



Catalog of Services

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MMPC Mission

Our mission is to advance medical and biological research by providing the scientific community with standardized, high quality metabolic and physiologic phenotyping services for mouse models of diabetes, diabetic complications, obesity and related disorders.

The six Centers are housed at outstanding academic institutions, staffed by experts in state-of-the-art technology. Researchers can ship mice to one of the Centers and obtain on a fee-for-service basis a range of complex exams used to characterize mouse metabolism, blood composition including hormones, energy balance and physical activity, eating and exercise, insulin resistance, organ function, metabolic fluxes and morphology, physiology, histology and measures of diabetic complications in heart, kidney, vasculature, eye, etc. Many tests are done in living animals and are designed to elucidate subtle to complex traits that would define models of metabolic disease.

The development of transgenic technology and gene targeting protocols has resulted in numerous mouse lines with specific phenotypes and well-defined DNA structural changes. Candidate genes for diabetes, obesity and other disorders of metabolism have been identified and transgenic mice are being generated using this technology. By broadening the availability of sophisticated metabolic phenotyping, we hope to help investigators identify and study new mouse models that will lead to an improved understanding of these complex diseases.

In 2006, the MMPC formed a collaboration with the NIH-sponsored Animal Models of Diabetic Complications Consortium (AMDCC) in order to more thoroughly phenotype putative new mouse models of disease for a range of complications including cardiovascular disease, nephropathy, neuropathy, retinopathy. The MMPC is committed to improving access to existing tests, and to developing new technologies for this purpose.

Goals

1. Broaden the scope of metabolic phenotyping tests for mice available to investigators.
2. Standardize key methodologies.
3. Expedite the completion of research.
4. Compile a database of information relevant to mouse models of diabetes, obesity, and diabetic complications.

Guidelines and Policies

The MMPC is sponsored by the National Institutes of Health as a resource to provide services to the community of scientists who use mice to study diabetes, obesity, diabetic complications, and other metabolic diseases. In order to accomplish this goal, the MMPC offers to researchers phenotyping tests that require specialized expertise or equipment. Modest fees for these tests are set at or below actual cost. Researchers can arrange to ship mice or murine tissues to the Centers for analysis. Complete information is available at www.mmmpc.org.

Center Structure & Steering Committees

Each Center has a structure that consists of an Executive Committee, an Administrative Core and Director, experimental and analytical Test Cores, an Animal Health and Welfare Core, and a Research & Development program. The MMPC program has a Coordinating and Bioinformatics Unit which houses the MMPC Pilot and Feasibility grant program, the MMPC website, and MMPC Database. This CBU is shared with the NIH-sponsored Animal Models of Diabetes Complications Consortium (AMDCC). Details for the structure and personnel at each MMPC can be obtained from the individual web sites. The six Centers share a National Steering Committee consisting of Center Directors, NIH personnel, and external advisors.

Application for Services

After identifying the appropriate Center(s) from the individual web pages or test catalog, www.mmmpc.org/shared/catalog.aspx, the applicant should first contact the Center Director or Core Director to discuss the mouse strain, determine the best set of tests to be conducted, and obtain an estimate of costs. The applicant then obtains a password protected account and completes an online request for services, www.mmmpc.org/shared/orderTest.aspx, which is targeted to the appropriate Center. The request is reviewed by the Center Executive Committee. Acceptance is based on Center workload, relevance of the available and/or requested tests to the mouse model, and the perceived value of the animal to diabetes, obesity, and metabolic disease research. The applicant will be contacted with the decision. Following consultation with the Center and/or Core Director(s), a written estimate for all tests agreed upon, including the number of mice required for each test and a timeline for receipt and testing of the mice at the MMPC, will be sent to the applicant for his/her approval.

Data

Tests will be conducted using the experimental protocols found on the individual MMPC web sites or in the catalog. Detailed descriptions will be provided upon request. Upon completion of the requested tests, data in an appropriate form will be stored in the MMPC database and posted on MMPC's password protected web site, www.mmmpc.org/secure/index.aspx, for viewing by the submitting investigator only. The Center personnel will be available to discuss experimental details, etc.

Data Ownership

All data generated from a submitted strain belongs to the submitting investigator and his/her institution.

Center personnel have no rights to use this data for personal or institutional research purposes unless a formal, documented arrangement of collaboration exists between Center personnel and the investigator.

The NIH strongly encourages the sharing of research data. NIH guidelines regarding data sharing can be found at <http://grants1.nih.gov/grants/guide/notice-files/NOT-OD-03-032.html>. This notice states,

We believe that data sharing is essential for expedited translation of research results into knowledge, products, and procedures to improve human health. The NIH endorses the sharing of final research data to serve these

and other important scientific goals. The NIH expects and supports the timely release and sharing of final research data from NIH-supported studies for use by other researchers... [The] definition of "the timely release and sharing" [is] to be no later than the acceptance for publication of the main findings from the final data set. NIH continues to expect that the initial investigators may benefit from first and continuing use but not from prolonged exclusive use.

All data collected at the Centers from background strains and standard disease models (i.e., those commercially available) will be stored in the publicly available MMPC database. It is anticipated that the complete set of data generated by the MMPCs on all newly generated strains, taken together in a database, will be valuable for understanding diabetes, obesity and other metabolic diseases.

Therefore, the NIH requests that investigators allow the data generated by an MMPC to be placed in a public MMPC database after the first of the following two conditions has been met:

1. The data have been published and are therefore in the public domain.
2. Two years have passed since the investigator received the data from the Center.

Because it is sometimes not possible to publish or even interpret data within a two-year timeframe, investigators may request that specific data be withheld from the public database for an additional period of time.

The Center personnel and the investigator must read and sign a Mouse / Tissue Transfer Agreement, www.mmmpc.org/documents/MTA.pdf, which clearly states these rights and responsibilities.

Fees

Price information for each test is available on the individual Center web pages or by request from each MMPC. Applicants will receive a written estimate that must be acknowledged by the submitting investigator before animals can be shipped for testing. Fees are set as a fraction of the total costs incurred by the MMPC for that test, and are calculated based on the obtainment of revenue neutrality.

Animal Guidelines

Please see animal care and shipping section of this catalog or www.mmmpc.org/shared/animalShipping.aspx. Specific instructions can be obtained from center personnel.

Acknowledgements

One index of success of the MMPC program is the contribution of data produced by the MMPC to publications and oral communications. Please acknowledge the MMPC when presenting data obtained using Center Services.

Animal Care and Shipping

Minimum testing required of mice received from other institutions

While each of the four institutions may require additional testing for mice received into their labs, below is an agreed upon list of the minimum testing that will be required prior to shipping. A health certificate, no older than 3 months, must be faxed/shipped to the receiving institution. This certificate must be received and reviewed before approval will be given to ship.

- Cilia-associated respiratory bacillus
- Clostridium piliforme (Tyzzer's disease)
- Ectromelia
- Lymphocytic choriomenengitis virus
- Mouse adenoviruses
- Mouse hepatitis virus
- Mouse parvoviruses
- Mouse rotavirus
- Mycoplasma pulmonis
- Pneumonia virus of mice
- Reovirus
- Sendai virus
- Theiler's virus (GD-7)
- Fur mites
- Pinworms

At this time, Vanderbilt routinely uses Fenbendazole medicated feed for four weeks and treats with Ivermectin as soon as the animals arrive. This center will continue to automatically treat their incoming animals. University of Cincinnati, Yale, and UT Southwestern will not treat unless testing indicates it is necessary.

Quarantine Testing

Below is an agreed upon list of tests to be performed while the mice are in quarantine. While each center will have additional tests that may vary, this is a list of the minimum testing to be performed for this program. As standard procedure, testing of incoming animals will include the storage of an aliquot of serum for retesting at a different lab in the case of positive test results. All researchers sending animals from a non-commercial source should attempt to send two additional animals, one to be tested upon arrival and the second to be tested in 3-4 weeks.

- **Serology:**
 - Cilia-associated respiratory bacillus
 - Clostridium piliforme (Tyzzer's disease)
 - Ectromelia
 - Lymphocytic choriomenengitis virus
 - Mouse adenoviruses
 - Mouse cytomegalovirus
 - Mouse hepatitis virus
 - Mouse parvoviruses
 - Mouse rotavirus
 - Mouse thymic virus
 - Mycoplasma pulmonis
 - Pneumonia virus of mice
 - Reovirus
 - Sendai virus

- Theiler's virus (GD-7)
- **Subgross examination of the pelt:**
 - Fur Mites
- **Subgross examination of the cecal and colon contents:**
 - Pinworms

Length of quarantine time for animals

Three to four weeks is the minimum amount of time required to release the animals into regular colonies. A one-week minimum period is appropriate for animals to recover from the stress of traveling and new environment prior to phenotyping. Vanderbilt does not allow researchers access to their animals before the 3-4 week quarantine period. After that time, they can remove the animals for terminal procedures only. UT Southwestern will not allow animals to leave quarantine before 8 weeks, but the researchers will be allowed access to them after the 3-4 week quarantine period. University of Cincinnati and Yale University will allow researchers access to the animals during the 3-4 week minimum quarantine, but the animals cannot be taken out of the facility except for terminal procedures.

Cages

- **Bedding:**
 - Bed-O-Cob bedding will be used, changed every two weeks.
- **Ventilation:**
 - Ventilated cages will be used.
- **Watering Systems:**
 - Not standardized.
- **Cage Capacity:**
 - Female: maximum 5/cage & minimum 2/cage
 - Male: maximum 2/cage (monitor for signs of stress)
- **Diet:**
 - Harland Teklad irradiated chow will be used at all centers. The product code is #7912, Tekland LM-485 Mouse/Rat Sterilizable Diet. In the cases of feeding studies, a semi-purified diet with defined composition will be used instead of the chow.

Shipping

- **Bax Global**
 - 1-800 CALL BAX
 - www.baxworld.com
- **World Courier**
 - 1-800-221-6600
 - www.worldcourier.com

Release of Protocol Information

All animal care protocols developed during the planning of this project will be shared with NIH.

- **Required Approvals:**

- All phenotyping tests carried out at the Centers must be done using protocols that have been previously approved by the University IACUC.
- **Current Center Capacities:**
 - University of Cincinnati Medical Center: 1,000 cages
 - University of Texas Southwestern Medical Center: 40 cages (anticipates increase)
 - Vanderbilt University Medical Center: 120 cages
 - Yale University School of Medicine: 120 cages (anticipates increase)

Committee on Animal Husbandry Issues

- Shawn Burgess, Ph.D. UT Southwestern
- Greg Hanley, DVM, Ph.D. Dipl. ACLAM Vanderbilt University
- Phil Howles, Ph.D. University of Cincinnati
- Todd Jackson, DVM, Dipl. ACLAM University of Cincinnati
- Robert Jacoby, DVM, Ph.D. Yale University
- James Macy, DVM, Dipl. ACLAM Yale University (11/02/01 meeting only)
- Patrick Tso, Ph.D. University of Cincinnati

Tests Listed by General Subject

The first letter of each Test No. denotes the center:

CA = Case Western Reserve University
 C = University of Cincinnati Medical Center
 T = University of Texas Southwestern Medical Center
 S = University of Washington, Seattle
 V = Vanderbilt University School of Medicine
 Y = Yale University School of Medicine

Amino Acid Metabolism

Test No.	Test Name	Keywords	
V3090	Full amino acid profiles by HPLC / PITC or HLPC / OPA		amino acids, HPLC
T2001	Sources of plasma glucose using 2H NMR		gluconeogenesis, glucose production, glycogenesis, hepatic, liver, metabolic spectroscopy
T2011	Intermediary metabolism in the isolated liver using NMR		hepatic, liver, metabolism, NMR, spectroscopy
V3009	Amino acid kinetics		amino acid flux, amino acid kinetics
V3091	Specific selected amino acid profiles		amino acids, HPLC
V3092	Radioactivity of specific individual amino acids		amino acids, chromatography, protein synthesis, proteolysis, specific activity
V3093	Specific activities for gluconeogenic and glycogenic assessment		amino acids, chromatography, protein synthesis, proteolysis, specific activity
CA2017	Tissue-specific protein synthesis using 2H ₂ O-labeled water	\$150.00 / sample	amino acids, metabolism, protein synthesis
CA2035	Metabolomic profile of amino acids in plasma		amino acids, metabolism, plasma
CA2036	Metabolomic profile of amino acids in urine		amino acids, metabolism, urine
CA2037	Metabolomic profile of amino acids in tissue		amino acids, metabolism, tissue

Body Composition

Test No.	Test Name	Keywords	
C1041	Body Composition		body composition, fat mass, obesity
CA2000	Body composition using 2H-labeled water	\$20.00 / sample	body composition, body weight, fat mass
CA2002	Body Weight	\$5.00 / animal / day	body composition, body weight, fat mass
S6101	Body Composition		body composition, energy balance, fat mass, obesity, water
V4004	Urine pH		pH, urine
Y4087	Blood Electrolytes-Na/Cl/K		electrolytes, metabolism, muscle, potassium, serum chemicals, serum panel, sodium
Y5004	Blood Calcium		serum chemicals
Y5005	Blood Inorganic Phosphorous		inorganic phosphate, phosphate, serum chemicals
Y5007	Magnesium		serum chemicals
Y5006	Urine Inorganic Phosphorous		inorganic phosphate, phosphate, urine

Test No.	Test Name	Keywords
Y5010	Apolipoprotein C3	lipids, lipoproteins, serum chemical
Y4088	Urine Electrolytes-Na/K/Cl	electrolytes, muscle, potassium, u

Carbohydrate Metabolism

Test No.	Test Name	Keywords
C1070	Glucose tolerance tests (intraperitoneal glucose tolerance, oral glucose tolerance)	diabetes, insulin action, insulin secretion
C1088	Plasma Glucose-dependent insulinotropic peptide (GIP) concentration	gut, hormone, lipids, metabolism
C1072	Insulin Sensitivity Test	diabetes, insulin action, insulin secretion, metabolism
C1087	Glucose enrichment and concentration	carbohydrate metabolism, diabetes
T2001	Sources of plasma glucose using 2H NMR	gluconeogenesis, glucose production, glycogenesis, hepatic, liver, metabolism, spectroscopy
T2002	Gluconeogenic and citric acid cycle pathways (relative fluxes using 2H, 13C and J-HSQC NMR)	citric acid cycle, gluconeogenesis, Krebs's cycle, liver, spectroscopy, T
T2003	Absolute gluconeogenic flux rates	gluconeogenesis, glucose production, glycogenolysis, hepatic, liver, metabolism
V3003	Glucose Tolerance Test (Oral and Intravenous)	glucose intolerance, glucose tolerance, insulin action
V3004	Glucose turnover	endogenous glucose production, glucose kinetics, glucose turnover, tracers
V3005	Hyperinsulinemic clamp	hyperinsulinemic clamp, insulin action, resistance
V3006	Hyperglycemic clamp	hyperglycemic clamp, insulin secretion, pancreas
V3007	Gluconeogenesis & glycogenolysis (from hepatic 14C-UDPglucose and PEP)	gluconeogenesis, glucose production, glycogenolysis, liver
V3008	Glycogen synthesis	glycogen synthesis, liver, muscle
V3010	Tissue specific glucose uptake	2-deoxyglucose, glucose metabolism, tissue specific glucose uptake
Y4001	Hyperinsulinemic-euglycemic clamp experiments	insulin action, insulin resistance
CA2004	Glucose tolerance tests (GTT)	\$52.00 / animal / test carbohydrate metabolism, diabetes
CA2005	Insulin concentrations at fasting and post intraperitoneal glucose administration	insulin, insulin secretion
CA2006	Plasma insulin measurement by ELISA	\$9.50 / mouse carbohydrate metabolism, insulin, action
CA2007	Insulin concentrations at fasting and post intraperitoneal insulin administration	
CA2008	Glucose concentrations at fasting and post intraperitoneal insulin administration - insulin tolerance test (ITT)	carbohydrate metabolism, diabetes, sensitivity
CA2013	Hyperinsulinemic Clamp (Hypoglycemic or Euglycemic) using stable isotopes	INQUIRE / animal / test

Test No.	Test Name	Keywords	
CA2024	Metabolomic profile of citric acid cycle and gluconeogenic intermediates	\$150.00 / sample [for concentration]	
C1071	Plasma glucose levels		carbohydrate, diabetes, metabolism
S6120	Intraperitoneal Glucose Tolerance Test		carbohydrate metabolism, diabetes intolerance, glucose tolerance, insulin
S6121	Insulin Sensitivity Test		carbohydrate metabolism, diabetes tolerance, insulin action, insulin resistance, insulin sensitivity
Y4002	Hyperglycemic clamp experiments		insulin secretion, pancreas
V4005	Glycemic Control using Minimed		glucose
Y4083	Blood Glucose		carbohydrate, diabetes, glucose, pancreatic serum chemicals, serum metabolism

Cardiac Function

Test No.	Test Name	Keywords	
C1003	Arterial baroreflex responses		cardiac function, vascular tone
C1010	Cardiac output		contractility, ejection fraction, heart volume
C1020	Cardiac contractility (left ventricular function in the isolated heart)		cardiac, heart, pressure, ventricular
C1021	Echocardiography		cardiac, heart, morphology
C1022	Left ventricular pressure measurements in intact mice		cardiac, heart, pressure, ventricular
T2010	Substrate oxidation and anaplerosis in the isolated heart		anaplerosis, cardiac, heart, metabolism spectroscopy, substrate oxidation
T2013	TCA cycle flux (VTCA) and alpha-ketoglutarate-glutamate exchange flux (Vx) in the isolated mouse heart using 1H NMR		citric acid cycle, heart, Krebs's cycle, metabolism, NMR, spectroscopy, T
T2012	Intracellular sodium or high-energy phosphates in the isolated perfused mouse liver or heart		heart, high-energy phosphates, liver, sodium, spectroscopy
V3030	In vitro Morphology, Morphometrics and Histology (isolated heart)		cardiac function, heart, morphology
V3031	Echocardiography, in vivo morphology, systolic and diastolic function; Stress echocardiography		diastolic, echocardiography, morphology, stress, systolic
V3032	Electrocardiography and telemetry		cardiac, ECG, EKG, electrocardiography, telemetry
V3095	Heart Rate Open Variability		
V3096	Ventricular Hemodynamics		
S6200	Echocardiography (non-invasive)		cardiac function, echocardiography, morphology
S6201	Electrocardiography - ECG (non-invasive)		echocardiography, heart
S6202	Invasive Hemodynamics - Left Ventricular Catheterization/Millar		cardiac function, cardiac output, echocardiography, heart, pressure, volume, ventricular
S6204	Telemetry (invasive)		cardiac, ECG, EKG, electrocardiography

Test No.	Test Name	Keywords
		telemetry
S6205	Blood Pressure (non-invasive)	blood flow, blood pressure, hypertension, vascular
S6206	Carotid Stenosis - Arterial response to injury	blood vessel, endothelial denudation, histology, neointimal hyperplasia, muscle, vascular
Y4091	Blood Albumin	liver, plasma, serum albumin, serum chemicals
Y5007	Magnesium	serum chemicals
S6207	Myocardial Infarction	cardiac, heart
S6208	Hindlimb Ischemia	blood vessel, cardiac, cardiac function, hypertension, hypotension, restenosis, vascular
S6209	Open Thoracotomy	surgery
S6210	Vein Catheter Insertion	catheterization, surgery, vascular, vein
S6211	Bone Marrow Transplantation	
S6212	Drug Treatment	
S6213	Ultrasound Imaging - Aortic	cardiac function, heart

Central Nervous System

Test No.	Test Name	Keywords
C1043	Hypothalamic Gene Expression	central nervous system, hormone, hypothalamus, neuroendocrine

Circulation

Test No.	Test Name	Keywords
C1002	Inter-arterial pressure	blood pressure, blood vessel, hypertension, vascular
C1001	Tail Cuff Blood pressure	blood pressure, blood vessel, hypertension, vascular
C1003	Arterial baroreflex responses	cardiac function, vascular tone
C1010	Cardiac output	contractility, ejection fraction, heart volume
C1011	Regional Blood Flow Measurements	blood flow, blood pressure, smooth muscle, vascular
C1013	Arterial response to injury (neointimal hyperplasia)	angioplasty, endothelial denudation, neointimal hyperplasia, restenosis
C1014	Vascular contractility measurements	aortic ring, contractility, vascular tone
C1020	Cardiac contractility (left ventricular function in the isolated heart)	cardiac, heart, pressure, ventricular function
C1021	Echocardiography	cardiac, heart, morphology
C1022	Left ventricular pressure measurements in intact mice	cardiac, heart, pressure, ventricular function
V3033	Blood pressure measurements	blood pressure, blood vessel, hypertension, vascular

Test No.	Test Name	Keywords
V3034	Vascular morphology	blood vessel, histology, intima, smooth muscle, vascular
C1012	Renal blood flow regulation (free flow measurements)	blood flow, kidney, renal, vascular
Y5007	Magnesium	serum chemicals
S6230	Myography - basic	
S6231	Myography - additional	

Diabetes

Test No.	Test Name	Keywords
C1070	Glucose tolerance tests (intraperitoneal glucose tolerance, oral glucose tolerance)	diabetes, insulin action, insulin secretion
C1072	Insulin Sensitivity Test	diabetes, insulin action, insulin secretion, metabolism
C1087	Glucose enrichment and concentration	carbohydrate metabolism, diabetes
T2001	Sources of plasma glucose using 2H NMR	gluconeogenesis, glucose production, glycogenesis, hepatic, liver, metabolic spectroscopy
T2002	Gluconeogenic and citric acid cycle pathways (relative fluxes using 2H, 13C and J-HSQC NMR)	citric acid cycle, gluconeogenesis, Krebs's cycle, liver, spectroscopy, T
T2003	Absolute gluconeogenic flux rates	gluconeogenesis, glucose production, glycogenolysis, hepatic, liver, metabolic
V3002	Jugular vein and carotid artery catheterization	blood vessel, chronic, surgery
V3004	Glucose turnover	endogenous glucose production, glucose kinetics, glucose turnover, tracers
V3005	Hyperinsulinemic clamp	hyperinsulinemic clamp, insulin action, resistance
V3006	Hyperglycemic clamp	hyperglycemic clamp, insulin secretion, pancreas
V3007	Gluconeogenesis & glycogenolysis (from hepatic 14C-UDPglucose and PEP)	gluconeogenesis, glucose production, glycogenolysis, liver
V3008	Glycogen synthesis	glycogen synthesis, liver, muscle
V3010	Tissue specific glucose uptake	2-deoxyglucose, glucose metabolism, tissue specific glucose uptake
V3082	Tissue microdissection	laser microdissection, pancreas
Y4001	Hyperinsulinemic-euglycemic clamp experiments	insulin action, insulin resistance
CA2004	Glucose tolerance tests (GTT)	\$52.00 / animal / test carbohydrate metabolism, diabetes
CA2005	Insulin concentrations at fasting and post intraperitoneal glucose administration	insulin, insulin secretion
CA2006	Plasma insulin measurement by ELISA	\$9.50 / mouse carbohydrate metabolism, insulin, action
CA2007	Insulin concentrations at fasting and post intraperitoneal insulin administration	
CA2008	Glucose concentrations at fasting and post	carbohydrate metabolism, diabetes

