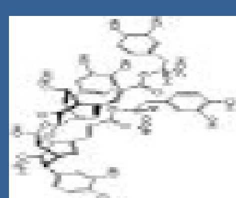


~100,000 transcripts

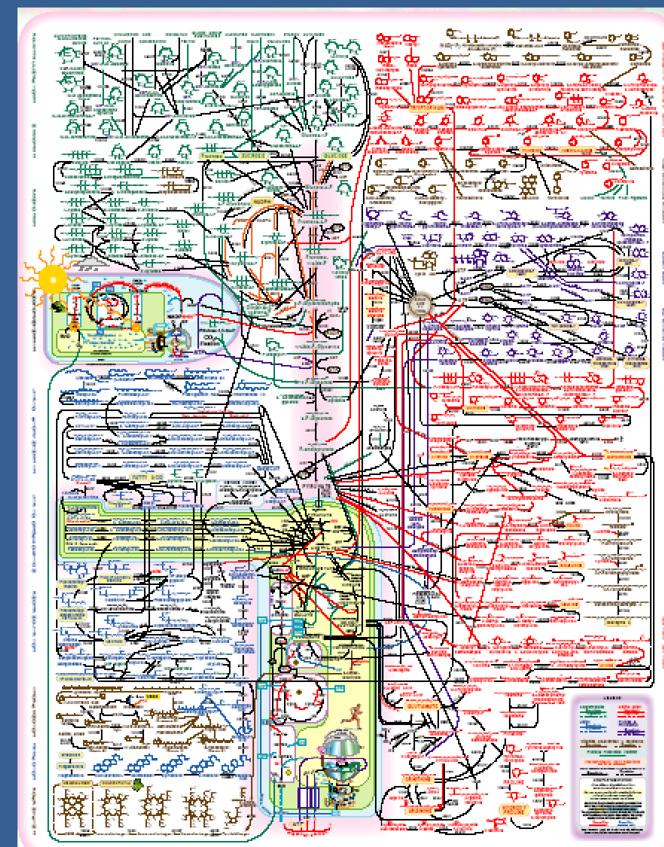
Proteome

~1,000,000 protein forms?

Metabolome



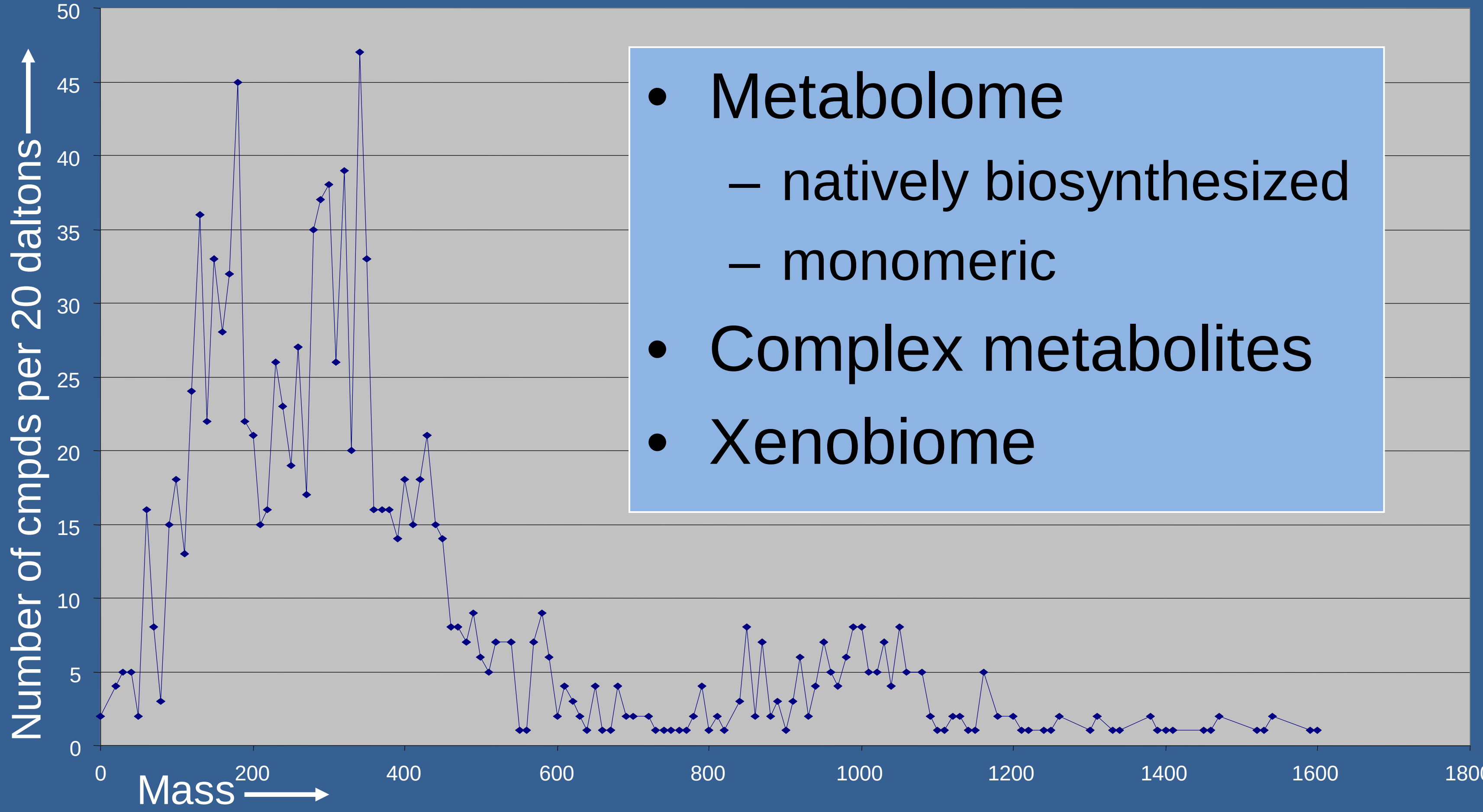
~2000 to 5,000 metabolites



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*

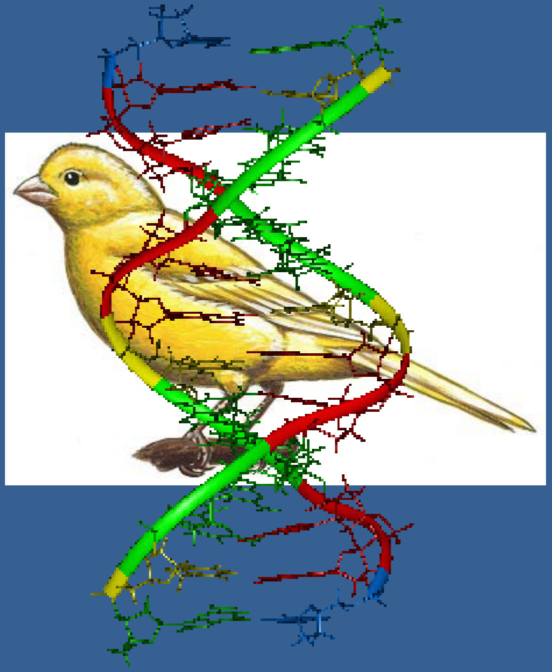
Mass Distribution of Compounds in the Human Metabolome



- 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

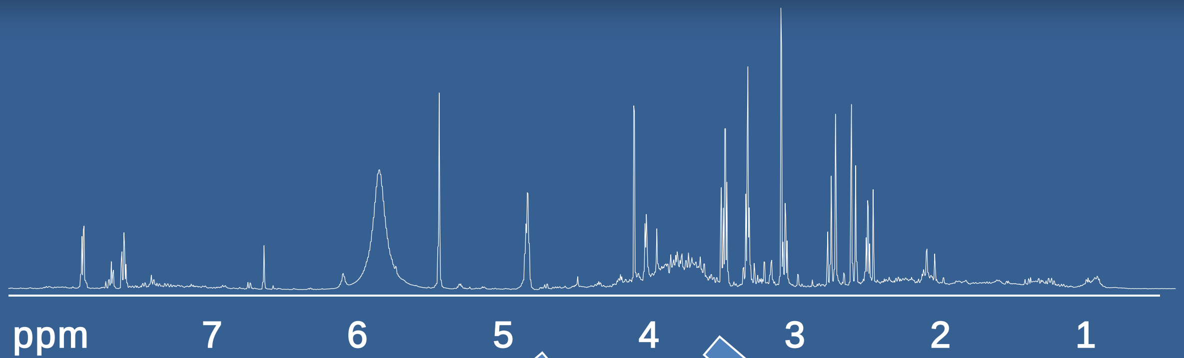


Metabolites are the Canaries of the Genome

Why Are Metabolites Relevant?

