Targeted Metabolomics

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Acknowledgements

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Targeted Approach:

- **Quantitative analysis**
- **Measure molarities of chemically cognate panels of small metabolites in diverse biological matrices (in our case - with emphasis on obesity, diabetes, and cardiovascular disease)**
- **µM determined via stable-isotope dilution**

Targeted Metabolomics Instrumentation

Waters Quattro micro API
Triple Quadrupole

Waters TQD
Triple Quadrupole

Waters Xevo TQ-S

Targeted Metabolomics Instrumentation

Agilent 6410
Triple Quadrupole

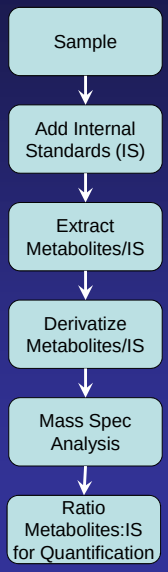
Agilent 5975B MSD GC/MS

Thermo Trace ISQ GC/MS

Experimental Design

- **Well defined groups (control vs. mutant/treated)**
- **Consistent conditions (media, serum, temperature, diet)**
- **Consistent collection of samples**
- **Proper storage**
- **Quality controls**
- **Proper number of replicates (technical and biological)**

Targeted Metabolomics Workflow



Established Targeted Modules

- Flow Injection MS/MS
  - Amino Acids (15 analytes)
  - Acylcarnitines (66 analytes)
  - Free and Total Carnitine
  - Acyl CoAs (57 analytes)
  - Ceramides (21 analytes)
  - Creatinine
- LC-MS/MS
  - Malonyl CoA
  - Tryptophan and Kynurenic Acid
- GC MS
  - Organic Acids (20 analytes)
  - Fatty Acids (free-9 and total-9 analytes)

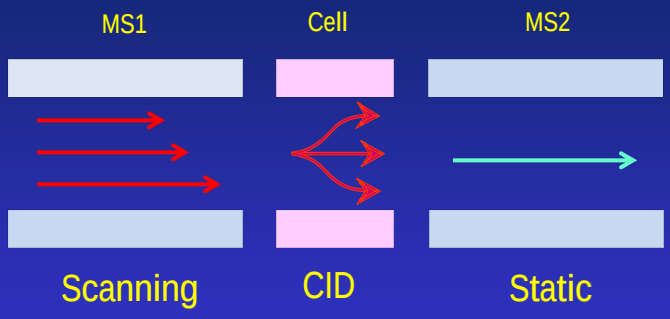
Active Development

- LC-MS/MS for Acylcarnitines
- Sphingomyelins (30 analytes)
- Nucleotides

Biological Matrices

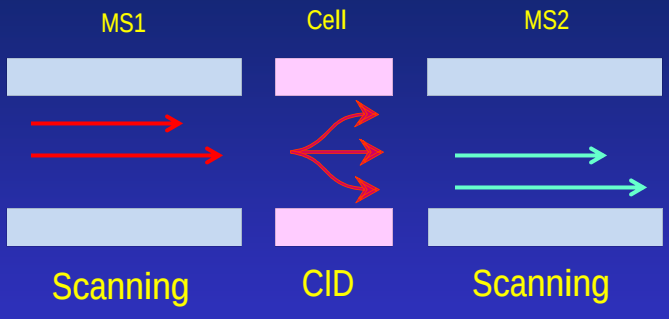
- Plasma/serum
- Blood spots
- Liver
- Muscle
- Adipose
- Kidney
- Brain
- Heart
- Cell culture
- Urine
- Cecal water
- Cerebral spinal fluid
- Lung lavage fluid
- Fly larva
- Worms

