Spotlight on Core Facilities

Metabolic Phenotyping Core

February 9th, 2022

Ruth Gordillo
Syann Lee

ACKNOWLEDGEMENTS

Philipp Scherer
Joel Elmquist
Jay Horton

Touchstone Diabetes Center
Center for Hypothalamic Research
Center for Human Nutrition
Department of Internal Medicine

Core Research Technical Personnel:
Shannon Hacker
Allison Walker
Jannine Gamayot
Sarah Rico
Arely Tinajero
Chelsea Limboy
Jenny Lee

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P01-DK088761, *Touchstone Center, Hypothalamic Research Center and Center for Human Nutrition.*

State of Texas funds

Continuous technical support and collaborative efforts

LEADERSHIP AND SUPPORT FACULTY

- **Philipp Scherer**

Director, Touchstone Diabetes Center

- **Joel Elmquist**

Director, Center for Hypothalamic Research

- **Jay Horton**

Director, Center for Human Nutrition

- **Ruth Gordillo *(Touchstone Diabetes Center)***

Mass spectrometry. Analytical assays

- **Syann Lee *(Center for Hypothalamic Research)***

Rodent metabolic chambers. Surgeries. RNAscope hybridization

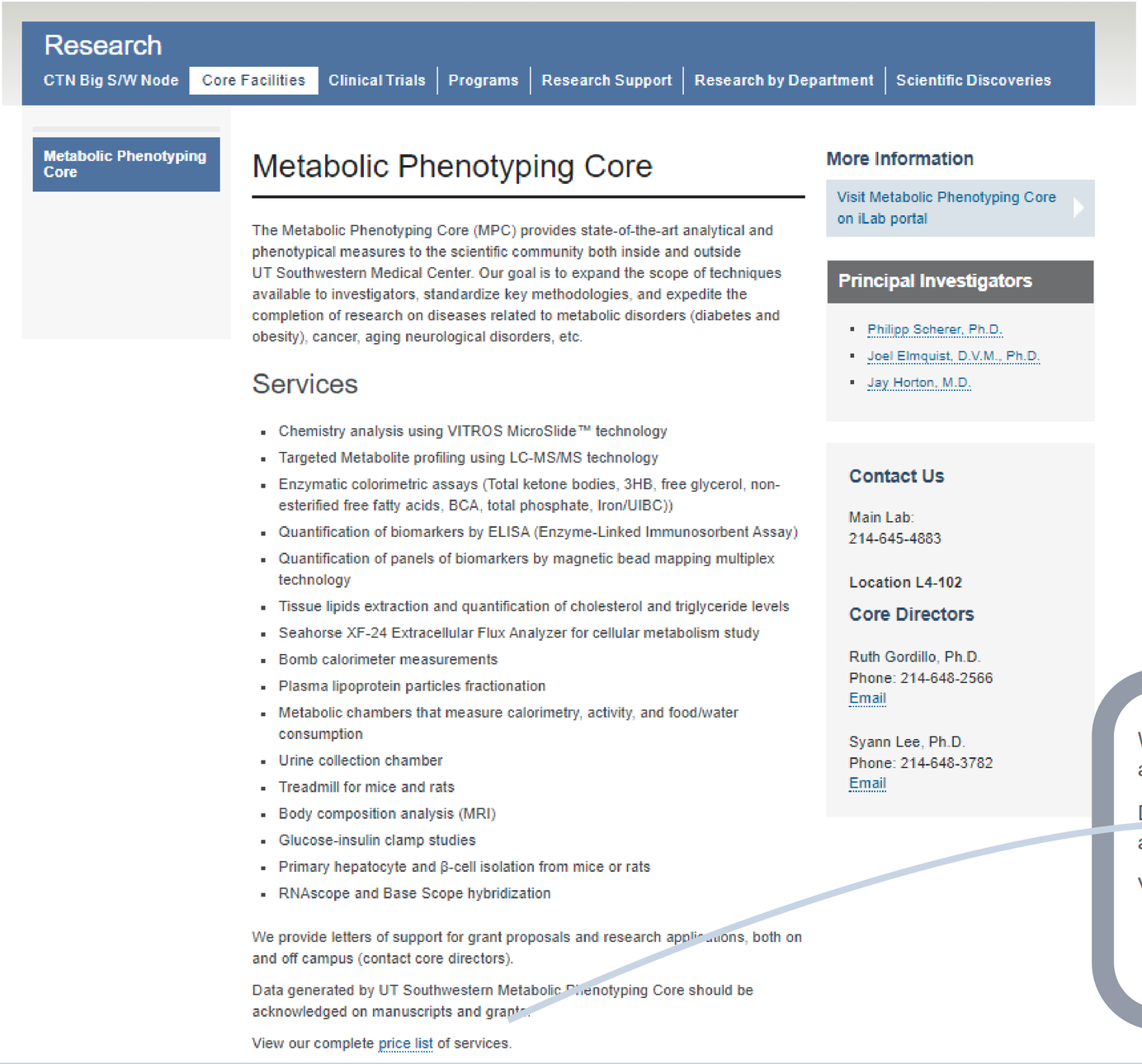
- **Laurent Gautron *(Center for Hypothalamic Research)***

Surgeries. RNAscope hybridization

- **Richard (Max) Wynn *(Biochemistry/Touchstone Diabetes Center)***

Analytical Assays

CORE MISSION



Research

CTN Big S/W Node | Core Facilities | Clinical Trials | Programs | Research Support | Research by Department | Scientific Discoveries

Metabolic Phenotyping Core

Metabolic Phenotyping Core

The Metabolic Phenotyping Core (MPC) provides state-of-the-art analytical and phenotypical measures to the scientific community both inside and outside UT Southwestern Medical Center. Our goal is to expand the scope of techniques available to investigators, standardize key methodologies, and expedite the completion of research on diseases related to metabolic disorders (diabetes and obesity), cancer, aging neurological disorders, etc.

Services

- Chemistry analysis using VITROS MicroSlide™ technology
- Targeted Metabolite profiling using LC-MS/MS technology
- Enzymatic colorimetric assays (Total ketone bodies, 3HB, free glycerol, non-esterified free fatty acids, BCA, total phosphate, Iron/UIBC))
- Quantification of biomarkers by ELISA (Enzyme-Linked Immunosorbent Assay)
- Quantification of panels of biomarkers by magnetic bead mapping multiplex technology
- Tissue lipids extraction and quantification of cholesterol and triglyceride levels
- Seahorse XF-24 Extracellular Flux Analyzer for cellular metabolism study
- Bomb calorimeter measurements
- Plasma lipoprotein particles fractionation
- Metabolic chambers that measure calorimetry, activity, and food/water consumption
- Urine collection chamber
- Treadmill for mice and rats
- Body composition analysis (MRI)
- Glucose-insulin clamp studies
- Primary hepatocyte and β-cell isolation from mice or rats
- RNAscope and Base Scope hybridization

We provide letters of support for grant proposals and research applications, both on and off campus (contact core directors).

Data generated by UT Southwestern Metabolic Phenotyping Core should be acknowledged on manuscripts and grants.

View our complete price list of services.

More Information

Visit Metabolic Phenotyping Core on iLab portal

Principal Investigators

- Philipp Scherer, Ph.D.
- Joel Elmquist, D.V.M., Ph.D.
- Jay Horton, M.D.

Contact Us

Main Lab:
214-645-4883

Location L4-102

Core Directors

Ruth Gordillo, Ph.D.
Phone: 214-648-2566
Email

Syann Lee, Ph.D.
Phone: 214-648-3782
Email

We provide letters of support for grant proposals and and off campus (contact core directors).

Data generated by UT Southwestern Metabolic Phen acknowledged on manuscripts and grants.

View our complete price list of services.

https://www.utsouthwestern.edu/research/core-facilities/mouse-metabolic-phenotyping/

Acknowledgement paragraph.

As per UTSW policy regarding core facilities supported by State funding, the following paragraph must be included in ALL the publications including data generated by UTSW Metabolic Phenotyping Core facility.

Acknowledgements.

a. The authors want to thank UT Southwestern Metabolic Phenotyping Core for the analysis of "XYZ" and expertise.

b. The authors want to thank UT Southwestern Metabolic Phenotyping core for performing "XYZ, surgeries, rodent metabolic chambers studies, etc." and expertise.

c. The authors want to thank UT Southwestern Metabolic Phenotyping core for their shared "NMR, Echo-MRI, scintillation counter, surgery room, etc." services, and expertise.

THE REAL HEROES

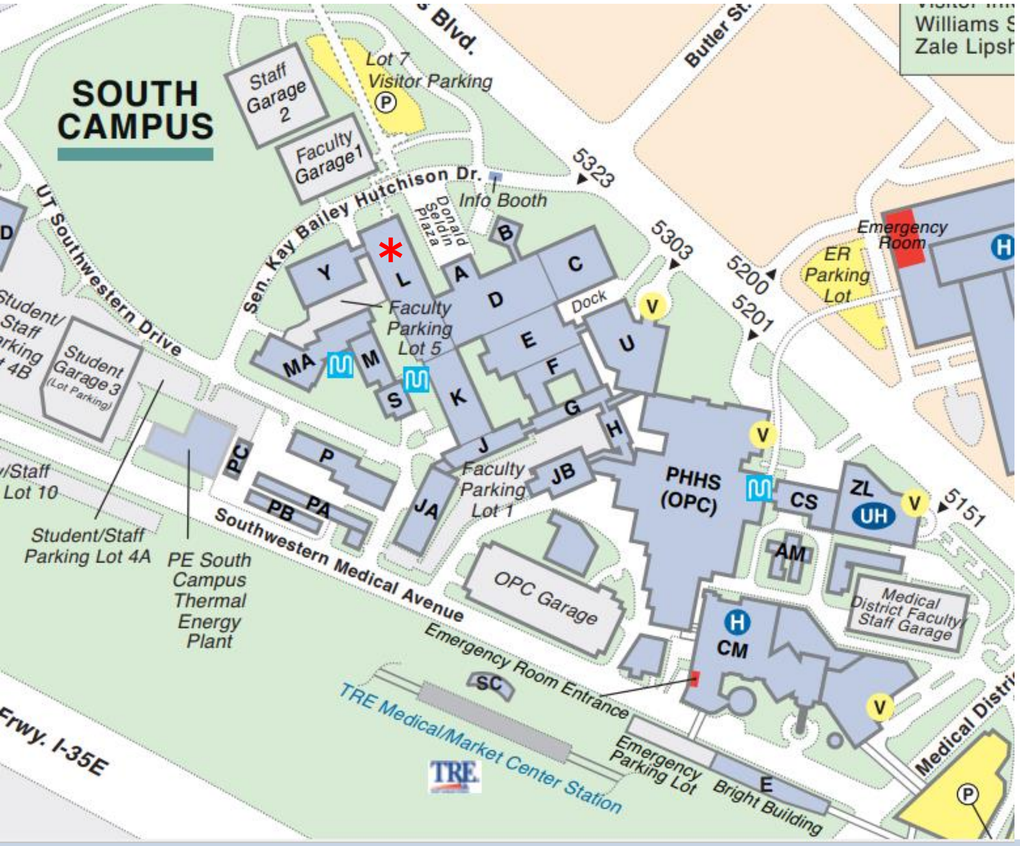
Shannon Hacker
Research Technician II

Allison Walker
Research Technician II

Jannine Irel Gamayot
Research Technician II

Sarah Rico
Research Technician II

MAIN LAB LOCATION AND CONTACTS



Location. L4. 102

Contacts:

Ruth Gordillo. Mass Spectrometry & Bioanalytical Assays

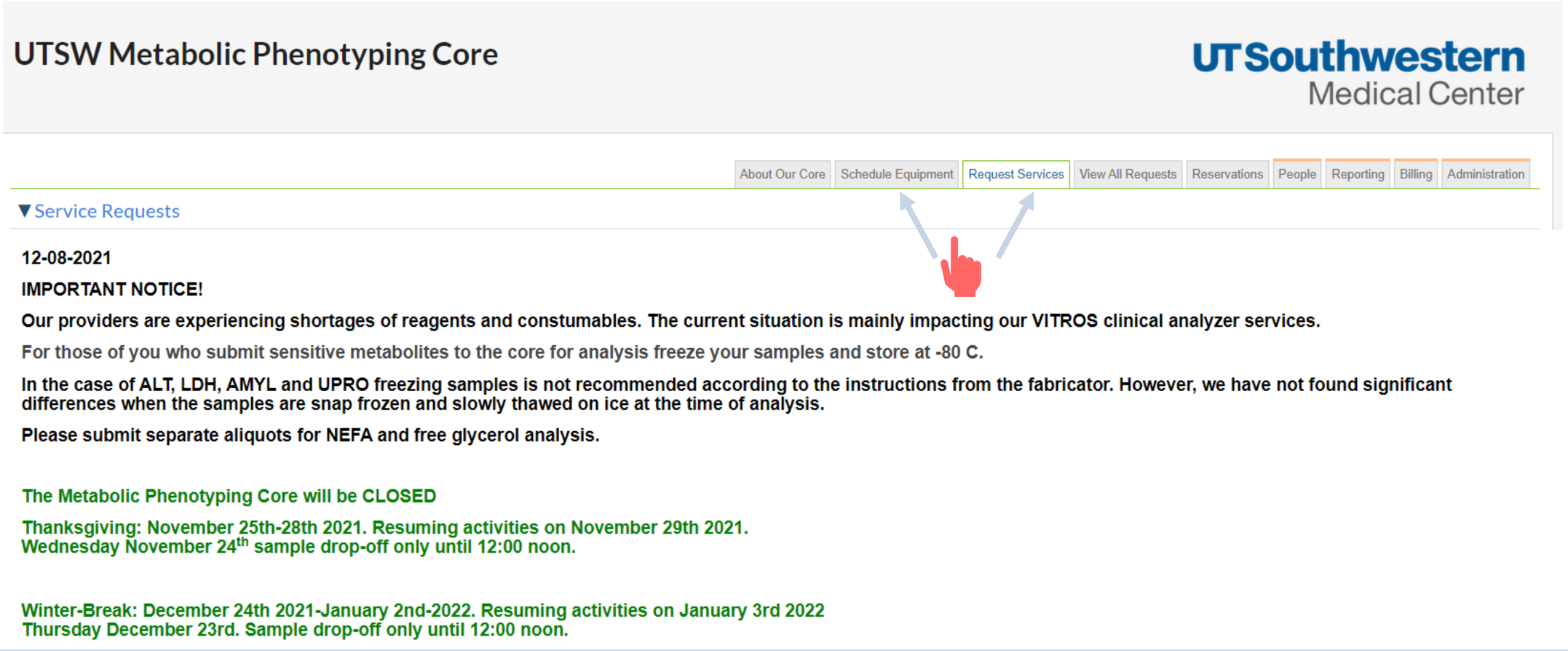
Ruth.Gordillo@utsouthwestern.edu

Syann Lee. Rodent metabolic chambers. Surgeries. RNAscope hybridization

Syann.lee@utsouthwestern.edu

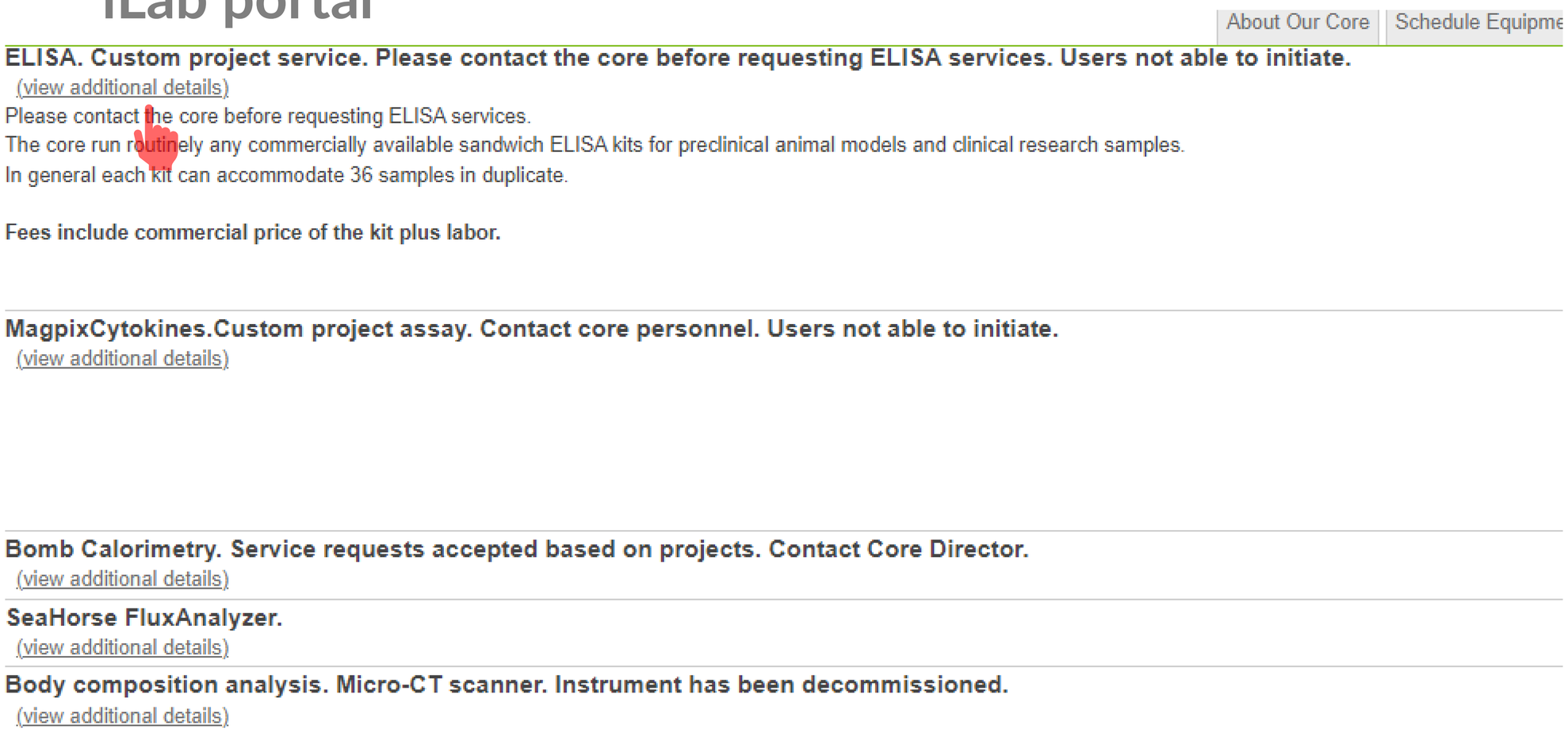
SERVICE REQUESTS AND EQUIPMENT RESERVATIONS

iLab portal



SERVICE REQUESTS AND EQUIPMENT RESERVATIONS

iLab portal



MPC USER OF THE YEAR AWARD

PART I

Bioanalytical Assays

Main laboratory L4.102

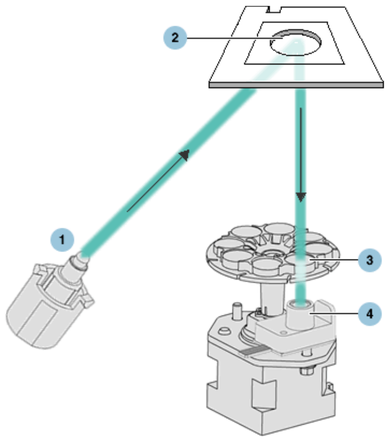
PART II

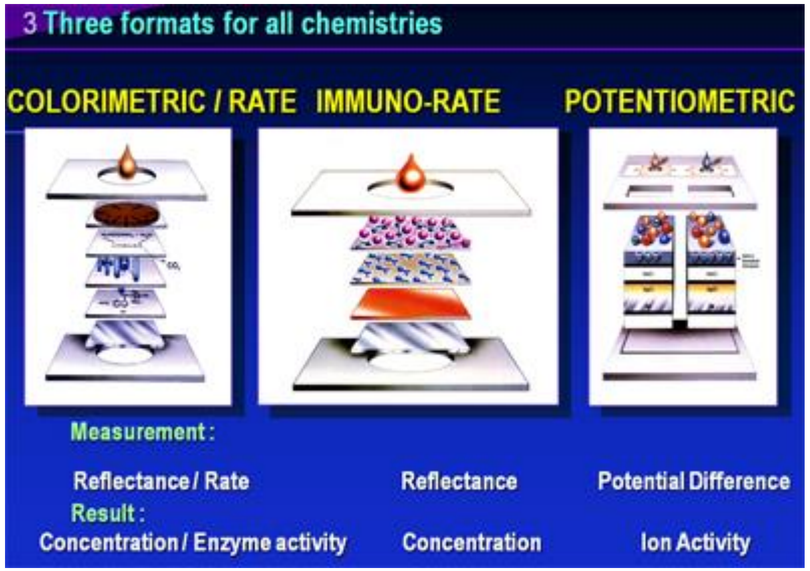
Rodent chambers

Rodent treadmills

RNAscope and Basescope hybridization

PART I. VITROS 350 CHEMISTRY SYSTEM. Clinical Analyzer



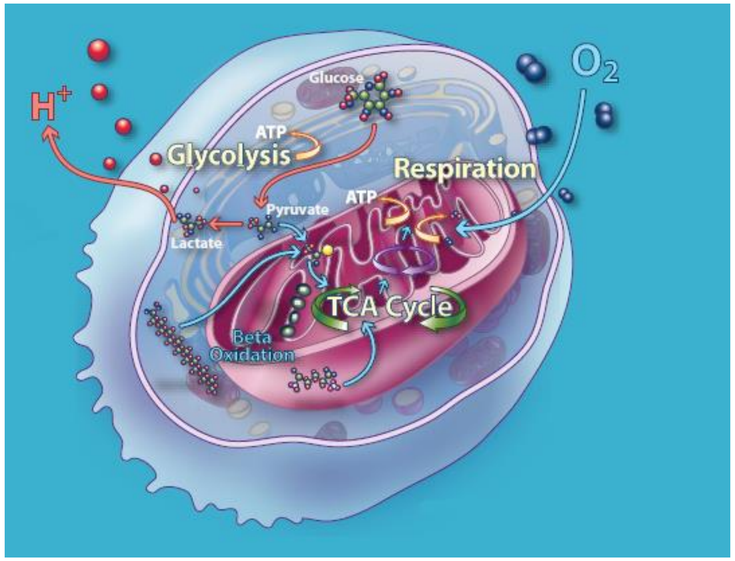


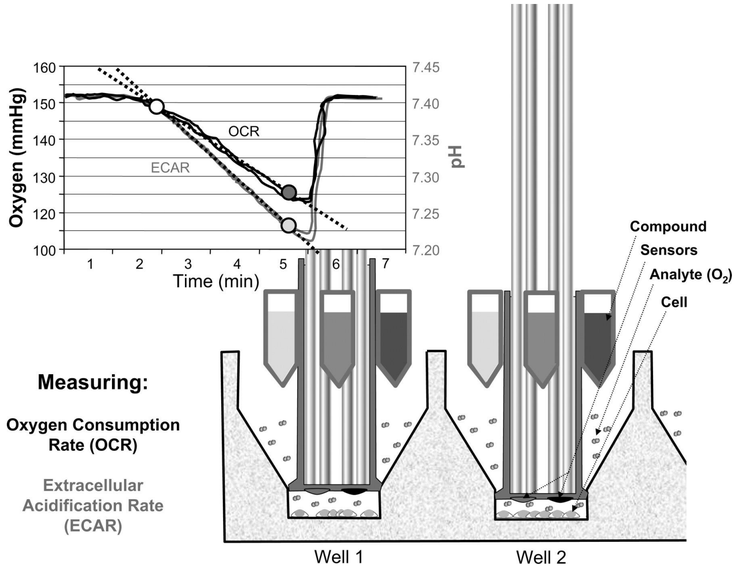
PART I. VITROS 350 CHEMISTRY SYSTEM. Clinical Analyzer

Albumin (ALB)
Alcohol (ALC)
Alkaline Phosphatase (ALKP)
Alanine Aminotransferase (ALT)
Ammonia (AMON)
Amylase (AMYL)
Aspartate Aminotransferase (AST)
Unconjugated/Conjugated Bilirubin (BuBc)
Blood Urea Nitrogen (Bun)
Calcium (Ca)
Cholinesterase (CHE)
Cholesterol (CHOL)/(HDLC)
Creatine Kinase (CK)
Creatine Kinase MB (CKMB)
Chloride (Cl)
Creatinine (CREA)
C-Reactive Protein (CRP)
Direct HDL (dHDL)

Carbon Dioxide (ECO2)
Iron (Fe)
Gamma Glutamyltransferase (GGT)
Glucose (GLU)
Potassium (K)
Lactate (LAC)
Lactate Dehydrogenase (LDH)
Lipase (LIPA)
Lithium (Li)
Magnesium (Mg)
Sodium (Na)
Phosphorus (PHOS)
CSF Protein (PROT)
Total Bilirubin (TBIL)
Total Protein (TP)
Triglyceride (TRIG)
Urine Protein (UPRO)
Uric Acid (URIC)

PART I. SEAHORSE XFe24 ANALYZER





BIOHAZARD

Biosafety Level 2

AUTHORIZED PERSONNEL ONLY

Assay running volume 500-1,000 µL per well
Sample requirements: 10,000-1,000,000 cells

Product Details

Features
Specifications
Technology
How It Works
Applications
Literature
Support
References
Tools
Videos
Training & Events
News
Related Products

Application Notes

XF Data Normalization by the Agilent Seahorse XF Imaging and Normalization System

This application note highlights how the XF Imaging and Normalization System provides an efficient and reliable method of normalization with a seamless and rapid workflow for XF Analyzer users.

Application Notes / English / 01 Nov 2018 / 719.16 KB / PDF

Evaluating Changes in Cell Metabolism in Neuroimmune and Neuropsychiatric Disorders

Application Brief- summarizes scientific Agilent-Seahorse XF research focused on neurodegeneration, immunology, and infectious disease.

Application Notes / English / 17 May 2017 / 689.46 KB / PDF

Metabolic Pathway Determination Using the XF24 Analyzer

How to measure the contribution of mitochondrial respiration and glycolysis to cellular bioenergetics.

Application Notes / English / 31 Mar 2017 / 493.83 KB / PDF

Normalization of Agilent Seahorse XF Data Data by In-situ Cell Counting Using a Biotek Cytation 5

Application Note - Normalization of Agilent Seahorse XF data by in-situ cell counting using a BioTek Cytation 5

Application Notes / English / 24 Mar 2017 / 931.07 KB / PDF

Identifying Metabolic Switches Using an Agilent Seahorse XFe Analyzer in Hypoxia

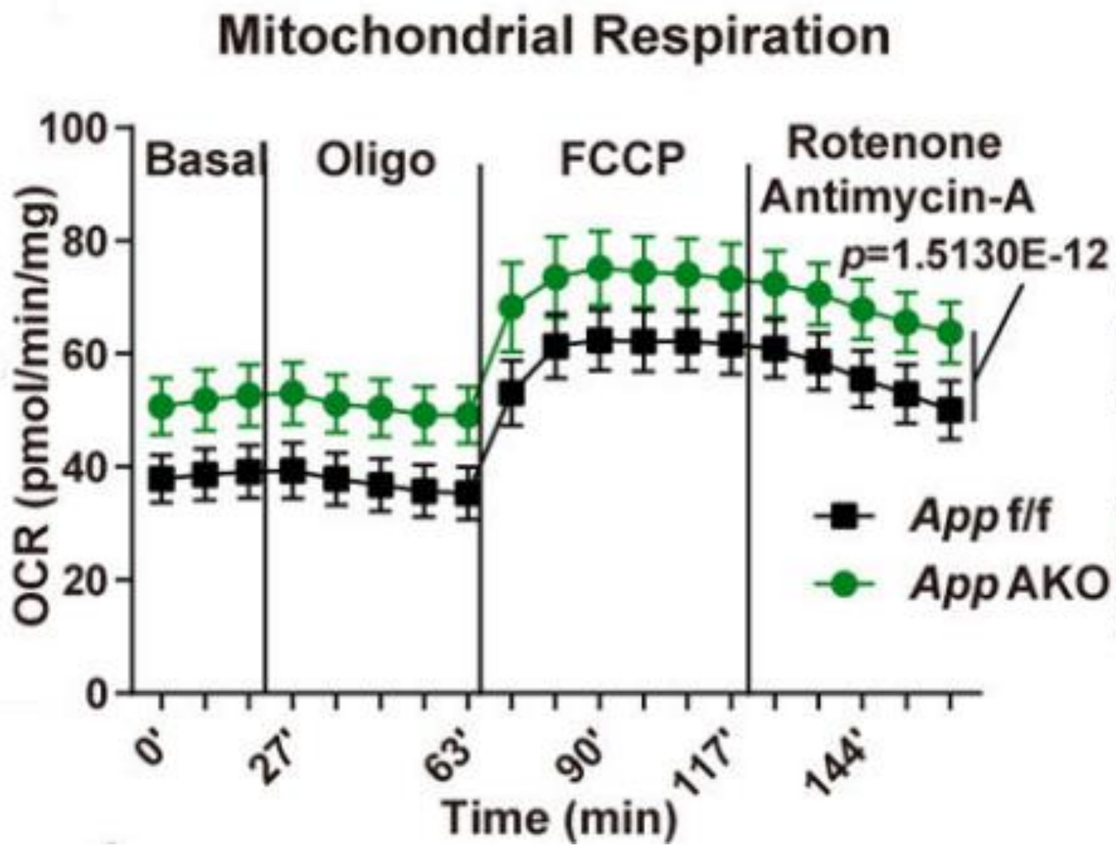
Identifying Metabolic Phenotype Switches in Cancer Cells Using the Agilent Seahorse XF/XFe Analyzer in an Hypoxic Environment