Test No.	Test Name	Keywords	
	intraperitoneal insulin administration - insulin tolerance test (ITT)		sensitivity
CA2013	Hyperinsulinemic Clamp (Hypoglycemic or Euglycemic) using stable isotopes	INQUIRE / animal / test	
C1071	Plasma glucose levels		carbohydrate, diabetes, metabolism
S6120	Intraperitoneal Glucose Tolerance Test		carbohydrate metabolism, diabetes intolerance, glucose tolerance, insulin
S6121	Insulin Sensitivity Test		carbohydrate metabolism, diabetes tolerance, insulin action, insulin resistance, insulin sensitivity
S6122	Drug treatment (Streptozotocin; other)		
Y4002	Hyperglycemic clamp experiments		insulin secretion, pancreas
Y4083	Blood Glucose		carbohydrate, diabetes, glucose, plasma, serum chemicals, serum metabolism
Y4098	HDL Cholesterol		cholesterol, lipids, lipoproteins
Y4099	LDL Cholesterol		cholesterol, lipids, plasma, serum
Y5000	Cholesterol		cholesterol, lipids, plasma, serum
Y5003	Beta-Hydroxybutyrate (COBAS)		diabetes, ketones, plasma, serum
S6230	Myography - basic		
S6231	Myography - additional		
S6105	Running Wheels Activity		
S6140	General Chemistry - Glucose		
S6141	Lipids - Lipid Extraction		
S6142	Lipids - Free Fatty Acids		
S6143	Lipids - HDL		
S6144	Lipids - Triglyceride TG		
S6145	Lipids - Cholesterol TC		
S6146	Lipids - FPLC		
S6147	Cytokines & Hormones - TNF Alpha		
S6148	Cytokines & Hormones - IL-6		
S6149	Cytokines & Hormones - IL-4		
S6150	Cytokines & Hormones - Leptin		
S6151	Cytokines & Hormones - Insulin		
S6152	Cytokines & Hormones - PAI-1 and others		
S6153	Cytokines & Hormones - Glucagon		
S6154	Cytokines & Hormones - Adiponectin		
S6155	Cytokines & Hormones - Urine Albumin		
S6156	Cytokines & Hormones - Urine Creatinine		
S6157	Cytokines & Hormones - BUN		
S6158	Cytokines & Hormones - Insulin (by Eliza)		
S6159	Cytokines & Hormones - Taqman PCR Quantification		

Energetics

Test No.	Test Name	Keywords	
T2002	Gluconeogenic and citric acid cycle pathways (relative fluxes using 2H, 13C and J-HSQC NMR)		citric acid cycle, gluconeogenesis, Krebs's cycle, liver, spectroscopy, T
T2010	Substrate oxidation and anaplerosis in the isolated heart		anaplerosis, cardiac, heart, metab spectroscopy, substrate oxidation
T2013	TCA cycle flux (VTCA) and alpha-ketoglutarate-glutamate exchange flux (Vx) in the isolated mouse heart using 1H NMR		citric acid cycle, heart, Krebs's cycl metabolism, NMR, spectroscopy, T
T2012	Intracellular sodium or high-energy phosphates in the isolated perfused mouse liver or heart		heart, high-energy phosphates, liv sodium, spectroscopy
CA2015	Turnover of glucose, lipid and/or protein	\$200.00 / 4 samples / mouse	
CA2022	13C-Labeling pattern of acetyl moiety of citrate (substrate oxidation)	\$110/sample	

Energy Expenditure & Exercise

Test No.	Test Name	Keywords	
C1042	Energy Expenditure Measurements		body weight, energy balance
V3012	Indirect calorimetry /energy expenditure		carbon dioxide, energy expenditure exchange, indirect calorimetry, ox
V3013	Exercise capacity (metabolic response to exercise)		endurance, exercise capacity, exe tolerance
V3014	Spontaneous exercise activity		spontaneous exercise activity, wh
V3016	Exploratory locomotor activity		energy expenditure, exploratory lo activity
CA2003	Continuous measurement of body temperature	\$5.00 / animal / day	energy expenditure, exercise
CA2011	Total Energy expenditure using 2H2O-labeled water	\$100.00 / 4 samples / mouse	energy expenditure, water
S6102	Energy Expenditure		body weight, energy balance, ene expenditure
S6104	Body Temperature		
Y5003	Beta-Hydroxybutyrate (COBAS)		diabetes, ketones, plasma, serum
S6105	Running Wheels Activity		

Enzymatic Activity

Test No.	Test Name	Keywords	
Y5008	Creatine Kinase		creatine kinase, serum chemicals

Food Intake

Test No.	Test Name	Keywords	
C1040	Food intake and body weight measurements		energy balance, energy expenditure
C1041	Body Composition		body composition, fat mass, obesi

Test No.	Test Name	Keywords	
C1043	Hypothalamic Gene Expression		central nervous system, hormone, hypothalamus, neuroendocrine
V3015	Food Consumption		spontaneous exercise activity, wh
CA2001	Food Consumption	\$5.00 / animal / day	food intake
CA2002	Body Weight	\$5.00 / animal / day	body composition, body weight, fo
C1044	DietMax Meal Pattern Analysis		food intake
S6103	Meal Pattern Analysis		food intake

Hormone Measurements

Test No.	Test Name	Keywords	
V3090	Full amino acid profiles by HPLC / PITC or HLPC / OPA		amino acids, HPLC
C1088	Plasma Glucose-dependent insulintropic peptide (GIP) concentration		gut, hormone, lipids, metabolism
C1081	C-Peptide		diabetes, hormone, insulin
C1082	Cholecystokinin (CCK)		CCK, gut, hormone, intestine
C1085	Plasma/serum concentrations glucagon		counterregulatory, hormone, panc
C1086	Plasma Glucagon-like peptide 1 (GLP-1) concentration		counterregulatory, hormone, panc
C1089	Insulin concentrations in plasma/serum/lymph/cerebrospinal fluid		diabetes, hormone, pancreas
C1090	Plasma/serum concentrations of leptin		eating behaviour, fat, hormone, lip
C1091	Somatostatin in plasma or tissue extracts		diabetes, hormone, pancreas
V3050	Insulin		hormone
V3051	Glucagon		hormone
V3052	Corticosterone		hormone
V3053	Catecholamines		hormone
V3054	Leptin		hormone
V3055	C-Peptide		hormone
V3056	Growth Hormone (GH)		hormone
V3058	TSH		hormone
V3059	PRL		hormone
V3060	ACTH		hormone
V3061	Insulin-like growth hormone-1 (IGF-1)		hormone
Y4080	Insulin		hormone
Y4081	Glucagon		hormone
Y4082	Leptin		hormone
V3062	Aldosterone		hormone
V3064	Resistin		hormone
V3065	Adiponectin		hormone
V3066	Estradiol		hormone
V3067	Testosterone		hormone

Imaging

Test No.	Test Name	Keywords	
V3090	Full amino acid profiles by HPLC / PITC or HLPC / OPA		amino acids, HPLC
V3017	Assess real time imaging of cellular metabolic events		islets, metabolism, microcirculation, real time imaging
V3018	In vivo optical imaging of gene expression		gene expression, GFP, luciferase

Immunology of Diabetes

Test No.	Test Name	Keywords	
V3082	Tissue microdissection		laser microdissection, pancreas

Insulin and Insulin Function

Test No.	Test Name	Keywords	
C1070	Glucose tolerance tests (intraperitoneal glucose tolerance, oral glucose tolerance)		diabetes, insulin action, insulin secretion
C1072	Insulin Sensitivity Test		diabetes, insulin action, insulin secretion, metabolism
V3002	Jugular vein and carotid artery catheterization		blood vessel, chronic, surgery
V3005	Hyperinsulinemic clamp		hyperinsulinemic clamp, insulin action, resistance
V3006	Hyperglycemic clamp		hyperglycemic clamp, insulin secretion, pancreas
V3082	Tissue microdissection		laser microdissection, pancreas
Y4001	Hyperinsulinemic-euglycemic clamp experiments		insulin action, insulin resistance
CA2004	Glucose tolerance tests (GTT)	\$52.00 / animal / test	carbohydrate metabolism, diabetes
CA2005	Insulin concentrations at fasting and post intraperitoneal glucose administration		insulin, insulin secretion
CA2006	Plasma insulin measurement by ELISA	\$9.50 / mouse	carbohydrate metabolism, insulin, action
CA2007	Insulin concentrations at fasting and post intraperitoneal insulin administration		
CA2008	Glucose concentrations at fasting and post intraperitoneal insulin administration - insulin tolerance test (ITT)		carbohydrate metabolism, diabetes, sensitivity
CA2013	Hyperinsulinemic Clamp (Hypoglycemic or Euglycemic) using stable isotopes	INQUIRE / animal / test	
S6120	Intraperitoneal Glucose Tolerance Test		carbohydrate metabolism, diabetes, intolerance, glucose tolerance, insulin
S6121	Insulin Sensitivity Test		carbohydrate metabolism, diabetes, tolerance, insulin action, insulin resistance, insulin sensitivity
Y4002	Hyperglycemic clamp experiments		insulin secretion, pancreas

Isolated Organ and Cell Perfusion

Test No.	Test Name	Keywords	
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Test No.	Test Name	Keywords
C1020	Cardiac contractility (left ventricular function in the isolated heart)	cardiac, heart, pressure, ventricular
T2010	Substrate oxidation and anaplerosis in the isolated heart	anaplerosis, cardiac, heart, metab spectroscopy, substrate oxidation
T2013	TCA cycle flux (VTCA) and alpha-ketoglutarate-glutamate exchange flux (Vx) in the isolated mouse heart using ¹ H NMR	citric acid cycle, heart, Kreb's cycl metabolism, NMR, spectroscopy, T
T2012	Intracellular sodium or high-energy phosphates in the isolated perfused mouse liver or heart	heart, high-energy phosphates, liv sodium, spectroscopy
T2011	Intermediary metabolism in the isolated liver using NMR	hepatic, liver, metabolism, NMR, s
V3030	In vitro Morphology, Morphometrics and Histology (isolated heart)	cardiac function, heart, morpholog
V3094	Perfusion-Fixation/Heart Dimension	

Kidney Function

Test No.	Test Name	Keywords
C1030	Micropuncture measurements	blood flow, kidney, renal, vascular
C1032	In situ microperfusion	kidney, renal, vascular
C1033	Control of Renal Perfusion Pressure	glomerular filtration, kidney, renal
C1034	Whole kidney clearance	glomerular filtration, kidney, renal
V3036	Metabolic panel	
C1031	Renal Blood Flow Regulation	blood flow, kidney, renal, vascular
C1012	Renal blood flow regulation (free flow measurements)	blood flow, kidney, renal, vascular
V3098	GFR-FITC-Inulin; HPLC Cr	glomerular filtration, HPLC, kidney
V3099	Albuminuria	kidney, urine
V4000	Renal Blood Flow (Doppler)	blood flow, blood pressure, kidney
Y4084	Blood Urea Nitrogen	renal, serum chemicals, serum me panel
Y4085	Blood Creatinine-HPLC	kidney, muscle, renal, serum cher serum metabolic panel
Y4086	Urine Creatinine-HPLC	kidney, muscle, renal, serum cher serum metabolic panel, urine
Y4087	Blood Electrolytes-Na/Cl/K	electrolytes, metabolism, muscle, potassium, serum chemicals, seru panel, sodium
Y5009	Lactate Dehydrogenase	serum chemicals

Lipid Metabolism

Test No.	Test Name	Keywords
C1051	Intestinal lipid absorption in the conscious mouse	absorption, fistula, gastrointestinal lipids, lymph
C1052	Plasma lipid profiles	fat, lipids, metabolism

Test No.	Test Name	Keywords	
C1053	Lipoprotein profiles		cholesterol, fat, metabolism
C1054	Lipoprotein fractionation by FPLC		cholesterol, fat, metabolism
C1055	Chylomicron metabolism (lymph)		absorption, chylomicron, fat, lipids, lipoproteins
C1056	Cholesterol synthetic rate		cholesterol, fat, lipids, synthesis
C1057	Plasma Free fatty Acid Levels		fat, lipids, metabolism
V3011	Tissue specific fatty acid uptake		125I-BMIPP, tissue specific fatty acid
V3070	Plasma lipids		fat, lipids, metabolism
V3071	Lipid extraction, separation, quantitation		fat, lipids, metabolism
V3072	Fatty acid profiles of lipid esters by gas liquid chromatography		fat, GCMS, lipids, metabolism
V3073	Quantitation of individual phospholipid classes		cholesterol, fat, lipids, metabolism, phospholipids
V3074	Short chain fatty acid analysis by gas liquid chromatography		fat, GCMS, lipids, metabolism, short chain fatty acid
V3075	Lipoprotein fractionation and characterization		fat, lipids, lipoproteins, metabolism
Y4060	Diacylglycerol concentration		fat, lipids, metabolism, signaling
Y4072	Lipid Panel		serum chemicals, serum metabolites
CA2010	Plasma triglycerides		plasma
CA2016	Fatty acid and cholesterol synthesis using 2H-labeled water	\$75.00 / sample	
CA2018	Profile of acylcarnitines in plasma/urine tissue samples	\$75.00/plasma or/urine sample \$90.00/tissue sample	
CA2019	Profile of long chain acyl-CoAs in tissue	\$150.00 per sample [for concentration]	
CA2020	Measurement of acetyl-CoA, propionyl-CoA and/or succinyl-CoA in tissue	\$225.00/sample (concentration) and C13 labeling pattern	
CA2021	Measurement of Methylmalonyl-CoA in tissue	\$225.00/sample (concentration) and C13 labeling pattern	
CA2023	Activity of acetyl-CoA carboxylase or malonyl-CoA decarboxylase in tissues	\$75.00 per assay	
C1059	Non-invasive measurement of fat absorption		absorption, fat, lipids, metabolism
C1060	Chemical determination of phospholipid		fat, lipids
C1061	Serum/Plasma Adiponectin		adipose, fat, hormone, lipids, metabolism
C1062	Serum/Plasma Resistin		adipose, fat, hormone, lipids
Y4061	Lysophosphatidic Acid		lipids
Y4098	HDL Cholesterol		cholesterol, lipids, lipoproteins
Y4099	LDL Cholesterol		cholesterol, lipids, plasma, serum
Y5000	Cholesterol		cholesterol, lipids, plasma, serum
Y5001	Triglycerides		lipids, plasma, serum chemicals
Y5002	Non-Esterified Fatty Acids		fatty acids, lipids, non-esterified fatty acids, serum chemicals

Test No.	Test Name	Keywords
Y5003	Beta-Hydroxybutyrate (COBAS)	diabetes, ketones, plasma, serum
Y5010	Apolipoprotein C3	lipids, lipoproteins, serum chemicals

Liver Function

Test No.	Test Name	Keywords
T2001	Sources of plasma glucose using 2H NMR	gluconeogenesis, glucose production, glycogenesis, hepatic, liver, metabolic spectroscopy
T2002	Gluconeogenic and citric acid cycle pathways (relative fluxes using 2H, 13C and J-HSQC NMR)	citric acid cycle, gluconeogenesis, Krebs's cycle, liver, spectroscopy, T
T2003	Absolute gluconeogenic flux rates	gluconeogenesis, glucose production, glycogenolysis, hepatic, liver, metabolic
T2012	Intracellular sodium or high-energy phosphates in the isolated perfused mouse liver or heart	heart, high-energy phosphates, liver, sodium, spectroscopy
T2011	Intermediary metabolism in the isolated liver using NMR	hepatic, liver, metabolism, NMR, spectroscopy
V3004	Glucose turnover	endogenous glucose production, glucose kinetics, glucose turnover, tracers
V3007	Gluconeogenesis & glycogenolysis (from hepatic 14C-UDPglucose and PEP)	gluconeogenesis, glucose production, glycogenolysis, liver
V3008	Glycogen synthesis	glycogen synthesis, liver, muscle
Y4091	Blood Albumin	liver, plasma, serum albumin, serum chemicals
Y4092	Alanine Aminotransferase	ALT, liver, plasma, serum chemicals
Y4093	Aspartate Aminotransferase	liver, plasma, serum chemicals
Y4094	Alkaline Phosphatase	liver, plasma, serum chemicals
Y4095	Total Bilirubin	bilirubin, liver, serum chemicals
Y4097	Total Protein	liver, plasma, serum chemicals
Y5000	Cholesterol	cholesterol, lipids, plasma, serum
Y5009	Lactate Dehydrogenase	serum chemicals

Metabolite Concentration and Enrichment

Test No.	Test Name	Keywords
V3090	Full amino acid profiles by HPLC / PITC or HLPC / OPA	amino acids, HPLC
C1057	Plasma Free fatty Acid Levels	fat, lipids, metabolism
C1087	Glucose enrichment and concentration	carbohydrate metabolism, diabetes
C1092	Plasma/Organ Triglycerides	fat, lipids, metabolism
T2010	Substrate oxidation and anaplerosis in the isolated heart	anaplerosis, cardiac, heart, metabolic spectroscopy, substrate oxidation
V3036	Metabolic panel	
V3070	Plasma lipids	fat, lipids, metabolism

Test No.	Test Name	Keywords	
V3075	Lipoprotein fractionation and characterization		fat, lipids, lipoproteins, metabolism
V3091	Specific selected amino acid profiles		amino acids, HPLC
V3092	Radioactivity of specific individual amino acids		amino acids, chromatography, protein synthesis, proteolysis, specific activity
V3093	Specific activities for gluconeogenic and glycogenic assessment		amino acids, chromatography, protein synthesis, proteolysis, specific activity
Y4050	Amino Acids		amino acids, enrichment, isotopes
Y4051	Beta-hydroxybutyrate		diabetes, enrichment, ketones, metabolism
Y4052	Free fatty acid		diabetes, enrichment, fat, lipids, metabolism
Y4053	Glucose		carbohydrate, diabetes, metabolism
Y4054	Glycerol		diabetes, enrichment, lipids, metabolism
Y4055	Glycogen		carbohydrate, diabetes, metabolism
Y4057	Long-chain fatty acyl CoA esters		fat, lipids, metabolism
Y4059	ADP, ATP		energetics, high-energy phosphate, mitochondria
Y4070	Chem 7		serum chemicals, serum metabolism
Y4071	Liver Function Tests		serum chemicals, serum metabolism
Y4072	Lipid Panel		serum chemicals, serum metabolism
Y4073	Divalent Ions		serum chemicals
CA2009	Triglycerides in liver		lipids, liver
CA2010	Plasma triglycerides		plasma
C1058	Plasma beta-hydroxybutyrate levels		diabetes, ketones, metabolism
C1083	Cholesterol (Total, HDL, LDL)		cholesterol, fat, lipids, metabolism
V4001	Urine Na/K		plasma, potassium, sodium, urine
V4002	Osmometer Plasma/Urine		osmolality, plasma, urine
V4003	Urine Ca/Phosphorus Excretion		urine
CA2025	Chronic arterial and jugular vein catheterization	\$51.00 / animal	catheterization, surgery
CA2026	Chronic arterial or jugular vein catheterization	\$28.00 / animal	catheterization, surgery
CA2028	Acute arterial or jugular vein catheterization	\$22.00 / animal / test	catheterization, surgery
CA2029	Acute portal vein catheterization	\$47.00 / animal / test	catheterization, surgery
CA2030	Implant [G2 E - Mitters™]	\$20.00 / animal	surgery
CA2032	Metabolomic profile of organic acids in plasma		metabolism, plasma
CA2033	Metabolomic profile of organic acids in urine		metabolism, urine
CA2034	Metabolomic profile of organic acids in tissue		metabolism, tissue
CA2035	Metabolomic profile of amino acids in plasma		amino acids, metabolism, plasma
CA2036	Metabolomic profile of amino acids in urine		amino acids, metabolism, urine
CA2037	Metabolomic profile of amino acids in tissue		amino acids, metabolism, tissue
CA2038	Metabolomic profile of free fatty acids in plasma		fatty acids, metabolism, plasma
CA2039	Metabolomic profile of free fatty acids in urine		fatty acids, metabolism, urine
CA2040	Metabolomic profile of free fatty acids in tissue		fatty acids, metabolism, tissue
CA2045	Measurement of ATP/ADP concentration in		ATP, tissue

Test No.	Test Name	Keywords
	tissue	

Modeling and Simulation

Test No.	Test Name	Keywords
V3090	Full amino acid profiles by HPLC / PITC or HLPC / OPA	amino acids, HPLC
T2020	Simulating the consequences of genetic manipulations	model, simulation

Magnetic Resonance Spectroscopy & Imaging

Test No.	Test Name	Keywords
T2001	Sources of plasma glucose using 2H NMR	gluconeogenesis, glucose production, glycogenesis, hepatic, liver, metabolism, spectroscopy
T2002	Gluconeogenic and citric acid cycle pathways (relative fluxes using 2H, 13C and J-HSQC NMR)	citric acid cycle, gluconeogenesis, Krebs's cycle, liver, spectroscopy, T
T2003	Absolute gluconeogenic flux rates	gluconeogenesis, glucose production, glycogenolysis, hepatic, liver, metabolism
T2013	TCA cycle flux (VTCA) and alpha-ketoglutarate-glutamate exchange flux (Vx) in the isolated mouse heart using 1H NMR	citric acid cycle, heart, Krebs's cycle, metabolism, NMR, spectroscopy, T
T2012	Intracellular sodium or high-energy phosphates in the isolated perfused mouse liver or heart	heart, high-energy phosphates, liver, sodium, spectroscopy
T2011	Intermediary metabolism in the isolated liver using NMR	hepatic, liver, metabolism, NMR, spectroscopy
T2020	Simulating the consequences of genetic manipulations	model, simulation

Pancreas, Islets and Beta Cells

Test No.	Test Name	Keywords
V3082	Tissue microdissection	laser microdissection, pancreas