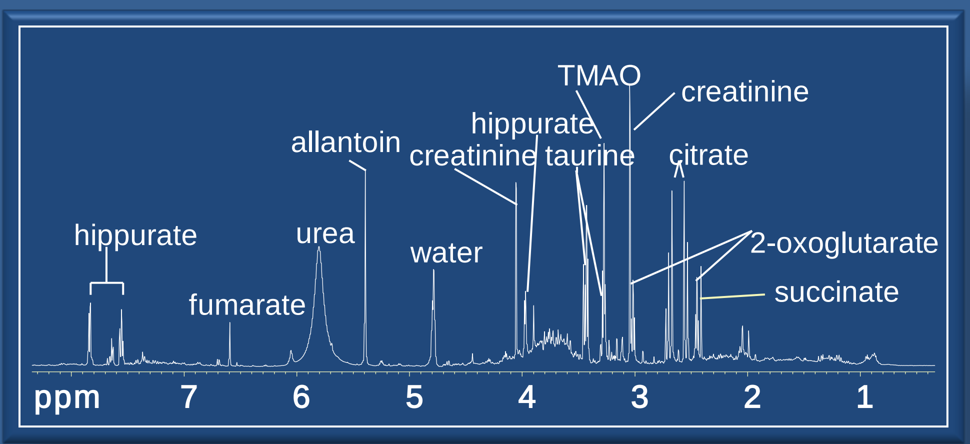
- Generate metabolic “signatures” for disease states or host responses
- Obtain a more “holistic” view of metabolism (and treatment)
- Accelerate assessment & diagnosis
- More rapidly and accurately (and cheaply) assess/identify disease phenotypes
- Monitor gene/environment interactions
- Rapidly track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

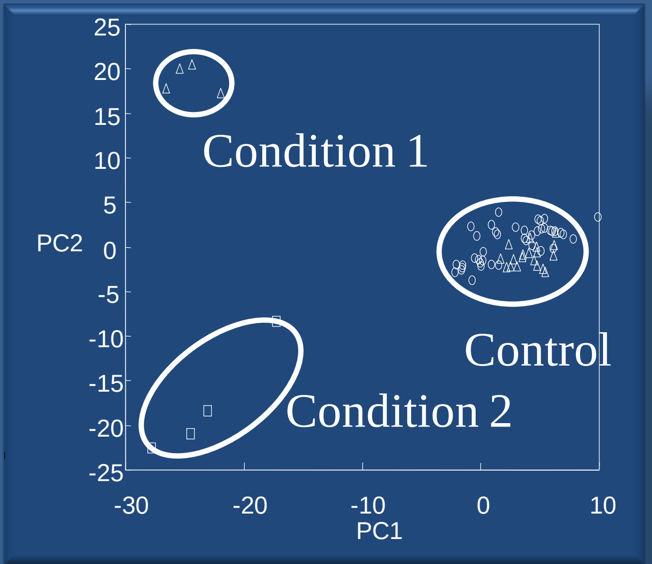
2 Routes to Metabolomics



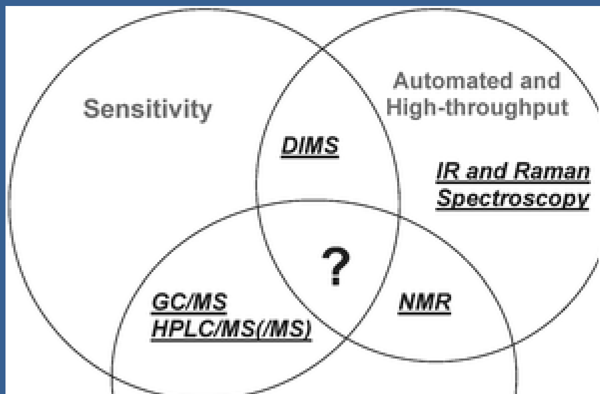
Quantitative Methods

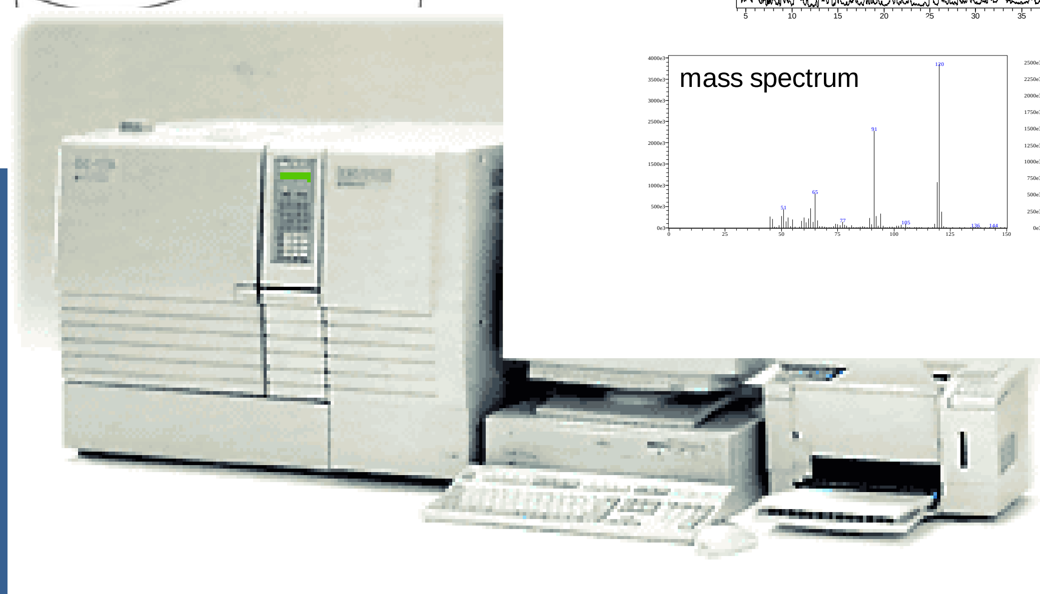
Chemometric (Pattern) Methods

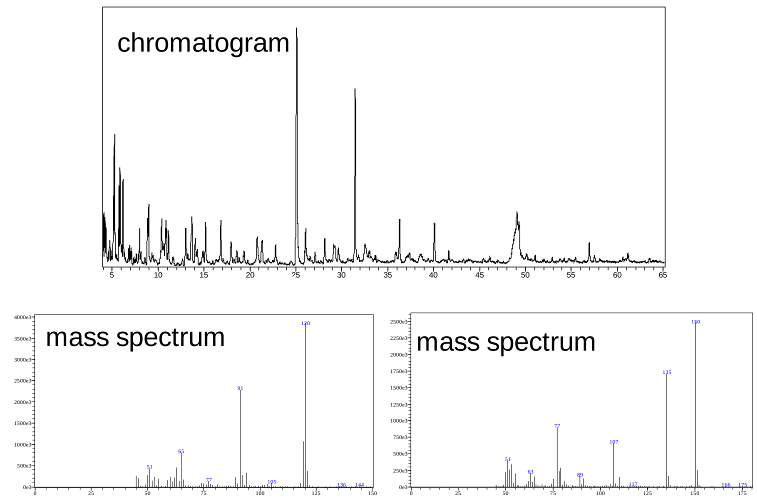




The Technology of Metabolomics







Separations Based Metabolomics Platforms

	CE-MS	GC-MS	LC-MS
AD	Small Injection Volumes High Resolution	High resolution Library ID	Soft ionization Full metabolome coverage
DA	Low capacity Difficult MS interface Requires charged analytes	Chemical derivitization Harsh ionization Limited metabolite applicability	Limited structural info Lower Resolution

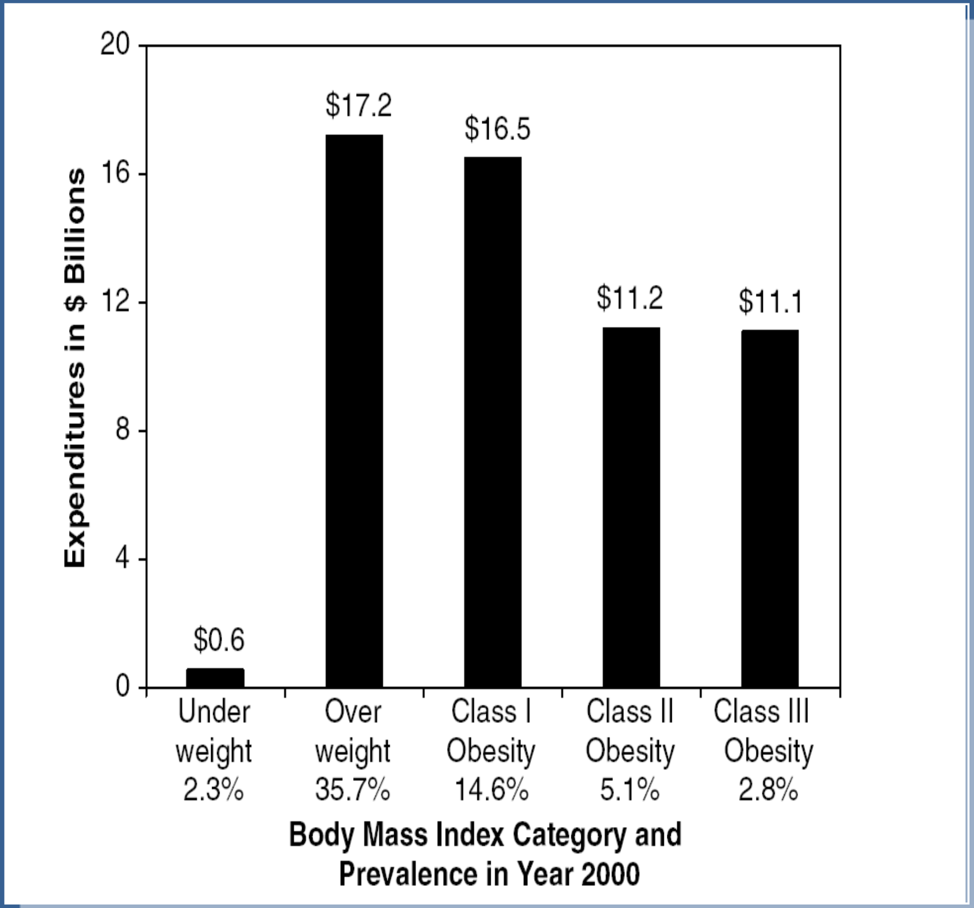
- **ALL ESI-MS Methods Are Subject to Ion Suppression**
- **Response Factors of Analytes are Not Equal**