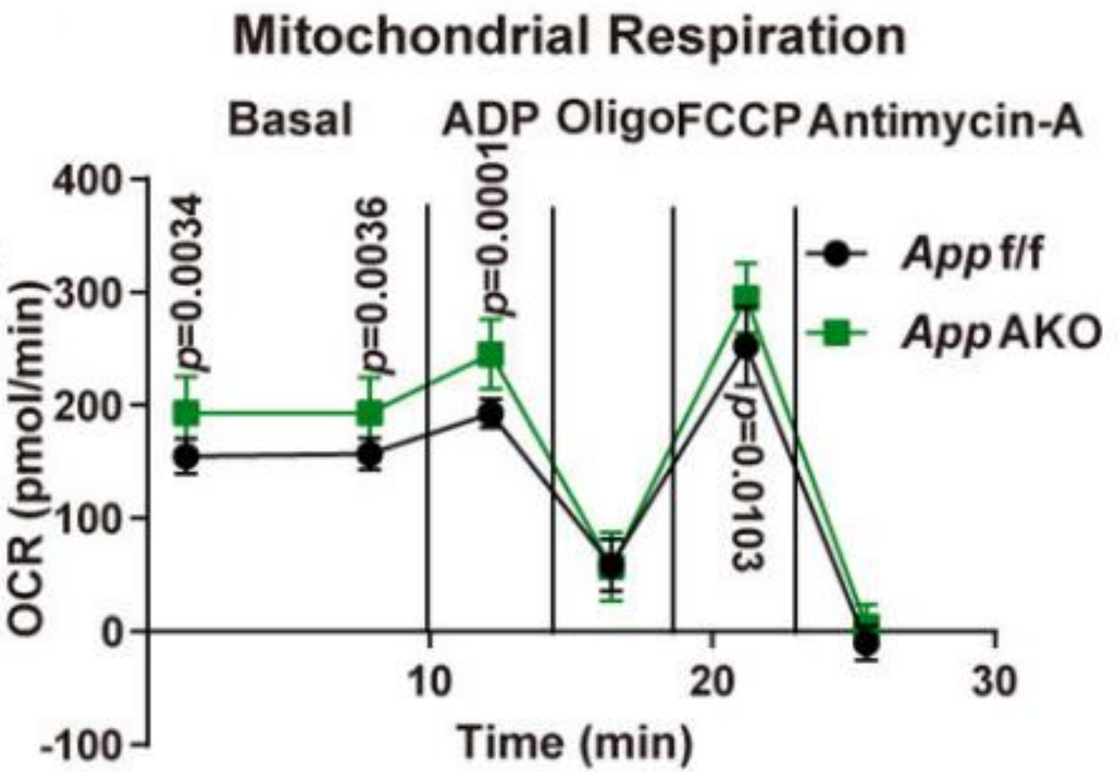
Application Notes / English / 03 Jan 2017 / 744.28 KB / PDF

Measure Mitochondrial Respiration in Skeletal Muscle Fibers with a Seahorse Analyzer

Measuring Mitochondrial Respiration in Intact Skeletal Muscle Fibers with an Agilent Seahorse XF24/XFe24 Analyzer

https://www.agilent.com/en/product/cell-analysis/real-time-cell-metabolic-analysis/xf-analyzers/seahorse-xfe24-analyzer-740878

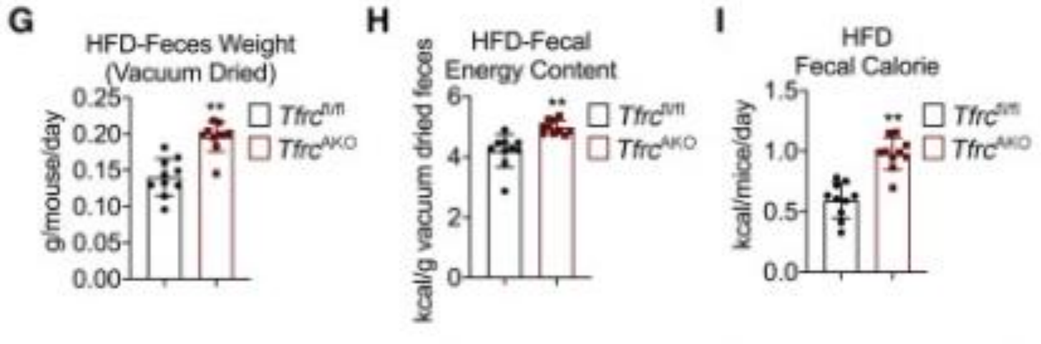

Mitochondrial Respiration
Basal
Oligo
FCCP
Rotenone
Antimycin-A
p=1.5130E-12
App f/f
App AKO
OCR (pmol/min/mg)
100
80
60
40
20
0
0'
27'
63'
90'
117'
144'
Time (min)


Mitochondrial Respiration
Basal
ADP
Oligo
FCCP
Antimycin-A
p=0.0034
p=0.0036
p=0.0001
p=0.0103
App f/f
App AKO
OCR (pmol/min)
400
300
200
100
0
-100
10
20
30
Time (min)

PART I. BOMB CALORIMETER

Semi-micro 25 – 200 mg of sample

Standard 0.6 – 1.2 g of sample

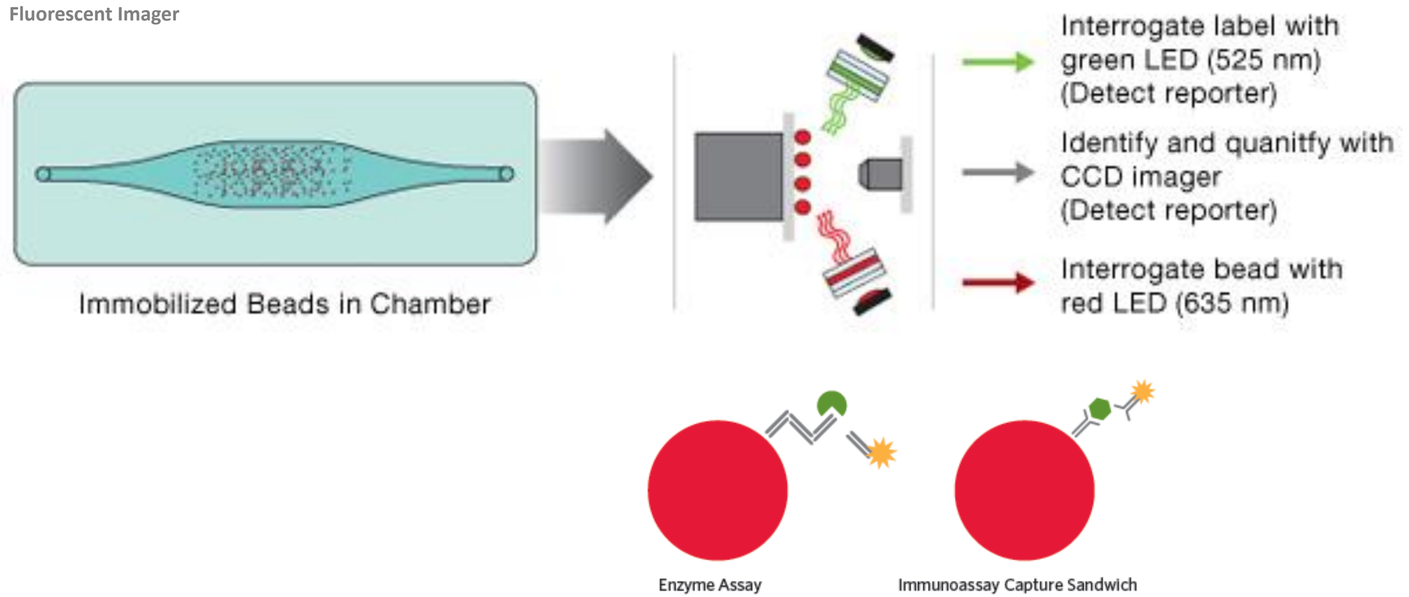


Zhang *et al.*
https://doi.org/10.1016/j.cmet.2021.06.001

Daily feces weight (G), fecal energy content (H), and daily fecal calorie content (I) of *Tfrc*$^{fl/fl}$ and *Tfrc*^{AKO} mice after 8 weeks of HFD

PART I. MULTIPLEXED IMMUNOASSAYS

MAGPIX READER
SIMULTANEOUSLY DETERMINATION UP TO 50 ANALYTES



Diabetes, Metabolic and Gut Hormones*:

Gut hormones regulate gut motility and secretion, pancreatic islet hormone secretion, food intake and energy expenditure.

Glucagon	GLP-1	Leptin
C-Peptide	GIP	Insulin
PYY	Ghrelin	Amylin

*only selected analytes listed

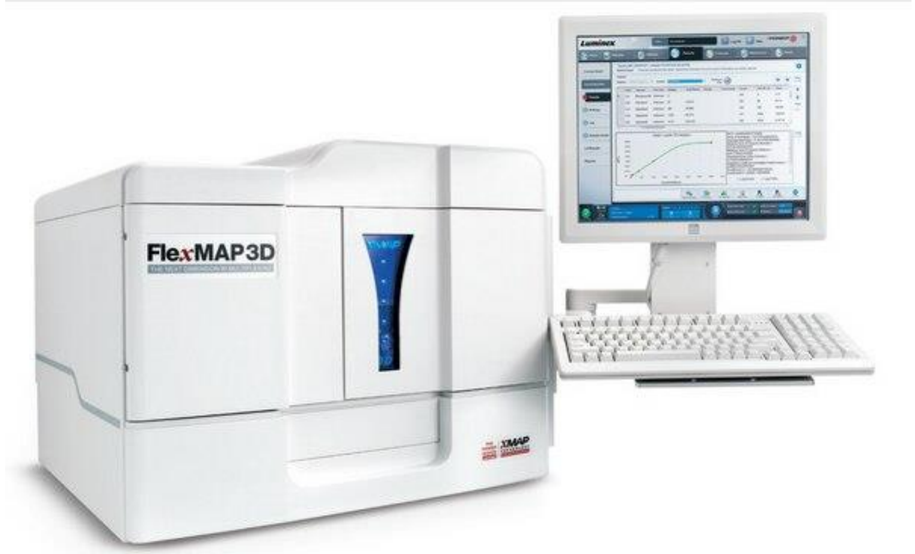
Adipokines*:

Hormones called adipokines regulate appetite and energy balance, insulin sensitivity, and lipid metabolism.

Adiponectin	IL-1β	IL-6
MCP-1	PAI-1	Resistin
TNF-α	Leptin	Adiponectin

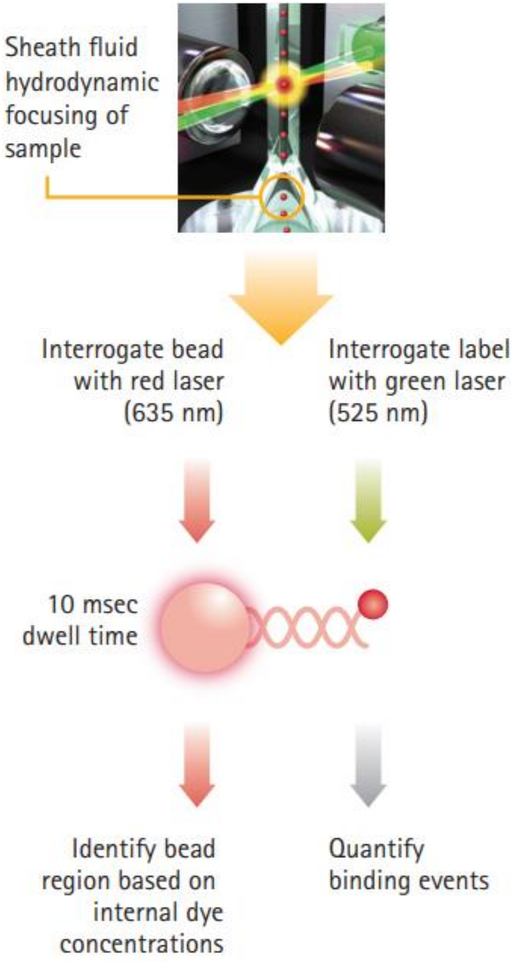
*only selected analytes listed

PART I. MULTIPLEXED IMMUNOASSAYS

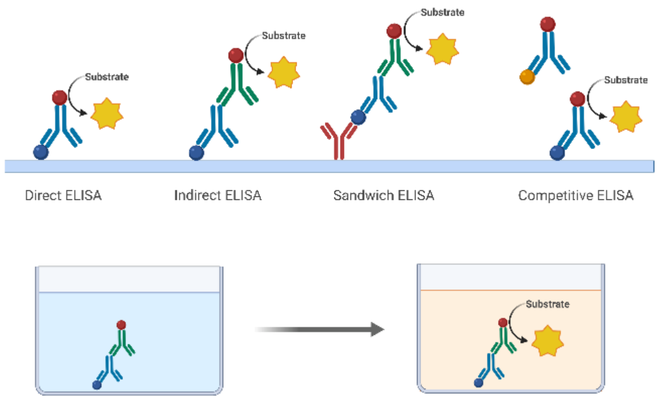


FLEXMAP 3D READER
SIMULTANEOUSLY DETERMINATION UP TO 500 ANALYTES

Flow cytometry-based analysis

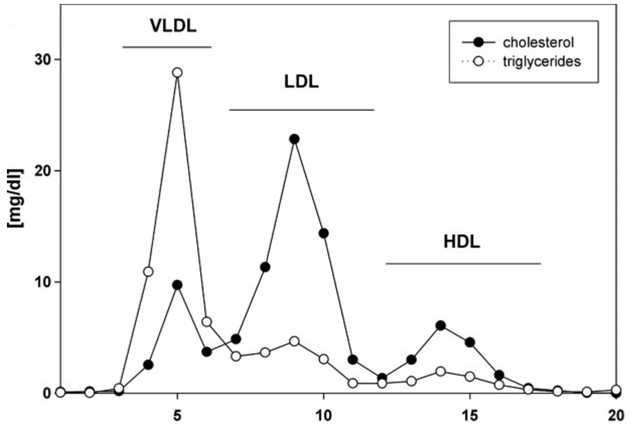


PART I. Enzyme Linked immunosorbent Assay (ELISA) Commercial Kits

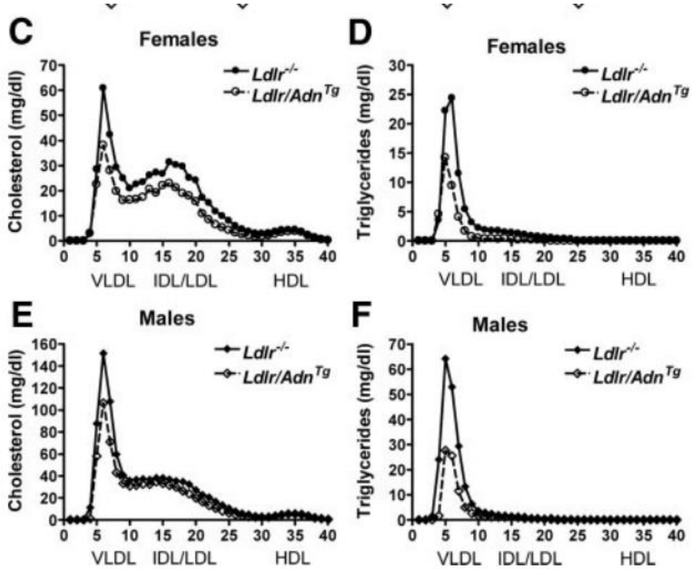


Multimode Plate Reader.
Absorbance, Luminescence, Fluorescence

PART I. Plasma Lipoprotein Fractionation by FPLC



Total cholesterol (TC) and triglyceride level of fast performance liquid chromatography (FPLC) fractions. Human Serum
Weisner *et al.*
https://doi.org/10.1194/jlr.D800028-JLR200



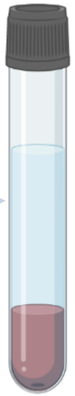
Total cholesterol (TC) and triglyceride level of fast performance liquid chromatography (FPLC) fractions. Mouse Serum. Adiponectin improves lipoprotein profile. Shown are total cholesterol (A) and triglycerides (B) in female and male Ldlr/AdnTg and Ldlr/ mice after feeding of WD for 4 months.