Pathology & Immunohistochemistry

Test No.	Test Name	Keywords
V3080	Gross examinations and necropsy	gross examination, necropsy
V3081	Tissue preparation, embedding, sectioning and routine staining	embedding, sectioning, staining, tissue preparation
V3082	Tissue microdissection	laser microdissection, pancreas
V3083	Screen/optimize immunohistochemical protocols for mouse-specific commercial and custom-designed antisera	histology, immunohistochemistry
V3097	Perfusion-Fixation/Histopathology/Quantify Sclerosis	

Test No.	Test Name	Keywords
CA2041	Tissue processing by Pathology Core (embedded in paraffin)	histology, tissue preparation
CA2042	Tissue processing by Pathology Core (H&E staining)	histology, staining, tissue prepara
CA2043	Protal vein injection and tissue collection (at 0 min. & 5 min.)	tissue preparation
S6300	Tissue Processing & Sectioning - Trim & Cassette Tissue	
S6301	Tissue Processing & Sectioning - Process & Embed Tissue, Paraffin	
S6302	Tissue Processing & Sectioning - Section Paraffin Block	
S6303	Tissue Processing & Sectioning - Section Frozen Block	
S6304	Tissue Processing & Sectioning - Additional Unstained Slides Sections	
S6305	Tissue Processing & Sectioning - Decalcification	
S6306	Tissue Processing & Sectioning - Serial Sections/Aortic Root	
S6307	Histology - H&E	
S6308	Histology - PAS	
S6309	Histology - Picrosirius Red	
S6310	Histology - Masson's Trichrome	
S6311	Histology - von Kossa	
S6312	Histology - Silver Methenamine	
S6313	Histology - MOVATS Pentachrome	
S6314	Histology - Oil Red O	
S6315	Histology - Other Stains	
S6316	Immunohistochemistry - IHC/FITC Staining	
S6317	Immunohistochemistry - Batch Staining	
S6318	Immunohistochemistry - New Antibody Workup	
S6319	Immunohistochemistry - TUNEL (Apoptag Plus)	
S6320	Electron Microscopy - Process & Embed	
S6321	Electron Microscopy - Thick Section	
S6322	Electron Microscopy - Thin Section	
S6323	Electron Microscopy - Scope Time	
S6324	In Situ Hybridization - Probe Labelling, Radioactive	
S6325	In Situ Hybridization - Plasmid Linearization	
S6326	In Situ Hybridization	
S6327	Morphometric Analysis - Atherosclerosis, Aortic Root w/MOVATS	

Test No.	Test Name	Keywords	
S6328	Morphometric Analysis - Atherosclerosis, Aortic Root w/Oil Red O		
S6329	Morphometric Analysis - Atherosclerosis, Aortic Root w/Oil Red O and MOVATS		
S6330	Morphometric Analysis - Atherosclerosis, Whole Aorta, En Face		
S6331	Morphometric Analysis - Atherosclerosis, Inominates		
S6332	Morphometric Analysis - Kidney, Glomerular Size & Percent Matrix		
S6333	Morphometric Analysis - Kidney, Interstitial Fibrosis		
S6334	Morphometric Analysis - Other		

Surgery

Test No.	Test Name	Keywords	
C1051	Intestinal lipid absorption in the conscious mouse		absorption, fistula, gastrointestinal lipids, lymph
V3001	Cannulation of cerebral ventricle		brain, central control, CSF
V3002	Jugular vein and carotid artery catheterization		blood vessel, chronic, surgery
S6123	Necropsy		necropsy, surgery
Y4089	Blood Bicarbonate/CO2		carbon dioxide, metabolism, serum, serum metabolic panel
CA2027	Acute arterial and jugular vein catheterization	\$40.00 / animal / test	catheterization, surgery
CA2028	Acute arterial or jugular vein catheterization	\$22.00 / animal / test	catheterization, surgery
CA2029	Acute portal vein catheterization	\$47.00 / animal / test	catheterization, surgery
CA2030	Implant [G2 E - Mitters™]	\$20.00 / animal	surgery
CA2031	Long-term analysis of surgery implantation on Min-Mitter™	\$20.00 / animal	surgery
CA2041	Tissue processing by Pathology Core (embedded in paraffin)		histology, tissue preparation
CA2042	Tissue processing by Pathology Core (H&E staining)		histology, staining, tissue preparation
CA2044	Brain uptake and bloodflow (includes femoral catheterization)		blood flow, brain

Vascular Function

Test No.	Test Name	Keywords	
C1002	Inter-arterial pressure		blood pressure, blood vessel, hypertension, vascular
C1001	Tail Cuff Blood pressure		blood pressure, blood vessel, hypertension, vascular
C1003	Arterial baroreflex responses		cardiac function, vascular tone
C1013	Arterial response to injury (neointimal hyperplasia)		angioplasty, endothelial denudation, neointimal hyperplasia, restenosis

Test No.	Test Name	Keywords	
C1014	Vascular contractility measurements		aortic ring, contractility, vascular
V3033	Blood pressure measurements		blood pressure, blood vessel, hypertension, hypotension, vascular
V3034	Vascular morphology		blood vessel, histology, intima, smooth muscle, vascular
V3076	Morphometric determinations (aorta)		blood vessel, histology, intima, smooth muscle, vascular
S6206	Carotid Stenosis - Arterial response to injury		blood vessel, endothelial denudation, histology, neointimal hyperplasia, smooth muscle, vascular
S6230	Myography - basic		
S6231	Myography - additional		

Miscellaneous

Test No.	Test Name	Keywords	
V3000A	Miscellaneous Tissue and Body Fluid Collection	Consult with Director	tissue
V3000B	Miscellaneous Implantation of Catheters, Sensors and Pellets	Consult with Director	catheterization
V3000C	Equipment Usage	Consult with Director	
V3000D	Personnel Training	Consult with Director	
V3000E	Cerebral Ventricle Cannulation	Consult with Director	cerebral ventricle
V3000F	Jugular Vein and Carotid Artery Catheterization	Consult with Director	catheterization

Protein

Test No.	Test Name	Keywords	
C1041	Body Composition		body composition, fat mass, obesity
T2020	Simulating the consequences of genetic manipulations		model, simulation
V3009	Amino acid kinetics		amino acid flux, amino acid kinetics
S6101	Body Composition		body composition, energy balance, mass, obesity, water

Tests Listed by Center

CASE WESTERN RESERVE UNIVERSITY

Analytical Core

Director: Colleen Croniger, Personnel: Michelle Puchowicz

The analytical Core conducts all assays involving the determination of isotope labeling and concentration profiles of metabolites including

- (i) the 2H-labeling of water, lipids and amino acids,
- (ii) the 18O-labeling of water,
- (iii) the concentration profile of acyl-CoAs (e.g. short, medium and long-chain species), and
- (iv) metabolic profiles of citric acid cycle intermediates, amino acids and fatty acids.

Test No.	Test Name	Keywords	
CA2011	Total Energy expenditure using 2H ₂ O-labeled water	\$100.00 / 4 samples / mouse	energy expenditure, water
CA2015	Turnover of glucose, lipid and/or protein	\$200.00 / 4 samples / mouse	
CA2016	Fatty acid and cholesterol synthesis using 2H-labeled water	\$75.00 / sample	
CA2017	Tissue-specific protein synthesis using 2H ₂ O-labeled water	\$150.00 / sample	amino acids, metabolism, protein
CA2018	Profile of acylcarnitines in plasma/urine tissue samples	\$75.00/plasma or/urine sample \$90.00/tissue sample	
CA2019	Profile of long chain acyl-CoAs in tissue	\$150.00 per sample [for concentration]	
CA2020	Measurement of acetyl-CoA, propionyl-CoA and/or succinyl-CoA in tissue	\$225.00/sample (concentration) and C13 labeling pattern	
CA2021	Measurement of Methylmalonyl-CoA in tissue	\$225.00/sample (concentration) and C13 labeling pattern	
CA2022	¹³ C-Labeling pattern of acetyl moiety of citrate (substrate oxidation)	\$110/sample	
CA2023	Activity of acetyl-CoA carboxylase or malonyl-CoA decarboxylase in tissues	\$75.00 per assay	
CA2024	Metabolomic profile of citric acid cycle and gluconeogenic intermediates	\$150.00 / sample [for concentration]	
CA2041	Tissue processing by Pathology Core (embedded in paraffin)		histology, tissue preparation
CA2042	Tissue processing by Pathology Core (H&E staining)		histology, staining, tissue prepara
CA2043	Protal vein injection and tissue collection (at 0 min. & 5 min.)		tissue preparation
CA2044	Brain uptake and bloodflow (includes femoral catherization)		blood flow, brain
CA2045	Measurement of ATP/ADP concentration in tissue		ATP, tissue

Metabolic Core

Director: Colleen Croniger, Associate Director: Michelle Puchowicz

The Metabolic Core conducts in vivo and ex vivo metabolic experiments on mice shipped to the Case MMPC. This core focuses on the following techniques and measurements:

- 1) Chronic or acute arterial, jugular and/or gastric catheterization
- 2) Acute catheterization of portal vein or urinary bladder
- 3) Energy expenditure integrated over four days by the "doubly-labeled water" method
- 4) Rates of fatty acid, cholesterol, triglycerides, or protein synthesis measured using 2H₂O
- 5) Insulin clamp, pancreatic clamp
- 6) Food intake
- 7) Body temperature
- 8) Urine and blood chemistry analysis
- 9) Glycosylated hemoglobin
- 10) Liver perfusion and heart perfusion
- 11) Tissue perfusion and fixation

Test No.	Test Name	Keywords	
CA2000	Body composition using 2H-labeled water	\$20.00 / sample	body composition, body weight, fat
CA2001	Food Consumption	\$5.00 / animal / day	food intake
CA2002	Body Weight	\$5.00 / animal / day	body composition, body weight, fat
CA2003	Continuous measurement of body temperature	\$5.00 / animal / day	energy expenditure, exercise
CA2004	Glucose tolerance tests (GTT)	\$52.00 / animal / test	carbohydrate metabolism, diabetes
CA2005	Insulin concentrations at fasting and post intraperitoneal glucose administration		insulin, insulin secretion
CA2006	Plasma insulin measurement by ELISA	\$9.50 / mouse	carbohydrate metabolism, insulin, action
CA2007	Insulin concentrations at fasting and post intraperitoneal insulin administration		
CA2008	Glucose concentrations at fasting and post intraperitoneal insulin administration - insulin tolerance test (ITT)		carbohydrate metabolism, diabetes sensitivity
CA2009	Triglycerides in liver		lipids, liver
CA2010	Plasma triglycerides		plasma
CA2013	Hyperinsulinemic Clamp (Hypoglycemic or Euglycemic) using stable isotopes	INQUIRE / animal / test	
CA2025	Chronic arterial and jugular vein catheterization	\$51.00 / animal	catheterization, surgery
CA2026	Chronic arterial or jugular vein catheterization	\$28.00 / animal	catheterization, surgery
CA2027	Acute arterial and jugular vein catheterization	\$40.00 / animal / test	catheterization, surgery
CA2028	Acute arterial or jugular vein catheterization	\$22.00 / animal / test	catheterization, surgery
CA2029	Acute portal vein catheterization	\$47.00 / animal / test	catheterization, surgery
CA2030	Implant [G2 E - Mitters™]	\$20.00 / animal	surgery
CA2031	Long-term analysis of surgery implantation on	\$20.00 / animal	surgery

Test No.	Test Name	Keywords
	Min-Mitter™	
CA2032	Metabolomic profile of organic acids in plasma	metabolism, plasma
CA2033	Metabolomic profile of organic acids in urine	metabolism, urine
CA2034	Metabolomic profile of organic acids in tissue	metabolism, tissue
CA2035	Metabolomic profile of amino acids in plasma	amino acids, metabolism, plasma
CA2036	Metabolomic profile of amino acids in urine	amino acids, metabolism, urine
CA2037	Metabolomic profile of amino acids in tissue	amino acids, metabolism, tissue
CA2038	Metabolomic profile of free fatty acids in plasma	fatty acids, metabolism, plasma
CA2039	Metabolomic profile of free fatty acids in urine	fatty acids, metabolism, urine
CA2040	Metabolomic profile of free fatty acids in tissue	fatty acids, metabolism, tissue

UNIVERSITY OF CINCINNATI MEDICAL CENTER

Cardiovascular and Renal Function Core

Director: David Hui

The Cardiovascular and Renal Function Core specializes in various blood pressure and flow parameters that can be affected by diabetes and/or obesity in the intact animal or isolated heart. In addition, changes in arterial response to vessel wall injury can be measured.

Test No.	Test Name	Keywords
C1002	Inter-arterial pressure	blood pressure, blood vessel, hypotension, vascular
C1001	Tail Cuff Blood pressure	blood pressure, blood vessel, hypotension, vascular
C1003	Arterial baroreflex responses	cardiac function, vascular tone
C1010	Cardiac output	contractility, ejection fraction, heart volume
C1011	Regional Blood Flow Measurements	blood flow, blood pressure, smooth muscle, vascular
C1013	Arterial response to injury (neointimal hyperplasia)	angioplasty, endothelial denudation, neointimal hyperplasia, restenosis
C1014	Vascular contractility measurements	aortic ring, contractility, vascular tone
C1020	Cardiac contractility (left ventricular function in the isolated heart)	cardiac, heart, pressure, ventricular function
C1021	Echocardiography	cardiac, heart, morphology
C1022	Left ventricular pressure measurements in intact mice	cardiac, heart, pressure, ventricular function
C1030	Micropuncture measurements	blood flow, kidney, renal, vascular
C1032	In situ microperfusion	kidney, renal, vascular
C1033	Control of Renal Perfusion Pressure	glomerular filtration, kidney, renal
C1034	Whole kidney clearance	glomerular filtration, kidney, renal
C1031	Renal Blood Flow Regulation	blood flow, kidney, renal, vascular
C1012	Renal blood flow regulation (free flow measurements)	blood flow, kidney, renal, vascular

Lipid, Lipoprotein and Glucose Metabolism Core

Director: Patrick Tso

Diabetes is defined by abnormalities in circulating metabolites; obviously glucose metabolism is impaired, but the presence of certain dyslipidemias can also lead to a predisposition for cardiovascular disease in diabetic patients.

This core is capable of measuring numerous metabolic parameters in mouse models pertaining to serum lipid profiles, glucose metabolism and plasma hormones.

Test No.	Test Name	Keywords
C1070	Glucose tolerance tests (intraperitoneal glucose tolerance, oral glucose tolerance)	diabetes, insulin action, insulin sensitivity
C1088	Plasma Glucose-dependent insulintropic peptide (GIP) concentration	gut, hormone, lipids, metabolism
C1051	Intestinal lipid absorption in the conscious mouse	absorption, fistula, gastrointestinal lipids, lymph
C1052	Plasma lipid profiles	fat, lipids, metabolism
C1053	Lipoprotein profiles	cholesterol, fat, metabolism
C1054	Lipoprotein fractionation by FPLC	cholesterol, fat, metabolism
C1055	Chylomicron metabolism (lymph)	absorption, chylomicron, fat, lipids, lipoproteins
C1056	Cholesterol synthetic rate	cholesterol, fat, lipids, synthesis
C1057	Plasma Free fatty Acid Levels	fat, lipids, metabolism
C1072	Insulin Sensitivity Test	diabetes, insulin action, insulin sensitivity, metabolism
C1081	C-Peptide	diabetes, hormone, insulin
C1082	Cholecystokinin (CCK)	CCK, gut, hormone, intestine
C1085	Plasma/serum concentrations glucagon	counterregulatory, hormone, pancreas
C1086	Plasma Glucagon-like peptide 1 (GLP-1) concentration	counterregulatory, hormone, pancreas
C1087	Glucose enrichment and concentration	carbohydrate metabolism, diabetes
C1089	Insulin concentrations in plasma/serum/lymph/cerebrospinal fluid	diabetes, hormone, pancreas
C1090	Plasma/serum concentrations of leptin	eating behaviour, fat, hormone, lipids
C1091	Somatostatin in plasma or tissue extracts	diabetes, hormone, pancreas
C1092	Plasma/Organ Triglycerides	fat, lipids, metabolism
C1058	Plasma beta-hydroxybutyrate levels	diabetes, ketones, metabolism
C1059	Non-invasive measurement of fat absorption	absorption, fat, lipids, metabolism
C1060	Chemical determination of phospholipid	fat, lipids
C1061	Serum/Plasma Adiponectin	adipose, fat, hormone, lipids, metabolism
C1062	Serum/Plasma Resistin	adipose, fat, hormone, lipids
C1071	Plasma glucose levels	carbohydrate, diabetes, metabolism
C1083	Cholesterol (Total, HDL, LDL)	cholesterol, fat, lipids, metabolism

Energy Metabolism, Food Intake & Body Weight Regulation Core

Co-Director: Randy Seeley, Co-Director: Steve Woods

Obesity is the major predisposing risk factor for type II diabetes. This core provides comprehensive set of measurements of food intake, energy expenditure (including use of indirect calorimetry) and body fat composition.

Test No.	Test Name	Keywords
C1040	Food intake and body weight measurements	energy balance, energy expenditure
C1041	Body Composition	body composition, fat mass, obesity
C1042	Energy Expenditure Measurements	body weight, energy balance
C1043	Hypothalamic Gene Expression	central nervous system, hormone, hypothalamus, neuroendocrine
C1044	DietMax Meal Pattern Analysis	food intake

UNIVERSITY OF TEXAS SOUTHWESTERN MEDICAL CENTER

Rogers NMR Center Metabolic Core

Director: Craig Malloy, Co-Director: Shawn Burgess

The Rogers NMR Center Mouse Metabolic Phenotyping Center specializes in the use of stable isotopes for investigations of metabolism, including a comprehensive analysis of gluconeogenesis. Our goal is to provide a repertoire of metabolic analyses that can be standardized and used to phenotype mouse models of type 2 diabetes. These tools are based on new concepts for probing intermediary metabolism using ^{13}C - or ^2H -enriched compounds. In some instances the studies are simple enough to perform in the referring investigator's lab; only partially processed tissue samples need be shipped to the Rogers NMR Center for full analysis. Procedures are available for determining the substrates contributing to plasma glucose, measurement of both the relative and absolute rates of the pathways involved in gluconeogenesis, substrate utilization in the heart, and

$^{23}\text{Na}/^{31}\text{P}$ NMR investigations of liver metabolism. In addition, software is made available to predict the potential effects of genetic manipulation on various pathways.

Test No.	Test Name	Keywords
T2001	Sources of plasma glucose using ^2H NMR	gluconeogenesis, glucose production, glycogenesis, hepatic, liver, metabolism, spectroscopy
T2002	Gluconeogenic and citric acid cycle pathways (relative fluxes using ^2H , ^{13}C and J-HSQC NMR)	citric acid cycle, gluconeogenesis, Krebs's cycle, liver, spectroscopy, T
T2003	Absolute gluconeogenic flux rates	gluconeogenesis, glucose production, glycogenolysis, hepatic, liver, metabolism
T2010	Substrate oxidation and anaplerosis in the isolated heart	anaplerosis, cardiac, heart, metabolism, spectroscopy, substrate oxidation
T2013	TCA cycle flux (VTCA) and alpha-ketoglutarate-glutamate exchange flux (V_x) in the isolated mouse heart using ^1H NMR	citric acid cycle, heart, Krebs's cycle, metabolism, NMR, spectroscopy, T
T2012	Intracellular sodium or high-energy phosphates in the isolated perfused mouse liver or heart	heart, high-energy phosphates, liver, sodium, spectroscopy
T2011	Intermediary metabolism in the isolated liver using NMR	hepatic, liver, metabolism, NMR, spectroscopy
T2020	Simulating the consequences of genetic manipulations	model, simulation

UNIVERSITY OF WASHINGTON, SEATTLE

Diabetes and Energy Balance Core

Director: Michael Schwartz, Co-Director: Gregory Morton, Personnel: Oanh Mai

Description of Services

* Precise, non-invasive, quantitative assessment of energy homeostasis in rodent models including energy expenditure, respiratory quotient, locomotor activity, body temperature, food intake, meal patterns and body composition.

* Assessment of a variety of metabolic disease phenotypes associated with obesity and type 2 diabetes such as glucose intolerance, insulin resistance and hyperlipidemia in mice.

* Quantification of the extent of insulinitis and other inflammatory parameters in mouse models of type 1 diabetes mellitus such as autoimmune NOD and hyperglycemic Akita mice and mice treated with pancreatic islet toxins.

* Quantification using PCR approaches of the expression of genes in the hypothalamus and other sites involved in food intake, energy expenditure, and tissue complications of diabetes.

* Quantification of circulating and/or tissue factors (e.g. hormones, proteins, lipids, and carbohydrates) associated with the metabolic syndrome, obesity, type 1 diabetes, or type 2 diabetes.

* Longitudinal studies: To perform longitudinal studies in single animals as a function of age, diet or special treatment regimes using specific drugs or islet toxins.

Test No.	Test Name	Keywords
S6102	Energy Expenditure	body weight, energy balance, energy expenditure
S6101	Body Composition	body composition, energy balance, mass, obesity, water
S6103	Meal Pattern Analysis	food intake
S6104	Body Temperature	
S6120	Intraperitoneal Glucose Tolerance Test	carbohydrate metabolism, diabetes intolerance, glucose tolerance, insulin
S6121	Insulin Sensitivity Test	carbohydrate metabolism, diabetes tolerance, insulin action, insulin resistance, insulin sensitivity
S6122	Drug treatment (Streptozotocin; other)	
S6123	Necropsy	necropsy, surgery
S6105	Running Wheels Activity	
S6140	General Chemistry - Glucose	
S6141	Lipids - Lipid Extraction	
S6142	Lipids - Free Fatty Acids	
S6143	Lipids - HDL	
S6144	Lipids - Triglyceride TG	
S6145	Lipids - Cholesterol TC	
S6146	Lipids - FPLC	
S6147	Cytokines & Hormones - TNF Alpha	
S6148	Cytokines & Hormones - IL-6	
S6149	Cytokines & Hormones - IL-4	
S6150	Cytokines & Hormones - Leptin	
S6151	Cytokines & Hormones - Insulin	
S6152	Cytokines & Hormones - PAI-1 and others	
S6153	Cytokines & Hormones - Glucagon	

Test No.	Test Name	Keywords
S6154	Cytokines & Hormones - Adiponectin	
S6155	Cytokines & Hormones - Urine Albumin	
S6156	Cytokines & Hormones - Urine Creatinine	
S6157	Cytokines & Hormones - BUN	
S6158	Cytokines & Hormones - Insulin (by Eliza)	
S6159	Cytokines & Hormones - Taqman PCR Quantification	

Cardiovascular Core

Director: Charles Murry, Co-Director: Elina Minami

Description of Services:

- 1) Cardiovascular injury models (myocardial infarction, hindlimb ischemia and collateralization.
- 2) Structural and physiological assessment of organ damage that reflect common complications of diabetes.
- 3) Flow cytometric quantitation of circulating progenitor cells (e.g. c-kit, sca-1, Flt-1, and CD31).
- 4) Bioimmunoassay analysis of growth factors, cytokines, and chemokines affecting the cardiovascular system in diabetes and in injury.

