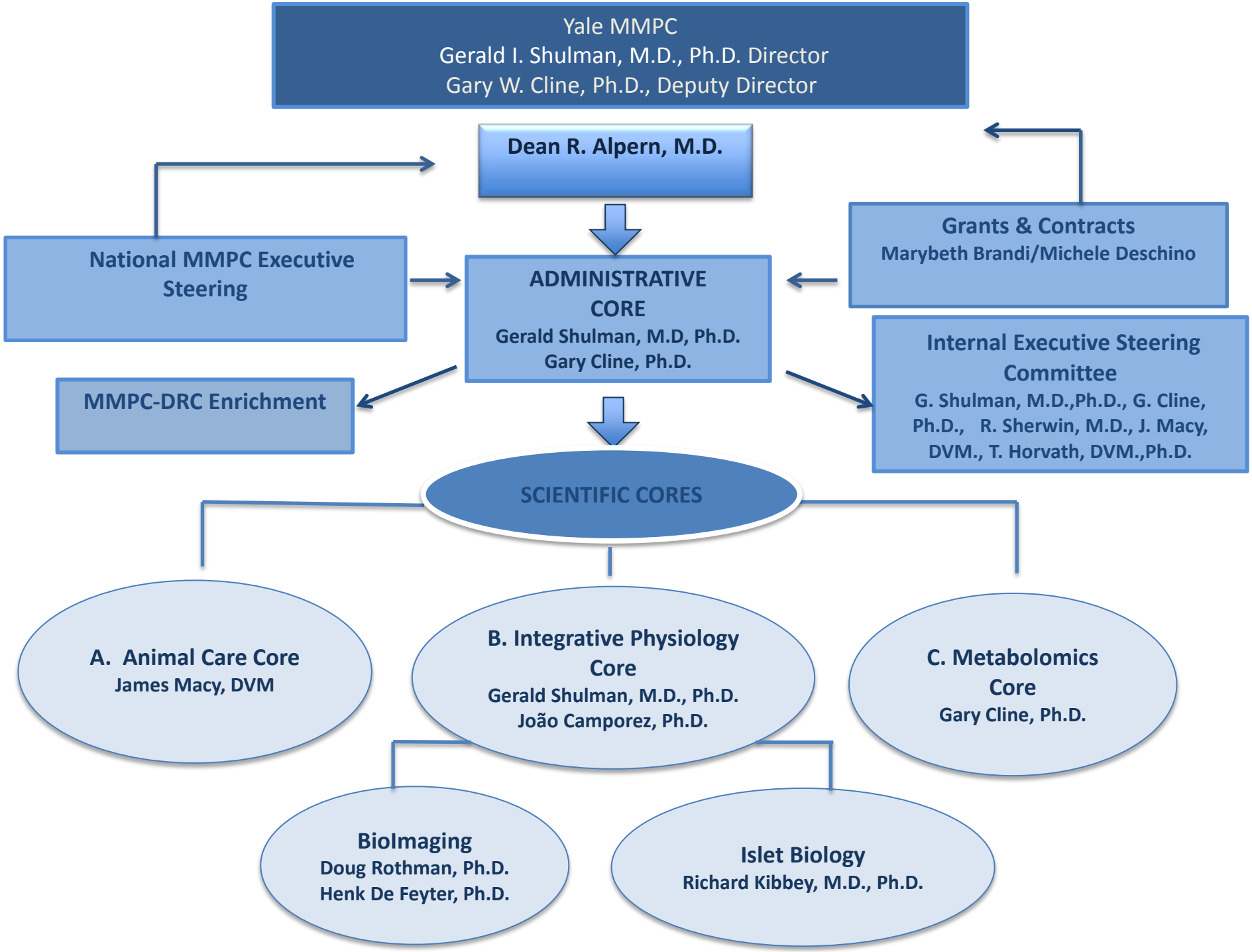
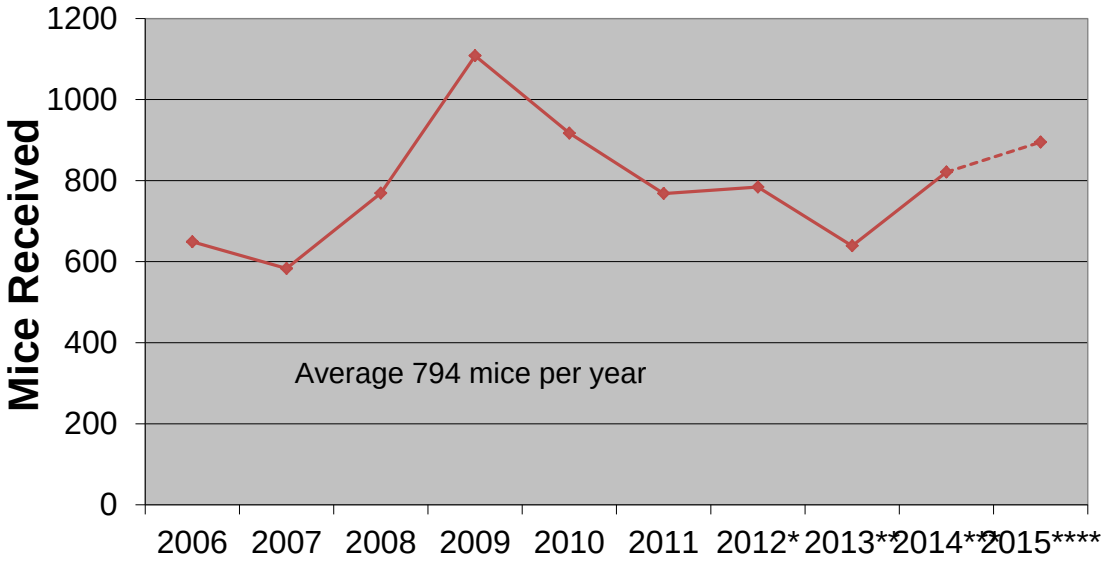