Nawrocki *et al.*
https://doi.org/10.1194/jlr.D800028-JLR200

PART I. Tissue Lipids Extractions. Enzymatic Colorimetric assay

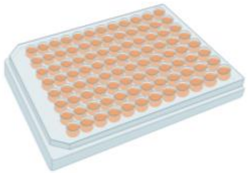
- Total Cholesterol
- Total Triacylglycerides
- Non-Esterified Fatty Acids (Free Fatty Acids and Acyl CoA)

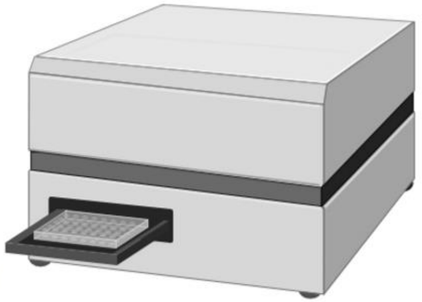
Homogenization



Single phase liquid extraction

Plating samples and standards, reagents addition



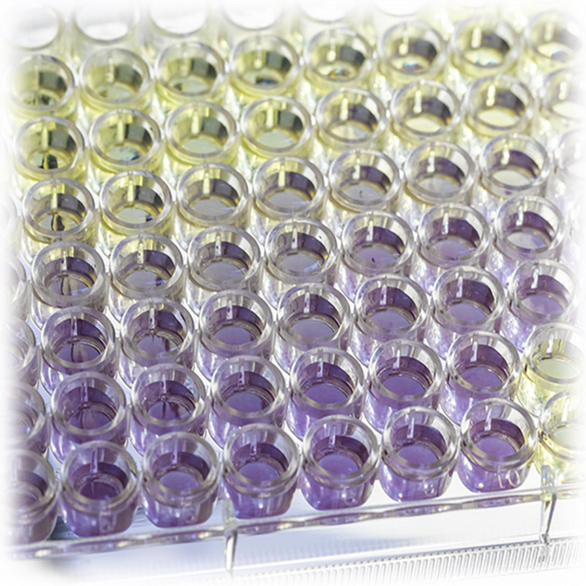


Absorbance reading and data processing

PART I. Colorimetric Assays.

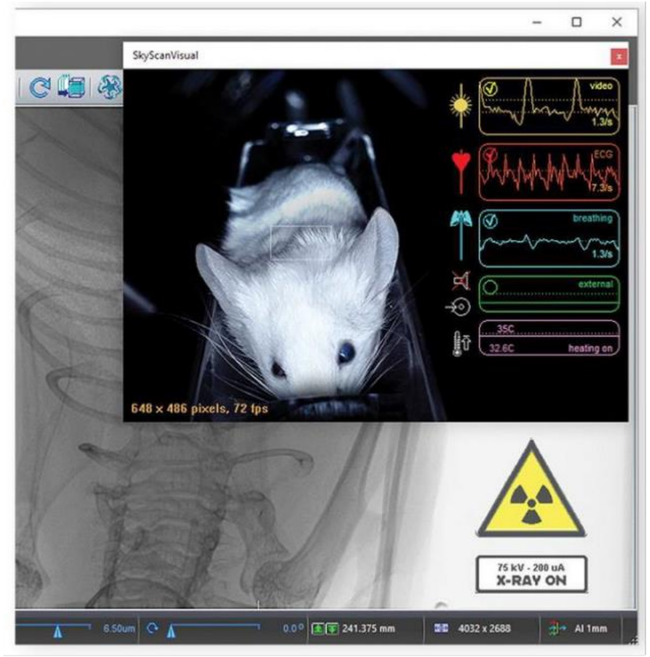
- NEFA serum and plasma samples
- Free glycerol
- Total ketone bodies and 3-hydroxybutyrate (3HB)
- Iron/Unsaturated iron-binding capacity (UIBC)
- Protein content

Other assays as requested. Custom design project



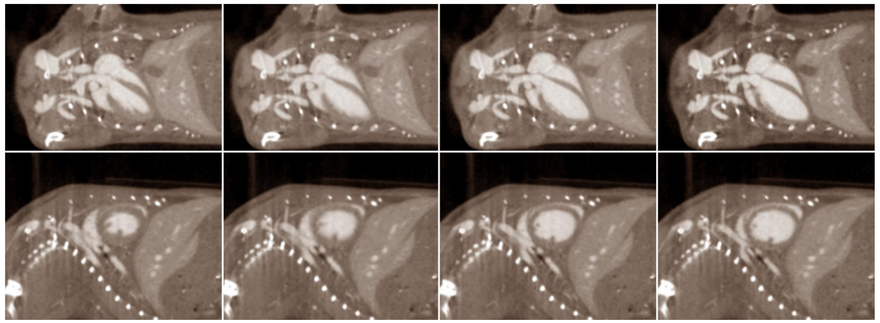
PART I. Micro-CT scanner. Coming Soon!!!

Bruker SKYSCAN 1276
JA barrier facility

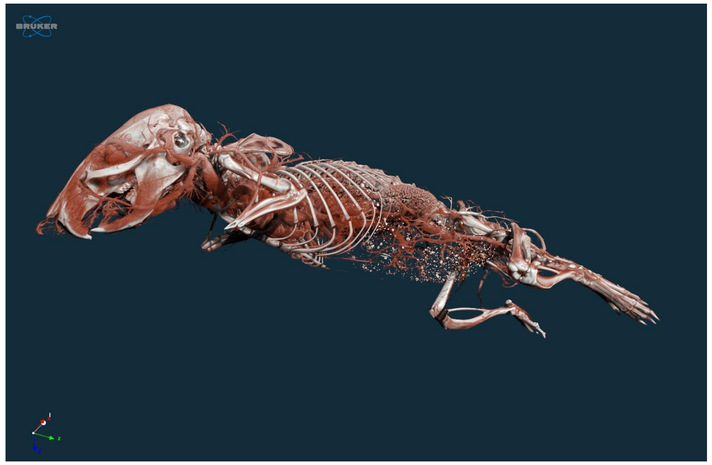


- Low-dose, fast scan → longitudinal studies
- Smallest pixel size 2.8 µm (highest resolution)
- Integrated Physiological monitoring

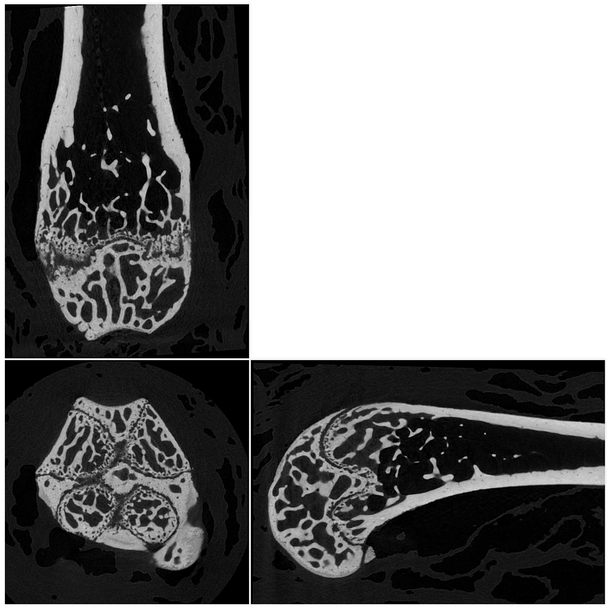
PART I. Micro-CT scanner. Coming Soon!!!



Cross-sectional images of a time sequence of the mouse heartbeat, scanned *in vivo* after contrast agent injection



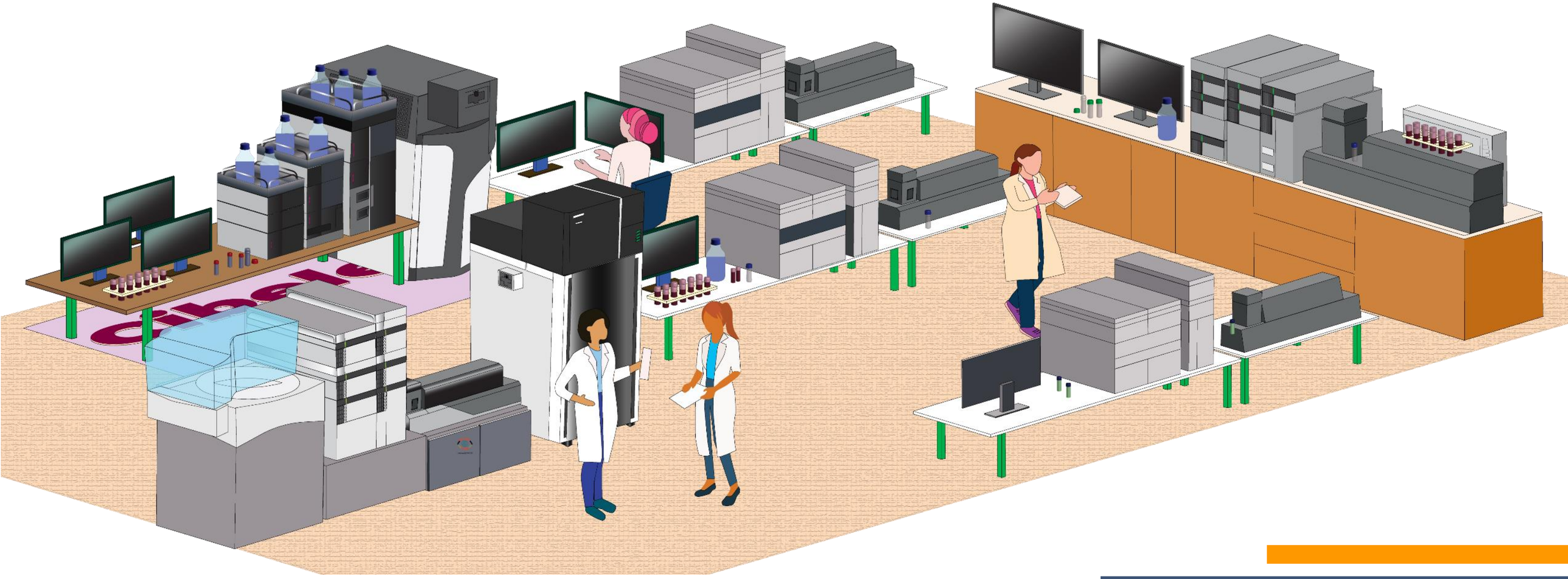
3D representation of the mouse vasculature, scanned in vivo at 7 µm voxel size after a bolus injection of vascular contrast agent.



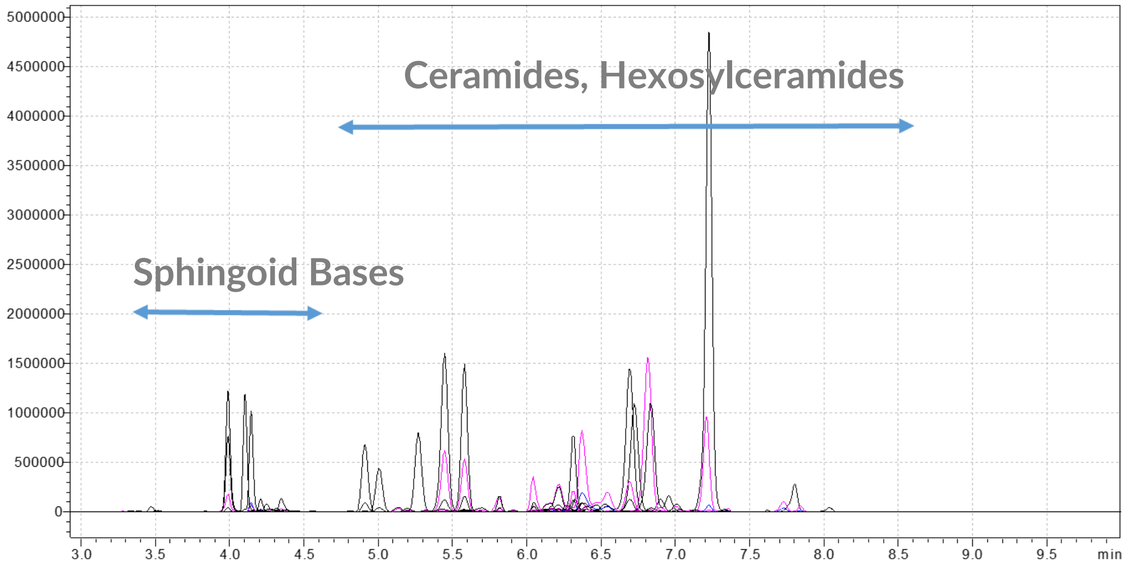
Orthogonal slices through a mouse femur, scanned at 2.8 µm voxel size

Images source: Bruker.com

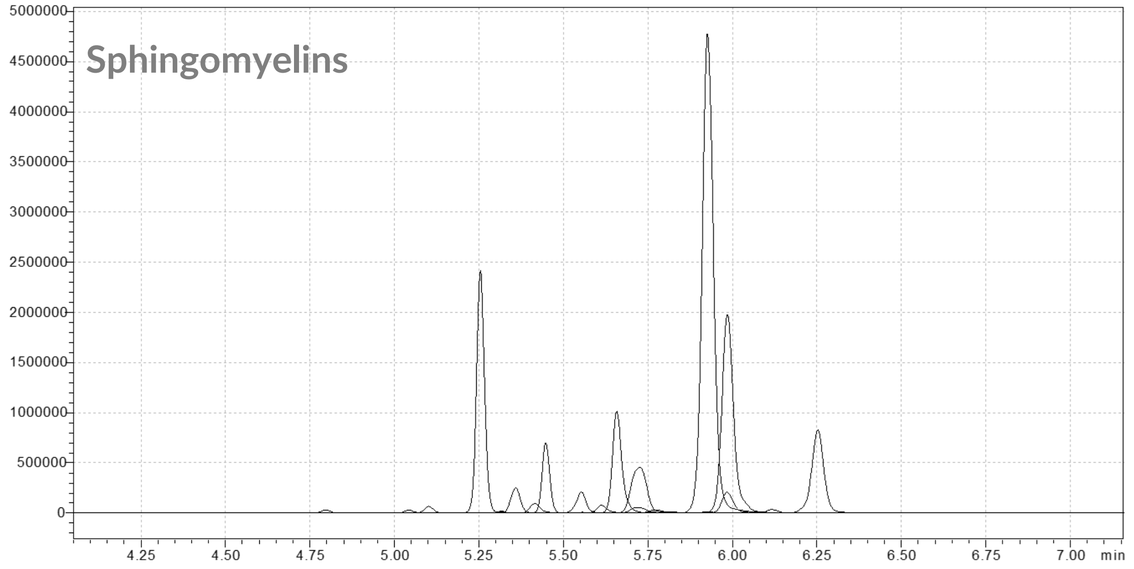
PART I. Mass Spectrometry Lab



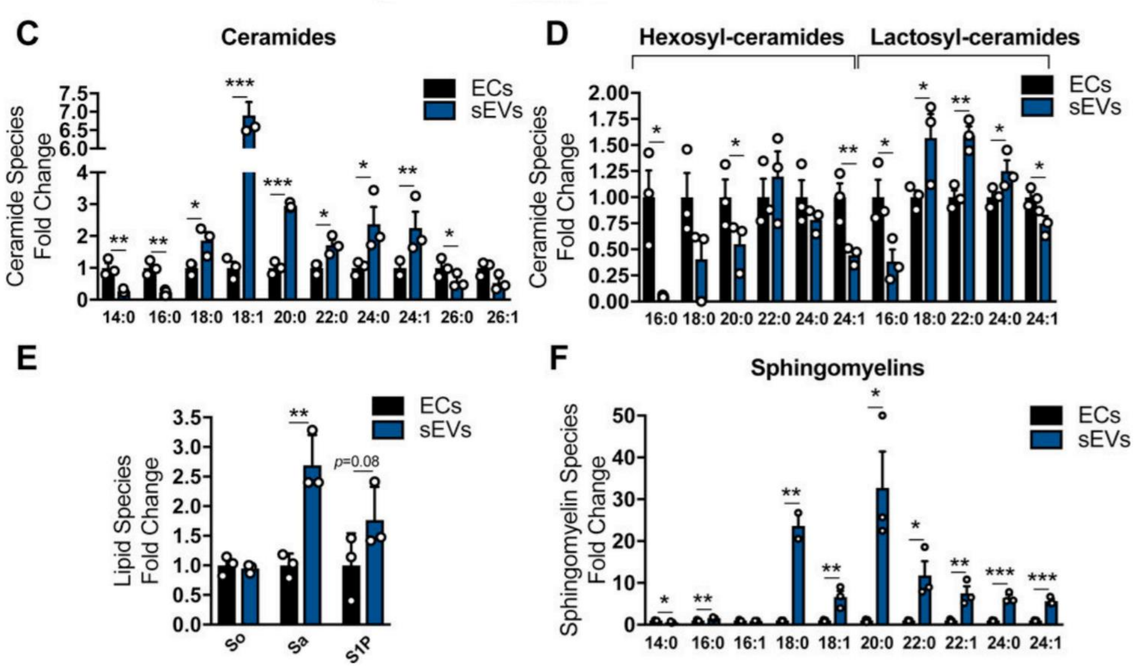
PART I. Mass spectrometry-based analysis. Sphingolipids Profiling