Test No.	Test Name	Keywords
S6200	Echocardiography (non-invasive)	cardiac function, echocardiography, morphology
S6201	Electrocardiography - ECG (non-invasive)	echocardiography, heart
S6202	Invasive Hemodynamics - Left Ventricular Catheterization/Millar	cardiac function, cardiac output, echocardiography, heart, pressure, volume, ventricular
S6204	Telemetry (invasive)	cardiac, ECG, EKG, electrocardiography, telemetry
S6205	Blood Pressure (non-invasive)	blood flow, blood pressure, hypertension, hypotension, vascular
S6206	Carotid Stenosis - Arterial response to injury	blood vessel, endothelial denudation, histology, neointimal hyperplasia, muscle, vascular
S6230	Myography - basic	
S6231	Myography - additional	
S6207	Myocardial Infarction	cardiac, heart
S6208	Hindlimb Ischemia	blood vessel, cardiac, cardiac function, hypertension, hypotension, restenosis, vascular
S6209	Open Thoracotomy	surgery
S6210	Vein Catheter Insertion	catheterization, surgery, vascular,
S6211	Bone Marrow Transplantation	
S6212	Drug Treatment	
S6213	Ultrasound Imaging - Aortic	cardiac function, heart

Nephrology, Macrovascular and Microvascular Core

Director: Charles Alpers, Co-Director: Kevin O'Brien

Description of Services

- 1) Blood pressure, plasma lipid and lipoprotein levels, urine albumin.
- 2) Processing (freezing and/or fixation and paraffin embedding) of tissue or cells.
- 3) Sectioning of frozen and paraffin-embedded tissue blocks appropriate for light, fluorescent and electron microscopy.
- 4) Diagnostic pathology expertise in the evaluation of local and systemic effects in tissues and target organs of diabetic animals.
- 5) Monoclonal and polyclonal antibodies to targeted gene products and reporter genes (e.g., p-galactosidase, alkaline phosphatase, green fluorescent protein).
- 6) RNA and DNA probes for in situ hybridization studies, which complement studies of expressed proteins.

Test No.	Test Name	Keywords
S6300	Tissue Processing & Sectioning - Trim & Cassette Tissue	
S6301	Tissue Processing & Sectioning - Process & Embed Tissue, Paraffin	
S6302	Tissue Processing & Sectioning - Section Paraffin Block	
S6303	Tissue Processing & Sectioning - Section Frozen Block	
S6304	Tissue Processing & Sectioning - Additional Unstained Slides Sections	
S6305	Tissue Processing & Sectioning - Decalcification	
S6306	Tissue Processing & Sectioning - Serial Sections/Aortic Root	
S6307	Histology - H&E	
S6308	Histology - PAS	
S6309	Histology - Picrosirius Red	
S6310	Histology - Masson's Trichrome	
S6311	Histology - von Kossa	
S6312	Histology - Silver Methenamine	
S6313	Histology - MOVATS Pentachrome	
S6314	Histology - Oil Red O	
S6315	Histology - Other Stains	
S6316	Immunohistochemistry - IHC/FITC Staining	
S6317	Immunohistochemistry - Batch Staining	
S6318	Immunohistochemistry - New Antibody Workup	
S6319	Immunohistochemistry - TUNEL (Apoptag Plus)	
S6320	Electron Microscopy - Process & Embed	
S6321	Electron Microscopy - Thick Section	

Test No.	Test Name	Keywords
S6322	Electron Microscopy - Thin Section	
S6323	Electron Microscopy - Scope Time	
S6324	In Situ Hybridization - Probe Labelling, Radioactive	
S6325	In Situ Hybridization - Plasmid Linearization	
S6326	In Situ Hybridization	
S6327	Morphometric Analysis - Atherosclerosis, Aortic Root w/MOVATS	
S6328	Morphometric Analysis - Atherosclerosis, Aortic Root w/Oil Red O	
S6329	Morphometric Analysis - Atherosclerosis, Aortic Root w/Oil Red O and MOVATS	
S6330	Morphometric Analysis - Atherosclerosis, Whole Aorta, En Face	
S6331	Morphometric Analysis - Atherosclerosis, Inominates	
S6332	Morphometric Analysis - Kidney, Glomerular Size & Percent Matrix	
S6333	Morphometric Analysis - Kidney, Interstitial Fibrosis	
S6334	Morphometric Analysis - Other	

VANDERBILT UNIVERSITY SCHOOL OF MEDICINE

Metabolic Pathophysiology Core

Director: Owen McGuinness, Associate Director: Kate Ellacott, Personnel: Emily Born

The Metabolic Pathophysiology conducts these major tests: vein/artery cannulations, glucose and insulin clamps, glucose tolerance test, tissue specific glucose/fatty acid uptake, calorimetry, exercise, gluconeogenesis/glycogenolysis, body composition, food consumption, optical imaging of gene expression/cellular events, isolation of pancreatic islets /insulin secretion.

Test No.	Test Name	Keywords
V3001	Cannulation of cerebral ventricle	brain, central control, CSF
V3002	Jugular vein and carotid artery catheterization	blood vessel, chronic, surgery
V3003	Glucose Tolerance Test (Oral and Intravenous)	glucose intolerance, glucose tolerance action
V3004	Glucose turnover	endogenous glucose production, glucose kinetics, glucose turnover tracers
V3005	Hyperinsulinemic clamp	hyperinsulinemic clamp, insulin resistance
V3006	Hyperglycemic clamp	hyperglycemic clamp, insulin secretion pancreas
V3007	Gluconeogenesis & glycogenolysis (from hepatic 14C-UDPglucose and PEP)	gluconeogenesis, glucose production, glycogenolysis, liver
V3008	Glycogen synthesis	glycogen synthesis, liver, muscle
V3009	Amino acid kinetics	amino acid flux, amino acid kinetics

Test No.	Test Name	Keywords	
V3010	Tissue specific glucose uptake		2-deoxyglucose, glucose metabolism, tissue specific glucose uptake
V3011	Tissue specific fatty acid uptake		125I-BMIPP, tissue specific fatty acid uptake
V3012	Indirect calorimetry /energy expenditure		carbon dioxide, energy expenditure, oxygen exchange, indirect calorimetry, oxygen consumption
V3013	Exercise capacity (metabolic response to exercise)		endurance, exercise capacity, exercise tolerance
V3014	Spontaneous exercise activity		spontaneous exercise activity, wheel running
V3015	Food Consumption		spontaneous exercise activity, wheel running
V3016	Exploratory locomotor activity		energy expenditure, exploratory locomotor activity
V3017	Assess real time imaging of cellular metabolic events		islets, metabolism, microcirculation, real time imaging
V3018	In vivo optical imaging of gene expression		gene expression, GFP, luciferase
V3000A	Miscellaneous Tissue and Body Fluid Collection	Consult with Director	tissue
V3000B	Miscellaneous Implantation of Catheters, Sensors and Pellets	Consult with Director	catheterization
V3000C	Equipment Usage	Consult with Director	
V3000D	Personnel Training	Consult with Director	
V3000E	Cerebral Ventricle Cannulation	Consult with Director	cerebral ventricle
V3000F	Jugular Vein and Carotid Artery Catheterization	Consult with Director	catheterization

Cardiovascular Pathophysiology & Complications Core

Director: Douglas Vaughan, Associate Director: Jeff Rottman

The Cardiovascular Pathophysiology and Complications Core conducts these major tests: morphology and histology, echocardiography, electrocardiography, blood pressure, vascular morphology, renal function, metabolic panel in state-of-the-art facilities.

Test No.	Test Name	Keywords	
V3030	In vitro Morphology, Morphometrics and Histology (isolated heart)		cardiac function, heart, morphology
V3031	Echocardiography, in vivo morphology, systolic and diastolic function; Stress echocardiography		diastolic, echocardiography, morphology, stress, systolic
V3032	Electrocardiography and telemetry		cardiac, ECG, EKG, electrocardiography, telemetry
V3033	Blood pressure measurements		blood pressure, blood vessel, hypertension, hypotension, vascular
V3034	Vascular morphology		blood vessel, histology, intima, smooth muscle, vascular
V3035	Electrolytes, indices of renal function		
V3036	Metabolic panel		
V3094	Perfusion-Fixation/Heart Dimension		
V3095	Heart Rate Open Variability		
V3096	Ventricular Hemodynamics		

Test No.	Test Name	Keywords	
V3097	Perfusion-Fixation/Histopathology/Quantify Sclerosis		
V3098	GFR-FITC-Inulin; HPLC Cr		glomerular filtration, HPLC, kidney
V3099	Albuminuria		kidney, urine
V4000	Renal Blood Flow (Doppler)		blood flow, blood pressure, kidney
V4001	Urine Na/K		plasma, potassium, sodium, urine
V4002	Osmometer Plasma/Urine		osmolality, plasma, urine
V4003	Urine Ca/Phosphorus Excretion		urine
V4004	Urine pH		pH, urine
V4005	Glycemic Control using Minimed		glucose

Analytical Resources Core

Director: Sergio Fazio

The Analytical Resources Core performs these major tests: plasma hormones, amino acids, lipids and lipoproteins, pathology and immunohistochemistry.

Test No.	Test Name	Keywords	
V3090	Full amino acid profiles by HPLC / PIRC or HLPC / OPA		amino acids, HPLC
V3050	Insulin		hormone
V3051	Glucagon		hormone
V3052	Corticosterone		hormone
V3053	Catecholamines		hormone
V3054	Leptin		hormone
V3055	C-Peptide		hormone
V3056	Growth Hormone (GH)		hormone
V3058	TSH		hormone
V3059	PRL		hormone
V3060	ACTH		hormone
V3061	Insulin-like growth hormone-1 (IGF-1)		hormone
V3070	Plasma lipids		fat, lipids, metabolism
V3071	Lipid extraction, separation, quantitation		fat, lipids, metabolism
V3072	Fatty acid profiles of lipid esters by gas liquid chromatography		fat, GCMS, lipids, metabolism
V3073	Quantitation of individual phospholipid classes		cholesterol, fat, lipids, metabolism, phospholipids
V3074	Short chain fatty acid analysis by gas liquid chromatography		fat, GCMS, lipids, metabolism, short chain fatty acid
V3075	Lipoprotein fractionation and characterization		fat, lipids, lipoproteins, metabolism
V3076	Morphometric determinations (aorta)		blood vessel, histology, intima, smooth muscle, vascular
V3080	Gross examinations and necropsy		gross examination, necropsy
V3081	Tissue preparation, embedding, sectioning and		embedding, sectioning, staining, tissue

Test No.	Test Name	Keywords	
	routine staining		preparation
V3082	Tissue microdissection		laser microdissection, pancreas
V3083	Screen/optimize immunohistochemical protocols for mouse-specific commercial and custom-designed antisera		histology, immunohistochemistry
V3091	Specific selected amino acid profiles		amino acids, HPLC
V3092	Radioactivity of specific individual amino acids		amino acids, chromatography, protein synthesis, proteolysis, specific activity
V3093	Specific activities for gluconeogenic and glycogenic assessment		amino acids, chromatography, protein synthesis, proteolysis, specific activity
V3062	Aldosterone		hormone
V3064	Resistin		hormone
V3065	Adiponectin		hormone
V3066	Estradiol		hormone
V3067	Testosterone		hormone

YALE UNIVERSITY SCHOOL OF MEDICINE

In Vivo Metabolism Core

Director: Varman Samuel, Co-Director: Cheol Soo Choi, Personnel: David Frederick, Personnel: Andreas Birkenfeld

The In Vivo Metabolism Core at the Yale Mouse Metabolic Phenotyping Center is designed to conduct in vivo experiments and analytical assays to characterize the metabolic phenotype of transgenic/knockout mouse models potentially useful for understanding diabetes, its complications, obesity, and related metabolic disorders.

Test No.	Test Name	Keywords	
Y4001	Hyperinsulinemic-euglycemic clamp experiments		insulin action, insulin resistance
Y4002	Hyperglycemic clamp experiments		insulin secretion, pancreas

Analytical Core

Director: Gary Cline, Personnel: Mario Kahn, Personnel: Christopher Carmean, Personnel: Xian-Man Zhang

Metabolic phenotyping of transgenic mice requires the ability to determine whole body, tissue specific, and cellular metabolic fluxes, and to delineate cellular mechanisms of signal transduction. The Analytical Core provides the expertise, technical resources, and instrumentation necessary to characterize perturbations in metabolism in transgenic mice strains, and serves as a resource lab for the analysis of samples generated during the course of experiments undertaken by the in vivo, in vitro, and NMR cores.

Test No.	Test Name	Keywords	
Y4060	Diacylglycerol concentration		fat, lipids, metabolism, signaling
Y4050	Amino Acids		amino acids, enrichment, isotopes
Y4051	Beta-hydroxybutyrate		diabetes, enrichment, ketones, metabolism
Y4052	Free fatty acid		diabetes, enrichment, fat, lipids, metabolism
Y4053	Glucose		carbohydrate, diabetes, metabolism
Y4054	Glycerol		diabetes, enrichment, lipids, metabolism
Y4055	Glycogen		carbohydrate, diabetes, metabolism

Test No.	Test Name	Keywords
Y4057	Long-chain fatty acyl CoA esters	fat, lipids, metabolism
Y4059	ADP, ATP	energetics, high-energy phosphate, mitochondria
Y4070	Chem 7	serum chemicals, serum metabolic
Y4071	Liver Function Tests	serum chemicals, serum metabolic
Y4072	Lipid Panel	serum chemicals, serum metabolic
Y4073	Divalent Ions	serum chemicals
Y4080	Insulin	hormone
Y4081	Glucagon	hormone
Y4082	Leptin	hormone
Y4061	Lysophosphatidic Acid	lipids
Y4083	Blood Glucose	carbohydrate, diabetes, glucose, p serum chemicals, serum metabolic
Y4084	Blood Urea Nitrogen	renal, serum chemicals, serum me panel
Y4085	Blood Creatinine-HPLC	kidney, muscle, renal, serum chem serum metabolic panel
Y4086	Urine Creatinine-HPLC	kidney, muscle, renal, serum chem serum metabolic panel, urine
Y4087	Blood Electrolytes-Na/Cl/K	electrolytes, metabolism, muscle, potassium, serum chemicals, seru panel, sodium
Y4089	Blood Bicarbonate/CO2	carbon dioxide, metabolism, serum serum metabolic panel
Y4091	Blood Albumin	liver, plasma, serum albumin, seru chemicals
Y4092	Alanine Aminotransferase	ALT, liver, plasma, serum chemical
Y4093	Aspartate Aminotransferase	liver, plasma, serum chemicals
Y4094	Alkaline Phosphatase	liver, plasma, serum chemicals
Y4095	Total Bilirubin	bilirubin, liver, serum chemicals
Y4097	Total Protein	liver, plasma, serum chemicals
Y4098	HDL Cholesterol	cholesterol, lipids, lipoproteins
Y4099	LDL Cholesterol	cholesterol, lipids, plasma, serum
Y5000	Cholesterol	cholesterol, lipids, plasma, serum
Y5001	Triglycerides	lipids, plasma, serum chemicals
Y5002	Non-Esterified Fatty Acids	fatty acids, lipids, non-esterified fa serum chemicals
Y5003	Beta-Hydroxybutyrate (COBAS)	diabetes, ketones, plasma, serum
Y5004	Blood Calcium	serum chemicals
Y5005	Blood Inorganic Phosphorous	inorganic phosphate, phosphate, s chemicals
Y5007	Magnesium	serum chemicals
Y5006	Urine Inorganic Phosphorous	inorganic phosphate, phosphate, u
Y5008	Creatine Kinase	creatine kinase, serum chemicals

Test No.	Test Name	Keywords
Y5009	Lactate Dehydrogenase	serum chemicals
Y5010	Apolipoprotein C3	lipids, lipoproteins, serum chemicals
Y4088	Urine Electrolytes-Na/K/Cl	electrolytes, muscle, potassium, u

Test Descriptions by Center

CASE WESTERN RESERVE UNIVERSITY

Analytical Core

CA2011 Total Energy expenditure using 2H₂O-labeled water \$100.00 / 4 samples / mouse

Keywords: energy expenditure, water

TEE is equal to the sum of basal metabolic metabolic, thermic effect of eating and physical activity. Following a single bolus injection of 2H and 18O-labeled water one can determine TEE via the elimination of 2H and 18O from body water. This test requires serial measurements of the labeling of body water over approximately 1 week, which necessitates the collection of blood or urine samples.

Note: This test does not require catheterized mice, nor does it require that mice be shipped to the MMPC. The isotopes are non-radioactive and no special safety precautions are required, the tracers will be shipped from the MMPC to the investigator. Investigators will be instructed on how to administer the isotopes, collect samples and then ship them back to the MMPC.

CA2015 Turnover of glucose, lipid and/or protein \$200.00 / 4 samples / mouse

Keywords:

During a constant tracer infusion, the dilution of the infused tracer yields a measure of that molecule's rate of appearance. One can measure the turnover of numerous molecules using this strategy. One can determine the kinetics glucose, glycerol and protein using [6,6-2H₂]glucose, [2H₅]glycerol and [2H₅]phenylalanine.

This test requires a catheterized animal.

CA2016 Fatty acid and cholesterol synthesis using 2H-labeled water \$75.00 / sample

Keywords:

Rates of fatty acid and cholesterol synthesis can be determined in tissues via the incorporation of 2H or 13C-labeled precursors. For example, following a bolus injection of 2H-labeled water one can collect samples (e.g. blood, liver and/or adipose tissue). The respective lipids are isolated and their 2H-labeling is determined. This test can be performed in 2 modes, short term vs long term. In a short term study, the tracer is administered and samples are collected within hours to determine the synthesis of lipids in plasma and/or liver. In a long term study, the tracer is continuously administered over several days. Samples of adipose tissue are collected. The difference in time scale is necessary since the pool of lipids in adipose tissue is relatively large and requires more time for label to appear.

Note: This test does not require catheterized mice, nor does it require that mice be shipped to the MMPC. The isotopes are non-radioactive and no special safety precautions are required, the tracers will be shipped from the MMPC to the investigator. Investigators will be instructed on how to administer the isotopes, collect samples and then ship them back to the MMPC.

CA2017 Tissue-specific protein synthesis using 2H₂O-labeled water \$150.00 / sample

Keywords: amino acids, metabolism, protein synthesis

Rates of protein synthesis can be determined from the incorporation of 2H-labeled water. For example, following a bolus injection of 2H-labeled water one can collect samples (e.g. blood, liver, muscle, etc). Total proteins are isolated and their 2H-labeling is determined. This test can be performed in 2 modes, short term vs long term. In a short term study, the tracer is administered and samples are collected within hours to determine the synthesis of proteins in plasma, liver, etc. This mode is well-suited for examining the acute response of protein synthesis to a perturbation (e.g. food intake). In a long term study, the tracer is continuously administered over several days. Samples are collected and the assays are performed. The long term design yields an integrative measure of protein synthesis, i.e. the isotope is present during the fed and the fasted state and accounts for all protein synthesis over such a transition.

Note: This test does not require catheterized mice, nor does it require that mice be shipped to the MMPC. The isotopes are non-radioactive and no special safety precautions are required, the tracers will be shipped from the MMPC to the investigator. Investigators will be instructed on how to administer the isotopes, collect samples and then ship them back to the MMPC.

CA2018 Profile of acylcarnitines in plasma/urine tissue samples \$75.00/plasma or/urine sample

\$90.00/tissue sample

Keywords:

These LC-MS-MS assays are routinely run in which acylcarnitines are identified as C_x, where x is the number of carbons in the acyl group. Samples are spiked with unlabeled and labeled internal standards. The mass isotopomer distribution of each peak is determined to characterize its labeling pattern.

This test, coupled with the assay of the profile of urinary organic acids helps in the characterization of a number of metabolic defects, such as inborn errors of fatty acid oxidation disorders.

CA2019 Profile of long chain acyl-CoAs in tissue \$150.00 per sample [for concentration]

Keywords:

Commercial preparations of CoA and acyl-CoA contain an unnatural analog of CoA, iso-CoA, in which the 3' phosphate has been moved to the 2' position of ribose. We can use the acyl-iso-CoA esters as internal standards to calculate the concentration and mass isotopomer distribution of acyl-CoAs from LC-MS data.

CA2020 Measurement of acetyl-CoA, propionyl-CoA and/or succinyl-CoA in tissue \$225.00/sample (concentration) and C13 labeling pattern

Keywords:

Commercial preparations of CoA and acyl-CoA contain an unnatural analog of CoA, iso-CoA, in which the 3' phosphate has been moved to the 2' position of ribose. We can use the acyl-iso-CoA esters as internal standards to calculate the concentration and mass isotopomer distribution of acyl-CoAs from LC-MS data.

CA2021 Measurement of Methylmalonyl-CoA in tissue \$225.00/sample (concentration) and C13 labeling pattern

Keywords:

Commercial preparations of CoA and acyl-CoA contain an unnatural analog of CoA, iso-CoA, in which the 3' phosphate has been moved to the 2' position of ribose. We can use the acyl-iso-CoA esters as internal standards to calculate the concentration and mass isotopomer distribution of acyl-CoAs from LC-MS data.

CA2022 13C-Labeling pattern of acetyl moiety of citrate (substrate oxidation) \$110/sample

Keywords:

A number of investigators, who use ¹³C-labeled precursors of acetyl-CoA -in vivo- in isolated organs or in cell incubations, have attempted to estimate the labeling of mitochondrial acetyl-CoA to calculate the contribution of the substrate to the acetyl-CoA oxidized in the citric acid cycle (CAC). The best proxy for mitochondrial acetyl-CoA is the acetyl moiety of citrate.

We developed an assay of the labeling of the acetyl moiety of citrate which involves (i) tissue extraction, (ii) alkaline hydrolysis of extant acetyl-CoA, (iii) after pH adjustment, cleavage of citrate with CoA + ATP-citrate lyase which we isolated from rat liver.