Precursor Ion Scan



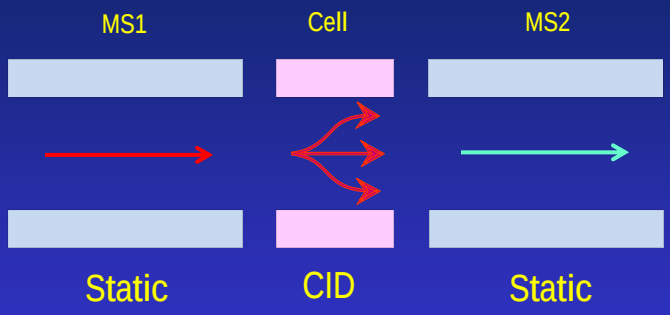
MS/MS : Compound Class Screening

Neutral Loss Scan



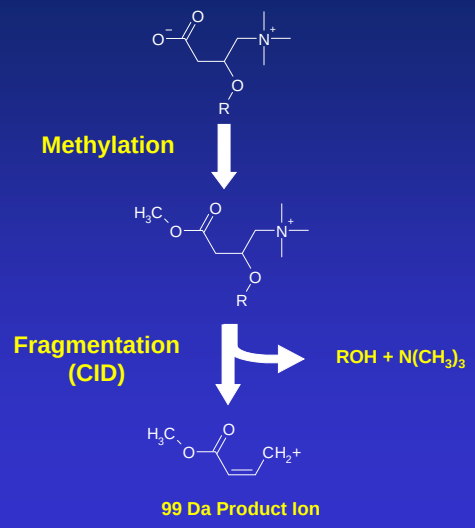
MS/MS : Compound Class Screening

Multiple Reaction Monitoring

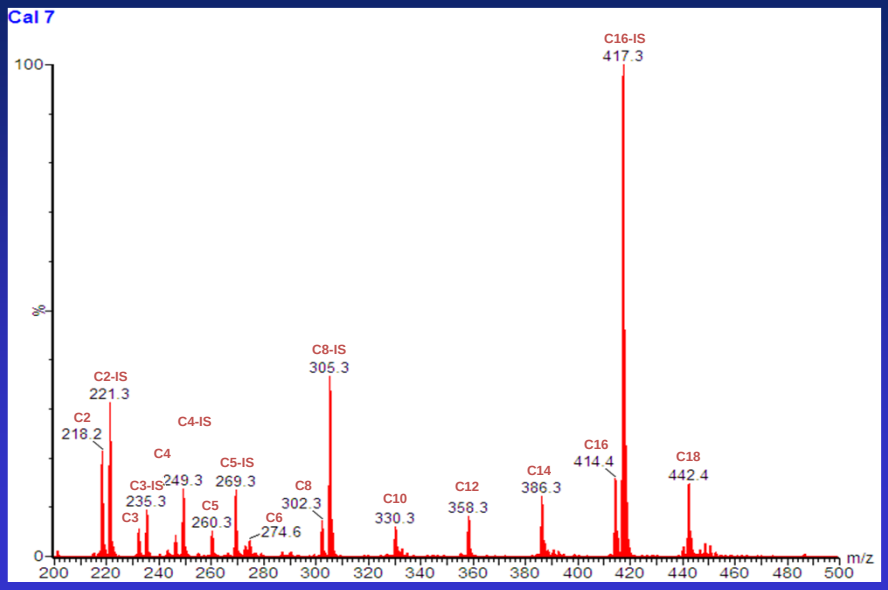


MS/MS : Compound-Specific Monitoring

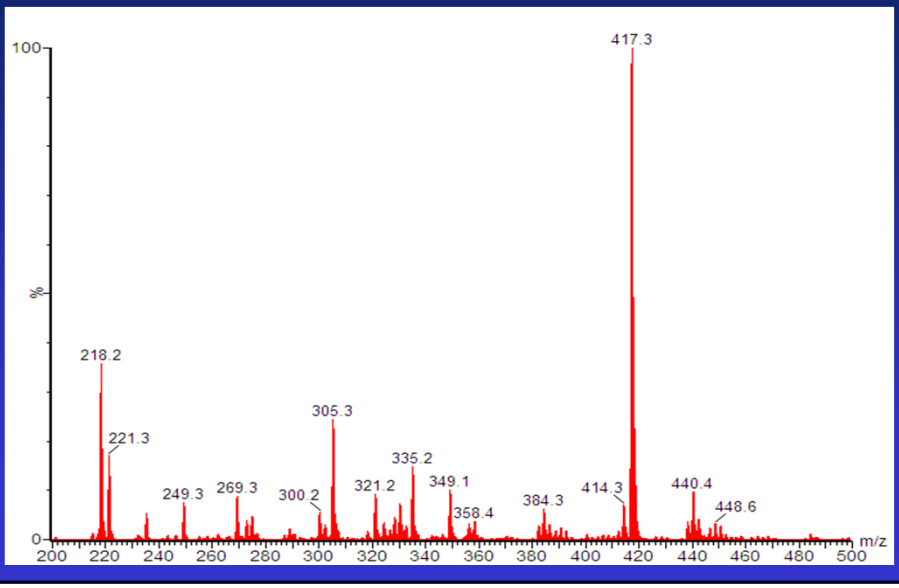
Targeted Metabolomics Example: Acylcarnitines



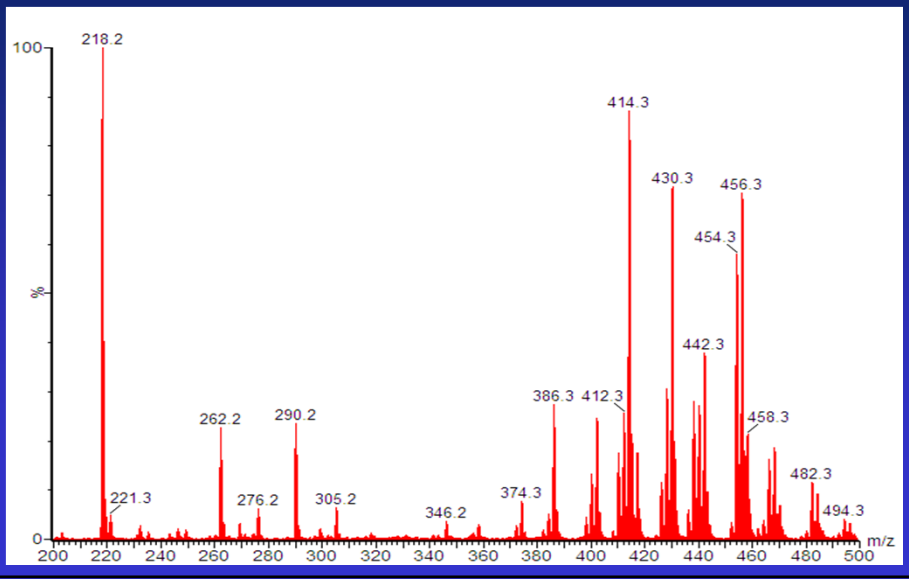
Typical Acylcarnitine Spectrum (calibrator)