Adapted From: Want, E. J.; Cravatt, B. F.; Siuzdak, G., *ChemBioChem* **2005**, 6, 1941 – 1951
Adapted From: Villas-Boas, S. G.; Mas, S.; Akesson, M.; Smedsgaard, J.; Nielsen, J., *Mass Spectrom Rev* **2005,** 24, (5), 613-46

Relative risk of health problems associated with obesity

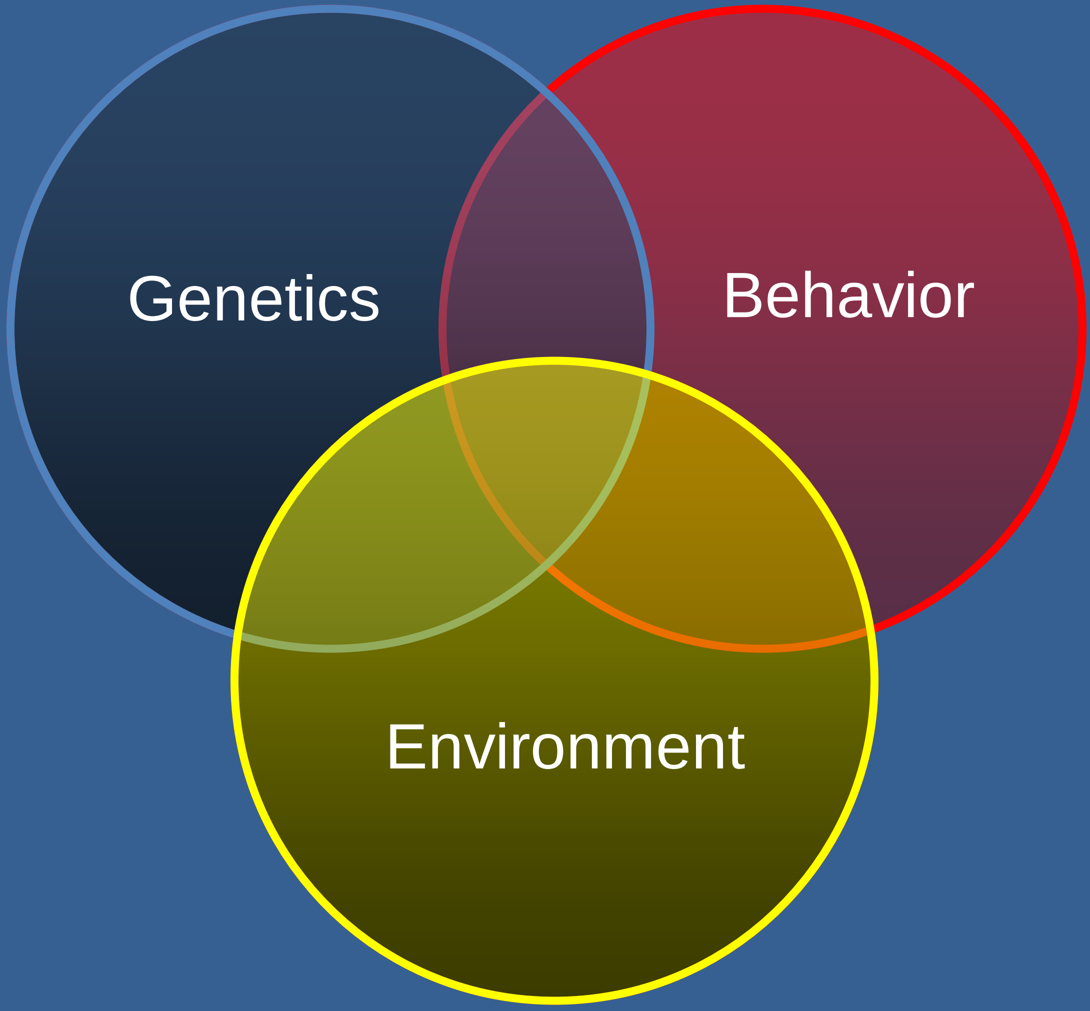
Greatly increased (relative risk >>3)	Moderately increased (relative risk 2-3)	Slightly increased (relative risk 1-2)
Diabetes	Coronary heart disease	Cancer (breast cancer in postmenopausal women, endometrial cancer, colon cancer)
Gall bladder disease	Osteoarthritis (knees)	Reproductive hormone abnormalities
Hypertension	Hyperuricemia and gout	Polycystic ovary syndrome
Dyslipidemia		Impaired fertility
Insulin resistance		Low back pain
Breathlessness		Increased anesthetic risk
Sleep apnea		Fetal defects arising from maternal obesity

Excess U.S. Medical Costs Related to Abnormal Body Weight

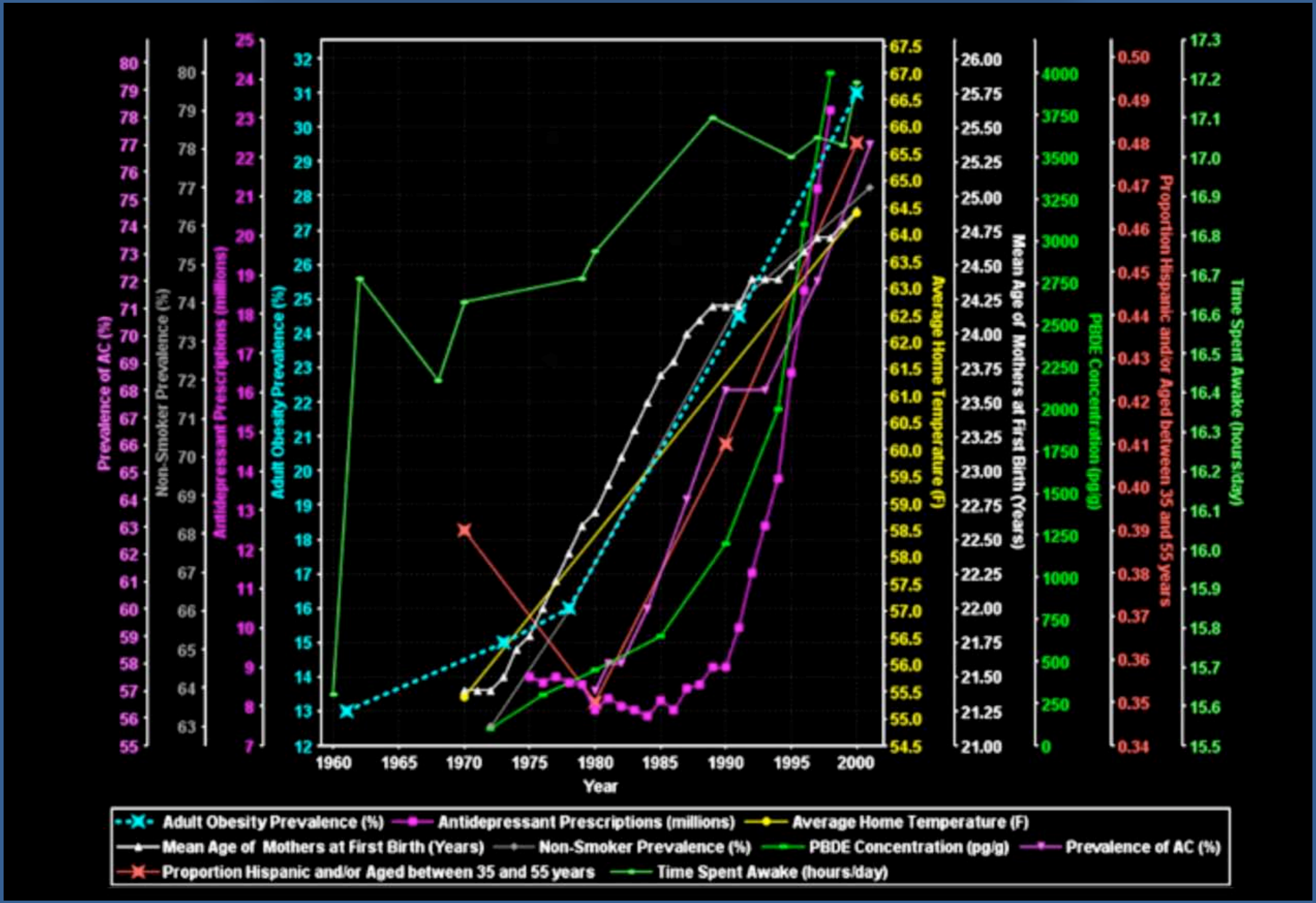


Int J Obesity 2005;29:334-339

Causes of Obesity



Environmental effects…



Keith SW, et al. *Int J Obes.* 2006;30:1585-1594.