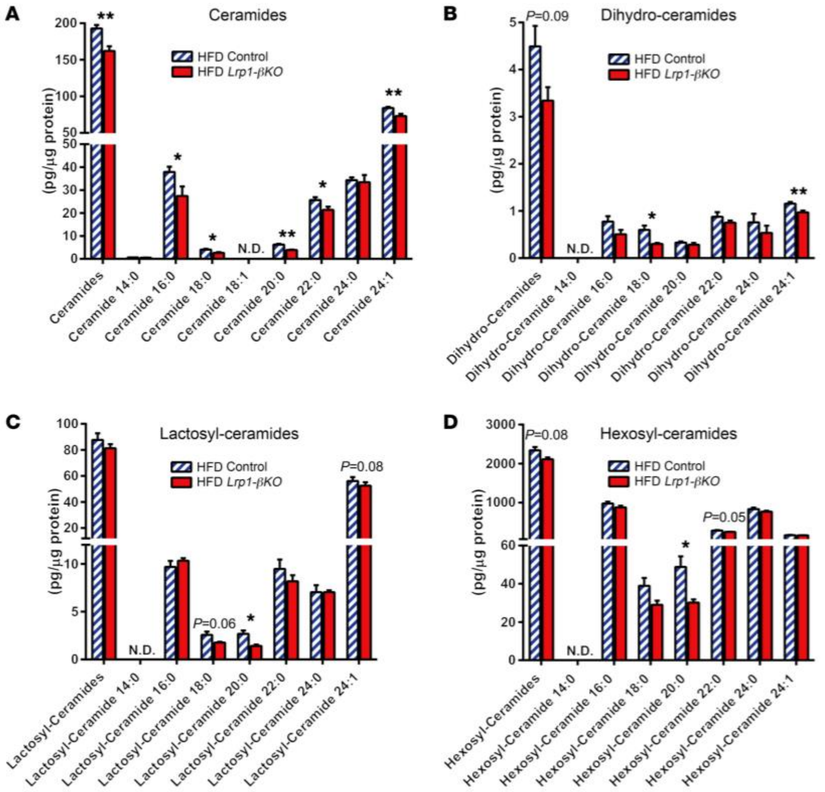
SRM Chromatogram Human Serum
Positive mode ESI



SRM Chromatogram Mouse Liver
Negative mode ESI
Product ion m/z 168.

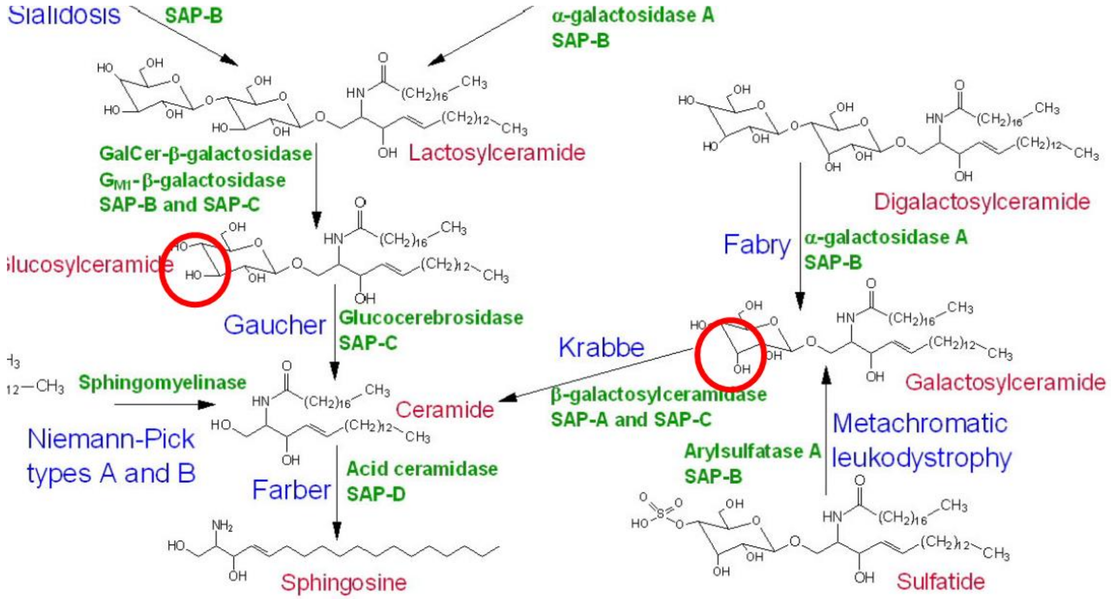


Crew *et al.* DOI: 10.1016/j.cell.2018.09.005
Small extracellular vesicles isolated from adipose tissue were found to be enriched in sphingolipids compared to endothelial cells.

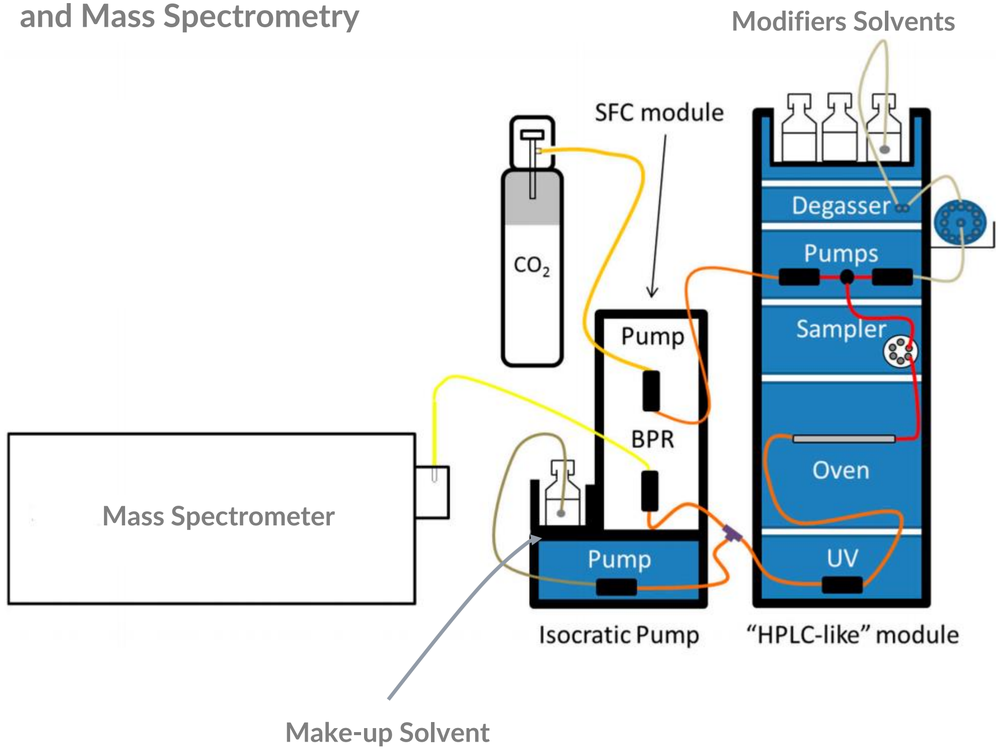


Ye *et al.* DOI: 10.1172/JCI97702
Overall decrease in ceramides and some of the lipotoxic precursors and derivatives of ceramides in Lrp1-βKO islets .

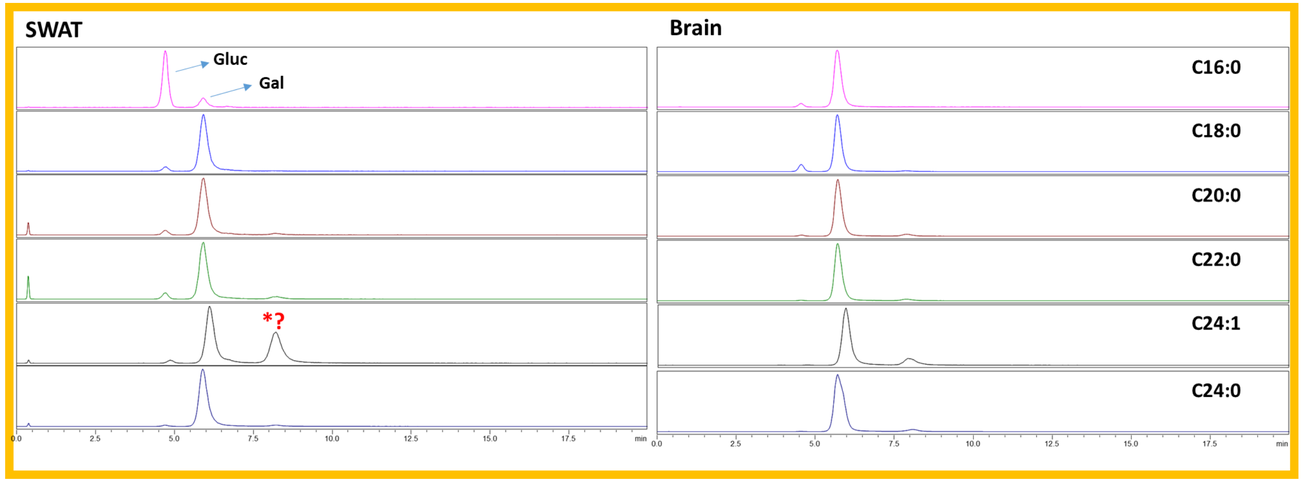
PART I. Mass spectrometry-based analysis. Supercritical Fluid Chrom.

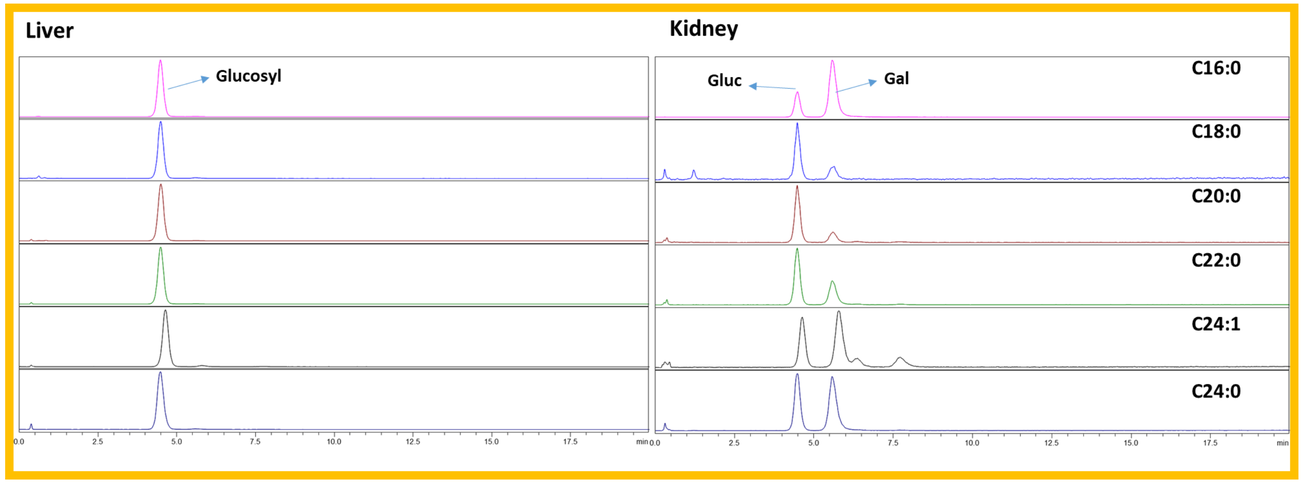


Hyphenation Between Supercritical Fluid Chromatography and Mass Spectrometry

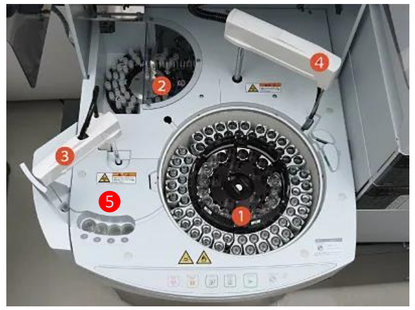


Adapted from Laboureur *et al.* DOI: 10.3390/ijms160613868


SWAT
Gluc
Gal
*?
Brain
C16:0
C18:0
C20:0
C22:0
C24:1
C24:0
0.0
2.5
5.0
7.5
10.0
12.5
15.0
17.5
min


Liver
Glucosyl
Kidney
Gluc
Gal
C16:0
C18:0
C20:0
C22:0
C24:1
C24:0
0.0
2.5
5.0
7.5
10.0
12.5
15.0
17.5
min

PART I. Mass Spectrometry-based analysis. Free Amino Acids



1. Carousel for samples and reagents. Temperature Controlled (9°C)
2. Racks for filtration and collection vials
3. Sample dispensing probe
4. Reagent dispensing probe
5. Rinsing solvents and detergents for sample probe