The acetyl-CoA formed is either assayed as such by LC-MS, or reacted with thiophenol, followed by GC-MS assay of acetylthiophenol. This assay allows one to calculate the contribution of two or three substrates to mitochondrial acetyl-CoA in the same experiment.

For example, consider a mouse heart perfused with unlabeled glucose + [1-¹³C]palmitate + [U-¹³C₄]acetoacetate.

These substrates yield acetyl-CoA that is unlabeled (M), singly labeled (M₁), or doubly labeled (M₂), respectively. So, the percent abundances of the M, M₁, and M₂ mass isotopomers of the acetyl moiety of citrate yield the contribution of each of the substrates to mitochondrial energy production.

CA2023 Activity of acetyl-CoA carboxylase or malonyl-CoA decarboxylase in tissues \$75.00 per assay

Keywords:

- For acetyl-CoA carboxylase, the tissue extract is incubated with unlabeled acetyl-CoA + NaH¹³CO₃ to form [¹³C]malonyl-CoA which, after hydrolysis, is assayed as its TMS derivative.

[U-¹³C₃]Malonyl-CoA is used as an internal standard.

- For malonyl-CoA decarboxylase, we developed two different assays. The first assay involves incubation with [2-14C]malonyl-CoA, to form [2-14C]acetyl-CoA which is reacted with carnitine to form [2-14C]acetyl-carnitine the radioactivity of which is counted after isolation.
- The second assay involves incubation with [U-13C3]malonyl-CoA to form [1,2-13C2]acetyl-CoA. The latter is reacted with thiophenol, and acetylthiophenol is assayed by GC-MS, [2H3, 1-13C]acetyl-CoA is used as internal standard.

CA2024 Metabolomic profile of citric acid cycle and gluconeogenic intermediates \$150.00 / sample [for concentration]

Keywords:

We will assay the relative concentration of citric acid cycle intermediates and those in the gluconeogenic pathway. Assays can be run using samples from mice/organs that have also been infused with a 13C-labeled tracer, e.g. 13C-lactate. This strategy allows one to determine flux rates (via the 13C-labeling patterns) and identify points of control of a pathway, e.g. gluconeogenesis (via the relative concentration profiles).

CA2041 Tissue processing by Pathology Core (embedded in paraffin)

Keywords: histology, tissue preparation

Tissue processing by Pathology Core (embedded in paraffin)

CA2042 Tissue processing by Pathology Core (H&E staining)

Keywords: histology, staining, tissue preparation

Tissue processing by Pathology Core (H&E staining)

CA2043 Portal vein injection and tissue collection (at 0 min. & 5 min.)

Keywords: tissue preparation

Portal vein injection and tissue collection (at 0 min. & 5 min.)

CA2044 Brain uptake and bloodflow (includes femoral catheterization)

Keywords: blood flow, brain

Brain uptake and blood flow (includes femoral catheterization)

CA2045 Measurement of ATP/ADP concentration in tissue

Keywords: ATP, tissue

Measurement of ATP/ADP concentration in tissues

Metabolic Core

CA2000 Body composition using 2H-labeled water \$20.00 / sample

Keywords: body composition, body weight, fat

The body is composed of 2 major compartments, fat and fat-free mass. Fat-free mass consists of water, inorganic matter (e.g. minerals) and organic matter (e.g. protein, DNA). One can determine total body water via the initial dilution of 2H-labeled water. Fat mass can be calculated assuming a constant relationship between water and inorganic and organic matter in fat-free mass.

This test requires a single injection of 2H-labeled water and the collection of 1 blood sample 2.5 hours post-injection.

Note: This test does not require catheterized mice, nor does it require that mice be shipped to the MMPC. The isotopes are non-radioactive and no special safety precautions are required, the tracers will be shipped from the MMPC to the investigator. Investigators will be instructed on how to administer the isotopes, collect samples and then ship them back to the MMPC.

CA2001 Food Consumption \$5.00 / animal / day

Keywords: food intake

CA2002 Body Weight \$5.00 / animal / day

Keywords: body composition, body weight, food intake

CA2003 Continuous measurement of body temperature \$5.00 / animal / day

Keywords: energy expenditure, exercise

CA2004 Glucose tolerance tests (GTT) \$52.00 / animal / test

Keywords: carbohydrate metabolism, diabetes, glucose

Glucose tolerance tests (GTT)

CA2005 Insulin concentrations at fasting and post intraperitoneal glucose administration

Keywords: insulin, insulin secretion

Insulin concentrations at fasting and post intraperitoneal glucose administration

CA2006 Plasma insulin measurement by ELISA \$9.50 / mouse

Keywords: carbohydrate metabolism, insulin, insulin action

Determination of the concentration of insulin in plasma.

CA2007 Insulin concentrations at fasting and post intraperitoneal insulin administration

Keywords:

Insulin concentrations at fasting and post intraperitoneal insulin administration.

CA2008 Glucose concentrations at fasting and post intraperitoneal insulin administration - insulin tolerance test (ITT)

Keywords: carbohydrate metabolism, diabetes, insulin sensitivity

Glucose concentrations at fasting and post intraperitoneal insulin administration

CA2009 Triglycerides in liver

Keywords: lipids, liver

Triglycerides in liver.

CA2010 Plasma triglycerides

Keywords: plasma

Plasma triglyceride concentration.

CA2013 Hyperinsulinemic Clamp (Hypoglycemic or Euglycemic) using stable isotopes INQUIRE / animal / test

Keywords:

Hyperinsulinemic Clamp (Hypoglycemic or Euglycemic) using stable isotopes

CA2025 Chronic arterial and jugular vein catheterization \$51.00 / animal

Keywords: catheterization, surgery

Chronic arterial and jugular vein catheterization

CA2026 Chronic arterial or jugular vein catheterization \$28.00 / animal

Keywords: catheterization, surgery

Chronic arterial or jugular vein catheterization

CA2027 Acute arterial and jugular vein catheterization \$40.00 / animal / test

Keywords: catheterization, surgery

Acute arterial and jugular vein catheterization

CA2028 Acute arterial or jugular vein catheterization \$22.00 / animal / test

Keywords: catheterization, surgery

Acute arterial or jugular vein catheterization

CA2029 Acute portal vein catheterization \$47.00 / animal / test

Keywords: catheterization, surgery

Acute portal vein catheterization

CA2030 Implant [G2 E – Mitters™] \$20.00 / animal

Keywords: surgery

Implant [G2 E – Mitters™]

CA2031 Long-term analysis of surgery implantation on Min-Mitter™ \$20.00 / animal

Keywords: surgery

Long-term analysis of surgery implantation on Min-Mitter™

CA2032 Metabolomic profile of organic acids in plasma

Keywords: metabolism, plasma

Metabolomic profile of organic acids in plasma

CA2033 Metabolomic profile of organic acids in urine

Keywords: metabolism, urine

Metabolomic profile of organic acids in urine

CA2034 Metabolomic profile of organic acids in tissue

Keywords: metabolism, tissue

Metabolomic profile of organic acids in tissue

CA2035 Metabolomic profile of amino acids in plasma

Keywords: amino acids, metabolism, plasma

Metabolomic profile of amino acids in plasma

CA2036 Metabolomic profile of amino acids in urine

Keywords: amino acids, metabolism, urine

Metabolomic profile of amino acids in urine

CA2037 Metabolomic profile of amino acids in tissue

Keywords: amino acids, metabolism, tissue

Metabolomic profile of amino acids in tissue

CA2038 Metabolomic profile of free fatty acids in plasma

Keywords: fatty acids, metabolism, plasma

Metabolomic profile of free fatty acids in plasma

CA2039 Metabolomic profile of free fatty acids in urine

Keywords: fatty acids, metabolism, urine

Metabolomic profile of free fatty acids in urine

CA2040 Metabolomic profile of free fatty acids in tissue

Keywords: fatty acids, metabolism, tissue

Metabolomic profile of free fatty acids in tissue

UNIVERSITY OF CINCINNATI MEDICAL CENTER

Cardiovascular and Renal Function Core

C1002 Inter-arterial pressure

Keywords: blood pressure, blood vessel, hypertension, hypotension, vascular

This is a more precise blood pressure measurement than tail cuff pressure method. Experiments and recording will be initiated at 24 to 48 hours following implantation of indwelling arterial catheters.

C1001 Tail Cuff Blood pressure

Keywords: blood pressure, blood vessel, hypertension, hypotension, vascular

The measurement of systolic pressure through tail sphygmomanometry in the mouse can be accomplished using computer controlled-pulse detection and data acquisition. Minimal training period of 4 to 7 days and measurements will be obtained over an extended period of 5 to 7 days.

C1003 Arterial baroreflex responses

Keywords: cardiac function, vascular tone

Baroreflex is a homeostatic feedback mechanism whereby acute changes in blood pressure result in compensatory alterations in cardiac function and vascular tone. Resetting of the baroreflex can be an important hallmark of cardiovascular dysfunction.

C1010 Cardiac output

Keywords: contractility, ejection fraction, heart, stroke volume

Dose response relationships of blood pressure and ascending aorta blood flow velocity will be determined for various vasoactive agents. Relative changes in total peripheral resistance in closed-chest, anesthetized mice will be evaluated with simultaneous measurements of arterial blood pressure and cardiac output.

C1011 Regional Blood Flow Measurements

Keywords: blood flow, blood pressure, smooth muscle, vascular

Transonic transit time flow probes will be used to provide high fidelity volumetric flow measurements.

C1013 Arterial response to injury (neointimal hyperplasia)

Keywords: angioplasty, endothelial denudation, neointimal hyperplasia, restenosis

Mouse carotid arteries will be denuded of the endothelium with a resin bead derivatized catheter probe to mimic balloon angioplasty. Histological sections will be made 14 days after arterial injury for measurement of neointimal hyperplasia, medial size and thickness and vessel wall remodeling.

C1014 Vascular contractility measurements

Keywords: aortic ring, contractility, vascular function

Isolated aorta preparations will be used to study vascular contractility. Aorta will be precontracted with phenylephrine

and exposed to increasing concentrations of acetylcholine. Isometric force will be measured to determine endothelium-dependant response to stimulation. In addition, spontaneous mechanical activity of the portal vein will be quantified in terms of frequency, on-time, off-time, the tension-time integral and the maximum positive and negative dF/dt .

C1020 Cardiac contractility (left ventricular function in the isolated heart)

Keywords: cardiac, heart, pressure, ventricular

Parameters measured: cardiac function (left ventricular pressure, rates of contraction and relaxation, tau), heart rate, mean arterial pressure, left atrial pressure, cardiac output, stroke volume, aortic and coronary flow, myocardial oxygen consumption.

The research will employ an ex-vivo, isolated work-performing heart methodology to study the fundamental contractile processes in the mouse heart. Heart muscle function is analyzed through length-tension, pressure-volume, and force frequency relationships of muscle, and the consequences of cardiac performance to particular pathological stresses such as hypoxia and ischemia-reperfusion injury can be assessed. All cardiovascular parameters are obtained independently of any neuronal or hormonal influence.

C1021 Echocardiography

Keywords: cardiac, heart, morphology

Transthoracic echocardiography will be used as index of myocardial performance. M-mode and pulsed-Doppler echocardiography will be used for noninvasive assessment of ventricular function. Left ventricular function will be determined under baseline conditions and during beta-adrenergic stimulation. Data to be collected include: left ventricular end-diastole and systole, septal wall thickness at end diastole, posterior wall thickness at end diastole, pressure gradient from ascending and descending aortic flow velocities, heart rate, stroke volume and cardiac output.

C1022 Left ventricular pressure measurements in intact mice

Keywords: cardiac, heart, pressure, ventricular

Ventricular pressure measurements will be made in intact animals as follows: cardiovascular performance in response to beta-adrenergic stimulation will be evaluated by infusing isoproterenol, with continuous monitoring of blood pressure wave. More precise evaluation of cardiac contractility may be made by combining left ventricular pressure measurements with M-mode echocardiography to obtain pressure-dimension relationships. Additional studies may be extended to directly assess cardiac functions using isolated work-performing heart preparations.

C1030 Micropuncture measurements

Keywords: blood flow, kidney, renal, vascular

Animals will receive a priming dose and maintenance infusion of FITC-inulin. Proximal and distal convolution of several surface nephrons will be identified and mapped by intraluminal injection of a small volume of dye. Late proximal puncture sites will be identified as the last surface segment to fill with the dye. Early distal puncture sites can be identified when the dye returns to the kidney surface.

C1032 In situ microperfusion

Keywords: kidney, renal, vascular

Early and late convolutions of a single proximal tubule having 3-5 loops on the kidney surface will be identified and mapped as described for other renal function tests. In a small population of nephrons, late proximal and early distal convolutions from a single nephron will be identified for microperfusion of the loop segment.

C1033 Control of Renal Perfusion Pressure

Keywords: glomerular filtration, kidney, renal, vascular

Hormones that influence renal function will be infused to produce a slight diuresis that permits sufficient urine collection for evaluation of pressure natriuresis responses.

C1034 Whole kidney clearance

Keywords: glomerular filtration, kidney, renal, vascular

Standard clearance techniques will be used to evaluate renal blood pressure, glomerular filtration rate and excretion of

sodium, potassium and chloride ions. Sodium and potassium concentrations will be measured in 4 microL plasma or urine by flame photometry. Chloride will be measured by chloridometer. Osmolality will be measured by freezing point depression.

C1031 Renal Blood Flow Regulation

Keywords: blood flow, kidney, renal, vascular

After identification of proximal and distal puncture sites, an oil block will be introduced and a complete tubular fluid sample will be collected. In those nephrons which have both proximal and distal convolutions, collections will be made from the distal site prior to the proximal site. When the same nephrons are re-punctured during the second experimental period, disappearance of the original oil block and normal fluid flow at the distal site will be used as verification of tubule patency. Tubular fluid samples will be obtained to measure Na⁺ and CL⁻ and FITC-inulin concentrations.

C1012 Renal blood flow regulation (free flow measurements)

Keywords: blood flow, kidney, renal, vascular

Renal blood flow regulation (free flow measurements)

Lipid, Lipoprotein and Glucose Metabolism Core

C1070 Glucose tolerance tests (intraperitoneal glucose tolerance, oral glucose tolerance)

Keywords: diabetes, insulin action, insulin sensitivity

Mice will be fasted for 4 hours and anesthetized. A fasting blood sample will be removed from the tail vein and a concentrated solution of glucose injected into the abdominal cavity of the mice through a needle passed through the abdominal skin. Blood samples will be removed from the tail vein 5, 10, 15, 20, 25 and 30 minutes later. Samples of plasma obtained during the test will be measured for concentrations of glucose and insulin.

C1088 Plasma Glucose-dependent insulinotropic peptide (GIP) concentration

Keywords: gut, hormone, lipids, metabolism

Glucose-dependent insulinotropic polypeptide (GIP) is measured by radioimmunoassay.

C1051 Intestinal lipid absorption in the conscious mouse

Keywords: absorption, fistula, gastrointestinal tract, lipids, lymph

This procedure enables the study of the intestinal digestion, absorption and lymphatic transport of dietary lipid in the conscious mouse. By infusing the lipid test meal intraduodenally at a constant rate, the lymphatic lipid output usually reaches a steady rate by the 3rd or 4th hour. Thus, lymphatic lipid output during the 5th or 6th hour represents the amount of lipid transported by the small intestine under steady-state conditions. At the end of the study, both luminal as well as mucosal radioactive lipids can be collected. Thus one can obtain a considerable amount of information regarding the digestion, uptake and mucosal esterification and the lymphatic transport of lipids.

C1052 Plasma lipid profiles

Keywords: fat, lipids, metabolism

Plasma lipid profiles

C1053 Lipoprotein profiles

Keywords: cholesterol, fat, metabolism

As part of the standard lipoprotein determination, plasma lipoproteins will be analyzed using agarose gel electrophoresis. Small aliquots of lymph or plasma can be used for the assay. It is particularly useful for identifying beta-VLDL and the analysis of lipoprotein classes when plasma triglycerides are high (>300 mg/dl)

C1054 Lipoprotein fractionation by FPLC

Keywords: cholesterol, fat, metabolism

To obtain a more precise determination of lipids and apolipoproteins in the different lipoprotein fractions, the

lipoproteins of plasma or lymph can be separated using Fast Protein Liquid Chromatography. This method is sometimes preferred over ultracentrifugation as the amount of blood required is much lower. The lipoproteins in each column fraction will be separated by agarose electrophoresis and stained to identify VLDL remnants, LDL, apo E-containing large HDL particles, small alpha migrating HDL and pre-beta HDL.

C1055 Chylomicron metabolism (lymph)

Keywords: absorption, chylomicron, fat, lipids, lipoproteins

The intestinal lymph duct and duodenum will be surgically cannulated. Chylomicrons are harvested and sized by negative staining. A small sample will be delipidated, and the apoprotein composition will be analyzed by polyacrylamide gel electrophoresis. Labeled CM is injected into mice and the plasma clearance rate is calculated as well as a determination of the uptake by the liver.

C1056 Cholesterol synthetic rate

Keywords: cholesterol, fat, lipids, synthesis

To measure sterol synthesis rates, mice will be injected with deuterated water i.p. One hour later, the animals are anesthetized and exsanguinated. Tissues are removed and saponified. The amount of digitonin-precipitable sterols will be determined and cholesterol synthesis can then be calculated.

C1057 Plasma Free fatty Acid Levels

Keywords: fat, lipids, metabolism

Determinations of substrates (free fatty acids) in blood will be made using specific biochemical reactions that generate a specific color in proportion to their concentration. Reactions will be run in microtiter plates and analyzed on a plate reader. Using this method assays can be performed on small (5-25ul) samples of plasma or serum.

C1072 Insulin Sensitivity Test

Keywords: diabetes, insulin action, insulin sensitivity, metabolism

Mice will be fasted for 4 hours and anesthetized. A fasting blood sample will be removed from the tail vein and insulin (0.5mU/g) will be injected into the abdominal cavity. Blood samples will be removed from the tail vein 5, 10, 15, 20, 25 and 30 minutes later. samples of plasma obtained during the test will be measured for concentrations of glucose and insulin.

C1081 C-Peptide

Keywords: diabetes, hormone, insulin

Plasma concentrations of C-peptide are determined using radioimmunoassay kits available fro Linco Inc. (St. Louis, MO)

C1082 Cholecystokinin (CCK)

Keywords: CCK, gut, hormone, intestine

This gastrointestinal hormone can be quantified by radioimmunoassay.

C1085 Plasma/serum concentrations glucagon

Keywords: counterregulatory, hormone, pancreas

Plasma concentrations of glucagon are determined using radioimmunoassay kits available fro Linco Inc. (St. Louis, MO)

C1086 Plasma Glucagon-like peptide 1 (GLP-1) concentration

Keywords: counterregulatory, hormone, pancreas

Glucagon-like peptide 1 (GLP-1) concentrations are measured using a specific radioimmunoassay.

C1087 Glucose enrichment and concentration

Keywords: carbohydrate metabolism, diabetes

Glucose enrichment and concentration

C1089 Insulin concentrations in plasma/serum/lymph/cerebrospinal fluid

Keywords: diabetes, hormone, pancreas

Concentrations of insulin in plasma or serum are determined using a sensitive radioimmunoassay that is specific for insulin but also reacts with proinsulin. The assay has been sensitized such that concentrations of insulin can be determined in small (10-25 ul) aliquots of serum/plasma. The assay has been used to detect insulin concentrations in other body fluids such as lymph and cerebrospinal fluid.

C1090 Plasma/serum concentrations of leptin

Keywords: eating behaviour, fat, hormone, lipids

Plasma concentrations of leptin are determined using radioimmunoassay kits available from Linco Inc. (St. Louis, MO)

C1091 Somatostatin in plasma or tissue extracts

Keywords: diabetes, hormone, pancreas

Somatostatin is measured with a radioimmunoassay that detects all of the prosomatostatin peptides.

C1092 Plasma/Organ Triglycerides

Keywords: fat, lipids, metabolism

Triglyceride concentrations in plasma or organs (including liver).

Blood samples will be collected in EDTA-containing tubes from the tails of mice after a 4 hour fast. Plasma will be prepared from these samples using centrifugation and the lipid levels will be determined using enzymatic methods.

C1058 Plasma beta-hydroxybutyrate levels

Keywords: diabetes, ketones, metabolism

Determinations of substrates (B-hydroxybutyrate) in blood will be made using specific biochemical reactions that generate a specific color in proportion to their concentration. Reactions will be run in microtiter plates and analyzed on a plate reader. Using this method assays can be performed on small (5-25ul) samples of plasma or serum.

C1059 Non-invasive measurement of fat absorption

Keywords: absorption, fat, lipids, metabolism

Non-invasive measurement of fat absorption

C1060 Chemical determination of phospholipid

Keywords: fat, lipids

Chemical determination of phospholipid

C1061 Serum/Plasma Adiponectin

Keywords: adipose, fat, hormone, lipids, metabolism

Serum/Plasma Adiponectin

C1062 Serum/Plasma Resistin

Keywords: adipose, fat, hormone, lipids

Serum/Plasma Resistin

C1071 Plasma glucose levels

Keywords: carbohydrate, diabetes, metabolism

Determinations of substrates (glucose) in blood will be made using specific biochemical reactions that generate a specific color in proportion to their concentration. Reactions will be run in microtiter plates and analyzed on a plate reader. Using this method assays can be performed on small (5-25ul) samples of plasma or serum.

C1083 Cholesterol (Total, HDL, LDL)

Keywords: cholesterol, fat, lipids, metabolism

Blood samples will be collected in EDTA-containing tubes from the tails of mice after a 4 hour fast. Plasma will be prepared from these samples using centrifugation and the lipid levels will be determined using enzymatic methods. The LDL-cholesterol will be estimated using the equation: $(VLDL+LDL\text{-cholesterol}) - (VLDL\text{- triglycerides})/5$.

Energy Metabolism, Food Intake & Body Weight Regulation Core

C1040 Food intake and body weight measurements

Keywords: energy balance, energy expenditure, nutrition

We will measure body weight and food intake on low fat diets to determine susceptibility to diet induced obesity. Mice will be placed on a high fat (40% calories from fat) or low fat (8% calories from fat) diet. Body weights and food intakes will be measured two times per week.

C1041 Body Composition

Keywords: body composition, fat mass, obesity

Total body composition in live, un-anesthetized small animals and carcasses will reveal absolute amounts of body fat, lean tissue and body water via quantitative magnetic resonance (QMR).

References:

Tinsley FC, Taicher GZ, Heiman ML. Evaluation of a quantitative magnetic resonance method for mouse whole body composition analysis. *Obes Res.* 2004 Jan;12(1):150-60.