Yale MMPC
Gerald I. Shulman, M.D., Ph.D. Director
Gary W. Cline, Ph.D., Deputy Director
Dean R. Alpern, M.D.
National MMPC Executive Steering
Grants & Contracts
Marybeth Brandi/Michele Deschino
ADMINISTRATIVE CORE
Gerald Shulman, M.D, Ph.D.
Gary Cline, Ph.D.
MMPC-DRC Enrichment
Internal Executive Steering Committee
G. Shulman, M.D.,Ph.D., G. Cline, Ph.D., R. Sherwin, M.D., J. Macy, DVM., T. Horvath, DVM.,Ph.D.
SCIENTIFIC CORES
A. Animal Care Core
James Macy, DVM
B. Integrative Physiology Core
Gerald Shulman, M.D., Ph.D.
João Camporez, Ph.D.
C. Metabolomics Core
Gary Cline, Ph.D.
BioImaging
Doug Rothman, Ph.D.
Henk De Feyter, Ph.D.
Islet Biology
Richard Kibbey, M.D., Ph.D.

Core B Animal Care	Project Year 1 2011	Project Year 2 2012	Project Year 3 2013	Project Year 4 2014	Project Year 5 2015	Project All Years
Users: Import, Quarantine, Housing & Husbandry (# internal,/#external)	32 (20/12)	22* (10/12)	31** (19/12)	16*** (9/7)	15**** (5/10)	116 (63/53)
Services: Import, Quarantine, Housing & Husbandry (# internal,/#external)	768 (480/288)	784* (501/283)	639** (345/294)	821 (617/204)	895**** (618/ 277)	3907 (2561/ 1346)



Yale MMPC Integrative Physiology Core: Services Provided Aug 2015-Jul 2016			
Investigator: Institution	**Mouse Model**	**Services**	**Phenotype**
Carlos Fernandez-Hernando: Yale*	mir33 KOmiR-33	Hyperinsulinemic-euglycemic clamp, Metabolic cage, Body composition	Impaired sensitivity, with reduced muscle and fat glucose uptake, and impaired EGP suppression.
Bruce Spiegleman: Harvard	Adipsin AAV (db/db mice)	Hyperinsulinemic-euglycemic clamps	No difference
Ricard Kibbey: Yale*	TABASCO	GTT	TABASCO-GTP mice had higher insulin secretion than TABASCO-ATP
Michael Jurczak: Univ Pittsburgh	Park2 KO, Park2 LKO	Fat tolerance test, GTT, Metabolic cages, Hyperinsulinemic-euglycemic clamps	Improved fat tolerance (lower plasma TG, consistent with increased fecal fat), Improved glucose tolerance, Improved suppression of EGP
Susan Kaech, Yale*	Tumor	U-13C glc infusion	Lung tumors had increased Vpdh/Vcs relative to normal lung

Yale MMPC Integrative Physiology Core: Services Provided Aug 2015-Jul 2016

Investigator: Institution	Mouse Model	Services	Phenotype
Elisa Einwallner: Univ Vienna	HMOX1 KO	Hyperinsulinemic-euglycemic clamp	No difference
Domenico Accili: Columbia	Gpr17 KO	Hyperinsulinemic-euglycemic clamp	No difference
Ruslan Medzhitov: Yale*	LPS injected ob/ob mice	Hyperinsulinemic-euglycemic clamp	Higher fasting glucose and EGP but surprisingly improved sensitivity
Anders Naar: Harvard	miR 128	Hyperinsulinemic-euglycemic clamp, Body composition	2 different micro RNAs were used: miR 11 lowered body fat and improved sensitivity in HFD mice. miR128 had no effect.
Roger Davis: Univ Massachusetts	IL6/JNK adipose specific KO	Hyperinsulinemic-euglycemic clamp	Adipose specific JNK KO, but not IL6 KO, had improved sensitivity (higher Ginf, higher Rd, improved suppression of lipolysis)