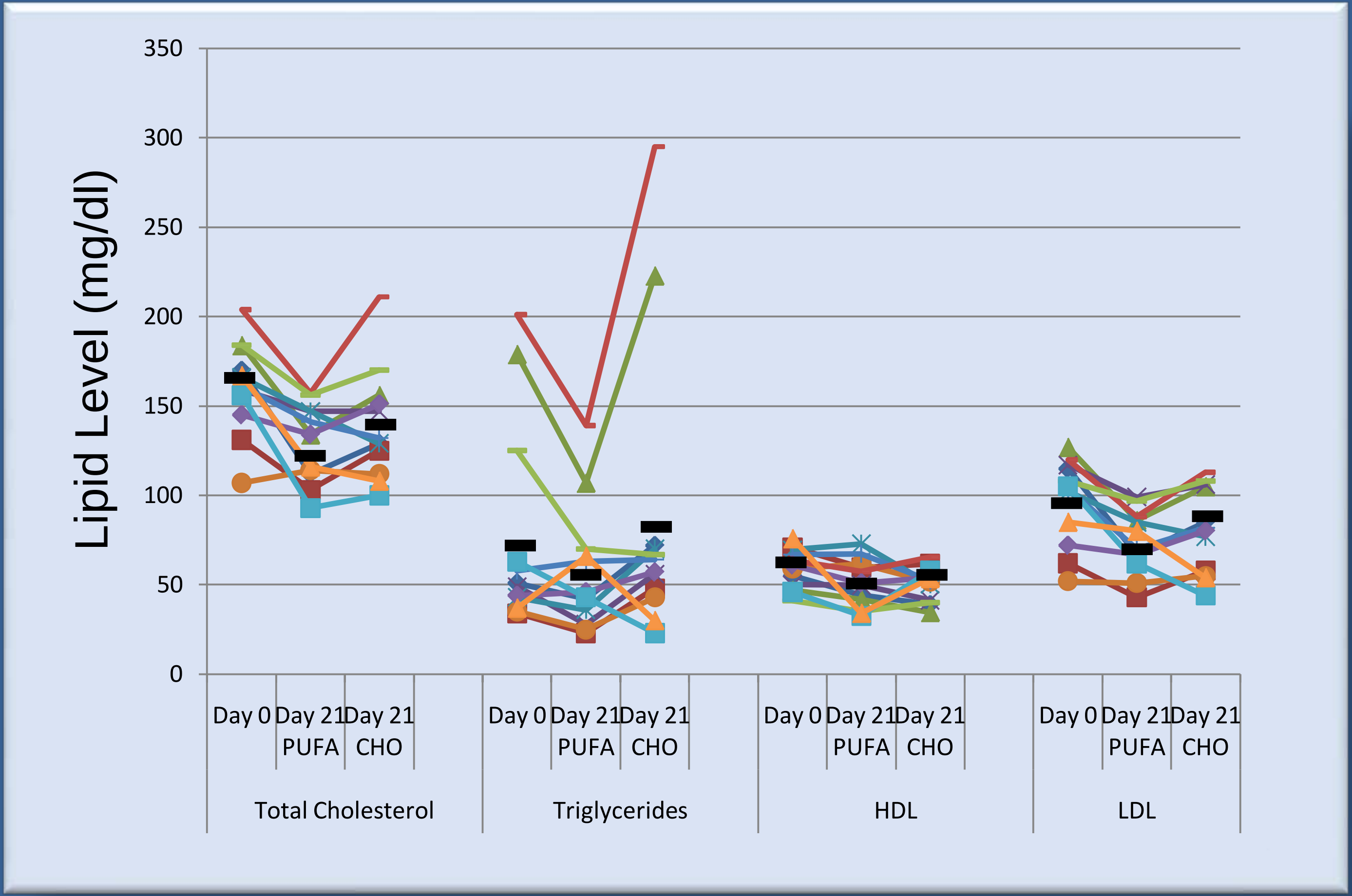
DBP: Metabolomics and Obesity

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 in100 lean (BMI < 27).

Phenotypic response to diets



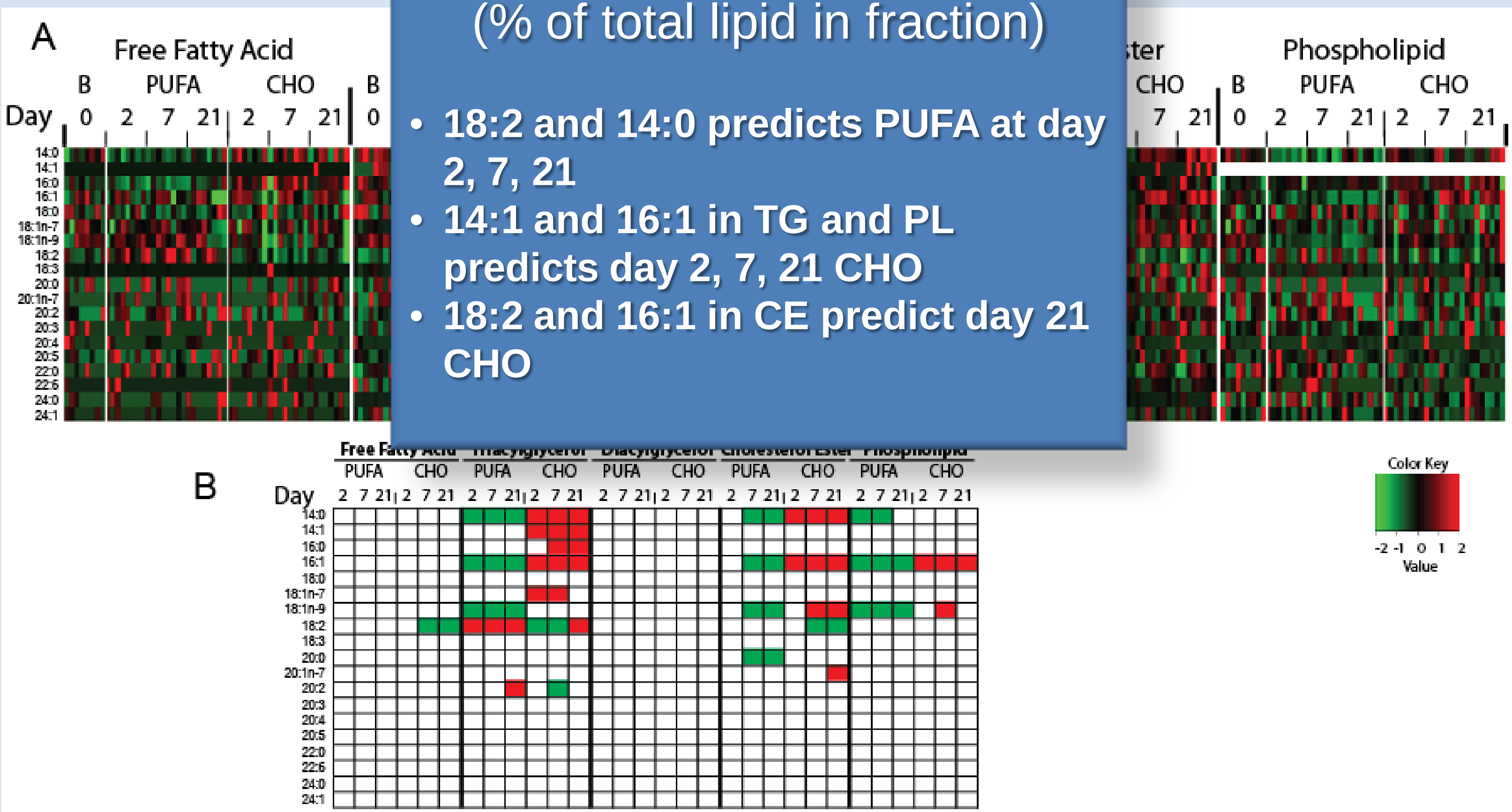
Lipomic assessment of plasma

Can macron ... atty acid profiles?

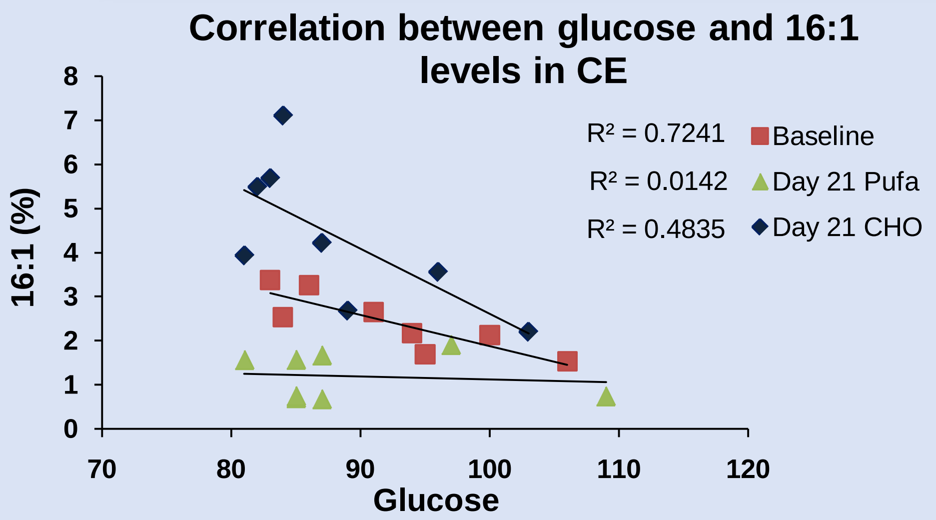
Principal Component Analysis

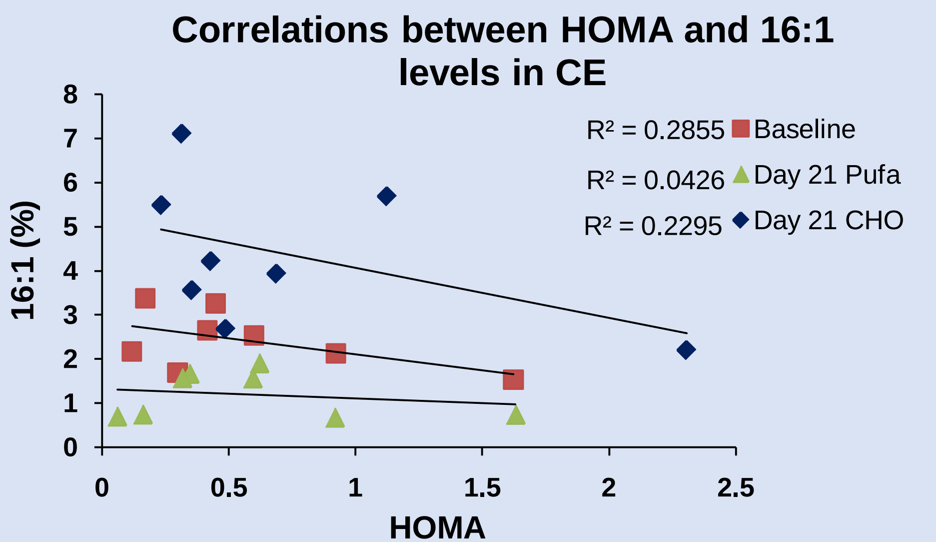
(% of total lipid in fraction)