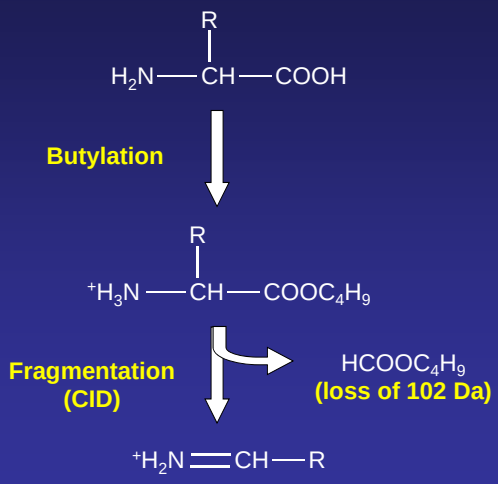
Typical Acylcarnitine Spectrum (plasma)



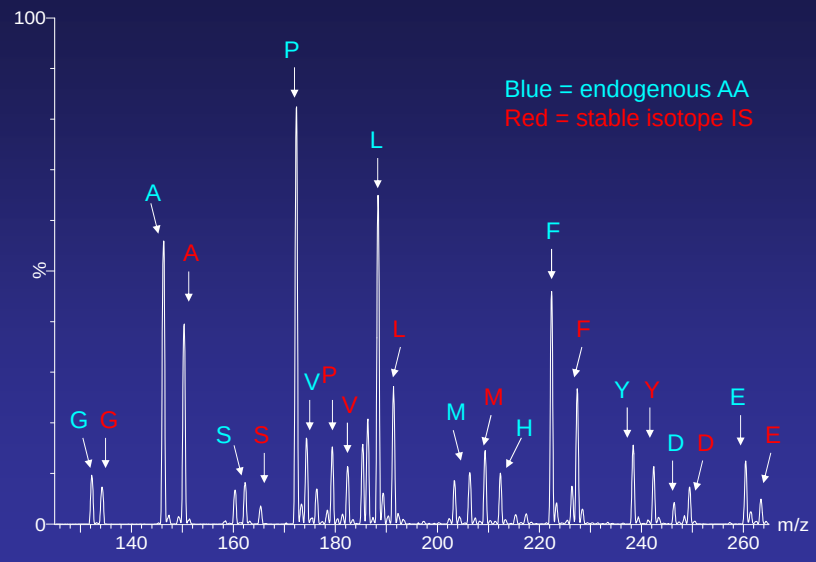
Typical Acylcarnitine Spectrum (mouse heart)



Targeted Metabolomics Example: Amino Acids



Typical Amino Acid Mass Spectrum



Amino Acids Quality Control

QC 1
(micromolar)

	Gly	Ala	Ser	Pro	Val	Leu/Ile	Met	His	Phe	Tyr	Asp	Glu	Orn	Cit	Arg
Nominal (Aug-05)	402	355	146	107	272	281	38	55	93	57	29	262	93	71	242
Mean (Sep-05 to Apr-07)	398	373	139	112	265	279	39	54	93	59	30	273	94	72	256
St Dev	34	18	15	12	20	19	5	5	3	3	3	26	9	6	22
% CV	9	5	11	11	7	7	12	10	4	5	9	10	9	8	9

QC 2
(micromolar)

	Gly	Ala	Ser	Pro	Val	Leu/Ile	Met	His	Phe	Tyr	Asp	Glu	Orn	Cit	Arg
Nominal (Aug-05)	711	674	205	170	334	351	103	119	158	122	93	329	161	135	312
Mean (Sep-05 to Apr-07)	723	693	203	175	334	344	103	119	159	124	94	337	161	137	321
St Dev	50	23	20	8	29	20	4	13	5	6	6	36	15	11	28
% CV	7	3	10	4	9	6	4	11	3	5	7	11	9	8	9

Acylcarnitines Quality Control

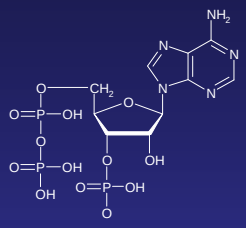
QC 1
(micromolar)

	C2	C3	C5	C6	C8	C10	C12	C14	C16	C18
Nominal (Aug-05)	8.76	1.88	0.40	0.34	0.33	0.35	0.34	0.38	0.42	0.45
Mean (Sep-05 to Apr-07)	8.39	1.85	0.41	0.37	0.20	0.37	0.33	0.35	0.40	0.44
St Dev	0.56	0.21	0.04	0.03	0.02	0.04	0.02	0.02	0.02	0.03
% CV	6.6	11.6	9.9	8.9	11.5	9.7	6.7	6.0	5.7	5.9

QC 2
(micromolar)

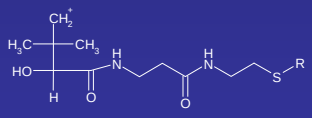
Nominal (Aug-05)	40.65	5.15	3.54	3.36	3.27	3.27	3.44	3.49	3.58	3.64
Mean (Sep-05 to Apr-07)	39.79	4.89	3.49	3.36	1.89	3.30	3.32	3.43	3.51	3.57
St Dev	1.92	0.53	0.22	0.29	0.10	0.19	0.22	0.14	0.13	0.14
% CV	4.8	10.7	6.3	8.5	5.1	5.7	6.5	4.1	3.7	3.9