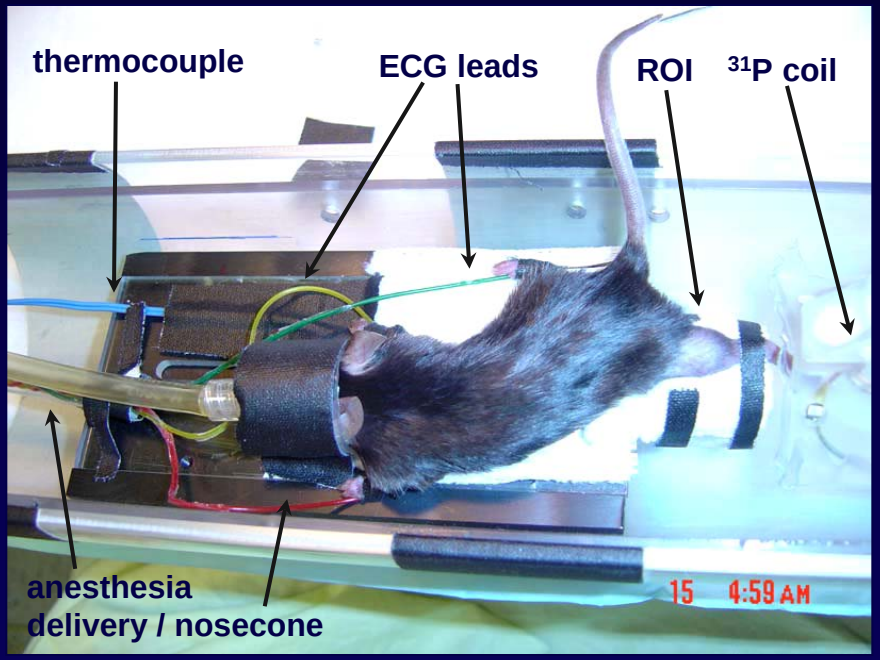
Yale MMPC Integrative Physiology Core: Services Provided Aug 2015-Jul 2016		
Investigator: Institution	**Mouse Model**	**Services**
C. Ron Kahn: Harvard	SB-GFAP KO	Hyperinsulinemic-euglycemic clamp
Timothy McGraw: Cornell	Adipose-Rab10 KO	Hyperinsulinemic-euglycemic clamp
Bill Sessa: Yale*	Clock gene	Metabolic Cage
Anton Bennet: Yale*	MKP5 KO	Hyperinsulinemic-euglycemic clamp
Myoung Sook Han: UMass	Zpf516	Metabolic Cage
Jonathan Bogan: Yale*	MTUG KO	Hyperinsulinemic-euglycemic clamp, Metabolic Cage
Barbara Kahn: Harvard	RBP4	Hyperinsulinemic-euglycemic clamp
Yasuko Iwakiri: Yale*	Nogo	Metabolic Cage
Tong Wang: Yale*	ROMK	Metabolic Cage
Terry Unterman: UIC	LIRKO+/-ATGL shRNA	Clamp w/U13C glc
Shingo Kajimura: UCSF	UCP1-/- PRDM-/-	Hyperinsulinemic-euglycemic clamp
Yingqun Huang: Yale*	H19	Metabolic Cage
Wajahat Mehal: Yale*	Digoxin	GTT
Rosalind Coleman: UNC	ACSL1 KO	Hyperinsulinemic-euglycemic clamp
Nancy Carrasco: Yale*	Low iodine diet	Metabolic Cage

***Animal Research at Yale:**
398 Principle Investigators w/ IACUC approved protocols, 481 protocols, ~125,000 mice as of July 2016.