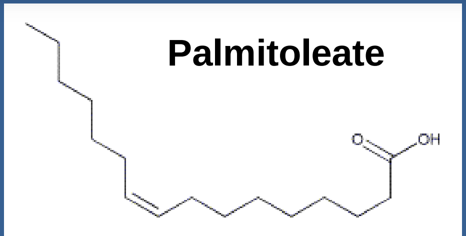
- 18:2 and 14:0 predicts PUFA at day 2, 7, 21
- 14:1 and 16:1 in TG and PL predicts day 2, 7, 21 CHO
- 18:2 and 16:1 in CE predict day 21 CHO



Palmitoleate, Glucose and Insulin Sensitivity







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

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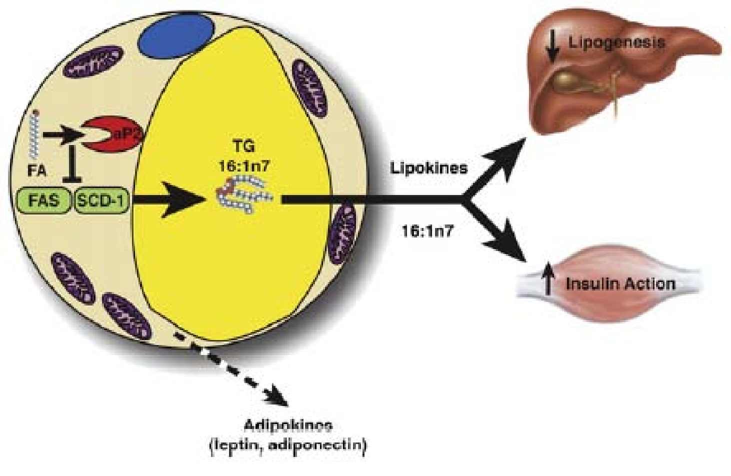
DOI 10.1016/j.cell.2008.07.048

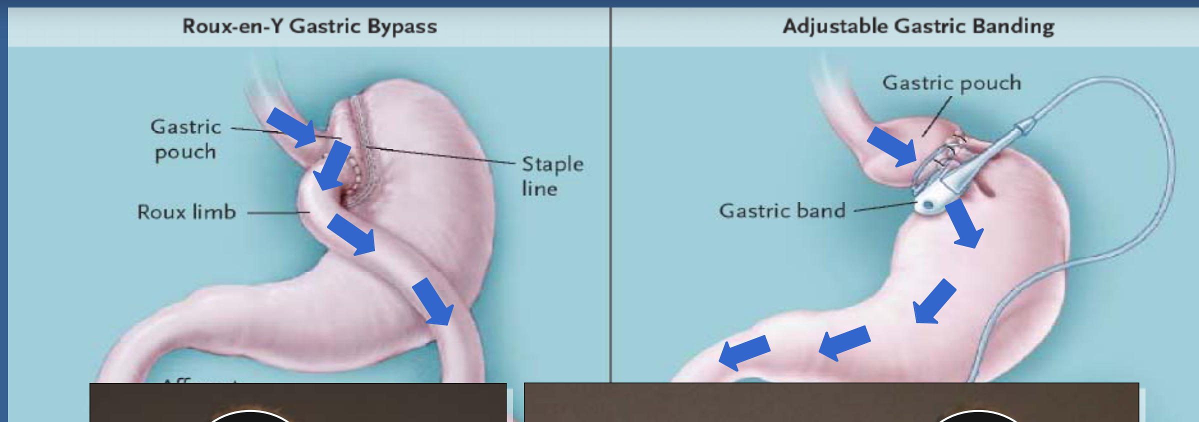


Phenotyping of Patients

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 Absorptiometery (DEXA, new).
- Metabolic Assessment. VO_2peak, 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).