C1042 Energy Expenditure Measurements

Keywords: body weight, energy balance

Both total energy expenditure and relative rates of carbohydrate versus fat oxidation will be determined via indirect calorimetry.

C1043 Hypothalamic Gene Expression

Keywords: central nervous system, hormone, hypothalamus, neuroendocrine

We will assess the relative activation of a number of hypothalamic peptidergic systems implicated in the control of body weight. The primary measure for the activation or inhibition of these systems will be measuring relative mRNA expression using in situ hybridization.

C1044 DietMax Meal Pattern Analysis

Keywords: food intake

DietMax Meal Pattern Analysis

UNIVERSITY OF TEXAS SOUTHWESTERN MEDICAL CENTER

Rogers NMR Center Metabolic Core

T2001 Sources of plasma glucose using 2H NMR

Keywords: gluconeogenesis, glucose production, glycogenesis, hepatic, liver, metabolism, NMR, spectroscopy

This study is based on Landau's 2H₂O method for measuring contributions to glucose production (1995, 1996). Using 2H NMR one can determine the fraction of plasma glucose derived from glycogen, glycerol or phosphoenolpyruvate (Jones et al., 2001). After a 24 hour fast, 2H₂O is injected i.p. into a mouse. After 20-30 min to allow for isotope equilibration, the animal is sacrificed, plasma is harvested and plasma glucose is purified and converted into

monoacetone glucose. The relative deuterium enrichments at positions 2, 5 and 6(s) measured

by ²H NMR are used to calculate the relative contributions to total serum glucose (Jones et al., 2001). This study could be performed at UTSWMC or an isotope kit may be shipped to the referring laboratory. Harvested, freeze dried plasma could then be shipped to UTSWMC for analysis. Typically, plasma from three mice are

pooled to generate one sample for NMR analysis; This requirement could be modified somewhat to accommodate animal availability, volume of blood obtainable, or plasma glucose concentration. This study is often performed simultaneously with study # T2002.

T2002 Gluconeogenic and citric acid cycle pathways (relative fluxes using ²H, ¹³C and J-HSQC NMR)

Keywords: citric acid cycle, gluconeogenesis, hepatic, Krebs's cycle, liver, spectroscopy, TCA cycle

After a fast, the majority of glucose production is fueled by flux of oxaloacetate out of the TCA cycle into phosphoenolpyruvate (PEP) via PEPCK. PEP may participate as a substrate in gluconeogenesis or return to the TCA cycle via pyruvate kinase and pyruvate carboxylase (e.g. pyruvate cycling). The activities of all these pathways relative to TCA cycle turnover (citrate synthase activity) may be quantified using the carbon-13 tracer [U-¹³C] propionate to enrich TCA cycle intermediates with ¹³C. The flux of carbon-13 from the TCA cycle to glucose can be followed by standard isotopomer analysis of plasma glucose (Jones et al., 1997) using either direct ¹³C observe NMR or J resolved heteronuclear single quantum coherence NMR (Burgess et al., 2001). Typically, this experiment will be combined with study #T2001 for quantitation of glucose sources relative to TCA cycle turnover (Jones et al., 2001). After a 24 hour fast, [U-¹³C] propionate (dissolved in 2H₂O if done in conjunction with T2001) is administered by i.p. injection. 20-30 minutes later (to allow equilibrium of the isotopes) the mouse is anesthetized by I.M. ketamine/xylazine injection and blood is harvested by cannulation of the descending aorta. Plasma glucose is extracted and converted to a suitable derivative for NMR analysis. This study may be performed at UTSWMC or an isotope kit may be shipped to the referring laboratory and freeze dried plasma returned to UTSWMC for analysis. Typically, plasma samples from 3 mice are pooled to generate one sample for NMR analysis; this requirement could be modified somewhat to accommodate animal availability, volume of blood obtainable, or plasma glucose concentration.

T2003 Absolute gluconeogenic flux rates

Keywords: gluconeogenesis, glucose production, glycogenolysis, hepatic, liver, metabolism

This study will provide the most comprehensive in vivo measurement of the mouse liver flux profile. The combination of T2001 and T2002 with measurement of glucose turnover provides absolute fluxes through key reactions in the liver: glycogenolysis, gluconeogenesis from glycerol, gluconeogenesis from PEP, pyruvate kinase, pyruvate carboxylase, PEP carboxykinase and citrate synthase. It requires administration of 2H₂O and [U-¹³C] propionate (or another suitable 3 carbon gluconeogenic substrate such as lactate or alanine) by i.v. infusion. Infusion of either [1,6-¹³C] or [3,4-¹³C] glucose allows glucose turnover to be calculated (Jones et al., 1998). Ideally, the mouse will have an indwelling venous catheter. On the day of the exam, the animal will be fasted for a standard period. An intravenous mixture of 3 tracers will be infused: [1,6-¹³C₂] glucose, 2H₂O, and [U-¹³C] propionate will be administered with a priming dose. After a 60-90 minute infusion under light general anesthesia the animal's blood will be harvested. Typically, 3 mouse samples are pooled to generate one sample for NMR analysis; This requirement could be modified somewhat to accommodate animal availability, volume of blood obtainable, or plasma glucose concentration. Metabolic flux calculations will be performed as follows. The rate of appearance of glucose (Ra) will be measured from the dilution of [1,6-¹³C₂] glucose based on proton and carbon NMR measurements of plasma and infused glucose (Jones et al., 1998). Hepatic glucose output is defined as Ra - infusion rate of [1,6-¹³C₂] glucose. The fraction of hepatic glucose derived from glycogen, PEP and gluconeogenesis will be estimated from the ratio of deuterium enrichment at positions 6(s), 5 and 2; all measured from their relative signal areas in the ²H NMR spectrum. Absolute flux from glycogen, PEP and glycerol will be calculated as the product of their fractional contributions and hepatic glucose output. All fluxes in the citric acid cycle will be referenced to the absolute rate of PEP production which will allow calculation of absolute fluxes in the citric acid cycle (Jones et al., 2001).

1. Jones, J.G., Carvalho, R.A., Franco, B., Sherry, A.D. and Malloy, C.R. (1998) Measurement of hepatic glucose output, krebs cycle, and gluconeogenic fluxes by NMR analysis of a single plasma glucose sample. *Analytical Biochemistry* 263, 39-45.

2. Jones, J.G., Solomon, M.A., Cole, S.M., Sherry, A.D. and Malloy, C.R. (2001) An integrated ²H and ¹³C NMR study of gluconeogenesis and TCA cycle flux in humans. *Am. J. Physiol.* 281, E848-56.

T2010 Substrate oxidation and anaplerosis in the isolated heart

Keywords: anaplerosis, cardiac, heart, metabolism, NMR, spectroscopy, substrate oxidation

The intact perfused mouse heart offers many advantages to the physiologist, among them the capability of full and continuous hemodynamic monitoring in response to interventions. Techniques are available in this laboratory that

allow simultaneous measurement of the rates of oxidation of long chain fatty acids, medium chain fatty acids, ketones, lactate, pyruvate or glucose in the mouse heart under various conditions (Malloy et al., 1990). This study provides the capacity to analyze up to four labeling patterns in one experiment. Typically, hearts are provided with [U-¹³C]long chain fatty acids, [1,3-¹³C]ketones, [3-¹³C]lactate, [3-¹³C]pyruvate, and unenriched glucose plus insulin, all at physiological concentrations. At the end of the perfusion period the heart is rapidly frozen and extracted with perchloric acid. ¹³C NMR of the heart extract provides the relative and absolute rates of oxidation of each substrate. Of course, the labeling patterns and concentrations of substrates can be modified to address specific questions. Anaplerosis is also provided in the analysis. Since the experiment involves only stable isotopes, the perfusions could be performed in any lab where there exists the necessary equipment and skilled personnel. The extract or frozen tissue could be sent back to UTSWMC for analysis. Only one heart is needed per NMR experiment.

T2013 TCA cycle flux (V_{TCA}) and alpha-ketoglutarate-glutamate exchange flux (V_x) in the isolated mouse heart using ¹H NMR

Keywords: citric acid cycle, heart, Krebs's cycle, metabolism, NMR, spectroscopy, TCA cycle

The main function of myocardial TCA cycle is to oxidize acetyl-CoA and supply NADH for ATP synthesis through oxidative phosphorylation. Measurement of flux through the TCA cycle (V_{tca}, umol/g/min) is therefore directly related to oxygen consumption (Jeffrey et al.,). V_{tca} may be altered from control mice when myocardial energetics are disturbed due to mechanical work load, metabolic efficiency, or substrate selectivity. We use ¹³C edited proton NMR spectroscopy to follow the time course of enrichment of glutamate C3 and C4 with a temporal resolution of ~20 s in mouse hearts perfused with ¹³C enriched substrates (Burgess et al., 2001b). A fit of the NMR data to a kinetic model of the tricarboxylic acid cycle and related exchange reactions yields V_{tca} and alpha-ketoglutarateglutamate exchange (V_x, umol/g/min) fluxes. The meaning of V_x is not well understood, but it has been shown to be sensitive to myocardial redox state and may be influenced by the malate-aspartate shuttle activity. Measurements of V_x may prove useful in situations where mitochondrial or transaminase function are thought abnormal. The experiment is elaborate and requires studies in both intact heart and tissue extracts. All studies must be performed at UTSWMC.

T2012 Intracellular sodium or high-energy phosphates in the isolated perfused mouse liver or heart

Keywords: heart, high-energy phosphates, liver, NMR, sodium, spectroscopy

This laboratory has a long history of measuring intracellular Na⁺ and phosphorus containing metabolites by NMR. We developed and pioneered the use of HTmDOTP4⁻ as a hyperfine shift reagent for resolving signals of intra- and extracellular Na⁺ by ²³Na NMR in isolated, perfused animal hearts and livers. We intend to make this expertise available to investigators who wish to better understand the roles of intracellular cations in diabetic models. In the same experiments, the ³¹P NMR resonances of dimethyl methylphosphonate and LaDOTP5⁻ may be used as markers of total tissue space and extracellular space, respectively. ³¹P NMR will be available for analysis of high energy phosphates, intracellular pH and other variables (such as free ADP) that are conventionally calculated from the ³¹P NMR spectrum. These studies must be performed at UTSWMC.

T2011 Intermediary metabolism in the isolated liver using NMR

Keywords: hepatic, liver, metabolism, NMR, spectroscopy

The isolated perfused liver offers major advantages in understanding intermediary metabolism. Isolated perfused mouse livers will be studied using a perfusate buffer containing physiological concentrations of lactate, pyruvate, ketones, and fatty acids. gluconeogenesis is measured directly (by arteriovenous difference) and by NMR studies of the liver and the effluent glucose. The perfusate will contain 2H₂O so that absolute fluxes from various glucose sources can be determined. The Rates associated with hepatic TCA cycle including pyruvate cycling can be determined simultaneously (Jones et al., 2001). This experiment returns the same parameters as in vivo experiment T2003. A variation of the experiment could include multiple labeled substrates for determining substrate selection by the liver. Since the experiment involves only stable isotopes, the perfusions could be performed in any lab where there exists the necessary equipment and skilled personnel. The extract or frozen tissue and perfusate effluent could be sent back to UTSWMC for analysis. Only one liver is needed per NMR experiment.

T2020 Simulating the consequences of genetic manipulations

Keywords: model, simulation

The metabolic consequences of pathway manipulation by genetic or other methods may not be easy to predict. Simulations can be performed on the behalf of physiologists or geneticists to determine whether our tracer methods are sensitive to activation or deactivation of particular pathways. A further important issue is selection of the optimal experimental approach (for example, tracer labeling patterns) to highlight the new metabolic state.

The answers to these questions impact the correct approach to phenotyping an animal, and could save significant animals and labor if addressed properly. Therefore, we have developed a software package, tcaSIM, which is available

to academic users free of charge over the Internet. This program allows the user to specify the relative fluxes, carbon labeling patterns of input molecules, and pool sizes of intermediates of the citric acid cycle and exchanging pools. It allows users to "delete" or "down-regulate" pathways such as pyruvate carboxylase or pyruvate dehydrogenase. The user can "switch on" less well studied pathways or conditions, such as the glyoxylate pathway, non-equilibration of the triose phosphate isomerase, orientation conserved transfer through succinate and fumarate, and the pentose phosphate pathway. The program provides isotopomers of all intermediates involved in the citric acid cycle, glycolysis, gluconeogenesis, and the pentose phosphate pathway.

Information about carbon isotopomers is valuable for teaching exercises, but it is usually not of interest to investigators. Therefore, the program also provides ¹³C NMR and HMQCTOCSY spectral data, relative enrichments (valuable for predicting ¹⁴C tracer results), and mass spectrometry results for simulated conditions. We will provide the program, train users as needed, and perform consultations for users.

UNIVERSITY OF WASHINGTON, SEATTLE

Diabetes and Energy Balance Core

S6102 Energy Expenditure

Keywords: body weight, energy balance, energy expenditure

Calorimetry (includes measurements of body composition, VO₂, VCO₂, RQ, food and water intake, physical activity, and data processing)

S6101 Body Composition

Keywords: body composition, energy balance, fat, fat mass, obesity, water

Body Composition

S6103 Meal Pattern Analysis

Keywords: food intake

Continuous Food Intake for meal pattern: meal size, meal frequency

S6104 Body Temperature

Keywords:

Includes data collection and analysis of body temperature

S6120 Intraperitoneal Glucose Tolerance Test

Keywords: carbohydrate metabolism, diabetes, glucose intolerance, glucose tolerance, insulin action

Intraperitoneal Glucose Tolerance Test

S6121 Insulin Sensitivity Test

Keywords: carbohydrate metabolism, diabetes, glucose tolerance, insulin action, insulin resistance, insulin sensitivity

Insulin Sensitivity Test

S6122 Drug treatment (Streptozotocin; other)

Keywords:

Drug treatment (Streptozotocin; other)

S6123 Necropsy

Keywords: necropsy, surgery

Necropsy

S6105 Running Wheels Activity

Keywords:

S6140 General Chemistry - Glucose

Keywords:

S6141 Lipids - Lipid Extraction

Keywords:

S6142 Lipids - Free Fatty Acids

Keywords:

S6143 Lipids - HDL

Keywords:

micro method

S6144 Lipids - Triglyceride TG

Keywords:

micro method

S6145 Lipids - Cholesterol TC

Keywords:

micro method

S6146 Lipids - FPLC

Keywords:

includes TC/TG & profile for fractions

S6147 Cytokines & Hormones - TNF Alpha

Keywords:

Quantified using Luminex and Linco Kits.

S6148 Cytokines & Hormones - IL-6

Keywords:

Quantified using Luminex and Linco kits.

S6149 Cytokines & Hormones - IL-4

Keywords:

Quantified using Luminex and Linco kits.

S6150 Cytokines & Hormones - Leptin

Keywords:

Quantified using Luminex and Linco kits.

S6151 Cytokines & Hormones - Insulin

Keywords:

Quantified using Luminex and Linco kits.

S6152 Cytokines & Hormones - PAI-1 and others

Keywords:

Quantified using Luminex and Linco kits.

S6153 Cytokines & Hormones - Glucagon

Keywords:

Quantified using Luminex and Linco kits.

S6154 Cytokines & Hormones - Adiponectin

Keywords:

Quantified using Luminex & Linco kits.

S6155 Cytokines & Hormones - Urine Albumin

Keywords:

S6156 Cytokines & Hormones - Urine Creatinine

Keywords:

S6157 Cytokines & Hormones - BUN

Keywords:

S6158 Cytokines & Hormones - Insulin (by Eliza)

Keywords:

Quantified using Eliza.

S6159 Cytokines & Hormones - Taqman PCR Quantification

Keywords:

Cardiovascular Core

S6200 Echocardiography (non-invasive)

Keywords: cardiac function, echocardiography, heart, morphology

basic measurements include:

fractional shortening and ejection fraction

heart rate

chamber dimensions and wall thickness

-additional measurements:

cardiac output

stroke volume

-data analysis and interpretation

S6201 Electrocardiography - ECG (non-invasive)

Keywords: echocardiography, heart

includes heart rate

S6202 Invasive Hemodynamics - Left Ventricular Catheterization/Millar

Keywords: cardiac function, cardiac output, contractility, echocardiography, heart, pressure, stroke volume, ventricular

-echocardiography required

-surgical model and basic measurements include:

blood pressure

chamber volume

ventricular pressure end-systole and end-diastole

stroke volume

-additional measurements:

cardiac output

stroke work and power

contractility

end-systolic pressure volume relationship and end-systolic relationship

-data analysis and interpretation

S6204 Telemetry (invasive)

Keywords: cardiac, ECG, EKG, electrocardiography, heart, telemetry

includes blood pressure and heart rate

S6205 Blood Pressure (non-invasive)

Keywords: blood flow, blood pressure, hypertension, hypotension, vascular

tail cuff blood pressure

S6206 Carotid Stenosis - Arterial response to injury

Keywords: blood vessel, endothelial denudation, histology, neointimal hyperplasia, smooth muscle, vascular

neointimal hyperplasia

vascular morphology

S6230 Myography - basic

Keywords:

basic protocol included one contraction curve and one relaxation curve in one vascular ring (minimum of 6 rings/day)

S6231 Myography - additional

Keywords:

additional complex concentration response curves: +/- inhibitors or with novel agonists. basic protocol required.

S6207 Myocardial Infarction

Keywords: cardiac, heart

permanent coronary ligation surgery

S6208 Hindlimb Ischemia

Keywords: blood vessel, cardiac, cardiac function, heart, hypertension, hypotension, restenosis, vascular

ischemia reperfusion (includes 2 doppler perfusion scans for blood flow)

S6209 Open Thoracotomy

Keywords: surgery

S6210 Vein Catheter Insertion

Keywords: catheterization, surgery, vascular, vein

S6211 Bone Marrow Transplantation

Keywords:

-irradiation of mice, isolation, preparation, and injection of bone marrow

-chimera determination (blood PCR, spleen, PCR, and FACS analysis)

S6212 Drug Treatment

Keywords:

injection and post-op animal care (body weight and blood pressure)

S6213 Ultrasound Imaging - Aortic

Keywords: cardiac function, heart

aortic valve, aortic velocity for aortic stenosis, aortic peak velocity for vascular stenosis, and mitral valve velocity for diastolic properties

Nephrology, Macrovascular and Microvascular Core

S6300 Tissue Processing & Sectioning - Trim & Cassette Tissue

Keywords:

S6301 Tissue Processing & Sectioning - Process & Embed Tissue, Paraffin

Keywords:

S6302 Tissue Processing & Sectioning - Section Paraffin Block

Keywords:

1st slide

S6303 Tissue Processing & Sectioning - Section Frozen Block

Keywords:

1st slide

S6304 Tissue Processing & Sectioning - Additional Unstained Slides Sections

Keywords:

S6305 Tissue Processing & Sectioning - Decalcification

Keywords:

S6306 Tissue Processing & Sectioning - Serial Sections/Aortic Root

Keywords:

Requires extra tech time.

S6307 Histology - H&E

Keywords:

S6308 Histology - PAS

Keywords:

S6309 Histology - Picrosirius Red

Keywords:

S6310 Histology - Masson's Trichrome

Keywords:

S6311 Histology - von Kossa

Keywords:

S6312 Histology - Silver Methenamine

Keywords:

S6313 Histology - MOVATS Pentachrome

Keywords:

S6314 Histology - Oil Red O

Keywords:

S6315 Histology - Other Stains

Keywords:

S6316 Immunohistochemistry - IHC/FITC Staining

Keywords:

S6317 Immunohistochemistry - Batch Staining

Keywords:

Up to 48 slides per batch. Charge for batch staining, plus price of primary antibody.

S6318 Immunohistochemistry - New Antibody Workup

Keywords:

Investigator must provide primary antibody.

S6319 Immunohistochemistry - TUNEL (Apoptag Plus)

Keywords:

S6320 Electron Microscopy - Process & Embed

Keywords:

S6321 Electron Microscopy - Thick Section

Keywords:

S6322 Electron Microscopy - Thin Section

Keywords:

S6323 Electron Microscopy - Scope Time

Keywords:

S6324 In Situ Hybridization - Probe Labelling, Radioactive

Keywords:

S6325 In Situ Hybridization - Plasmid Linearization

Keywords:

S6326 In Situ Hybridization

Keywords:

Up to 48 slides.

S6327 Morphometric Analysis - Atherosclerosis, Aortic Root w/MOVATS

Keywords:

S6328 Morphometric Analysis - Atherosclerosis, Aortic Root w/Oil Red O

Keywords:

S6329 Morphometric Analysis - Atherosclerosis, Aortic Root w/Oil Red O and MOVATS

Keywords:

S6330 Morphometric Analysis - Atherosclerosis, Whole Aorta, En Face

Keywords:

S6331 Morphometric Analysis - Atherosclerosis, Inominates

Keywords:

S6332 Morphometric Analysis - Kidney, Glomerular Size & Percent Matrix

Keywords:

S6333 Morphometric Analysis - Kidney, Interstitial Fibrosis

Keywords:

S6334 Morphometric Analysis - Other

Keywords:

VANDERBILT UNIVERSITY SCHOOL OF MEDICINE

Metabolic Pathophysiology Core

V3001 Cannulation of cerebral ventricle

Keywords: brain, central control, CSF

Implantation of a cerebral ventricle cannula allows investigators to evaluate physiological responses following central administration of various compounds. Anesthetized mice are placed in a digital stereotaxic apparatus (0.001 mm accuracy, Cartesian Instruments) specifically designed for mice. The dorsal scalp will be shaved, wiped with a betadine solution, and then a small midline incision over the dorsal surface is made to allow access to the cranium. After the affixed centering scope is used to "zero" lambda and bregma landmarks, a single guide cannula (2.5 mm length, 26-gauge, Plastics One) is positioned 1.0 mm above the lateral ventricle (coordinates: 0.6 mm posterior to bregma, 1.5 mm lateral to midline, 1.4 mm below the surface of the skull) and fixed to the skull using two stainless steel screws and dental cement. The incision in the scalp is then closed with surgical thread. Animals are removed to a post-surgical warming bed, and then individually housed for several hours until fully awake. Animals will be allowed to recover from surgery for a minimum of 7 days prior to testing, during which time a 30-gauge dummy cannula is left inside the guide cannula to prevent blockage.