Acyl-CoA MS/MS Fragmentation

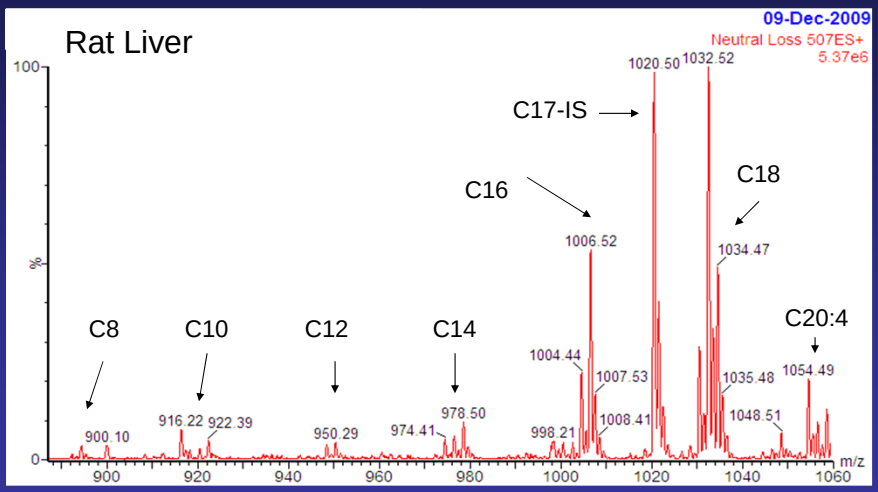


Neutral loss of 507 Da

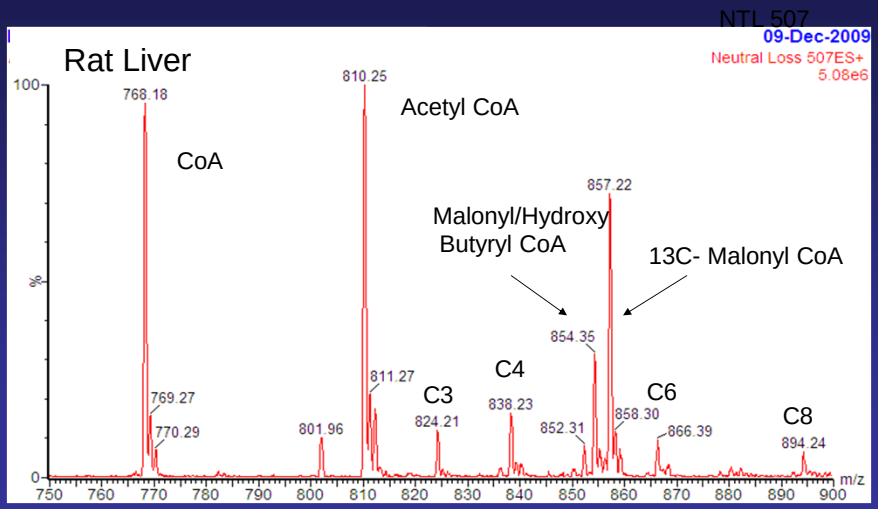
Fragmentation (CID)