In vivo MRS studies of mouse muscle

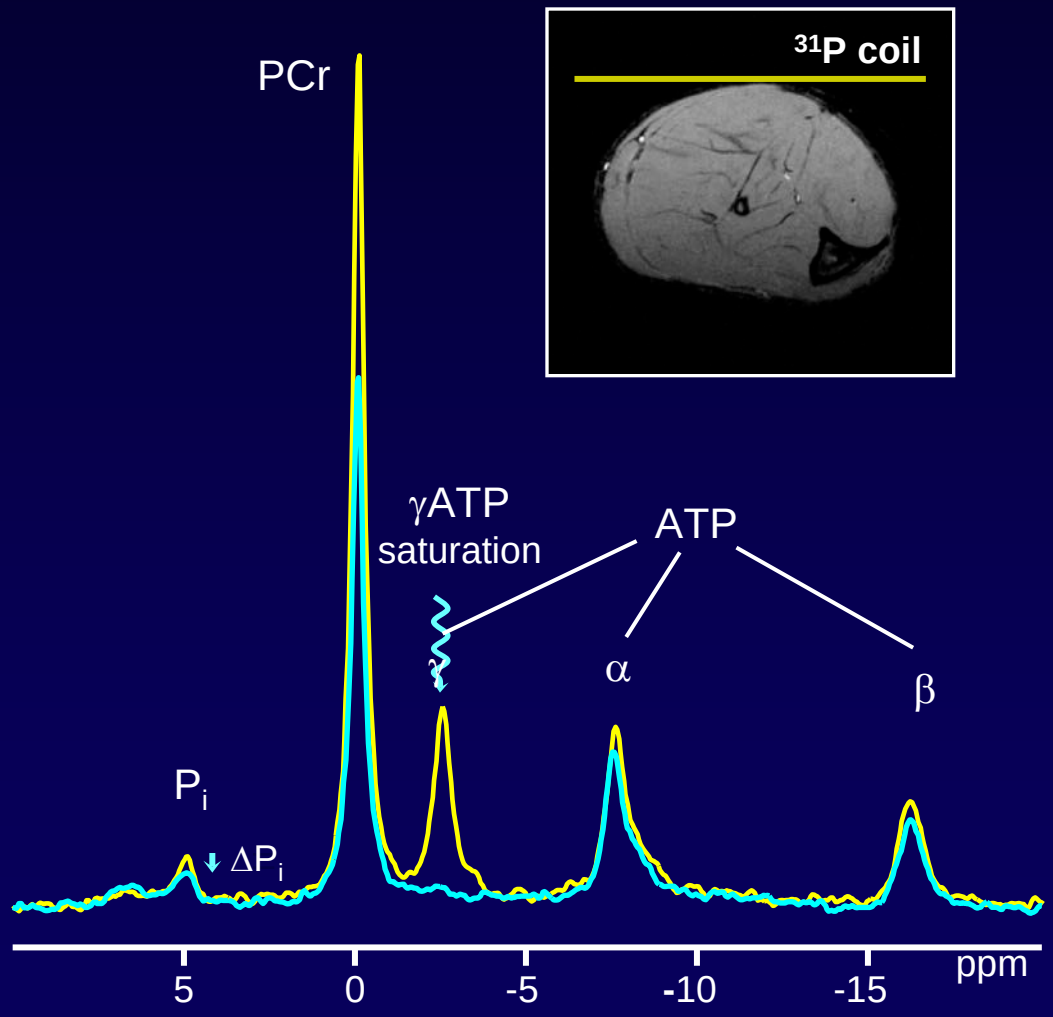
Animal Preparation

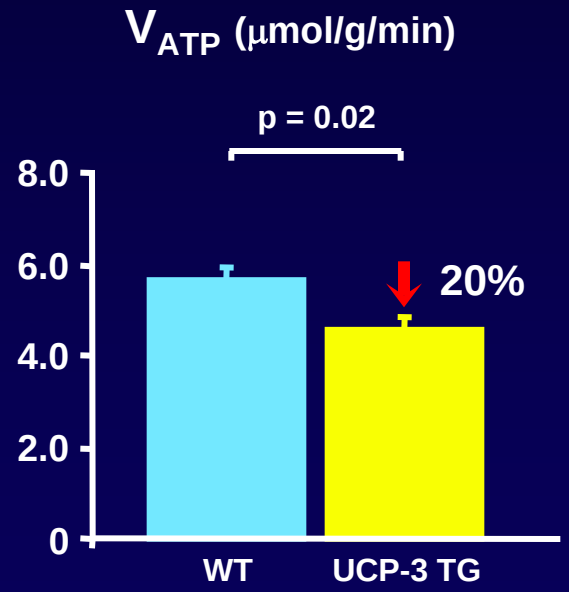
- mice anesthetized w/ ~1% isoflurane in air / O_2
- positioned prone w/ MR coils over the hindlimb muscles
- body temperature maintained w/ heated water pad
- physiological monitoring of bpm / respiration / body temp



- anesthesia adjusted to maintain stable physiological function

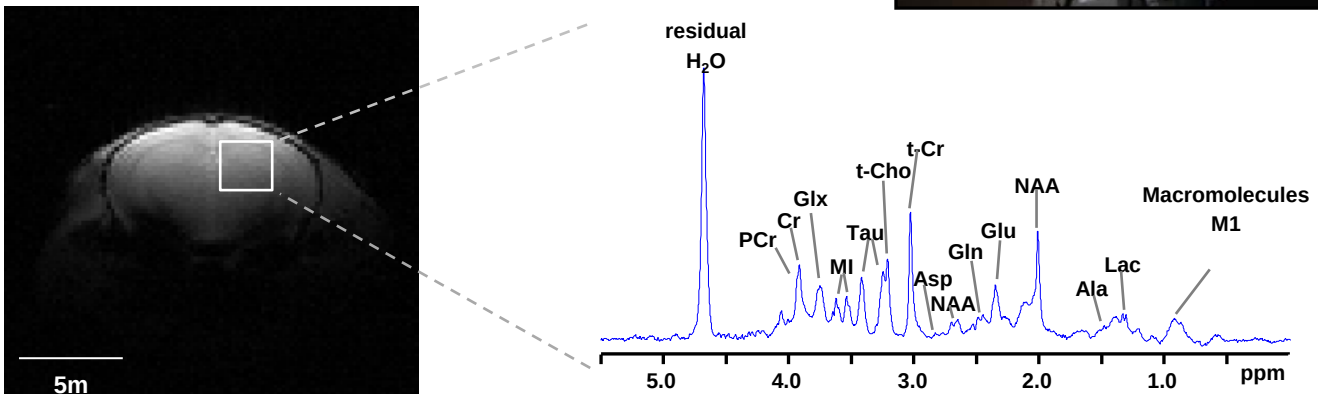
$P_i \rightarrow$ ATP flux in mouse muscle @ 9.4T