V3002 Jugular vein and carotid artery catheterization

Keywords: blood vessel, chronic, surgery

Arterial catheterization allows investigators to sample arterial blood as required for adequate glucose clamping (Niswender et al. *J. Biol. Chem.*, 1997, Halseth et al. *Am. J. Physiol.* 1999) or other infusion/sampling purposes (Rottman et al. *Am. J. Physiol.* 1999) Catheterization of the right jugular vein allows the infusion of hormones, substrates, and tracers into the systemic circulation. The jugular venous catheter can be used to sample venous blood in long-term experiments because the jugular vein catheter will work for almost a month.

Arterial catheters are made from polyethylene tubing (PE-10) that is connected to silicone tubing (0.3 mm I.D., and 0.64 mm O.D.), 25 mm long. Jugular vein catheters are made from silicone tubing (0.3 mm I.D., and 0.64 mm O.D.). These catheters are connected to stainless steel tubes (0.3 mm I.D., 0.41 mm O.D., 15 mm) bent into an L shape. On the free end of the L shaped stainless steel tube a 20 mm piece of micro-renathane tubing (0.36 mm I.D., and 0.84 mm O.D.) is attached. The L shaped stainless steel tubes, attached to an arterial and a jugular vein catheter, are bundled together with silicone tubing (0.76 mm I.D. and 1.65 mm O.D.) and anchored with silastic medical adhesive (Silicone Type A). The catheters and the micro-renathane-stainless steel tubing will be heat sterilized.

The mouse is anesthetized and its skin on the interscapula and ventral surface of the neck is depilated by plucking. The depilated area is sterilized with 10% povidone-iodine. A small longitudinal incision (about 5 mm) is made in the skin over where the anterior jugular, acromiodeltoid, and cephalic veins join together. The connective tissues surrounding this junction are carefully removed. Two thin threads of silk (6-0 Silk, Davis+Gech) are passed under the jugular vein below the level of the junction. They are separated by about 3 mm. The cephalic thread, placed just below the joint, is tied to prevent bleeding. A small incision is then made just below the ligature, and the catheter is pushed 13 mm into the lumen. The catheter is fixed with the second thread and the thread previously used to tie the jugular vein. The common carotid artery is separated from the vagus nerve and muscle, and then two thin threads of silk (6-0 Silk, Davis+Gech) are passed under the artery. The cephalic thread is tied to prevent bleeding and then the artery is clamped by small bulldog clamp. A small incision is made just below the ligature, and the catheter is inserted into the lumen. The clamp is taken off and the catheter is pushed in 10 mm. The catheter is fixed with a second thread and the thread previously used to prevent bleeding. A blunt needle (16 gauge) is carefully inserted through the incision on the interscapula and pushed subcutaneously until the end comes out through the incision in the neck. The catheters will be carefully seized and pulled slowly through the needle. The incisions in the skin are then sutured. The catheters are connected to the stainless steel tubes. The bent portion of the stainless steel tubing is implanted under the skin and the incision is sutured. The implanted catheter is flushed with saline containing 200 U heparin/ml and 1 mg ampicillin/ml. Then the micro-renathane tubing is closed with a stainless steel wire. The mouse is injected subcutaneously with 150 mg/kg ampicillin. The total duration of the operation is about 50 min. Animals are removed to a post-surgical warming bed, and monitored until fully awake. Postoperative body weight and food intake are measured daily.

V3003 Glucose Tolerance Test (Oral and Intravenous)

Keywords: glucose intolerance, glucose tolerance, insulin action

Oral glucose tolerance tests are performed on conscious mice with catheters chronically implanted directly in the stomach and the carotid artery. Intravenous glucose tolerance tests are performed on conscious mice with catheters chronically implanted in the jugular vein and carotid artery. Glucose will be given at 1g/kg or 2g/kg. These doses lead to peak blood glucose levels of approximately 250 mg/dl and 400 mg/dl in wild type C57/bl/6 mice.

V3004 Glucose turnover

Keywords: endogenous glucose production, glucose flux, glucose kinetics, glucose turnover, isotopes, tracers

A primed (2 μ Ci) continuous infusion of [3-3H]glucose (0.4 μ Ci/min) is used to assess the rates of glucose appearance (Ra) and disappearance (Rd). Tracer is infused to allow a steady state to be reached then blood samples are taken to assess arterial glucose specific activity. Ra will be estimated as the ratio of the rate of infusion of [3-3H]glucose and the steady state plasma [3H] glucose specific activity (dpm/mg). Under steady state conditions, the rate of glucose disappearance (Rd) equals the rate of glucose appearance. The rate of glucose clearance is calculated by dividing the Rd by the arterial glucose concentration. Application of this technique is described by Niswender et al. *J. Biol. Chem.* 1997, and She et al. *Mol. Cell. Biol.* 2000.

V3005 Hyperinsulinemic clamp

Keywords: hyperinsulinemic clamp, insulin action, insulin resistance

The hyperinsulinemic clamp is used to measure insulin action in vivo. Hyperinsulinemic clamps are performed on conscious mice with catheters chronically implanted in the jugular vein and carotid artery. A continuous infusion of insulin is given. Glucose levels are monitored in arterial samples every 5-10 min using a Hemocue glucose analyzer that allows the analysis of glucose with only 8 μ l of blood. Glucose is infused in the jugular vein catheter at rates

necessary to achieve the desired glucose level, based on feedback from arterial glucose measurements. These methods allow assessment of the responsiveness of the body to insulin (Halseth et al. Am. J. Physiol. 1999). By combining this technique with the tracer method one can also examine the impact of insulin on suppression of endogenous glucose production.

V3006 Hyperglycemic clamp

Keywords: hyperglycemic clamp, insulin secretion, pancreas

The responsiveness of the pancreas to glucose is assessed using the hyperglycemic clamp. Hyperglycemic clamps are performed on conscious mice with catheters chronically implanted in the jugular vein and carotid artery. A defined hyperglycemic stimulus is created using a primed variable glucose infusion to raise the glucose level to twice basal for 120 min. An established priming algorithm is used to elevate glucose quickly. Mice with extra copies of the glucokinase gene locus were demonstrated to have a blunted insulin response to hyperglycemia using this technique (Niswender et al. J. Biol. Chem. 1997).

V3007 Gluconeogenesis & glycogenolysis (from hepatic 14C-UDPglucose and PEP)

Keywords: gluconeogenesis, glucose production, glycogenolysis, liver

The contribution of gluconeogenesis to the rate of glucose appearance is estimated from the specific activities of 14C-labeled hepatic uridine diphosphoglucose (this is assumed to reflect the specific activity of hepatic glucose 6-phosphate), and hepatic phosphoenolpyruvate (PEP) following the infusion of [U-14C]lactate. [3-3H]glucose is used to measure the rate of glucose appearance. Gluconeogenesis is equal to the rate of glucose appearance \times [14C]UDP-glucose specific activity/[14C]PEP specific activity \times 2. Glycogenolysis is equal to the difference between rates of glucose appearance and gluconeogenesis. The contribution of the kidney to these measurements is assumed to be small.

V3008 Glycogen synthesis

Keywords: glycogen synthesis, liver, muscle

Using [U-14C]glucose, the incorporation of the carbon of glucose into glycogen can be measured. If the ratio of [14C]UDP-glucose to blood glucose specific activity is assessed the fraction of glycogen formation from direct and indirect pathways can be calculated.

V3009 Amino acid kinetics

Keywords: amino acid flux, amino acid kinetics, isotopes

The turnover of phenylalanine (3H ring 2,3,4,5,6 phenylalanine), glutamine (U-14C-glutamine) and leucine (1-14C-leucine) is assessed by a primed continuous infusion of their respective isotopes for 2 hours (0.2-0.4 μ Ci/min). Blood samples (20 μ l) are taken after a steady state is reached to assess plasma amino acid specific activity. Blood samples are mixed with an equal volume of 6% sulfosalicylic acid. Incorporation of tracer in tissue protein is used to assess tissue specific protein synthesis.

V3010 Tissue specific glucose uptake

Keywords: 2-deoxyglucose, glucose metabolic index, tissue specific glucose uptake

Tissue specific glucose uptake is assessed by measuring the tissue specific uptake of [2-3H]-deoxyglucose([2-3H]DG). [2-3H]DG is infused (0.2 μ Ci/min) for 40 minutes or injected (12 μ Ci). Arterial plasma samples are taken to determine the time course of [2-3H]DG during the 40 min period. [2-3H]DG is transported into cells and phosphorylated to yield [2-3H]DG-6-phosphate which is trapped in muscle. After 40 min mice are anesthetized with an intravenous infusion of pentobarbital and tissues of interest are rapidly removed and frozen in liquid nitrogen. This method has been applied during insulin- and exercise-stimulated conditions (Halseth et al. Am. J. Physiol. 1999).

V3011 Tissue specific fatty acid uptake

Keywords: 125I-BMIPP, tissue specific fatty acid uptake

Tissue fatty acid uptake is assessed by measuring tissue-specific incorporation of circulating 125I-BMIPP (Rottman et al. Am. J. Physiol. 2002). The beta-methyl modification of the long-chain fatty acid BMIPP (beta-methyl-p-iodophenylpentadecanoic acid) causes terminal trapping in the TCA cycle. Studies in man and a variety of small animal models, including rodents, have shown that BMIPP uptake and metabolism closely tracks that of endogenous long-chain fatty acids in a variety of normal and pathophysiologic states.

BMIPP is dissolved in proprionic acid, and incorporation of 125I is catalyzed with CuSO₄. After extraction, the purified 125I-BMIPP is dissolved in ursodeoxycholic acid, filtered, and adjusted to defined activity. This stable compound is suitable for direct intravascular injection. Serum levels are stable in tracer amounts after injection, and tissue incorporation is measured by gamma counting of freeze-clamped samples in protocols compatible with the simultaneous assay of, for example, [2-3H]DG.

V3012 Indirect calorimetry /energy expenditure

Keywords: carbon dioxide, energy expenditure, gas exchange, indirect calorimetry, oxygen

Whole body VO₂ and VCO₂ is measured continuously in conscious mice using a very flexible system. The core of this system is the Oxymax Deluxe System. Oxymax software allows for calibration, experiment execution and the review of obtained data. Data can be exported or reviewed within the Oxymax System.

The system used is flexible and sensitive enough to measure small changes in VO₂ and VCO₂. It can be used to measure resting or exercising gas exchange and energy expenditure.

V3013 Exercise capacity (metabolic response to exercise)

Keywords: endurance, exercise capacity, exercise tolerance

Exercise is an integrated measure of fitness. Abnormal exercise capacity and decreased activity are a hallmark of most severe cardiovascular and metabolic diseases, and changes in exercise capacity are sensitive and early markers of cardiac and metabolic dysfunction. Thus abnormalities can be revealed with exercise that may not otherwise be manifested. Gas exchange techniques can be used during treadmill exercise in the mouse to describe the metabolic cost of exercise. Substrate fluxes and metabolism can be assessed isotopically during exercise in chronically catheterized mice (Halseth et al. Am. J. Physiol. 1999).

Treadmill exercise can be used to quantify the capacity of a mouse for either endurance or high intensity exercise. Peak exercise capacity and VO₂ max will be measured using a closed gas exchange treadmill. Acclimated mice will exercise will at 3.5 m/min, 0° grade, increased to 5 m/min, 2° grade 3 minutes later and then increased by 2.5 m/min and 2° grade every 3 min thereafter up to 20 m/min and 14° grade.

V3014 Spontaneous exercise activity

Keywords: spontaneous exercise activity, wheel running

Spontaneous exercise activity is measured using a recording wheel placed in the cage during a 48 h period. The light dark cycle will be stringently controlled to minimize diurnal variations and training effects will be minimized by placing an identical wheel in the cage for the 24 hrs preceding the test measurement. Variables recorded include total distance traveled, peak speed and exercise duration.

V3015 Food Consumption

Keywords: spontaneous exercise activity, wheel running

Food consumption is assessed using an automated feeding apparatus that continually measure feeding behavior in an unobtrusive manner by allowing animals free access to food cups that are mounted on balances. The apparatus currently is capable of measuring and time-stamping individual weights from 16 balances simultaneously every 30 seconds and downloading the data directly to a computer for subsequent analysis. Therefore, cumulative food consumed and the time at which feeding bouts occur are continuously monitored. All feeding studies are done after the animal has acclimatized to the facility for at least 24 hours.

V3016 Exploratory locomotor activity

Keywords: energy expenditure, exploratory locomotor activity

Exploratory locomotor activity

V3017 Assess real time imaging of cellular metabolic events

Keywords: islets, metabolism, microcirculation, muscle, real time imaging

V3018 In vivo optical imaging of gene expression

Keywords: gene expression, GFP, luciferase

V3000A Miscellaneous Tissue and Body Fluid Collection Consult with Director

Keywords: tissue

Miscellaneous Tissue and Body Fluid Collection

V3000B Miscellaneous Implantation of Catheters, Sensors and Pellets Consult with Director

Keywords: catheterization

Miscellaneous Implantation of Catheters, Sensors and Pellets

V3000C Equipment Usage Consult with Director

Keywords:

Equipment Usage

V3000D Personnel Training Consult with Director

Keywords:

Personnel Training

V3000E Cerebral Ventricle Cannulation Consult with Director

Keywords: cerebral ventricle

Cerebral Ventricle Cannulation

V3000F Jugular Vein and Carotid Artery Catheterization Consult with Director

Keywords: catheterization

Jugular Vein and Carotid Artery Catheterization

Cardiovascular Pathophysiology & Complications Core**V3030 In vitro Morphology, Morphometrics and Histology (isolated heart)**

Keywords: cardiac function, heart, morphology

A limited necropsy is conducted, noting gross observations, and removing and weighing the heart and lungs separately. After fixation, the heart is sectioned in a standard four-chamber view. Digital photographs on each heart is recorded and archived in a web-accessible format. Chamber and mural dimensions will be measured. The fixed hearts will be maintained in a physical archive, while one slice will be paraffin embedded. 4-5 sections will be cut and prepared for H& E and Masson trichrome. Digital photomicrographs will be recorded and archived together with summary evaluations. A cardiologist with special expertise in mouse cardiac development and histology reviews all gross and microscopic sections.

V3031 Echocardiography, in vivo morphology, systolic and diastolic function; Stress echocardiography

Keywords: diastolic, echocardiography, morphology, stress, systolic

Echocardiography can detect the presence of localized or generalized hypertrophy or thinning of the myocardium of the left ventricle and the presence of regional or global wall motion abnormalities. In addition to assessment of left ventricular systolic dysfunction, the application of transmitral Doppler analysis allows the detection of abnormal filling patterns associated with left ventricular diastolic dysfunction. The presence of congenital or acquired structural abnormalities of the mitral valve, as well as obstructions of left ventricular outflow (at the muscular, subvalvular, valvular or supra-valvular level) can also be evident on echocardiographic screening.

Mice are maintained at an ambient temperature of 37°C prior to imaging. Electrodes are adhered to mice using Redux creme™ to help with conductivity. Mice are imaged with transducers of at least 12 MHz using a standoff generated with Aquasonic gel™ (that is pre-centrifuged to remove air bubbles) placed in the tip of a late glove finger pulled over the tip of the transducer. The transducer is pressed gently against the mouse.

Mice are placed in a left lateral decubitus position. They are imaged initially in a parasternal long axis view to study the septum, posterior wall, apex and left ventricular outflow tract. Short axis views at the chordal level to study symmetry of wall thickness and contraction and from the supraclavicular window to examine the aortic arch and the ascending

and descending aorta is obtained subsequently. For Doppler studies a mid-precordial long-axis view of the heart is acquired for color-flow and pulsed-Doppler recording. Two-dimensional and Doppler images are recorded on video tape; M-mode images are recorded on a strip chart at a speed of 100 mm/sec. Stress echocardiography is assessed following the administration of dobutamine 1.0 to 1.5 µg/g body weight by intraperitoneal injection.

The process of acclimation of the mouse to the desired temperature and imaging will take a total of 2-3 hours per mouse, though total technician attention is not required throughout this period. The stress component of the study following the administration of dobutamine is additional 10-30 minutes per mouse. All data are recorded on videotape, indexed by animal code number. A summary interpretation, as well as dimensions from the M-mode tracings, is entered in the database. A trained echocardiographer reviews data quality, raw data, and interpretation on each mouse.

V3032 Electrocardiography and telemetry

Keywords: cardiac, ECG, EKG, electrocardiography, heart, telemetry

Because of difficulties in detecting arrhythmias in anesthetized mice, a commercially-available system for recording ECG data in unanesthetized, unrestrained animals is used. Small (< 4 gm) telemetry devices (Data Sciences, Inc.) are implanted under anesthesia in the peritoneum and the wires tunneled to appropriate locations (generally left shoulder and right leg). The animals recover within a day, and ECG data can then be obtained for many hours via a telemetry receiver placed under the cage. The system has been adapted to allow detection of periods of irregular heart beat (as is seen with ventricular ectopic activity or atrial fibrillation), and analysis of heart rate variability. Data acquisition procedure consists of recording ECG data for 30 minutes from each animal typically followed by intra-peritoneal administration of isoproterenol (100 µg). An electrophysiologist examines raw and analyzed data and insures quality control.

V3033 Blood pressure measurements

Keywords: blood pressure, blood vessel, hypertension, hypotension, vascular

Blood pressure represents an integrated measure of overall cardiovascular function, and is affected by stroke volume, heart rate, inotropic state, and vascular tone. Abnormalities of blood pressure regulation (primarily hypertension, but also hypotension) are associated with major cardiovascular morbidity and mortality, and are epidemiologically associated with diabetes. Basal measurements of blood pressure are performed after mice are acclimated to the tail cuff apparatus during 3 sessions on successive days. This regimen has been shown to reduce stress-related perturbations and artifacts in measurement. Systolic and diastolic pressures are then measured over 3 sequential determinations. To mimic a particularly important human stress, we can also apply a vascular pathology model in which we induce a hypertrophic phenotype. Measurements can also be made in a model of vascular pathology in which a hypertrophic phenotype is induced dissolving 1 mg/ml of L-NAME in drinking water for 30 days. The systolic arterial pressure and heart rate of each mouse is measured before treatment and after the second and fourth weeks of treatment.

V3034 Vascular morphology

Keywords: blood vessel, histology, intima, smooth muscle, vascular

A variety of tissues (heart, aorta, kidney, brain) can be processed in four µm sections. Aortic sections are examined for wall thickening, perivascular fibrosis, and fibrin deposition. The inner border, the lumen outer border, the tunica media are traced in each arterial image with Masson's trichrome stain and imaged at a magnification of 200X. The lumen ratio (the medial thickness to internal diameter and the area fibrosis (collagen deposition stained with aniline blue) surrounding blood vessels are calculated and compared. Perivascular fibrosis is determined as the ratio of the area of fibrosis surrounding the vessel wall to the total vessel area.

V3035 Electrolytes, indices of renal function

Keywords:

V3036 Metabolic panel

Keywords:

V3094 Perfusion-Fixation/Heart Dimension

Keywords:

Perfusion-Fixation/Heart Dimension

V3095 Heart Rate Open Variability

Keywords:

Heart Rate Open Variability

V3096 Ventricular Hemodynamics

Keywords:

Ventricular Hemodynamics

V3097 Perfusion-Fixation/Histopathology/Quantify Sclerosis

Keywords:

Anesthetized mouse will be perfused with phosphate buffered saline (PBS) at 150-160 mmHg via a butterfly 23G needle inserted into left ventricle. One kidney can be harvested and the other kidney can be further perfused with 4% paraformaldehyde.

Mouse kidney perfused with 4% paraformaldehyde will be embedded in parafine. Four-micrometer section can be processed and stained with hematoxylin-eosin (HE) or periodic acid Schiff (PAS).

Glomerulosclerotic score can be calculated in PAS stained section. Mesangial matrix expansion occupying <25, 25-50, 50-75, or >75% of tuft is scored 1, 2, 3, and 4+, respectively, and no mesangial expansion was scored as 0.

V3098 GFR-FITC-Inulin; HPLC Cr

Keywords: glomerular filtration, HPLC, kidney

GFR will be measured in conscious mice based on the decay rate of plasma FITC-inulin following a single bolus intravenous injection of FITC-inulin. This method does not require urine collection, and GFR can be periodically measured in same mouse.

Additional approach for determining GFR in conscious mouse is based on creatinine clearance rate. Mouse 24-hour urine will be collected using metabolic cage. Plasma and urinary creatinine concentration will be determined using HPLC approach.

V3099 Albuminuria

Keywords: kidney, urine

Mouse urinary albumin excretion rate can be determined by two methods: (1) measuring the albumin to creatinine ratio in spot urine sample; (2) measuring albumin concentration in urine collected over 24 hours using metabolic cage. Urinary albumin and creatinine concentration will be determined using cartridge-based DCA2000 (Bayer Diagnostics) or ELISA kits (Exocell Inc).

V4000 Renal Blood Flow (Doppler)

Keywords: blood flow, blood pressure, kidney

Mouse renal cortical and medullary blood flow can be measured using a laser-doppler flowmeter (Tansonic Systems Inc). This system will also monitor blood pressure and heart rate. Renal function including urinary electrolyte excretion can be studied.

V4001 Urine Na/K

Keywords: plasma, potassium, sodium, urine

Sodium and potassium concentration in mouse plasma and urine will be determined using an automatic flame photometer (Instrumentation laboratory Inc). This measurement requires 20 µl of plasma or urine.

V4002 Osmometer Plasma/Urine

Keywords: osmolality, plasma, urine

Plasma and urine osmolality will be determined using a freezing point osmometer (Precision System Osmette). This measurement requires 50 µl of plasma or urine.

V4003 Urine Ca/Phosphorus Excretion

Keywords: urine

Calcium and phosphorus are two important electrolytes in the urine. The concentration of urinary calcium and phosphorus will be determined using colorimetric assay (BioAssay Systems). The measurement for calcium and phosphorus requires 5 μ l and 50 μ l of urine, respectively.

V4004 Urine pH

Keywords: pH, urine

The pH can be determined in as little as 5 μ l of urine (or other body fluid) using a Mini Combo pH Electrode (World Precision Instruments).

V4005 Glycemic Control using Minimed

Keywords: glucose

Blood glucose levels over 72 hours can be monitored in conscious mice using Medtronic MiniMed CGMS System (Medtronic). In this system, blood glucose level is determined based on glucose concentration in interstitial fluid. A correlation between glucose levels in the blood and interstitial fluid in mice has been previously demonstrated. A fiber probe will be implanted subcutaneously. This probe will detect interstitial glucose levels every ten seconds over three days and the signals will be stored in a glucose monitor. The probe will be removed after the experiment and the mice can be sent back to the researcher.