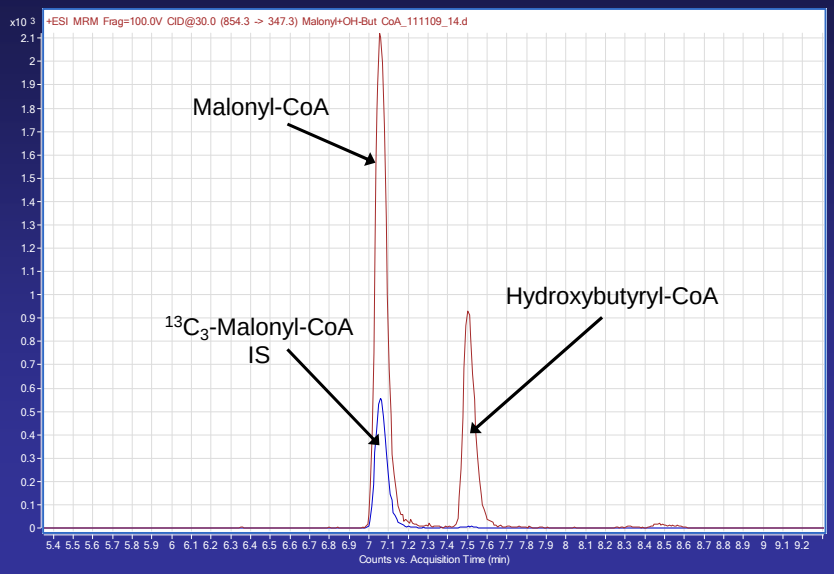
Long Chain Acyl CoA



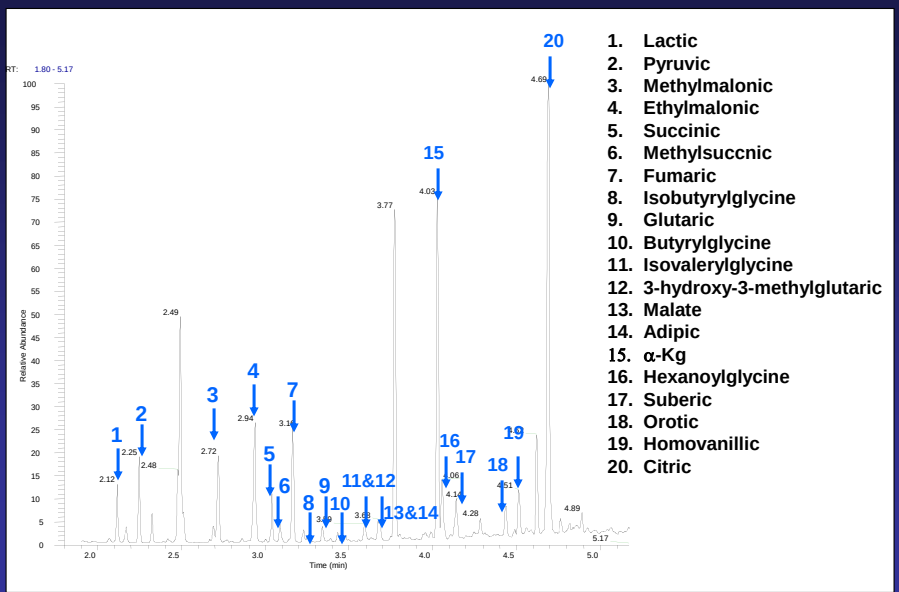
Short Chain Acyl CoA



LC-MS/MS Method for Malonyl-CoA

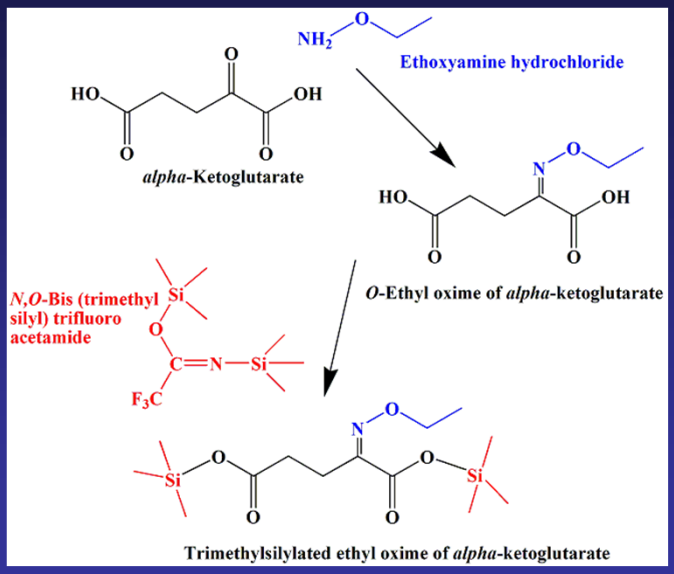


Chromatogram of organic acids extracted from urine

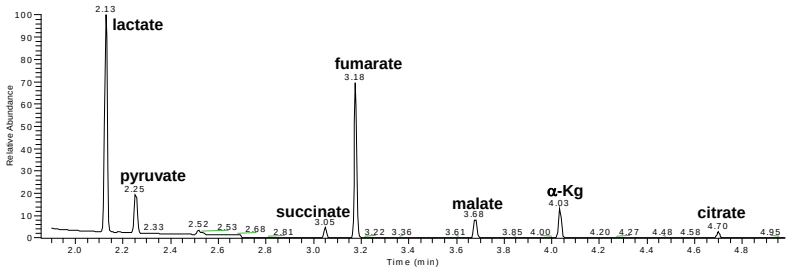


1. Lactic
2. Pyruvic
3. Methylmalonic
4. Ethylmalonic
5. Succinic
6. Methylsuccnic
7. Fumaric
8. Isobutyrylglycine
9. Glutaric
10. Butyrylglycine
11. Isovalerylglycine
12. 3-hydroxy-3-methylglutaric
13. Malate
14. Adipic
15. α-Kg
16. Hexanoylglycine
17. Suberic
18. Orotic
19. Homovanillic
20. Citric

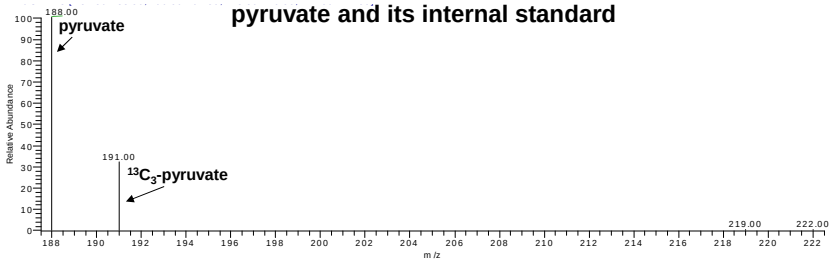
Organic Acid Derivatization



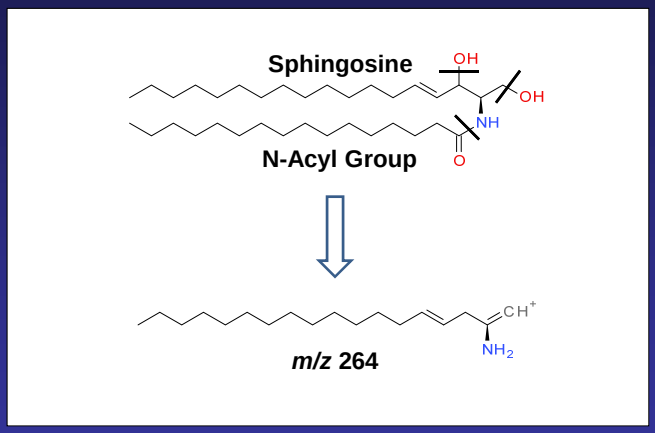
Chromatogram of organic acids extracted from rat liver homogenate