In vivo brain metabolomics profiling by ^{1}H MRS:

- 20-wk old mice: C57BL/6 and Ldlr -/-
- 12 weeks on either
  Low-fat diet (Harlan - TD08485, or
  High-fat diet (Harlan – TD88137
- Localized ^{1}H MR spectroscopy of
  left and right hemispheres
- Quantitation of *in vivo* MR spectra:
  Provencher LC Model.



Representative ^{1}H MR spectroscopy 9.4T of cerebral metabolites in 18.75µl voxel of a 20-wk old mouse after 12 weeks on a low-fat diet (Harlan-TD.08485).

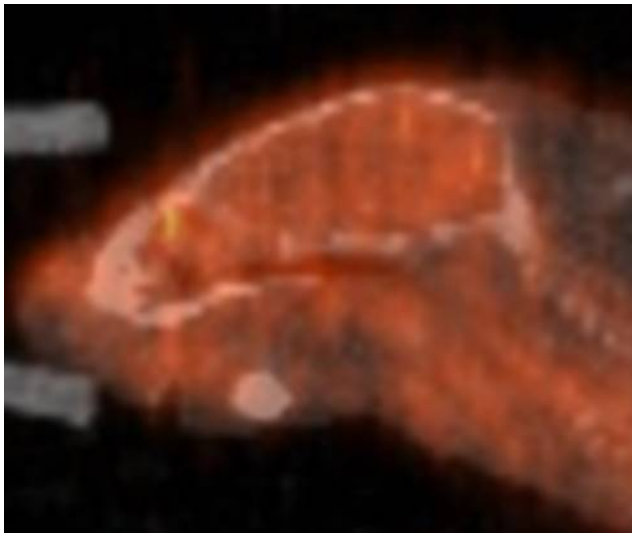
Provencher LC Model: Quantitation of *in vivo* MR spectra.