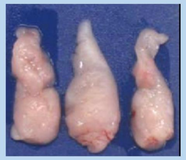
Dedicated Filtration Vial

Dedicated Collection Vial

Saliva

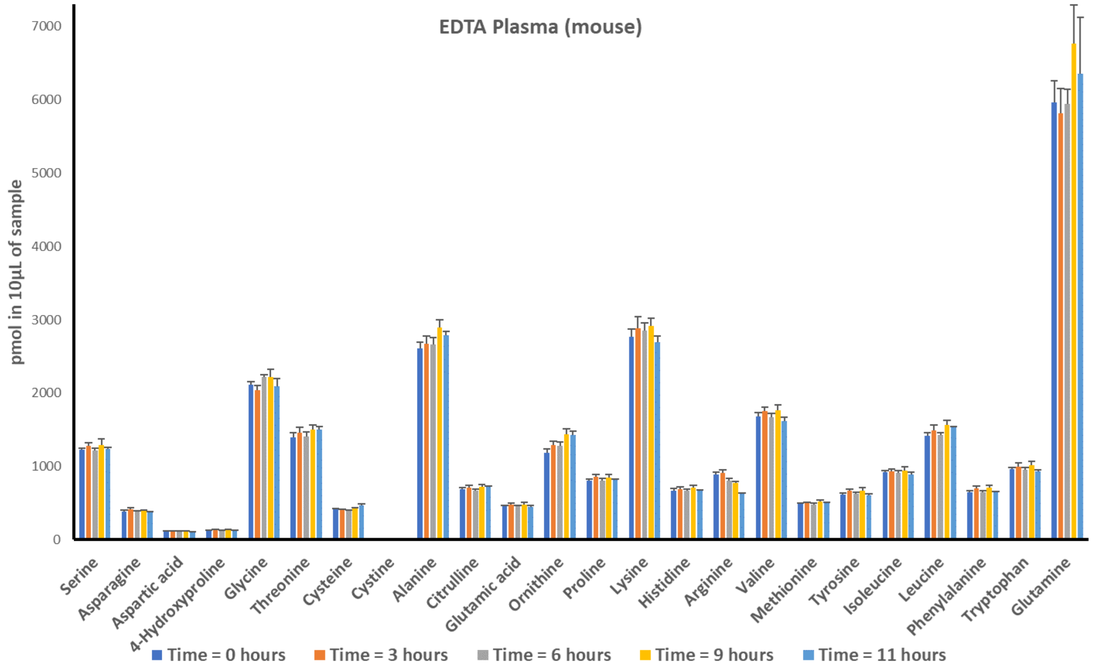
Urine

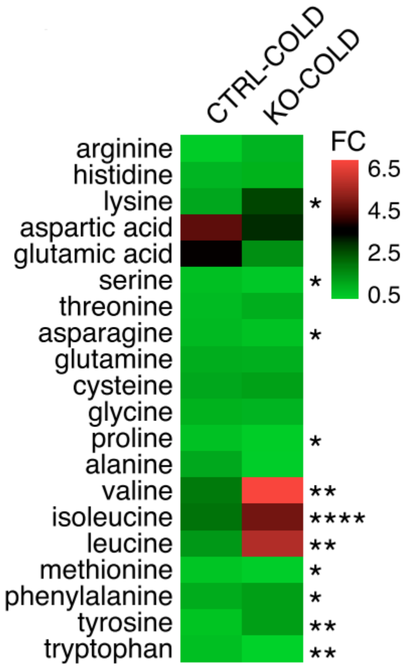
Blood, serum, plasma



Tissue homogenates and supernatants

Dispense Reagent 60 µL MeOH → Sample Dispense 10 µL (Serum Plasma) → Dispense IS Mix 10 µL → Stirring 90 seconds 1,900 rpm → Dispense Reagent 50 µL MeOH → Stirring 60 seconds 1,900 rpm → Filtration 70 seconds → Injection to LC-MS/MS

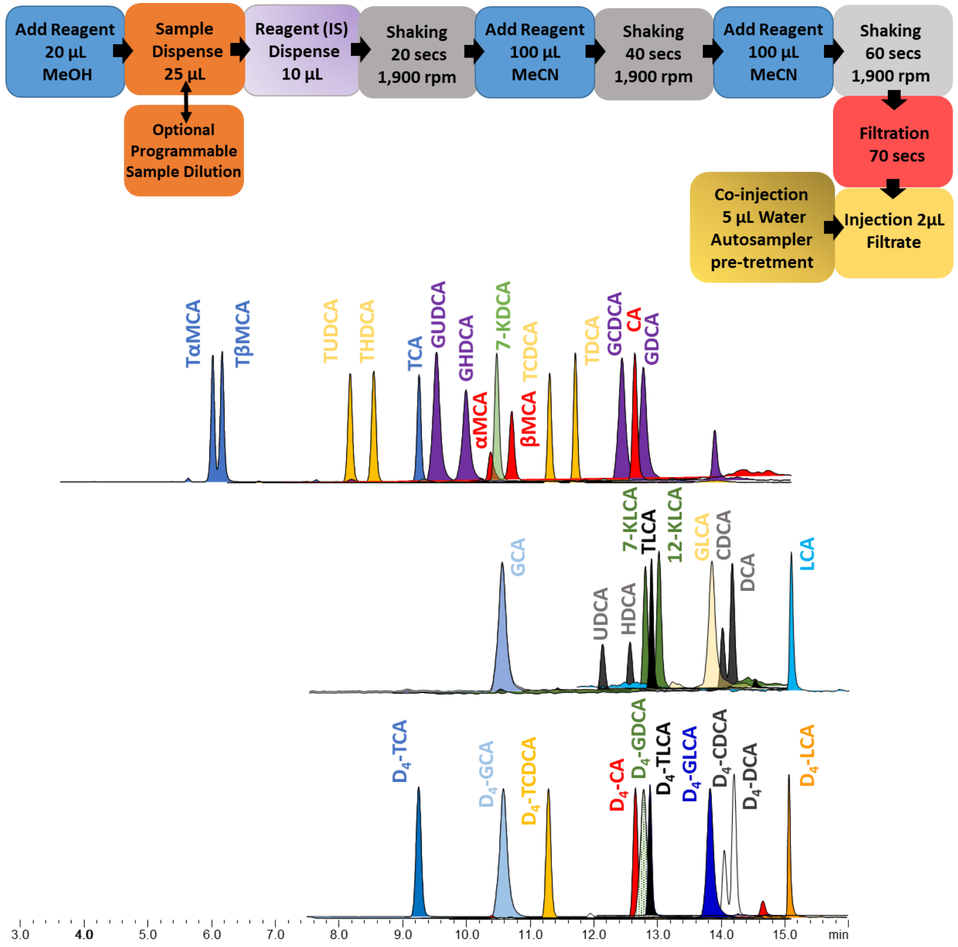


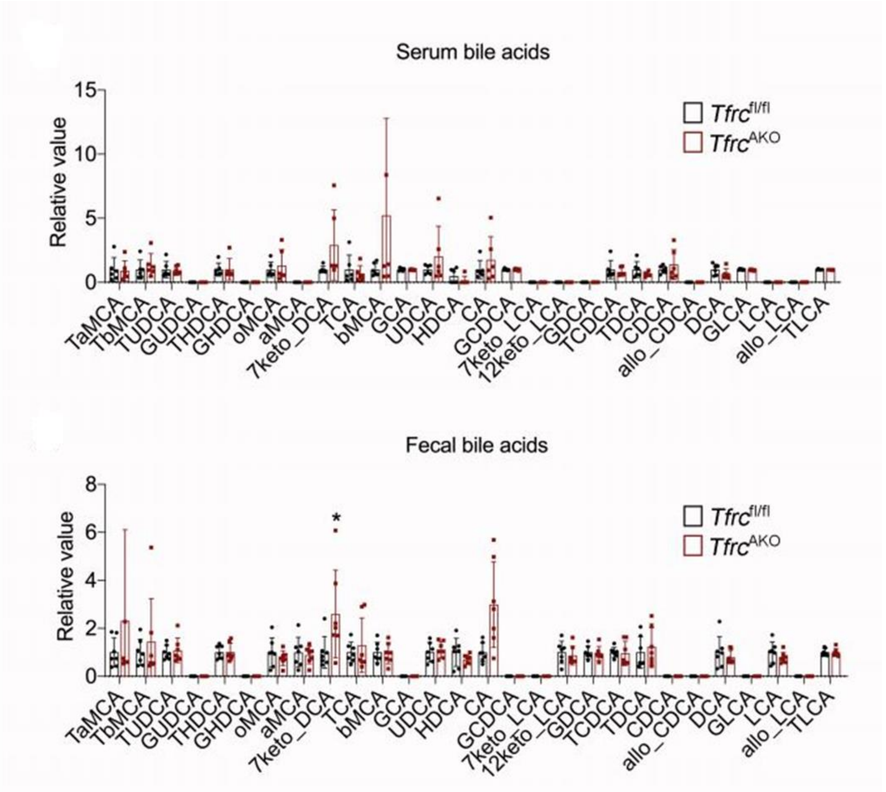


Cannavino et al. DOI: 10.1073/pnas.2104650118

Heat map showing fold change of BCAA and amino acid content in plasma from CTRL and FAM195A KO animals after cold exposure.

PART I. Mass Spectrometry-based analysis. Bile Acids

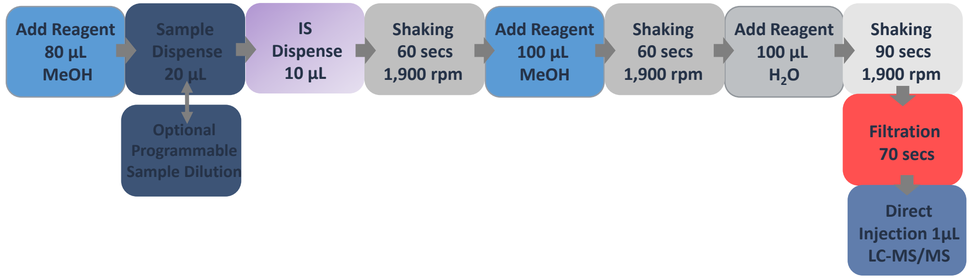


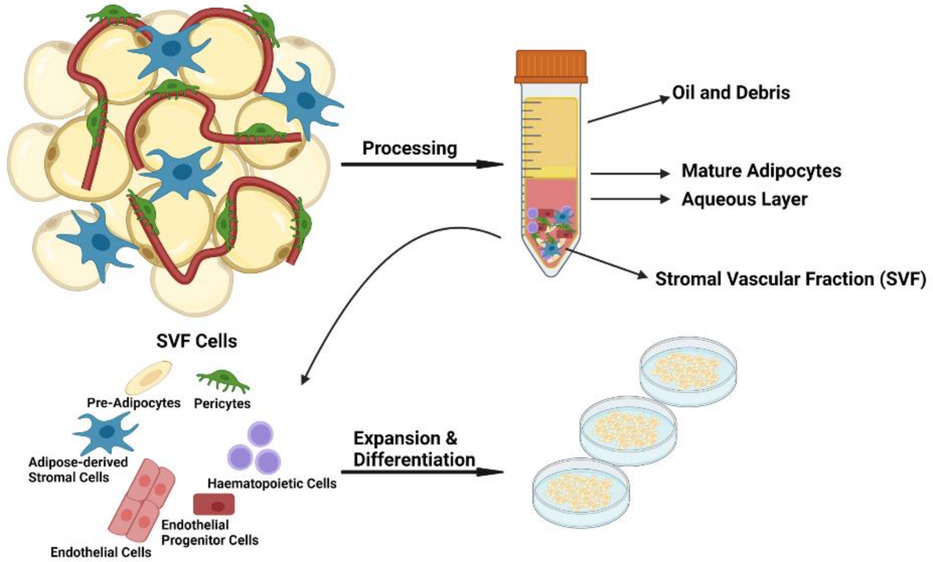


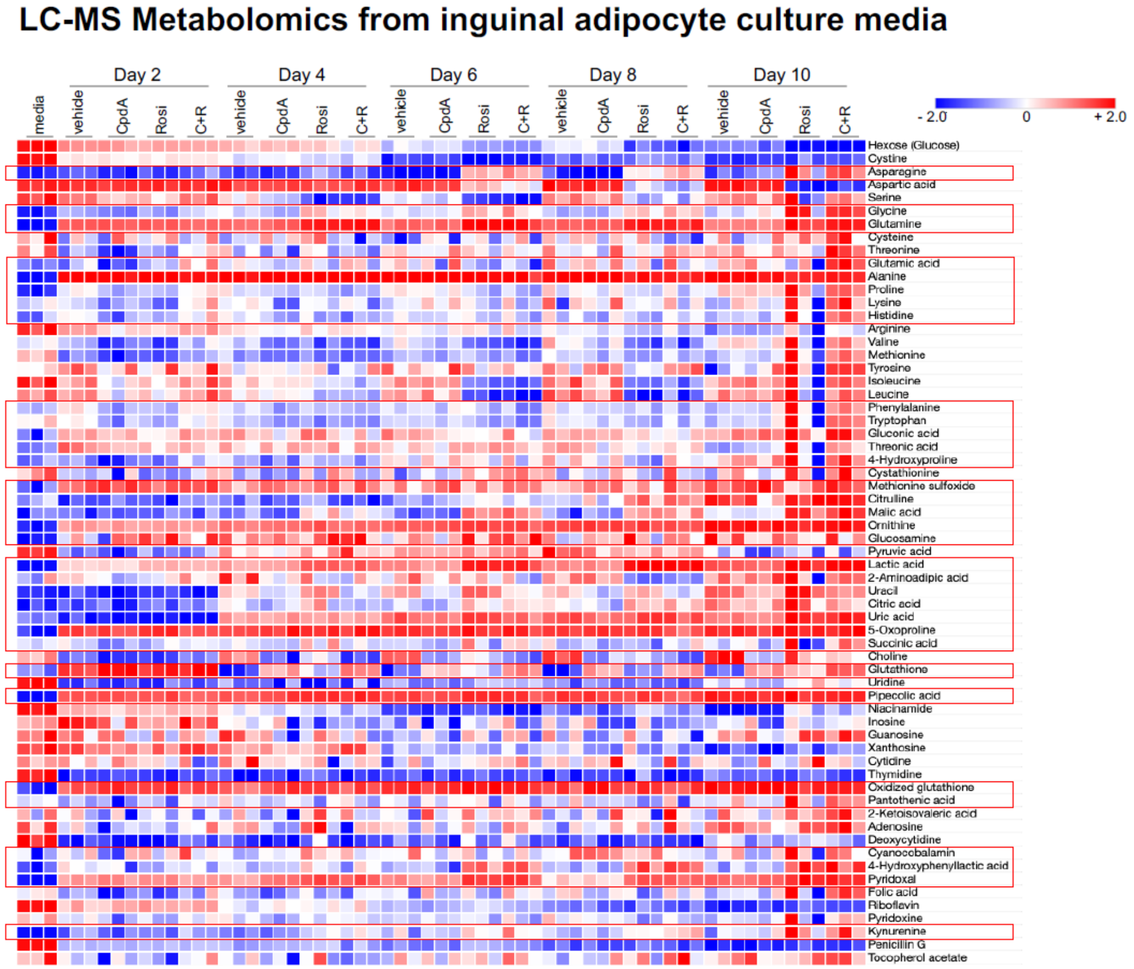
Zhang et al. DOI: 0.1016/j.cmet.2021.06.001

Bile Acids analysis in mouse serum and mouse feces.