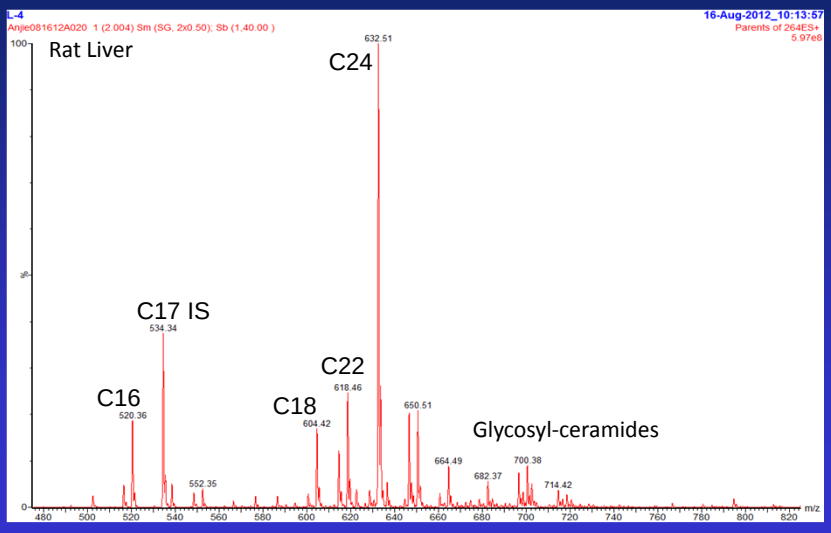
Single ion monitoring (SIM) of derivatized pyruvate and its internal standard



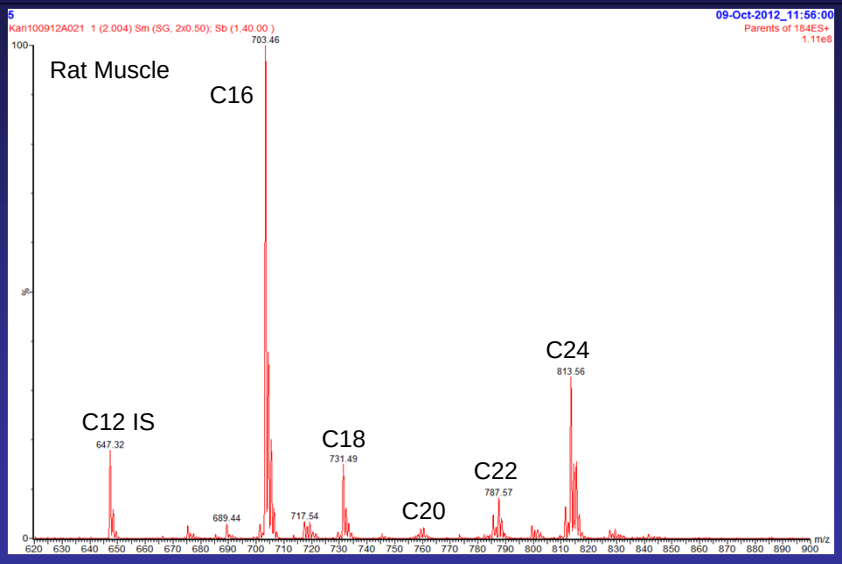
Ceramide MS/MS Fragmentation



Ceramides



Sphingomyelins



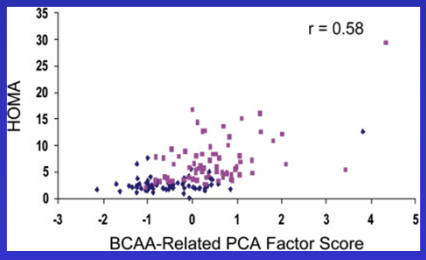
Utility of targeted panels

- Acyl carnitines - FA oxidation, BCAA catabolism
- Amino Acid Panel - Amino acids and Urea cycle
- Acyl CoA - FA oxidation, Lipid synthesis
- Ceramides - Cell structure, signaling and function
- Organic acids - FA oxidation, TCA cycle, amino acid catabolism
- Fatty Acid - Lipid synthesis, signaling

A BCAA-Related Metabolic "Signature" Correlates with Insulin Sensitivity

Measure	Obese (n=74)	Lean (n=67)	P value
Age (years)	52.0	50.0	
BMI (kg/m²)	36.6	23.2	< 0.0001
Fat mass (kg)	36.45	18.65	< 0.0001
Lean mass(kg)	55.09	45.45	< 0.0001
HOMA	5.73	2.51	< 0.0001
Si	2.12	4.44	<0.0001

The relationship between insulin sensitivity (HOMA) and a principal component comprised of BCAA-related metabolites, including the BCAAs valine, leucine, and isoleucine; Glx (glutamate and glutamine); the aromatic amino acids phenylalanine and tyrosine; and C3 and C5 acylcarnitines



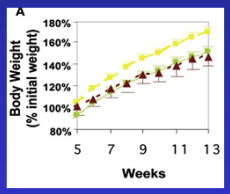
Newgard CB, An J, Bain JR, Muehlbauer MJ, Stevens RD, Lien LF, Haqq AM, Shah SH, Arlotto M, Slentz CA, Rochon J, Gallup D, Ilkayeva O, Wenner BR, Yancy WS Jr, Eisenson H, Musante G, Surwit RS, Millington DS, Butler MD, Svetkey LP.
A branched-chain amino acid-related metabolic signature that differentiates obese and lean humans and contributes to insulin resistance.

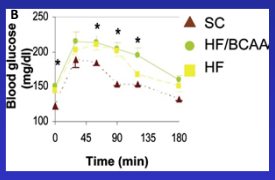
Cell Metab 9, 311-26, 2009

Effect of dietary BCAA supplementation

- **Performed feeding study in rats:**
  - **standard chow or SC**
  - **high-fat or HF**
  - **HF/BCAA**

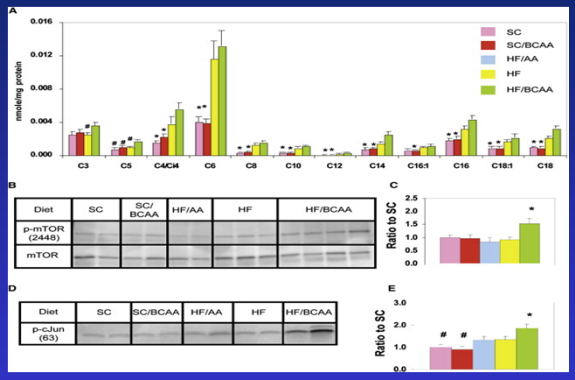
Rats fed HF/BCAA are insulin resistant despite normal body weight