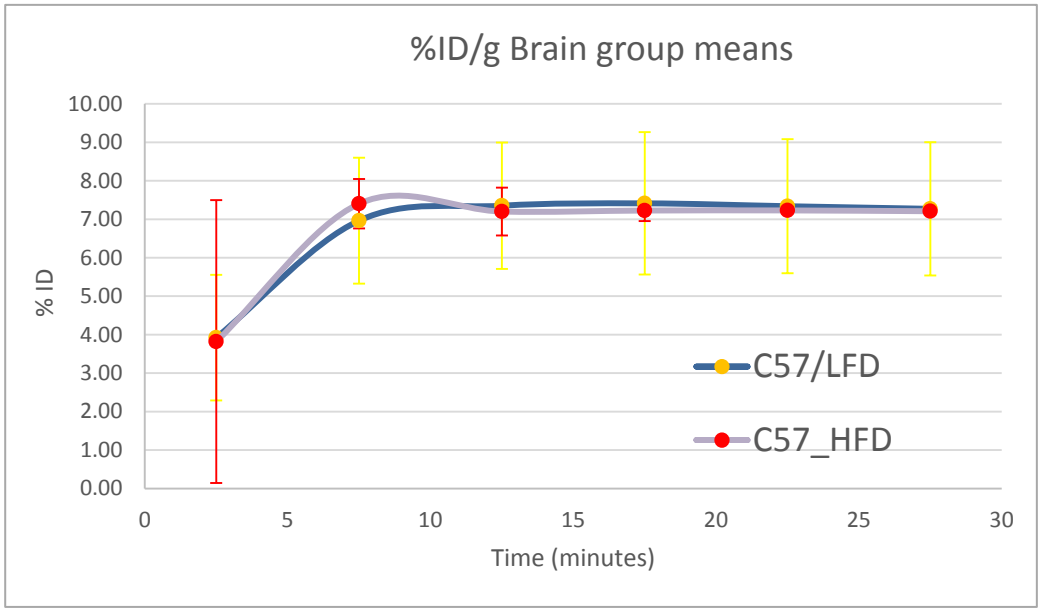
	C57Bl6/J LFD				C57Bl6/J HFD				LDLr$^{-/-}$ LFD				LDLr$^{-/-}$ HFD			
Metabolite	n	Avg	SD	SEM	n	Avg	SD	SEM	n	Avg	SD	SEM	n	Avg	SD	SEM
Ala	7	0.14	0.10	0.04	14	0.15	0.06	0.02	11	0.12	0.07	0.02	11	0.14	0.09	0.03
Asp	7	0.39	0.10	0.04	14	0.40	0.16	0.04	11	0.45	0.11	0.03	11	0.47	0.12	0.04
BHB	12	0.00	0.01	0.00	16	0.01	0.02	0.00	14	0.01	0.02	0.01	14	0.00	0.01	0.00
Cho	8	0.07	0.03	0.01	13	0.09	0.05	0.01	11	0.08	0.03	0.01	12	0.07	0.03	0.01
Cr	12	0.65	0.06	0.02	16	0.63	0.04	0.01	14	0.64	0.05	0.01	14	0.62	0.06	0.02
GABA	12	0.18	0.06	0.02	16	0.21	0.06	0.01	13	0.21	0.07	0.02	13	0.19	0.03	0.01
GlcA	12	0.20	0.08	0.02	16	0.19	0.07	0.02	14	0.18	0.04	0.01	14	0.18	0.08	0.02
GlcB	12	0.27	0.11	0.03	16	0.26	0.09	0.02	14	0.24	0.06	0.02	14	0.24	0.08	0.02
Glu	12	0.99	0.12	0.03	16	1.02	0.05	0.01	14	1.03	0.10	0.03	14	1.04	0.13	0.03
Gln	12	0.49	0.22	0.06	16	0.45	0.19	0.05	14	0.39	0.06	0.02	14	0.32	0.06	0.02
GSH	12	0.22	0.04	0.01	16	0.21	0.04	0.01	14	0.22	0.05	0.01	14	0.21	0.04	0.01
Lac	12	0.07	0.11	0.03	16	0.12	0.09	0.02	14	0.17	0.13	0.03	14	0.11	0.11	0.03
MI	12	0.65	0.10	0.03	16	0.65	0.11	0.03	14	0.65	0.04	0.01	14	0.68	0.08	0.02
NAA	12	0.75	0.06	0.02	16	0.75	0.07	0.02	14	0.73	0.06	0.02	14	0.73	0.04	0.01
PCho	12	0.12	0.03	0.01	15	0.11	0.03	0.01	14	0.12	0.04	0.01	14	0.12	0.03	0.01
PCr	12	0.35	0.06	0.02	16	0.37	0.04	0.01	14	0.36	0.05	0.01	14	0.38	0.06	0.02
Tau	12	0.81	0.14	0.04	16	0.81	0.10	0.02	14	0.89	0.09	0.02	14	0.85	0.13	0.03
MM_C57	12	0.008	0.001	0.000	16	0.009	0.001	0.000	14	0.009	0.001	0.000	14	0.009	0.001	0.000
Cr+PCr	12	1.00	0.00	0.00	16	1.00	0.00	0.00	14	1.00	0.00	0.00	14	1.00	0.00	0.00
Glu+Gln	12	1.48	0.24	0.07	16	1.48	0.19	0.05	14	1.43	0.09	0.03	14	1.36	0.14	0.04
Lip13a	10	0.62	0.20	0.06	13	0.56	0.19	0.05	11	0.54	0.17	0.05	12	1.18	0.88	0.25
Lip13b																
Lip9	11	0.23	0.13	0.04	14	0.19	0.08	0.02	13	0.18	0.10	0.03	14	0.29	0.12	0.03
Lip20	10	0.14	0.06	0.02	14	0.12	0.07	0.02	11	0.14	0.07	0.02	12	0.14	0.04	0.01
Lip13a+Lip13b	11	0.62	0.19	0.06	14	0.56	0.18	0.05	13	0.52	0.18	0.05	14	1.17	1.00	0.27

Provencher LC Model:
Quantitation of *in vivo* MR spectra.

	STATS: 2-tail T-test			
	C57Bl6	LFD: $LDLr^{-/-}$	$LDLr^{-/-}$	HFD: $LDLr^{-/-}$
Metabolite	HFD vs LFD	vs C57Bl6/J	HFD vs LFD	vs C57Bl6/J
Ala	0.82	0.77	0.80	0.73
Asp	0.49	0.21	0.74	0.47
BHB				
Cho	0.29	0.66	0.66	0.24
Cr	0.28	0.76	0.32	0.70
GABA	0.29	0.40	0.46	0.33
GlcA	0.81	0.35	0.77	0.70
GlcB	0.83	0.35	0.92	0.52
Glu	0.29	0.30	0.78	0.51
Gln	0.66	0.13	**0.003**	**0.02**
GSH	0.56	0.97	0.94	0.54
Lac				
MI	0.99	0.96	0.34	0.48
NAA	0.87	0.51	0.83	0.44
PCho	0.18	0.82	0.78	0.14
PCr	0.28	0.76	0.32	0.70
Tau	0.94	0.12	0.39	0.34
MM_C57	0.17	0.31	0.66	0.37
Cr+PCr				
Glu+Gln	1.00	0.48	0.19	0.08
Lip13a	0.48	0.21	**0.02**	**0.02**
Lip13b				
Lip9	0.32	0.31	**0.02**	**0.02**
Lip20	0.36	0.99	0.90	0.34
Lip13a+Lip13b	0.40	0.20	0.03	0.03