PART I. Mass spectrometry-based analysis. Cell Culture metabolites

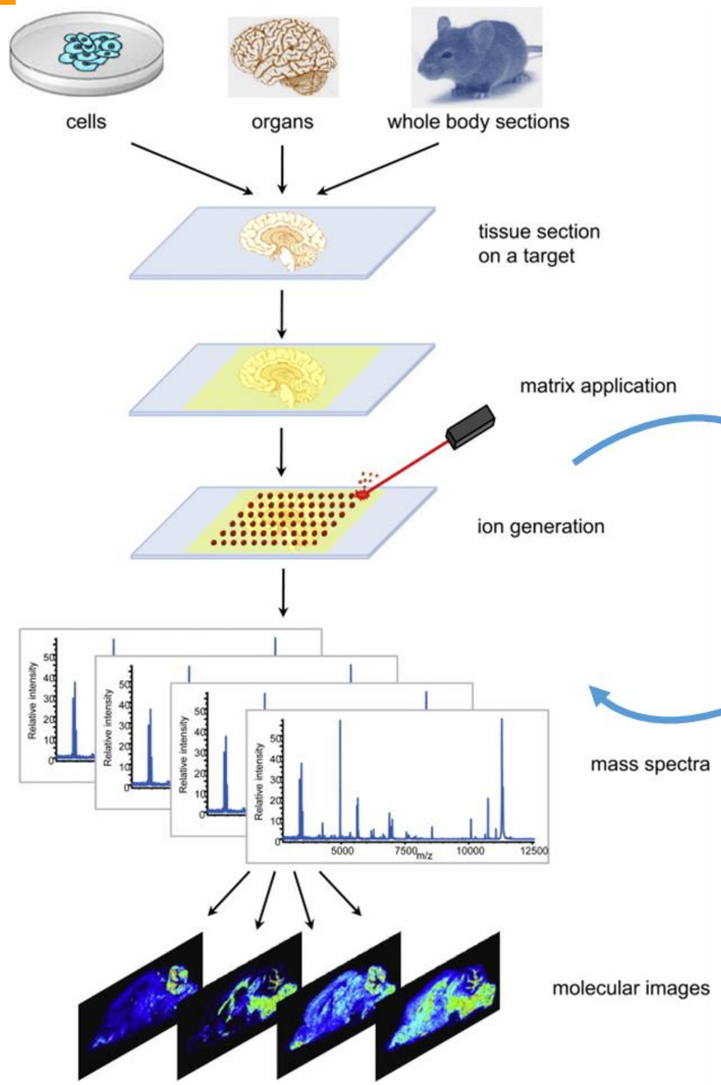






Collaboration with Dr. Oh's lab. Unpublished results

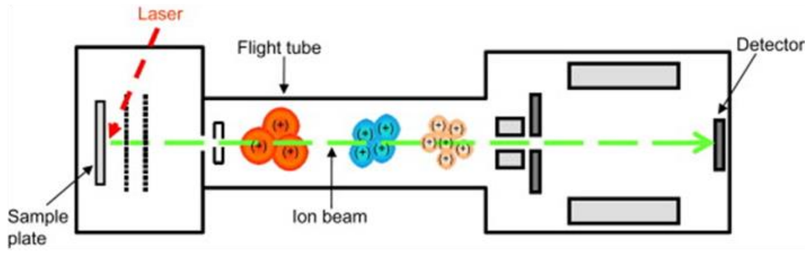
PART I. Mass spectrometry-based analysis. MALDI-TOF Imaging. Coming in the Future

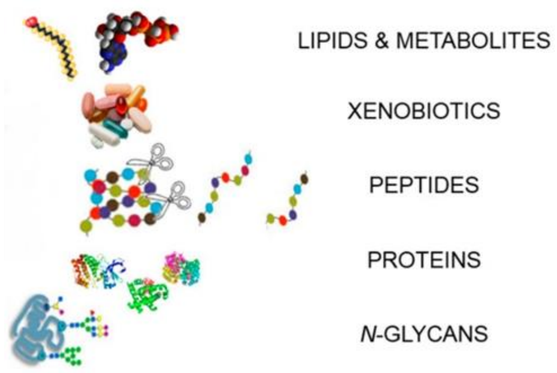


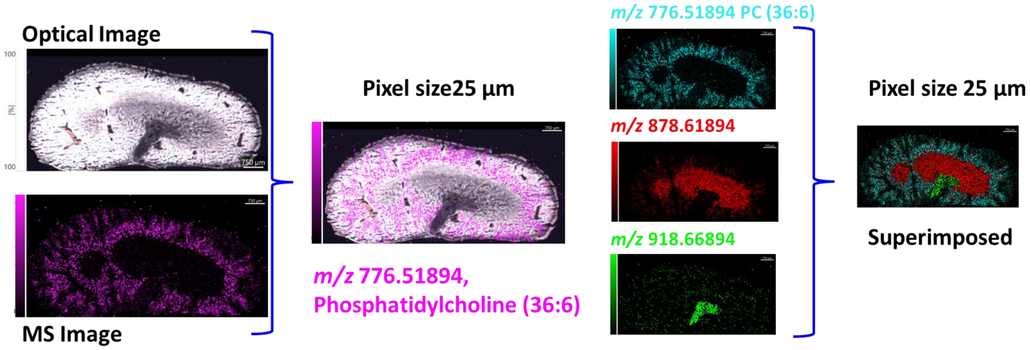
Schwamborn *et al.* DOI: 10.1016/j.molonc.2010.09.002

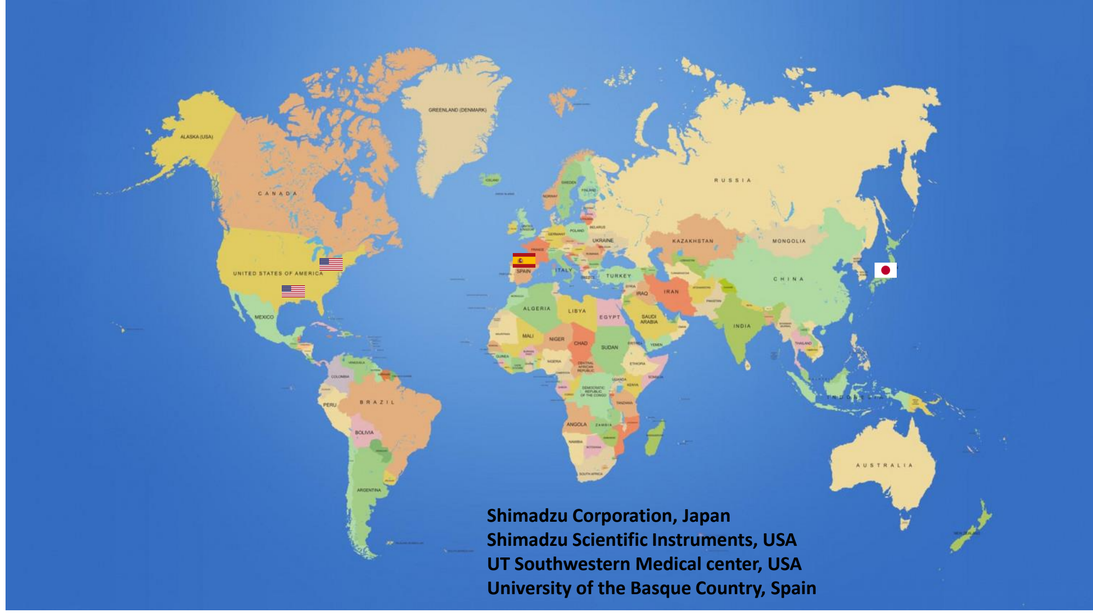
Smith *et al.* DOI: 10.3390/ijms18122588

MALDI-TOF Mass Spectrometer

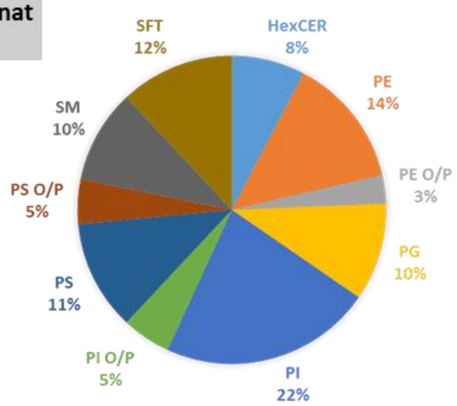


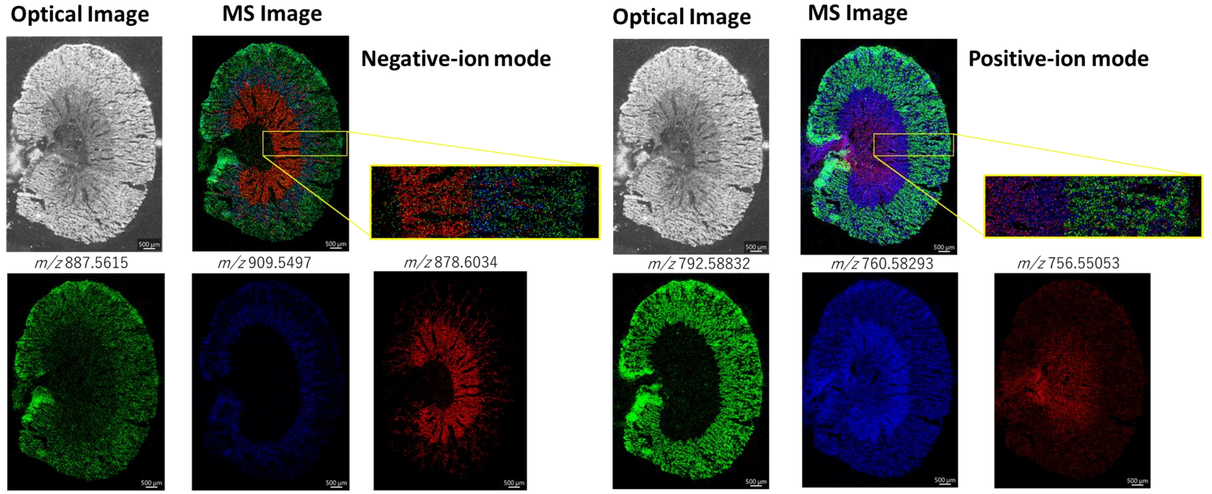






Lipid class	nº of species	Predominanation
HexCER	18	$-H^-$
PE	32	$-H^-$
PE O/P	7	$-H^-$
PG	24	$-H^-$
PI	52	$-H^-$
PI O/P	12	$-H^-$
PS	27	$-H^-$
PS O/P	11	$-H^-$
SM	23	$-CH3^-$
SFT	28	$-H^-$
TOTAL	234	





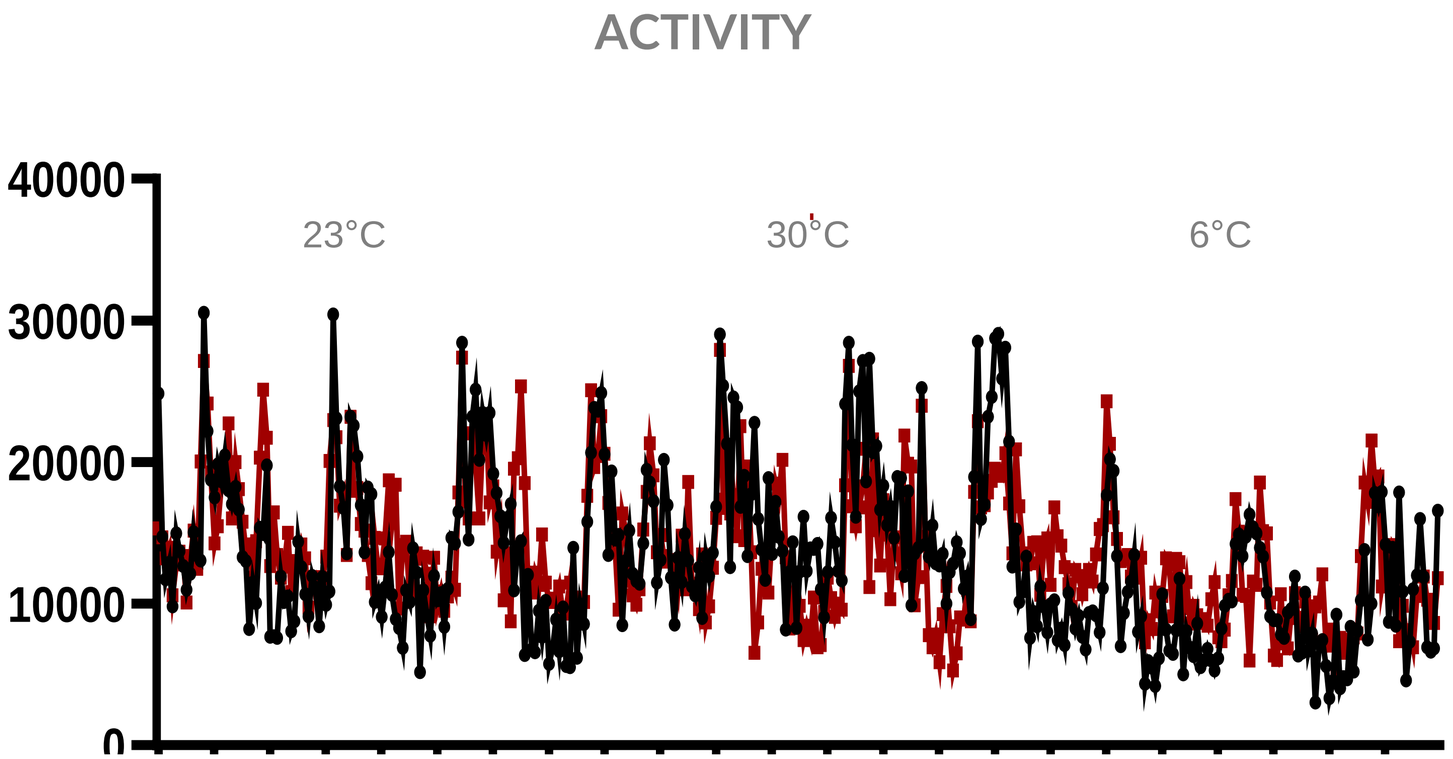
Odonera, Scherer *et al.* . Unpublished results

PART II. Metabolic chambers

Example experimental designs

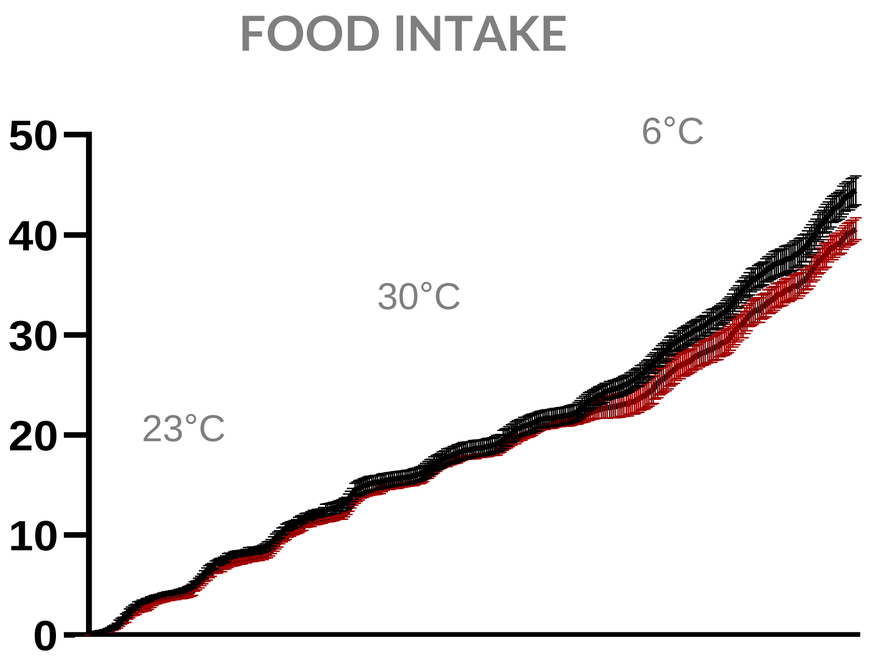
- Control vs KO mouse
- Fasting
- HFD challenge
- Dynamic temperature challenge
- Meal pattern analysis
- Drug treatment