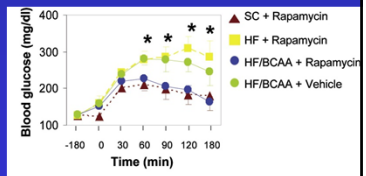




- **Found that BCAA supplementation of HF diet, but not SC diet, decreases food intake but maintains complete state of insulin resistance.**
- **Insulin resistance not seen upon pair-feeding of HF diet.**

BCAA Supplementation of HF Diet Causes Accumulation of Acylcarnitines in Skeletal Muscle and Chronic Activation of mTOR and JNK





Branched-chain amino acids alter neurobehavioral function in rats

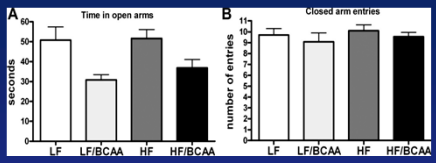
- **BCAA and AAA are strongly associated with obesity and insulin resistance in humans.**
- **BCAA and AAA are transported from the blood into the CNS by the large neutral amino acid transporter 1(LAT1). Their uptake is competitive.**
- **Tyr is the precursor of NE and DA. Trp is the precursor of serotonin and Kynurenic Acid.**

Does long-term supplementation with BCAA lead to behavioral changes?

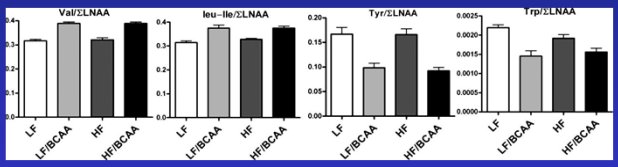
Anna Coppola, Brett R. Wenner, Olga Ilkayeva, Robert D. Stevens, Mauro Maggioni, Theodore A. Slotkin, Edward D. Levin and Christopher B. Newgard

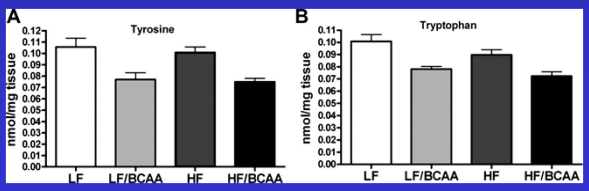
Am J Physiol Endocrinol Metab 304:E405-E413, 2013.

The effect of BCAA-supplemented diets on behavior

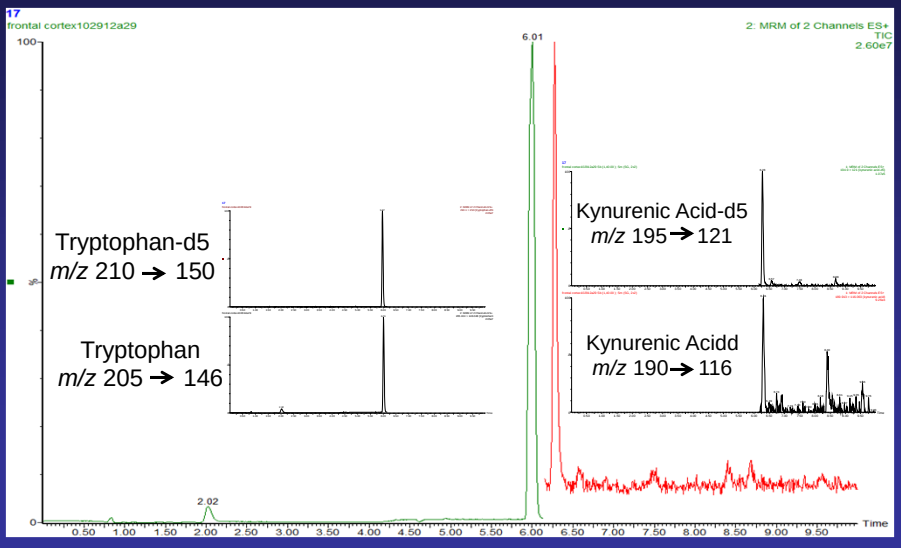


AAA levels in plasma and CNS are reduced by BCAA supplementation

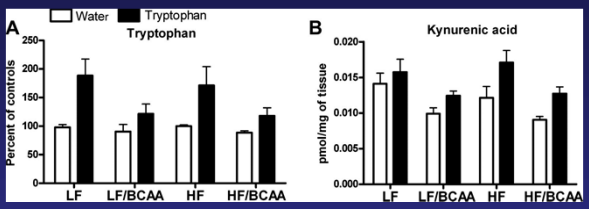


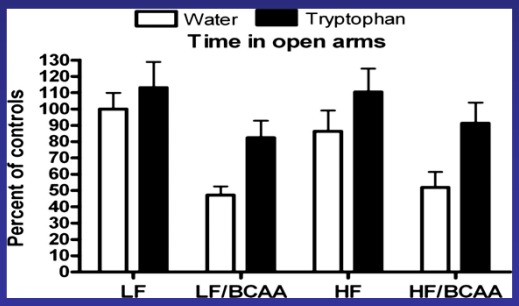


Chromatogram of Tryptophan and Kynurenic Acid Extracted from Rat Frontal Cortex



Tryptophan supplementation rescues the stressed behavior in rats fed BCAA-supplemented diets and increases brain KYNA levels