Mouse Brain Metabolism: ^{18}F-FDG Uptake

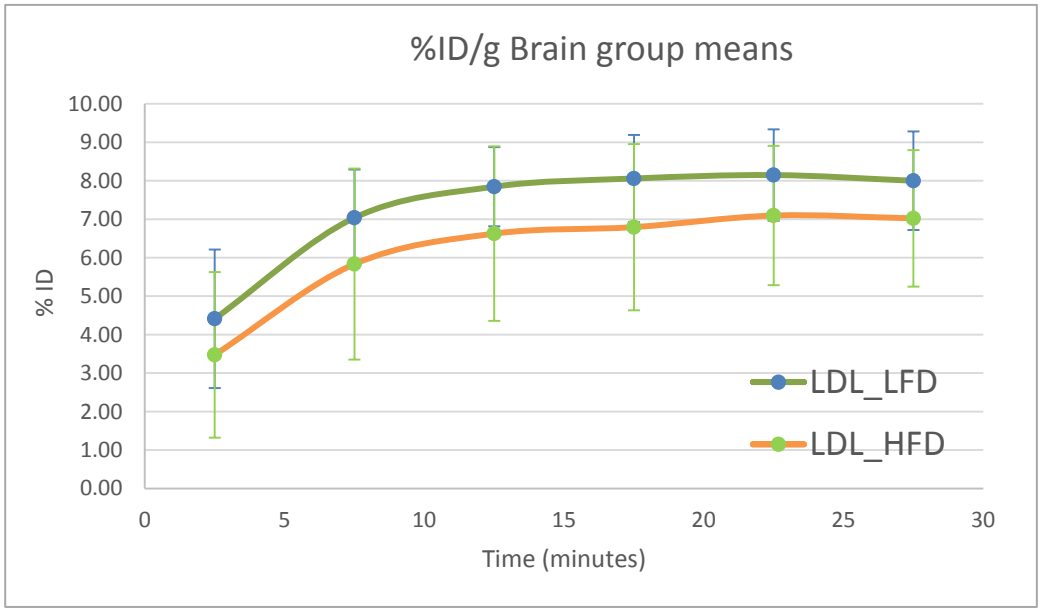




Percent Uptake of Injected Dose of 18F-FDG (% ID) into mouse.

C57Bl/6:
low-fat diet, high-fat diet,

LDLr-/-:
low-fat diet, high-fat diet



Yale-MMPC: Metabolomics Core

Assays: ~8000/yr, Users: ~110/yr

Metabolite Profiles

- GC-MS and LC/MS/MS
  - Carbohydrates
  - Protein, amino acids
  - Lipids
  - Intermediary metabolites of carbohydrates and lipids
  - Nucleotides and Redox state
  - Analysis of Kreb's cycle intermediates
- Metabolite Assays (enzymatic and electrolytic assays)
  - Chem 7
  - Liver Function Tests
  - Lipid profiles
  - Divalent Ions

Substrate Kinetics

- Metabolite deuterium and ^{13}C enrichments by GC-MS, LC/MS/MS, and NMR.
  - Amino Acid and Protein Metabolism
  - Carbohydrate Metabolism
  - Lipid Metabolism
  - Mitochondrial Metabolism

Yale-MMPC: Metabolomics Core

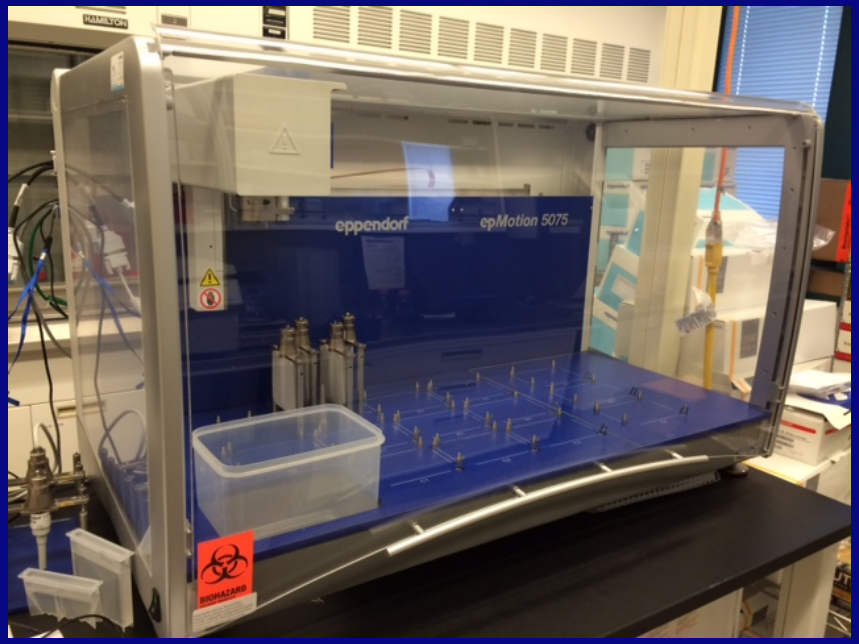
Method Development Initiatives during 2015-16