PART II. Metabolic chambers



Wyler and Elmquist, unpublished results

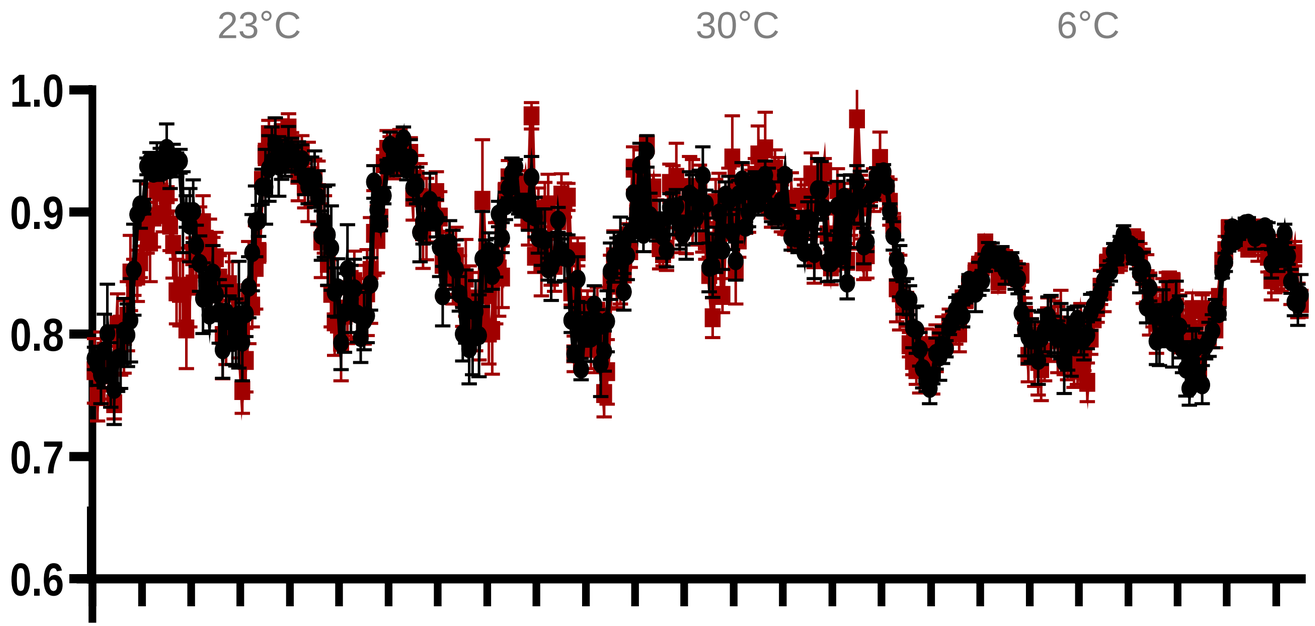
PART II. Metabolic chambers



Wyler and Elmquist, unpublished results

PART II. Metabolic chambers

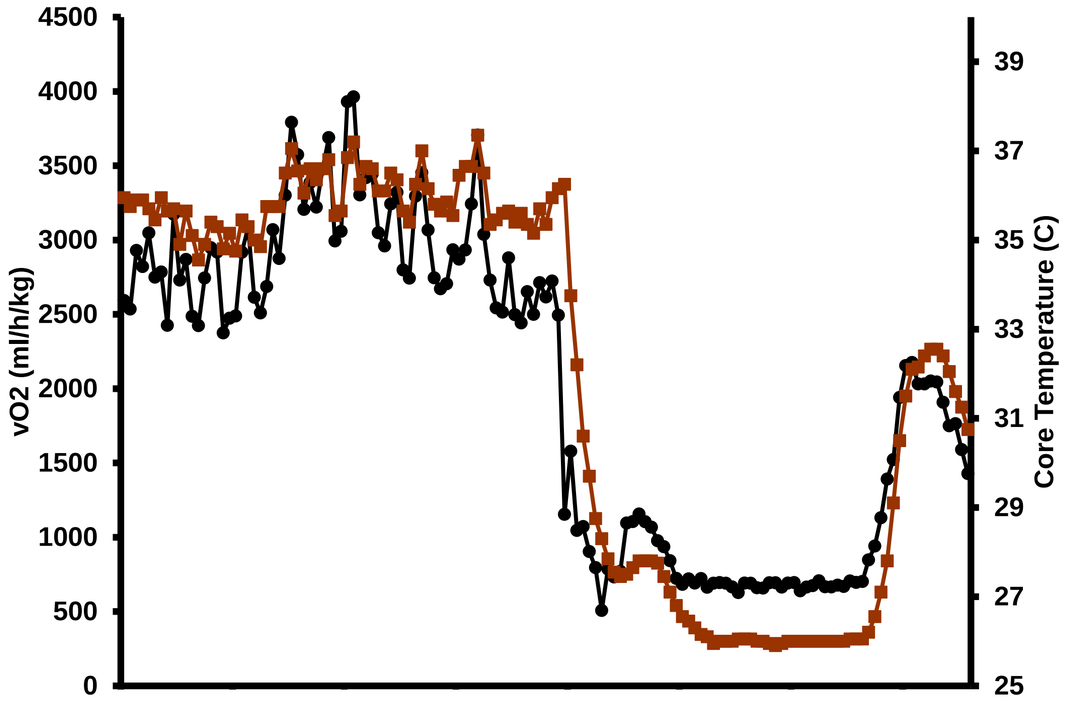
RESPIRATORY EXCHANGE RATIO (RER)



Wyler and Elmquist, unpublished results

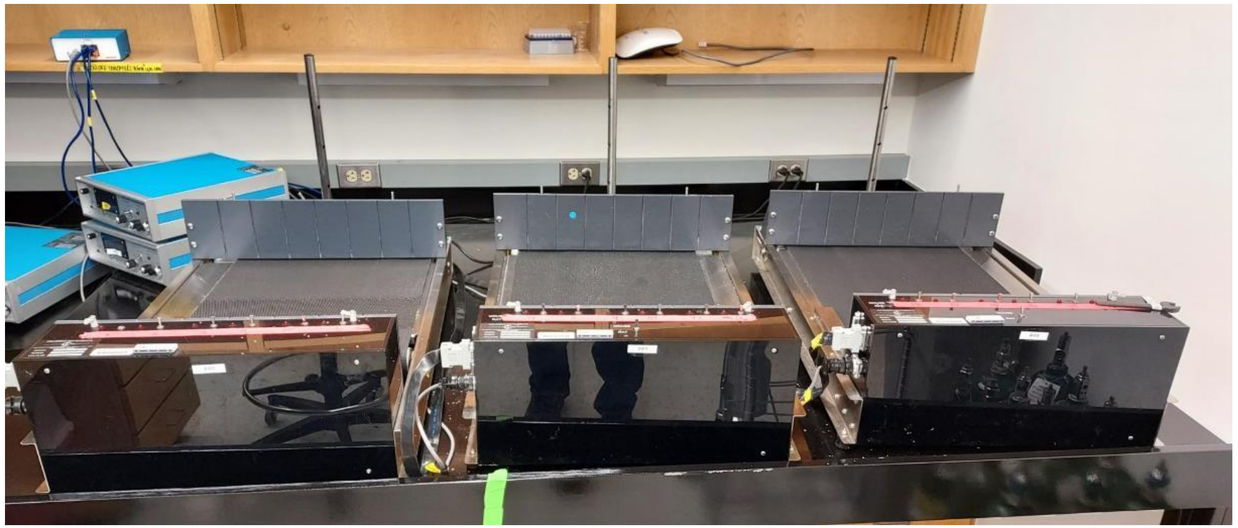
PART II. Metabolic chambers

OXYGEN CONSUMPTION WITH TELEMETRY



Gautron, unpublished results

PART II. Rodent Treadmills

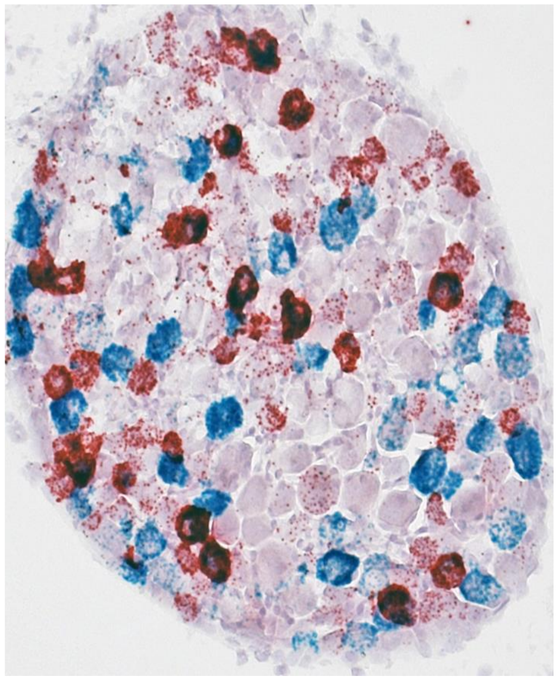


Example experimental designs

- Endurance Test
- Chronic Exercise Training
- Lactate and glucose before and after exerice

PART II. RNAscope and BaseScope Hybridization

- Fluorescent and chromogenic *in situ* hybridization
- Uniplex, duplex, triplex hybridization
- Includes a range of services from tissue preparation to hybridization

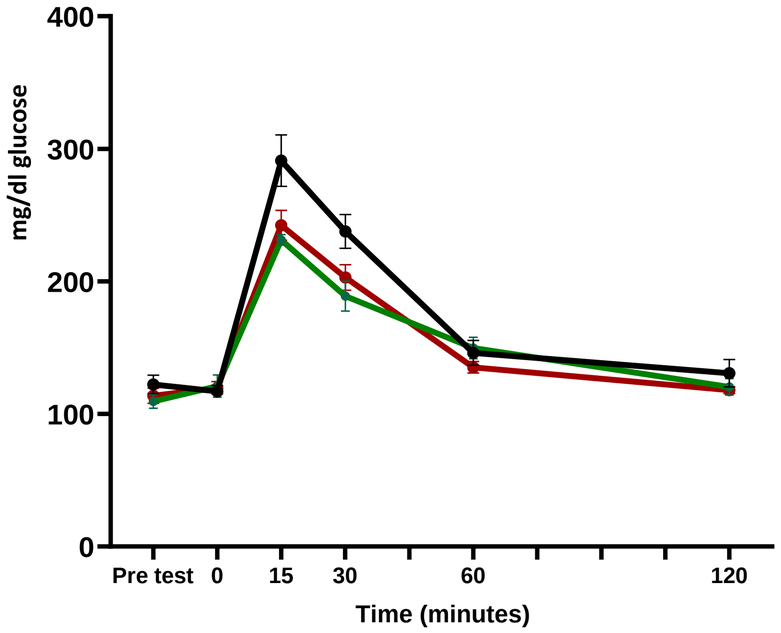


Detection of Ckkar (red) and Gpr65 (blue) by chromogenic duplex RNAScope *in situ* hybridization of the mouse nodose ganglion

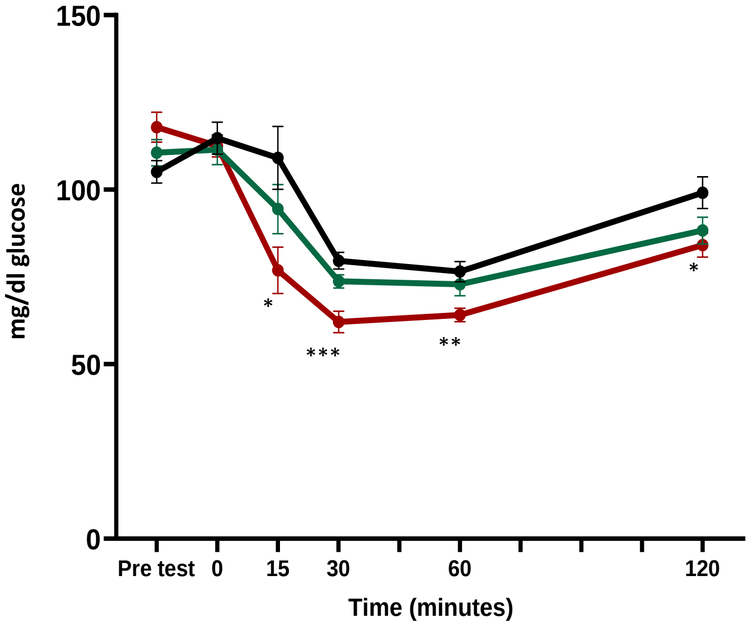
Gautron, unpublished results

PART II. Glucose homeostasis studies

Glucose Tolerance Test (GTT)

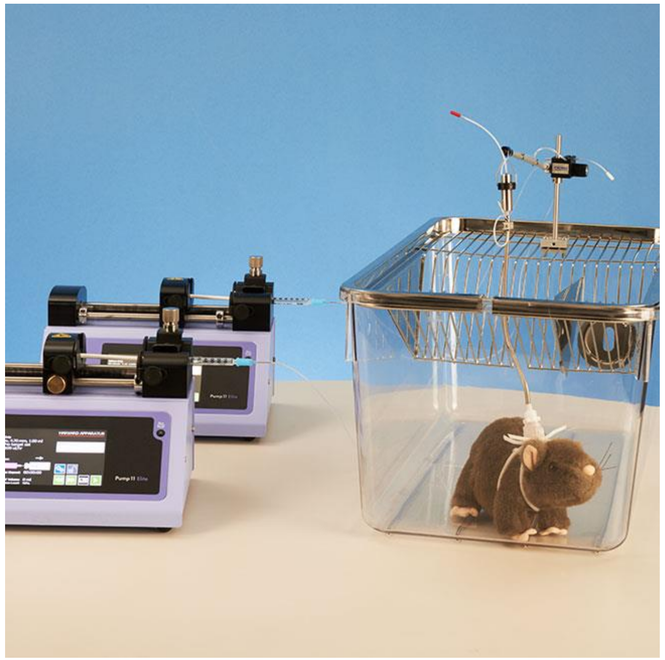


Insulin Tolerance Test (ITT)

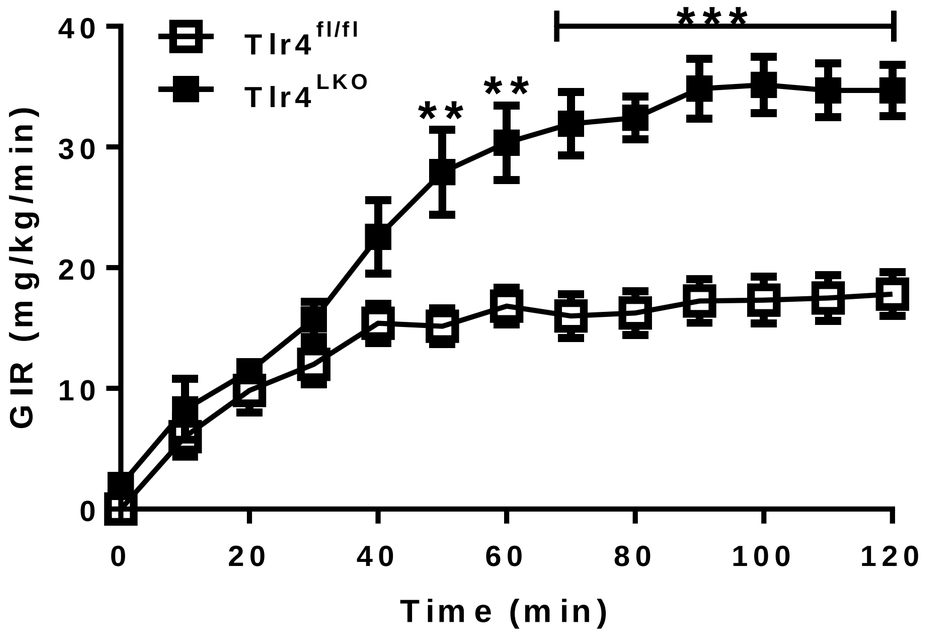


Wyler and Elmquist, unpublished results

PART II. Glucose homeostasis studies



Hyperinsulinemic-euglycemic clamps



Jia et al. doi: 10.1038/ncomms4878.

PART II. Body composition

Quantitative magnetic resonance imaging (qMRI)/
Nuclear Magnetic Resonance (NMR) analyzer

- Fat mass
- Lean mass
- Free water