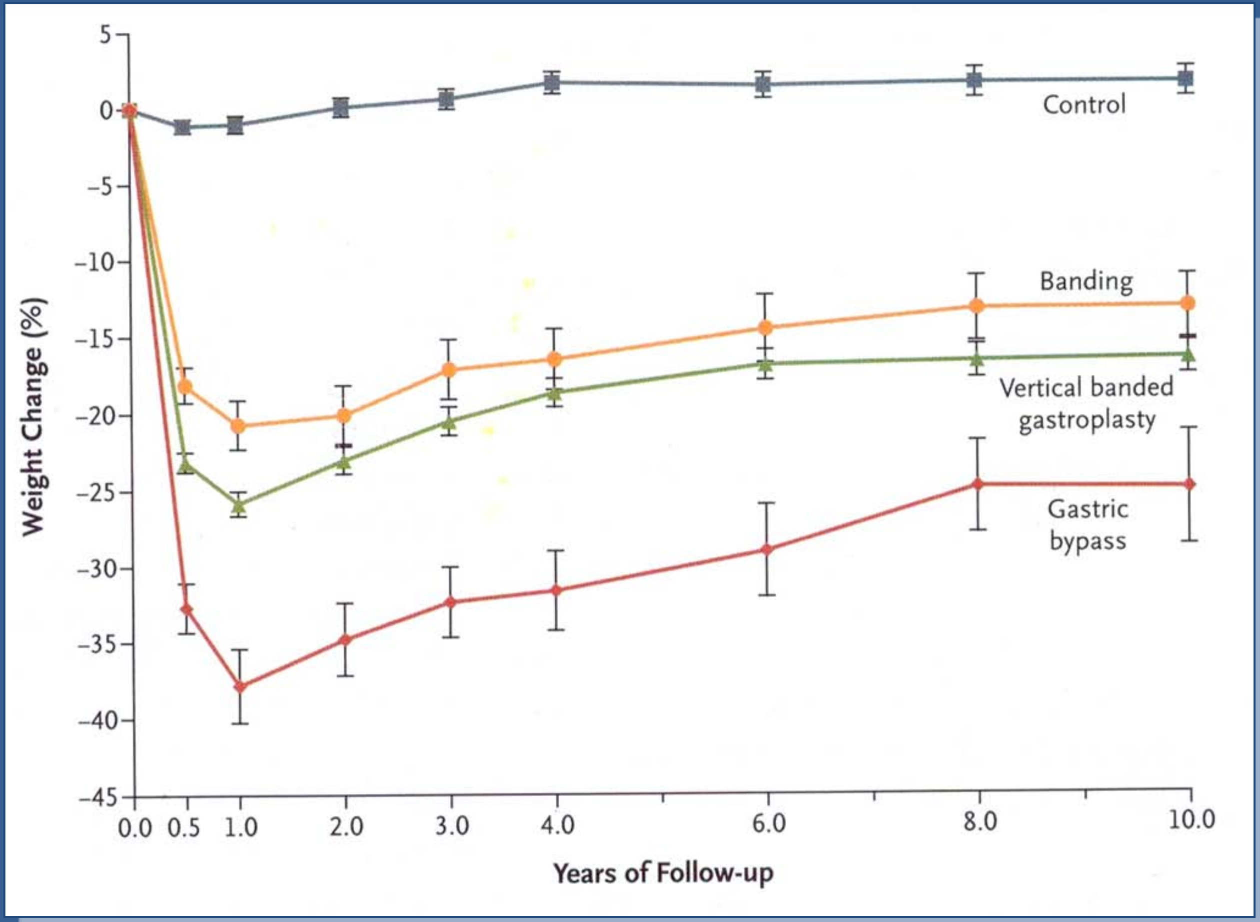
Gastric Bypass and Gastric Banding



Pre-operative Medications

Post-operative Medications

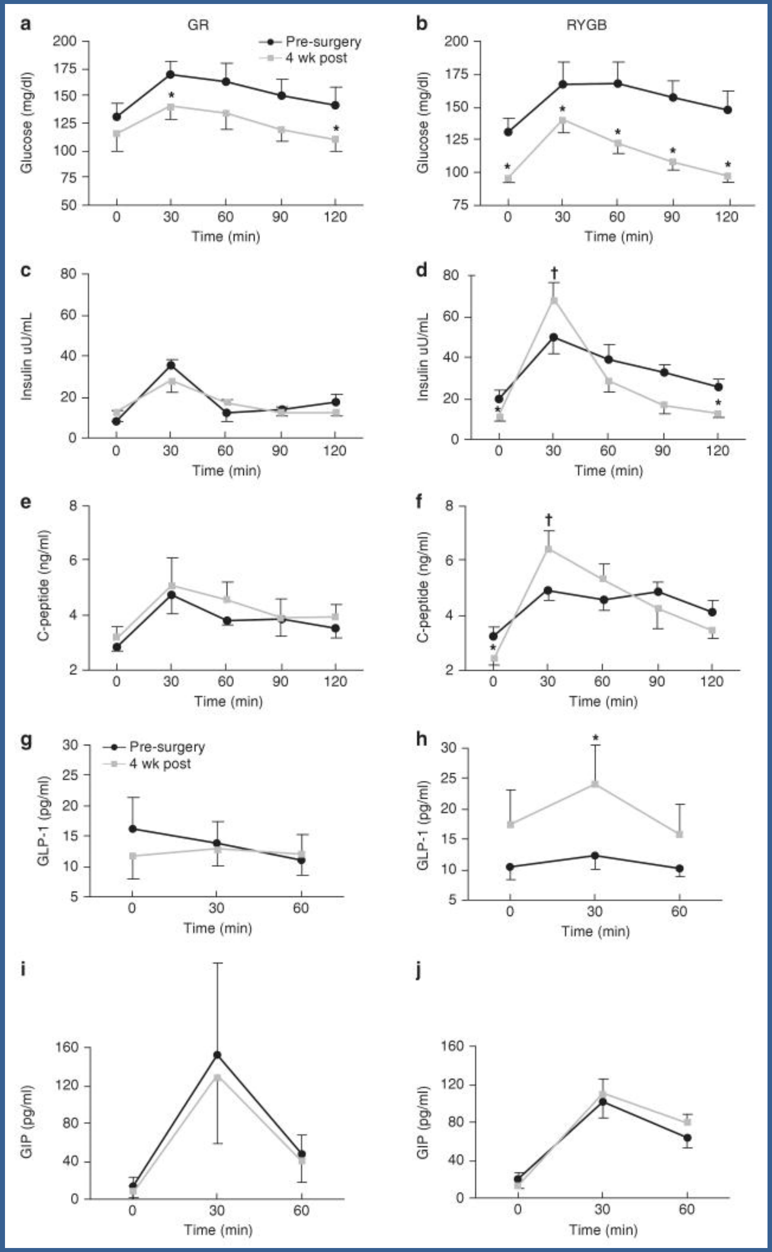
Weight Maintenance after Bariatric Surgery



N Engl J Med. 2004;351:26

Gastric Bypass and Gastric Banding

Early Clinical Effects

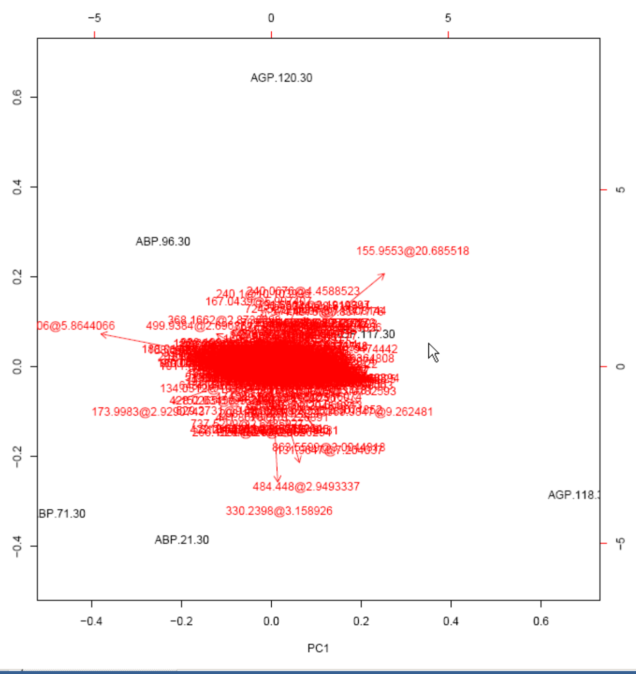


Int J Obes (Lond). 34:462-471, 2010

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

Administer 250 cc Ensure + 650 mg acetaminophen

0 15 30 60 90 120 150 180

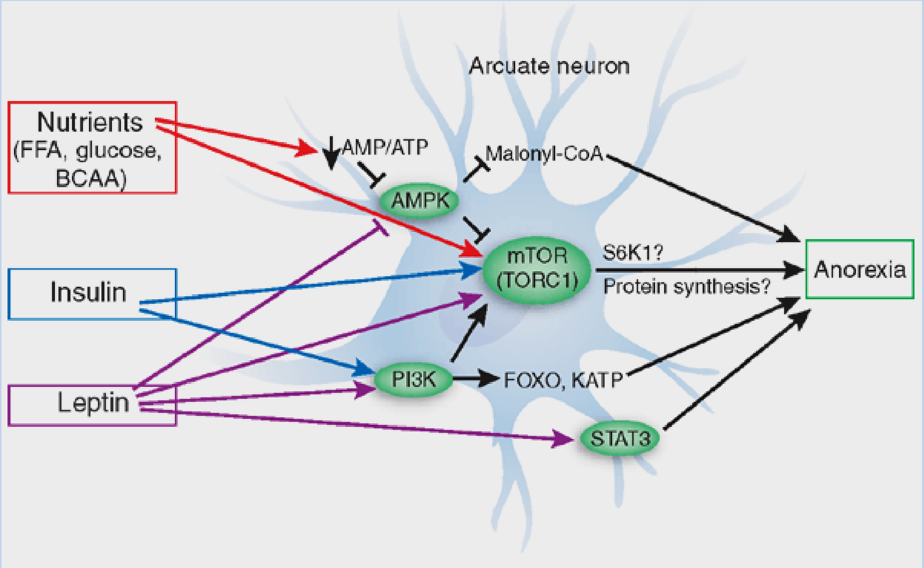


RnY/GB Before Sx	RnY/GB After Sx	Metabolite
1.176728	14.33873	Asparagine
2.852039	6.798669	Phenyl sulfate
1.31608	2.222061	5-[2-(hydroxymethyl)-5-methylphenoxy]-2,2-dimethyl-Pentanoic acid (Gemfibrozil M4)
1.966002	2.107524	2-Hydroxyethinylestradiol
1.079641	2.097368	docusate
0.932653	1.984876	beta-D-Fucose
0.786977	1.876784	Lactate
0.782593	1.874516	D-Glucose
1.323149	1.744332	Citric acid
4.808725	1.620464	trihydroxyoctadecenoic acid
1.340463	1.536745	1D-Myo-inositol 1,3,4,5-tetrakisphosphate
1.703495	1.519994	2-Aminopropiophenone
0.91429	1.479224	hydroxy capric acid
1.844499	1.468781	2-Hydroxymestranol
1.77825	1.444315	Pro Lys Pro
1.656207	1.142015	Glu His
0.461833	1.083051	Creatine
1.271909	1.054801	Mono-N-depropylprobenecid
1.152962	1.050375	GPEtn(16:0/22:4(7Z,10Z,13Z,16Z))
1.479468	1.041754	Ribitol
1.221211	1.035339	1-eicosanoyl-2-(11Z,14Z-eicosadienoyl)-sn-glycerol
0.829258	0.947206	GPEtn(18:0/18:3(9Z,12Z,15Z))[U]
1.020315	0.943904	D-Glucose
2.105966	0.764784	6,9-hexadecadienoic acid
1.014266	0.763771	N-(2-phenoxy-ethyl) arachidonoyl amine
0.886469	0.75287	Allopregnanalone sulfate
0.872243	0.722102	GPEtn(18:1(11Z)/18:1(9Z))[U]
0.668707	0.67994	Dihydrodipicolinic acid
0.672242	0.637524	Amiloride
0.706845	0.633441	undecenoic acid
0.660295	0.600171	Glutamic Acid
1.681267	0.596823	Arginine
1.637326	0.583346	Lauric acid
0.286136	0.569132	2-Hydroxy-3-(4-methoxyethylphenoxy)-propanoic acid
0.93842	0.533196	GPIns(18:1(9Z)/18:1(9Z))
1.992268	0.510135	GPCho(O-12:0/O-12:0[U])
2.034236	0.487977	1-(9Z-hexadecenoyl)-2-(9Z,12Z-heptadecadienoyl)-sn-glycerol
0.602441	0.428638	Methylprednisolone succinate
0.191259	0.410316	2,4-Dihydroxybutyric acid