- DAG, Diasterioisomeric Analysis (1,2-DAG, 2,3-DAG, and 1,3 DAG)
- Nuclear and Mitochondrial DNA turnover (from D-labeling from D_2O)
- Phosphoglycerolipids
- Microbiome specific metabolites (e.g. trimethylamine N-oxide, SCFA concentration and flux)
- MIMOSA: a novel mass spectroscopic platform to quantitate the essential mitochondrial metabolic fluxes present in most living cells. A key advance is the ability to unambiguously identify NMR-validated position-specific enrichments of multiple TCA cycle metabolites

New Initiative for 2015-16

Integration of germ-free and gnotobiotic mice studies with MMPC Animal, Integrative Physiology and Metabolomics Cores

Isolator Housing to maintain Germ Free and Gnotobiotic mice.



EP motion robot for Microbiome Analysis

Concentration-normalized DNA is used for 16S rRNA-targeted microbiome profiling or shotgun DNA sequencing

New Initiative for 2015-16

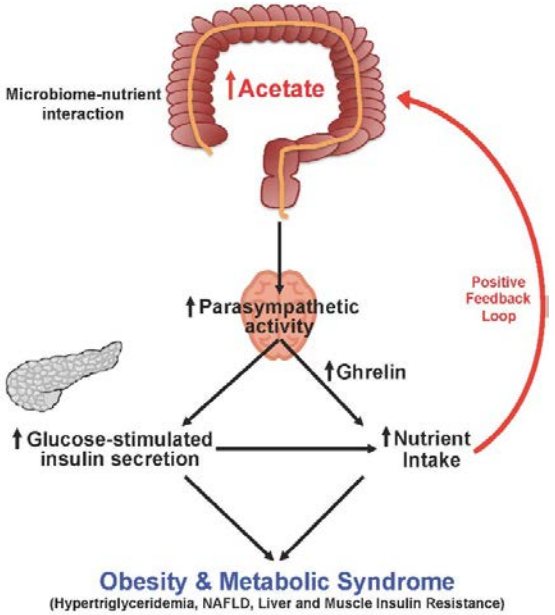
Microbiome Core

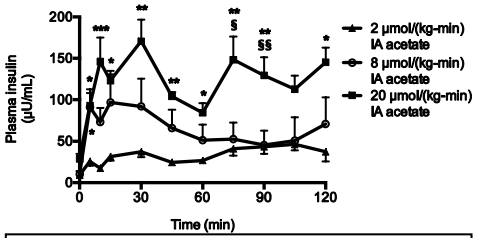
Director: Andrew Goodman, Ph.D.

Gnotobiotic mouse facility to support:

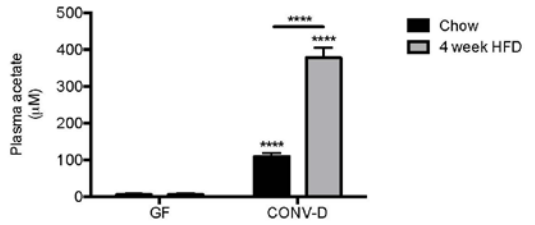
- breeding and maintenance of numerous germfree mouse lines,
- germfree rederivations by C-section and cross-fostering,
- BL-1 and BL-2 gnotobiotic experiments, and
- out-of-the-isolator gnotobiotics in static microisolator caging.
- Defining the composition and metagenomic content of the gastrointestinal microbiome (16s rRNA)
- Fecal microbiome transplantation
- Integration with core services of Animal, Integrative Physiology and Metabolomics Cores

Collaborative effort of Yale MMPC
Integrative Physiology Core, Metabolomics Core, and Microbiome Core:
Novel hypothesis linking gut microbiome and Metabolic Syndrome

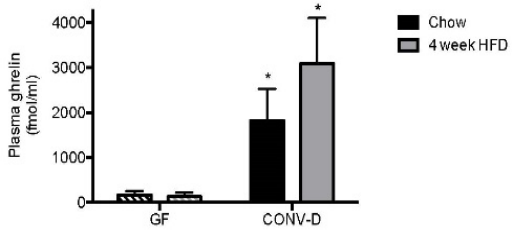




Higher rates of acetate delivery led to higher levels of plasma insulin during a hyperglycemic clamp.



GF-mice colonized (CONV-D) with feces from conventional mice have higher plasma acetate that increased ~4-fold with HFD.



Colonizing GF-mice feces from normal mice led to substantially higher plasma ghrelin. HFD raised plasma ghrelin higher.

Acetate mediates a microbiome-brain-β-cell axis to promote metabolic syndrome. Perry RJ, Peng L, Barry NA, Cline GW, Zhang D, Cardone RL, Petersen KF, Kibbey RG, Goodman AL, Shulman GI. ***Nature***. 2016 8;534(7606):213-7