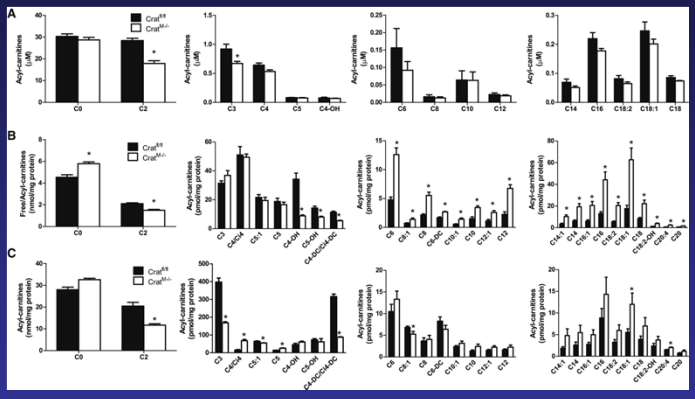


Free Carnitine and Acylcarnitine Profiling in Crat $^{M-/-}$ Mice

plasma

muscle

heart



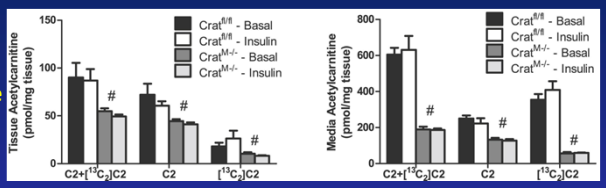
Muscle-Specific Deletion of Carnitine Acetyltransferase Compromises Glucose Tolerance and Metabolic Flexibility

Muoio DM, Noland RC, Kovalik JP, Seiler SE, Davies MN, DeBalsi KL, IlkayevaOR, Stevens RD, Kheterpal I, Zhang J, Covington JD, Baipevi S, Ravussin E, Kraus W, Koves TR, Mynatt RL

Cell Metab. 2012,15(5):764-77

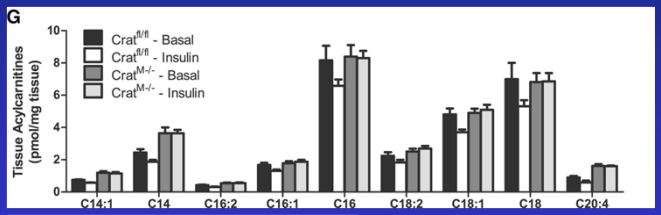
Acylcarnitine Profiling in Tissue, Mitochondria, and Cell Culture

Isolated Soleus Muscle

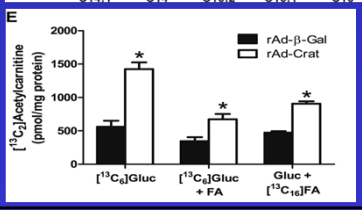


Media

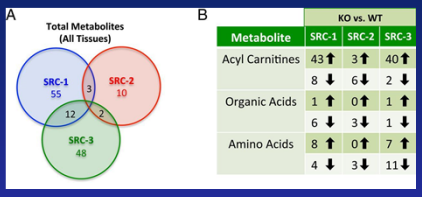
Isolated Mitochondria



Primary Human Skeletal Myocytes



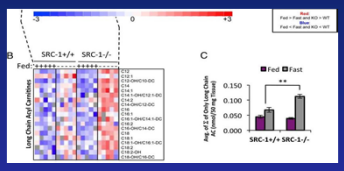
Tissue- and Pathway-Specific Metabolomic Profiles of the Steroid Receptor Coactivator (SRC) Family



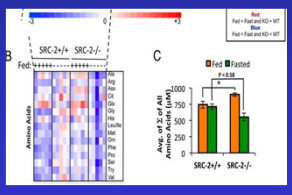
B

	KO vs. WT		
Metabolite	SRC-1	SRC-2	SRC-3
Acyl Carnitines	43↑	3↑	40↑
	8↓	6↓	2↓
Organic Acids	1↑	0↑	1↑
	6↓	3↓	1↓
Amino Acids	8↑	0↑	7↑
	4↓	3↓	11↓

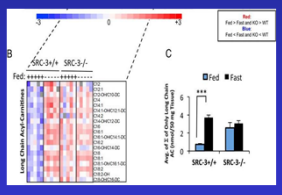
Liver



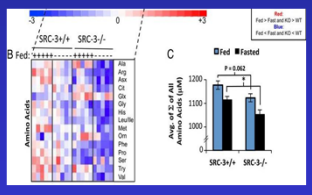
Heart



Skeletal Muscle



Brain



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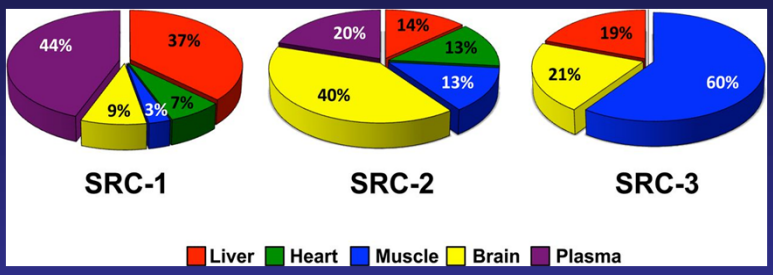
Tissue-specific metabolic contributions of the SRC family



- **The highly conserved SRC family of transcription coactivators shows very limited metabolic redundancy.**
- **Each SRC differentially controls metabolism of distinct tissues.**