Potential effects of increased dietary protein to enhance weight loss

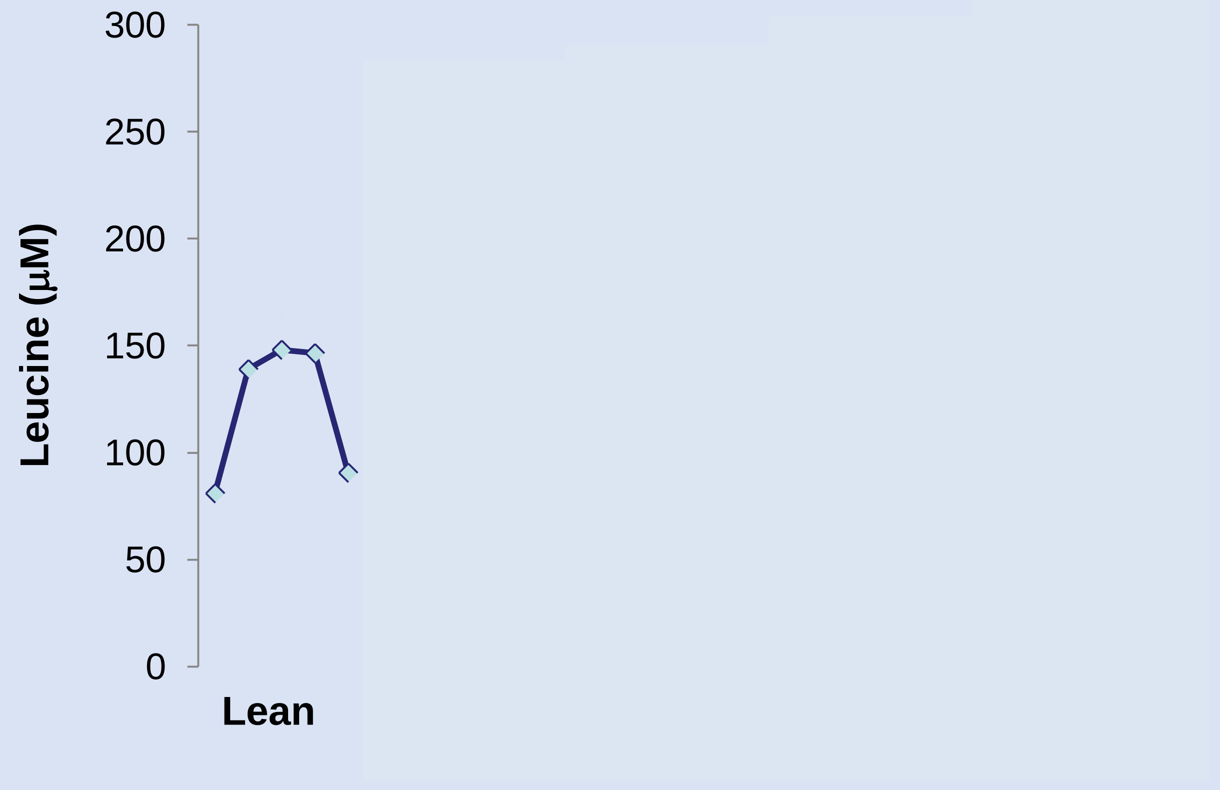
Amino Acid Effects

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



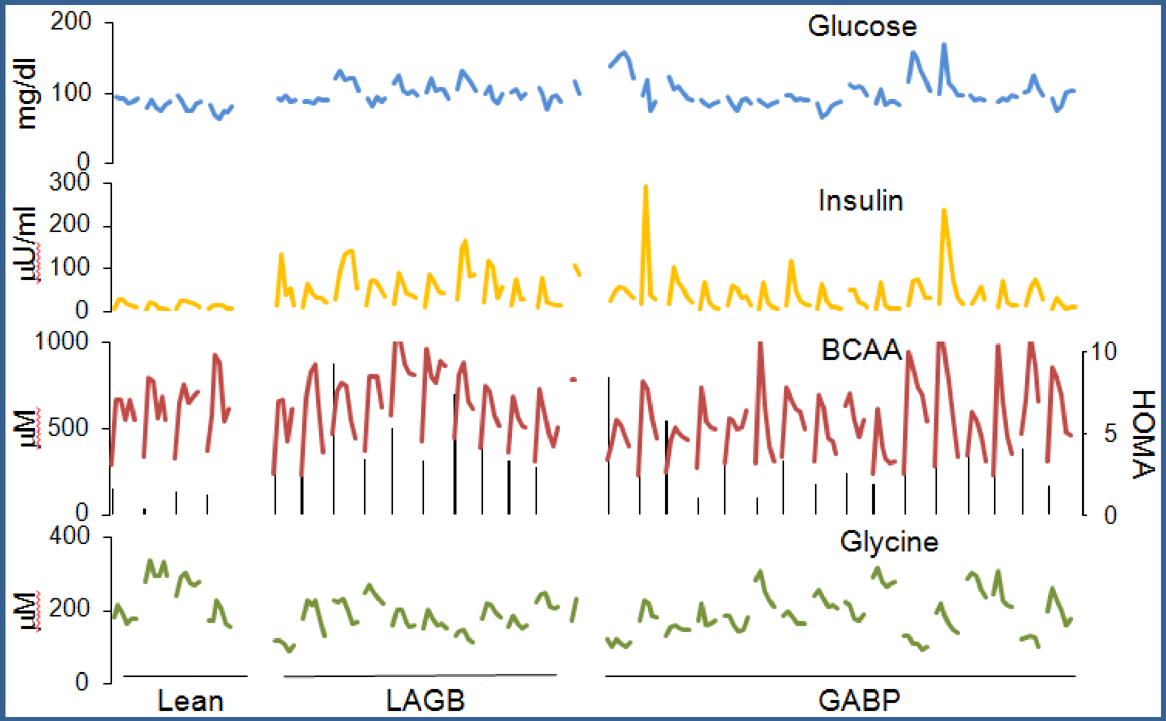
Tome et al. Am J Clin Nutr 2009;90(suppl):838S–43

Mixed Meal Tolerance Test: Pre and Post Weight Loss

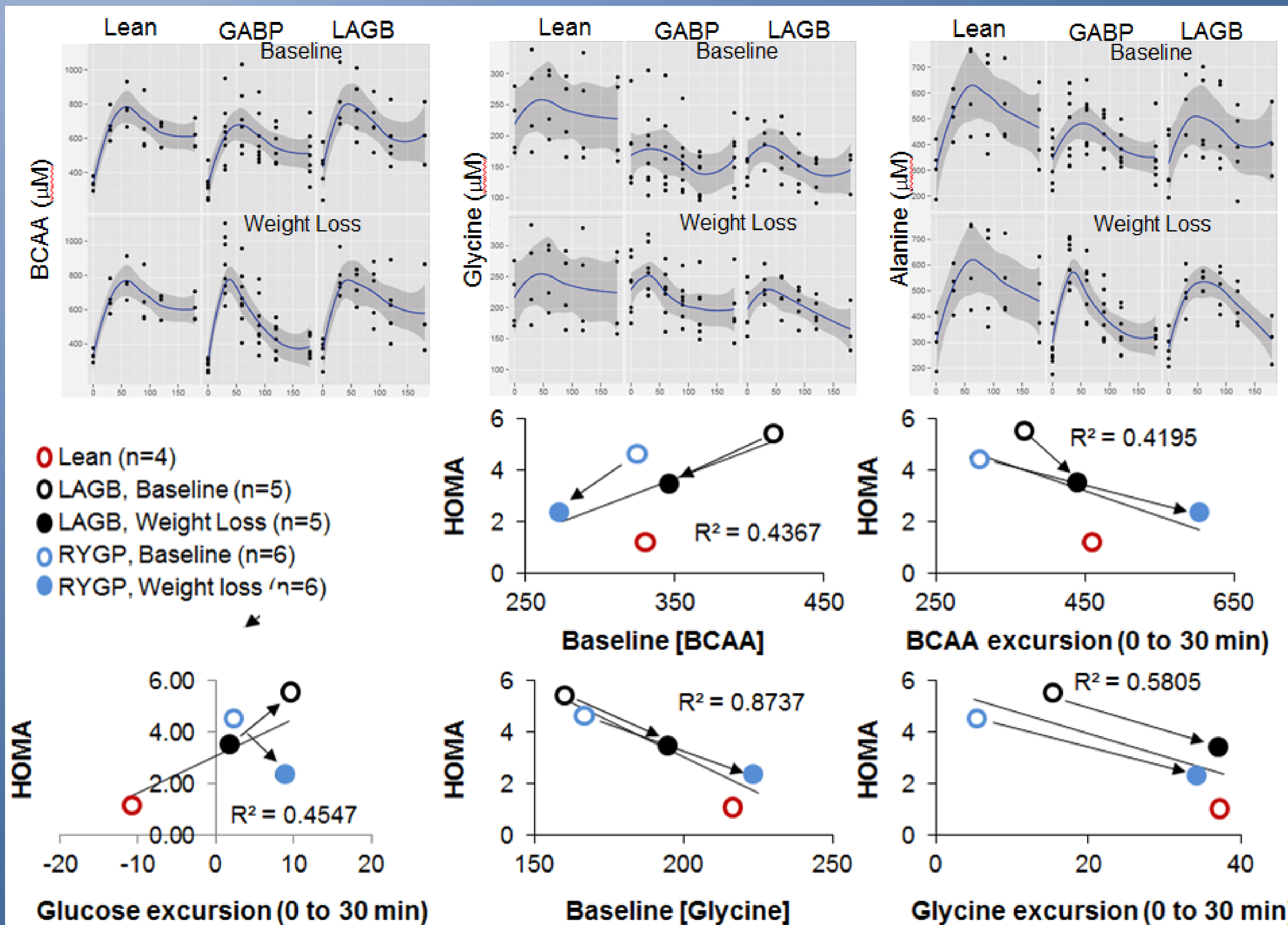


Each represents the time course of the indicated metabolite/hormone following administration of 250 ml of Ensure as a mixed meal tolerance test (0,30,60,90,150 minutes)

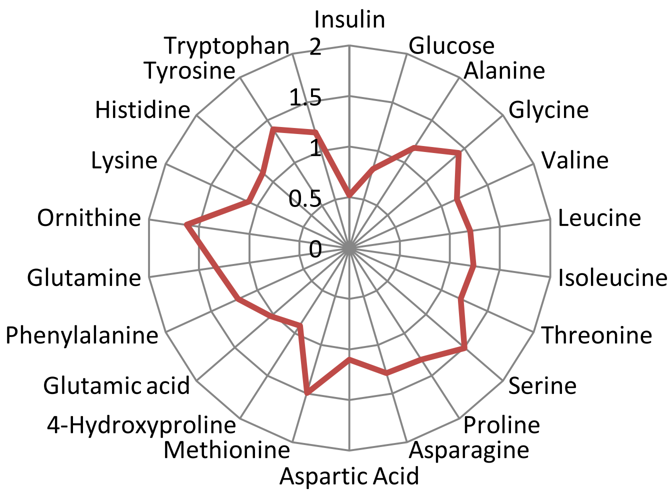
Mixed Meal Tolerance Test: Pre and Post Weight Loss

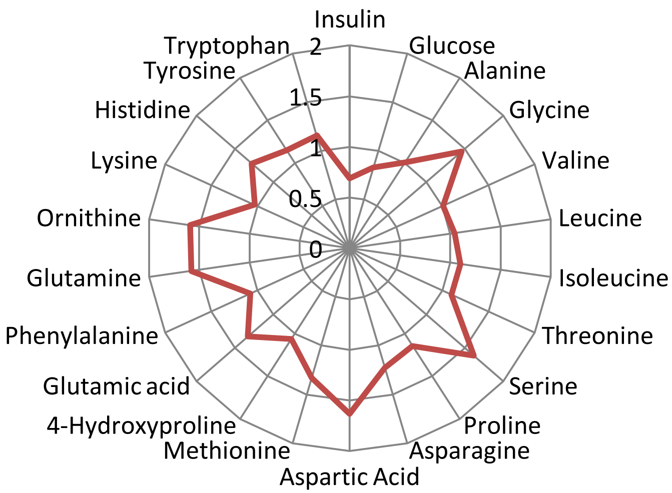


Mixed Meal Tolerance Test: Amino Acid Dynamics

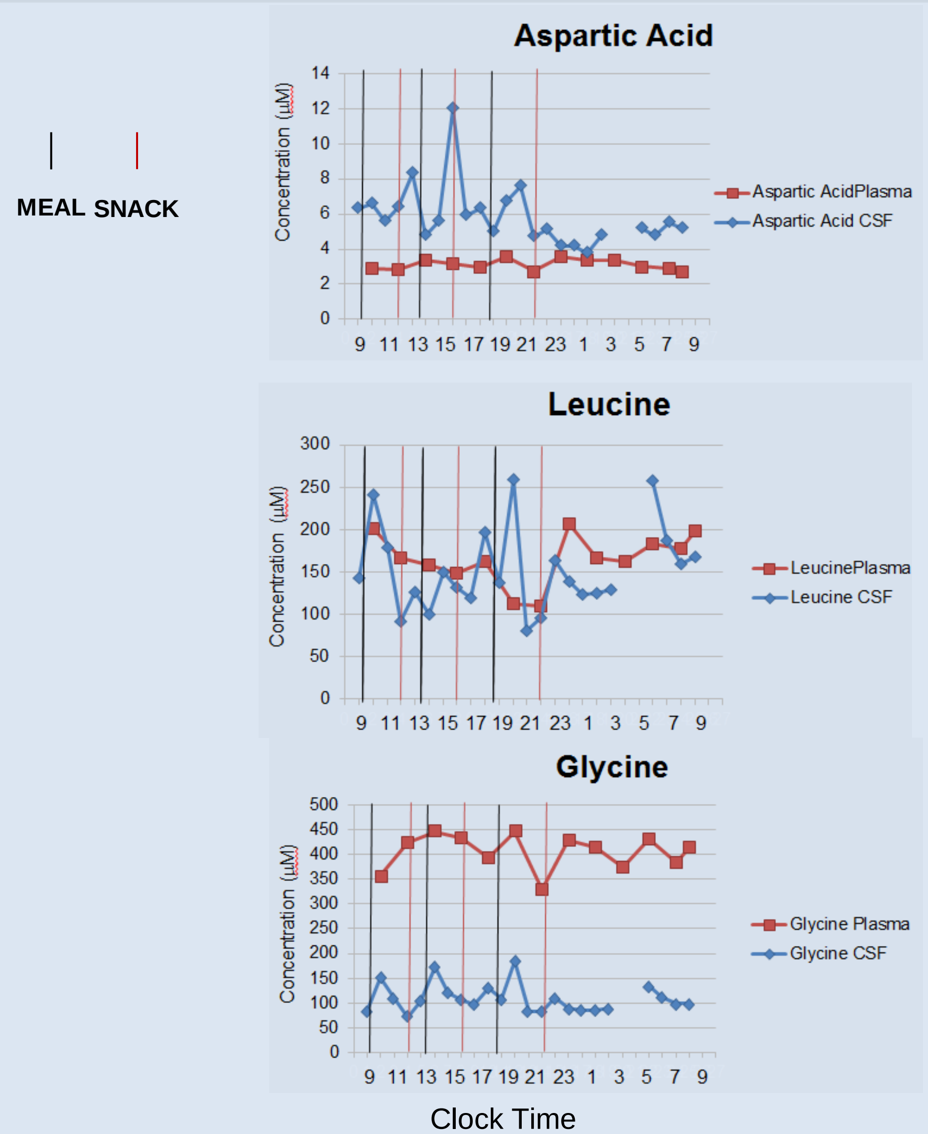


Change in Amino Acid dynamics following Roux-en-Y gastric bypass



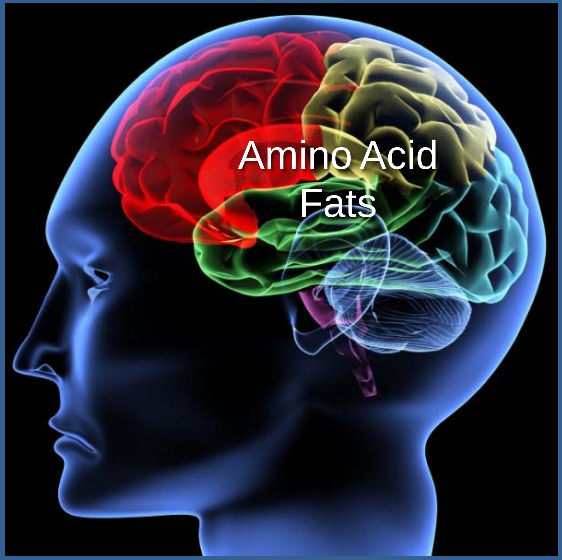


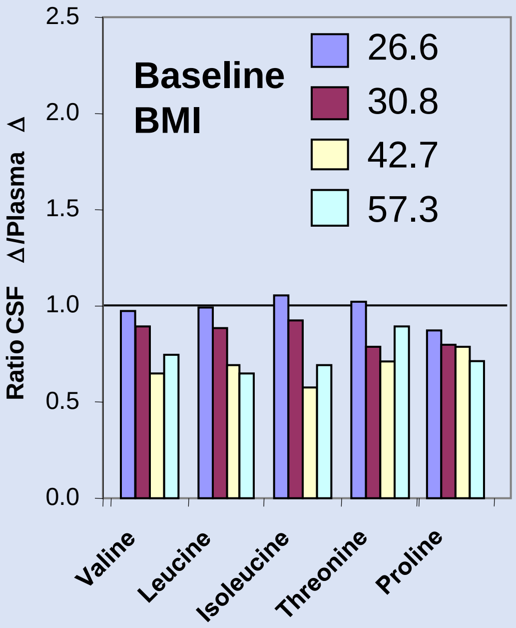
Cerebral Spinal Fluid Amino Acids



Can the CSF protect its amino acid levels?

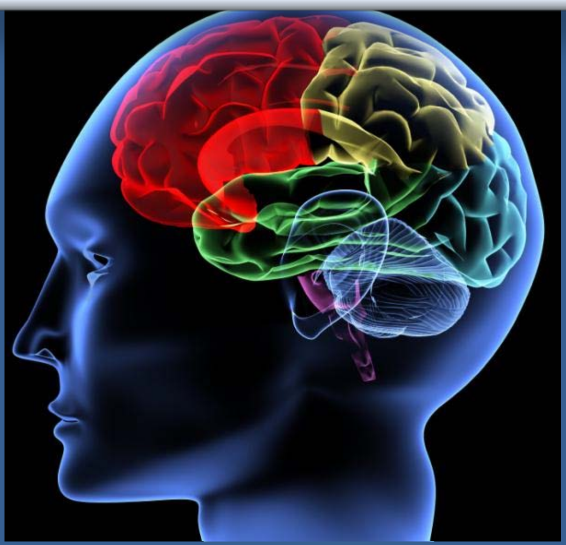
- Assess Plasma and CSF Amino Acid and Lipid Profiles at baseline and following 10% weight loss.
- Defined Diet for 72 hrs. prior to sampling.





Obese individuals have elevated plasma levels of amino acids (and other nutrients)

	Obese	Lean	P value
Amino acids (μM)	n = 74	n = 67	
Valine	281.4 (249.2, 332.9)	235.3 (204.1, 257.0)	<0.0001
Leucine/Isoleucine	170.0 (150.2, 200.8)	149.0 (132.5, 176.6)	<0.0001
Glutamate/Glutamine	118.4 (91.4, 143.7)	81.2 (66.7, 95.2)	<0.0001
Glycine	282.6 (245.6, 319.6)	328.4 (265.6, 403.0)	0.0007
Alanine	433.4 (394.5, 492.3)	367.3 (297.1, 420.0)	<0.0001
Phenylalanine	72.6 (66.3, 78.9)	61.6 (55.1, 68.8)	<0.0001
Tyrosine	79.5 (68.5, 90.0)	67.1 (56.7, 73.5)	<0.0001
Aspartate/Asparagine	20.1 (17.3, 23.8)	16.5 (13.5, 19.7)	<0.0001
Arginine	135.2 (116.5, 148.5)	115.3 (101.6, 137.0)	0.0007
Citrulline	32.0 (27.9, 40.3)	36.3 (30.5, 40.7)	0.04



Summary

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