Analytical Resources Core**V3090 Full amino acid profiles by HPLC / PITC or HLPC / OPA**

Keywords: amino acids, HPLC

The laboratory primarily measures full amino acid profiles using reverse-phase, HPLC and either derivatized with phenylisothiocyanate (PITC) or orthophthalaldehyde (OPA). The minimal sample requirement is 5 μ l injection volume.

V3050 Insulin

Keywords: hormone

The insulin assay is a double-antibody/PEG RIA. The lower limit of detection is 0.02 ng/ml. Ten μ l of sample are required for a single analysis.

V3051 Glucagon

Keywords: hormone

The glucagon assay is a double-antibody/PEG RIA. The lower limit of detection is 5 pg/ml. The volume of sample required for a single analysis is 10 μ l.

V3052 Corticosterone

Keywords: hormone

The mouse corticosterone is a solid phase RIA procedure. The lower limit of detection is 20 ng/ml. The volume of sample for a single analysis is 10 μ l.

V3053 Catecholamines

Keywords: hormone

The method employed is an HPLC procedure. The lower limits of detection for norepinephrine and epinephrine are 20 pg/ml. The volume of sample required for a single analysis is 100 μ l.

V3054 Leptin

Keywords: hormone

Mouse leptin is assayed using a double antibody/PEG RIA. The lower limit of detection is 0.5 ng/ml. The volume required a single measurement is 20 μ l.

V3055 C-Peptide

Keywords: hormone

C-Peptide is a five-day double antibody procedure. Requires 25 micro liters of plasma for duplicate analysis.

V3056 Growth Hormone (GH)

Keywords: hormone

Growth hormone is assayed in a duplicate analysis five-day double antibody procedure using 25 micro liters.

V3058 TSH

Keywords: hormone

TSH is assayed in a double-antibody RIA format. 55 micro liters of plasma is required for duplicate analysis. It involves a dilution step and is a 5-day double antibody procedure. The lower limit of the assay is 0.4ng/ml.

V3059 PRL

Keywords: hormone

Prolactin is assayed in a double-antibody RIA format. 25 micro liters of plasma is required for the assay. A dilution step is required and is 5-day double antibody assay. The lower limit of detection if 1.5ng/ml.

V3060 ACTH

Keywords: hormone

ACTH is assayed in a duplicate analysis five-day double antibody procedure using 55 micro liters of plasma.

V3061 Insulin-like growth hormone-1 (IGF-1)

Keywords: hormone

IGF-1 is a five-day double antibody procedure. 30 micro liters of plasma is used for extraction and duplicate analysis.

V3070 Plasma lipids

Keywords: fat, lipids, metabolism

Total plasma cholesterol and triglyceride are measured by standard enzymatic assays. HDL cholesterol is measured with the enzymatic method after precipitation of VLDL and LDL using dextran sulfate and Mg^{++} . Using these data LDL cholesterol can be calculated using the Friedewald equation, if triglyceride levels are below 400 mg/dl. Investigators may request a total plasma lipid profile or specific plasma lipid measurements.

Free fatty acids are extracted from plasma using heptane/isopropanol. The heptane layer containing FFA is removed, plated on silica gel plates and developed in petroleum ether, ethyl ether, and acetic acid. The FFA band is scraped from the plate and FFA are eluted with heptane /isopropanol. The solvent is removed, and the FFAs are methylated. Methylated fatty acids are analyzed by gas chromatography. Depending on the assay a variety of chromatograph conditions and columns are utilized. A computer identifies each fatty acid peak and can provide data in a number of different ways including quantitation of mass of fatty acid, percent distribution of fatty acids present, quantitation of total lipid in the sample.

V3071 Lipid extraction, separation, quantitation

Keywords: fat, lipids, metabolism

Lipids are extracted from tissue, cells, or plasma. Individual lipid classes are separated by thin layer chromatography. Lipid classes are visualized by either iodine vapors or rhodamine 6G, scraped from the plates and eluted from the silica gel. For colorimetric analysis of lipid classes, the individual lipids are eluted from the TLC silica gel. Phospholipids are analyzed either in total lipid extract or on phospholipid fraction eluted from TLC plates. Cholesterol esters and unesterified cholesterol are analyzed by the method of Babson. Alternatively, cholesterol is analyzed by gas chromatography. Lipid classes containing fatty acids are also quantitated by gas chromatography.

V3072 Fatty acid profiles of lipid esters by gas liquid chromatography

Keywords: fat, GCMS, lipids, metabolism

Total lipids are extracted and lipid classes separated by TLC as described above. Lipid ester spots are scraped from the plates and methylated. Fatty acids of lipid esters can be methylated without removal of the lipid from the silica gel. However, in some applications, we have found it advantageous to elute the lipid from the silica gel prior to methylation. The fatty acid profile of the lipid class is also determined. By this method total lipid mass and fatty acid profile for each lipid is determined.

V3073 Quantitation of individual phospholipid classes

Keywords: cholesterol, fat, lipids, metabolism, phospholipids

Individual phospholipid classes are isolated by one dimensional TLC. A total lipid extract is applied to high performance TLC plates. To quantitate the individual classes, the spots are scraped from the plate, eluted and phosphorus is determined. To determine the fatty acid composition of the individual phospholipid classes, the spots are scraped from the plates and fatty acids methylated.

V3074 Short chain fatty acid analysis by gas liquid chromatography

Keywords: fat, GCMS, lipids, metabolism, short chain fatty acid

Plasma short chain fatty acids are analyzed by the following procedure: to 200 μ l of EDTA plasma in a 1.5 ml Eppendorf microfuge tube is added 20 μ l of internal standard and 1 ml of absolute ethanol. The sample is mixed thoroughly, centrifuged, and the supernatant is recovered. The sample is evaporated using a Speed Vac and dissolved in 15 μ l water, and prior to injection 5 μ l of orthophosphoric acid (25%) is added. The short chain fatty acids are separated on a 6' x 2 mm glass column packed with SP-1200/1%H₃PO₄ on 80/100 Chromosorb W AW.

V3075 Lipoprotein fractionation and characterization

Keywords: fat, lipids, lipoproteins, metabolism

Lipoprotein fractions are isolated using columns arranged in tandem to achieve complete resolution of the major lipoprotein classes from 1-2 ml of plasma. The columns are equilibrated in 50 mM phosphate-buffered saline and calibrated using lipoprotein fractions isolated by ultracentrifugation. Fractions (0.5 ml) are collected and the appropriate tubes containing the desired lipoprotein fraction(s) combined. The position of the major lipoprotein classes are determined by cholesterol (or triglyceride) assay on the column fractions using a microtiter plate enzyme-based assay. As an alternative method lipoproteins can be isolated by fast protein liquid chromatography.

This includes analysis of the composition of the fraction (protein and lipid) as well as morphologic analysis (sizing) by negative stain electron microscopy. For compositional analysis the lipoprotein fractions protein is analyzed using the BCA method with a modification to eliminate lipid interference. The samples are then lyophilized and delipidated using ethanol and ether. Lipid components are separated by TLC and analyzed by GLC and/or colorimetric assays.

V3076 Morphometric determinations (aorta)

Keywords: blood vessel, histology, intima, smooth muscle, vascular

Mice are sacrificed and flushed with 30 ml saline. The heart with ascending aorta is embedded and snap-frozen in liquid N₂. Cryosections of 10 μ m thickness are taken from the region of the proximal aorta. Cryosections are stained with Oil Red O and counterstained with hematoxylin. In addition to the aortic cross-sections, whole aortas will be analyzed in "en face" preparations to evaluate the distribution and characteristics of atherosclerotic lesions in the distal aorta. After the removal of the heart and the aortic arch, the entire remainder of the thoracic and abdominal aorta is dissected from the carcass. An incision is then performed longitudinally and for the total length of the specimen, so to expose the inside face. The open face aorta is pinned out on a black cardboard submerged in saline, and then stained with Oil Red O.

V3080 Gross examinations and necropsy

Keywords: gross examination, necropsy

The standard necropsy procedure for diabetic mice includes an examination of the pancreas, heart, liver, eyes, peripheral nerves, peripheral vasculature, fat, and kidneys. Complete, intermediate, or limited necropsies will be performed with or without the aid of a dissecting microscope. Gross pathologic findings will be described, documented by digital photography and organs will be weighed.

V3081 Tissue preparation, embedding, sectioning and routine staining

Keywords: embedding, sectioning, staining, tissue preparation

The default fixative will be 4% paraformaldehyde. This offers investigators the option of later performing in situ hybridization or laser microdissection and capture of protein, DNA or RNA from select populations. This fixative provides a minimal degree of cross-linking of the proteins, thereby rendering antigenic sites more accessible. Other fixatives are used as appropriate. If frozen sections are needed, tissues will be snap frozen in liquid nitrogen and sectioned on a cryostat. When molecular analyses are required, tissue samples can also be rapidly placed in Trizol or RNA extraction solutions and stored at -80°C until investigators retrieve their samples.

V3082 Tissue microdissection

Keywords: laser microdissection, pancreas

Potential applications of laser dissection include the selective microdissection of islet cells for gene expression studies, harvesting and analysis of specific glomerular cells in diabetic nephropathy, and harvesting endothelial cells from diseased microvasculature. This Core has available a PixCell II™ laser capture microdissection device (Arcturus Engineering) and a PALM microdissection scope. These workstations perform laser capture microdissection simply, quickly and precisely. They are capable of locating single cells or large groups of cells and, using a simple aim-and-shoot method to extract them for subsequent molecular analysis (DNA, RNA, or protein).

V3083 Screen/optimize immunohistochemical protocols for mouse-specific commercial and custom-designed antisera

Keywords: histology, immunohistochemistry

To stain mouse tissues with mouse monoclonal antisera we utilize Mouse-on-Mouse (MOM) kits (Vector Labs), Ark kits (DAKO Corp) or HistoMouse Kits. Although peroxidase-based protocols will be the mainstay of the Subcore with visualization by brown DAB or red AEC chromagens, staff is familiar with the alkaline phosphatase-based kit and its detection with the fuschin chromagen. Fluorescently-tagged primary, secondary, or tertiary antibodies in frozen sections, cultured cells, or for co-localization studies will be used as required to tailor protocols to address investigator needs. Non-fluorescent double immunostaining will also be performed in the Pathology Subcore using the Double Label Kit provided by DAKO. When prospective antisera fail to recognize antigenic sites in paraffin embedded sections or produce non-specific staining patterns, we advise investigators to proceed with frozen sectioning.

V3091 Specific selected amino acid profiles

Keywords: amino acids, HPLC

BCAA and phenylalanine, gluconeogenic amino acids, and glutamate and GABA are measured using OPA derivatization and reverse-phase HPLC. These methods offer the advantages of speed, small sample sizes (<20 µl) and high sensitivity.

V3092 Radioactivity of specific individual amino acids

Keywords: amino acids, chromatography, protein synthesis, proteolysis, specific activity

a. Alanine and glutamine: Deproteinized blood will be injected onto a cation exchange column; separating the amino acids. As the amino acids elute, a portion of the eluent is diverted to a fraction collector for counting of radioactivity. A portion of the eluent is derivatized with ninhydrin. The color complex produced is measured to determine amino acid concentrations.

b. Leucine and phenylalanine: An HPLC method, requiring 20 µl of blood is used to measure specific activities in blood and tissue in tissues.

V3093 Specific activities for gluconeogenic and glycogenic assessment

Keywords: amino acids, chromatography, protein synthesis, proteolysis, specific activity

UDP glucose, UDP -galactose and PEP specific activities in percholoric acid extracts of liver will be obtained by sequential chromatographic separations as described by Rossetti.

V3062 Aldosterone

Keywords: hormone

The aldosterone assay is a non-extraction double-antibody RIA. The lower limit of detection is 2 pg/ml. 45 micro liters of plasma for duplicate analysis. Involves a dilution step and is a 2-day double antibody procedure.

V3064 Resistin

Keywords: hormone

Resistin may be analyzed in a double - antibody RIA format. It is a two day assay at room temperature. Sample volume: 50 μ L Plasma, serum or tissue culture medium. The lower limit of detection is 0.78 ng/mL. Can also be run as single-plexed assays using luminex instrumentation. A dilution step is required for the adiponectin requiring a minimum of 5 ul of sample. The resistin does not require a dilution and may be multiplexed with other adipokines with a required sample volume of 25 ul for duplicate analysis.

V3065 Adiponectin

Keywords: hormone

Adiponectin may be analyzed in a double-antibody RIA format. It requires an overnight incubation at room temperature. Sample volume < 2 μ L serum or plasma, or < 100 μ L tissue culture media. A 1:400 x dilution is required. The lower limit of detection is 1.56 pg/mL. Can also be run as single-plexed assays using luminex instrumentation. A dilution step is required for the adiponectin requiring a minimum of 5 ul of sample. The resistin does not require a dilution and may be multiplexed with other adipokines with a required sample volume of 25 ul for duplicate analysis.

V3066 Estradiol

Keywords: hormone

Estradiol is assayed in a double- antibody RIA in an overnight incubation at 37C + 15-20 minutes at room temperature. Fifty micro liters of sample are required for duplicate analysis. The lower limit of detection is 4.7 pg/ml.

V3067 Testosterone

Keywords: hormone

Testosterone is assayed in a Double Antibody RIA. Fifty μ L of serum or plasma is required for duplicate analysis. An overnight incubation at 37C + 10-15 minutes at room temperature are required. The lower limit of detection is 0.05ng/ml.

YALE UNIVERSITY SCHOOL OF MEDICINE

In Vivo Metabolism Core

Y4001 Hyperinsulinemic-euglycemic clamp experiments

Keywords: insulin action, insulin resistance

The surgery is performed at 4-5 days prior to the hyperinsulinemic-euglycemic clamp to establish a chronic catheter for intravenous infusion of substances (e.g., glucose, insulin) during the clamp. For this, a mouse is anesthetized with an intraperitoneal injection of ketamine and xylazine, and a catheter is inserted in the right jugular vein. On the day of clamp experiment, an overnight-fasted mouse is placed in an over-sized restrainer (i.e., rat-sized) for the experiment to be conducted in awake and minimally-stressed state. The tail is tethered using a tape for 2 hours prior to the start of experiment for acclimatization. A 3-way connector is attached to the jugular vein catheter for intravenous infusion, and the blood samples are obtained from the tail vessels requiring a small tail cut. A 2-hour hyperinsulinemic-euglycemic clamp is conducted with a primed-continuous infusion of human insulin at a rate of 15 pmol/kg/min to raise plasma insulin within a physiological range (~300 pM). Blood samples (20 ml) are collected at 10-20 min intervals for the immediate measurement of plasma glucose concentration, and 20% glucose is infused at variable rates to maintain glucose at basal concentrations (~6 mM). Insulin-stimulated whole body glucose metabolism is assessed with a continuous infusion of [3-3H]glucose (0.1 mCi/min) throughout the clamps. Basal rates of whole body glucose turnover are assessed using a primed-continuous infusion of [3-3H]glucose for 2 hours prior to the start of clamp. All infusions are performed using the microdialysis pumps, and all procedures are approved by Yale University Animal Care and Use Committee. To estimate insulin-stimulated glucose uptake in individual tissues, 2-deoxy-D-[1-14C]glucose (2-[14C]DG) is administered as a bolus (10 mCi) at 75 min after the start of clamp. Blood samples (20 ml) are taken at -5, 80, 85, 90, 100, 110, and 120 min of clamp for the measurement of plasma [3H]glucose, 3H₂O, and/or 2-[14C]DG concentrations. Additional blood samples (20 ml) are collected before and at the end of clamp for the measurement of plasma insulin concentrations. At the end of clamp, mouse is anesthetized with sodium pentobarbital injection, and tissues are taken and stored for biochemical/molecular analysis. The clamp experiment measures tissue-specific insulin action and glucose metabolism and includes the following measurements: 1) basal and insulin-stimulated hepatic glucose production, 2) insulin-stimulated whole body glucose uptake, glycolysis, and glycogen plus lipid synthesis, and 3) insulin-stimulated glucose uptake, glycolysis, and glycogen synthesis in individual tissues (e.g., skeletal muscle, adipose tissue, heart). Additionally, biochemical/molecular assays may be performed to assess tissue-specific insulin signaling activities (e.g., insulin-stimulated tyrosine phosphorylation

of IRS, PI 3-kinase activity) and tissue-specific triglyceride contents. Further details of the clamp experiment can be found in the following references: Diabetes 53:1060 (2004), J. Clin. Invest. 114:823 (2004).

Y4002 Hyperglycemic clamp experiments

Keywords: insulin secretion, pancreas

The procedure involves chronic cannulation of the jugular vein which is necessary for The surgery is performed at 4-5 days prior to the hyperglycemic clamp to establish a chronic catheter for intravenous infusion of substances (e.g., glucose, insulin) during the clamp. For this, a mouse is anesthetized with an intraperitoneal injection of ketamine and xylazine, and a catheter is inserted in the right jugular vein. On the day of clamp experiment, an overnight-fasted mouse is placed in an over-sized restrainer (i.e., rat-sized) for the experiment to be conducted in awake and minimally-stressed state. The tail is tethered using a tape for 2 hours prior to the start of experiment for acclimatization. A 3-way connector is attached to the jugular vein catheter for intravenous infusion, and the blood samples are obtained from the tail vessels requiring a small tail cut. A 2-hour hyperglycemic clamp is conducted with a variable infusion of 20% glucose to raise and maintain plasma glucose concentrations at ~16 mM. Blood samples (20 ml) are collected at 10-20 min intervals for the immediate measurement of plasma glucose concentrations using Beckman Glucose Analyzer. The area under curve of plasma glucose and insulin profiles is assessed to determine glucose-induced insulin secretion in vivo (i.e., pancreatic β -cell function).

Analytical Core

Y4060 Diacylglycerol concentration

Keywords: fat, lipids, metabolism, signaling

Diacylglycerols (DAGs) are extracted from 100mg frozen tissue with chloroform/methanol (2:1, vol/vol) containing 0.01% BHT (butylated hydroxytoluene). Prior to the extraction, known amount of 1,3-dipentadecanoin and triheptadecanoin are added as internal standard. Extracted samples were evaporate to dryness and redissolved in 1ml of hexane:ethyl acetate (85:15, vol/vol). DAGs were isolated from triglycerides (TGs) by use of a diol bondedphase SPE column (Waters, Inc., Milford, MA) under vacuum. The SPE column was preconditioned with 4 ml hexane, the lipid extract was placed on the column and TGs eluted with 8 ml of hexane-methylene chloride-ethyl ether (89:10:1, vol/vol/vol). DAGs were eluted with 8 ml of hexane-ethyl acetate (85:15, vol/vol) into a second set of collection tubes. The solvent were evaporated to dryness under vacuum and redissolved in 0.5 ml of hexane:ethyl acetate (85:15, vol/vol) for LC/MS/MS analysis. Separation of TGs from DAGs was assessed by monitoring for the presence of triheptadecanoin in the DAG fraction. Analysis is performed on a bench top tandem mass spectrometer API3000 (Perkin- Elmer Sciex) interfaced with an APCI (Atmospheric Pressure Chemical Ionization) source in flow injection mode. DAG species are readily ionized in APCI mode, and are quantified by monitoring $_M+H-18_+$ /product ions from corresponding fatty acid moiety.

Y4050 Amino Acids

Keywords: amino acids, enrichment, isotopes, metabolite

BCAA and phenylalanine, gluconeogenic amino acids, and glutamate and GABA concentration, and ^{13}C or 2H isotopic enrichments, are measured (as the trifluoro-acetamide n-butyl ester derivatives) using GC-MS (HP 5973MSD, Hewlett-Packard Instrument Corp., Palo Alto, CA). These measurements can be made in 20 μ l plasma, or 50 mg tissue.

Y4051 Beta-hydroxybutyrate

Keywords: diabetes, enrichment, ketones, metabolite

Plasma beta-hydroxybutyrate concentration, and ^{13}C or 2H isotopic enrichments, are measured (as the TMS derivative) using GC-MS (HP 5973MSD, Hewlett-Packard Instrument Corp., Palo Alto, CA). These measurements can be made in 20 μ l plasma.

Y4052 Free fatty acid

Keywords: diabetes, enrichment, fat, lipids, metabolite

Total serum non-esterified fatty acid concentrations are measured using an acyl-CoA oxidase-based colorimetric kit (Wako NEFA-C, Wako Pure Chemical Industries, Osaka, Japan). For measurements of free fatty acid profiles, and isotopic enrichments, ^{13}C , or 2H , the lipids are extracted from plasma with heptane/isopropanol. This solvent is evaporated and the free fatty acids are methylated using diazomethane, and analyzed by GC-MS (HP 5973MSD, Hewlett-Packard Instrument Corp., Palo Alto, CA). These measurements can be made in 20 μ l plasma.

Y4053 Glucose

Keywords: carbohydrate, diabetes, metabolite

Plasma glucose concentrations are measured by the glucose oxidase method (Glucose Analyzer II; Beckman Instruments, Fullerton, CA). ¹³C, or ²H, isotopic enrichments, are measured (as the penta-acetate derivative) using GC-MS (HP 5973MSD, Hewlett-Packard Instrument Corp., Palo Alto, CA). These measurements can be made in 20 µl plasma.

Y4054 Glycerol

Keywords: diabetes, enrichment, lipids, metabolite

Plasma glycerol concentrations, ¹³C, or ²H, isotopic enrichments, are measured (as the tri-acetate derivative) using GC-MS (HP 5973MSD, Hewlett-Packard Instrument Corp., Palo Alto, CA). These measurements can be made in 20 µl plasma.

Y4055 Glycogen

Keywords: carbohydrate, diabetes, metabolite

Liver, and muscle glycogen is extracted with 0.9 N perchloric acid and 99% ethanol to precipitate glycogen. The glycogen from the pellet is dialysed, and digested to free glucose with amyloglucosidase. Concentrations are determined as described above for glucose. These measurements can be made in 10 mg tissue. Specific carbon labeling is determined by ¹³C-NMR (Avance 500, Bruker, Inc. Billerica, MA). Total ¹³C, or ²H, isotopic enrichments are determined (as the penta-acetate derivative) using GC-MS (HP 5973MSD, Hewlett-Packard Instrument Corp., Palo Alto, CA). These measurements can be made in 100 mg tissue.

Y4057 Long-chain fatty acyl CoA esters

Keywords: fat, lipids, metabolism

Frozen tissue (~100mg), liver or muscle, is ground under liquid nitrogen and homogenized in 100mM KH₂PO₄, pH 4.9 and 2-propanol. Heptadecanoyl CoA was added as internal standard. Saturated (NH₄)₂SO₄ and acetonitrile are added for phase separation solid phase extraction using Oligonucleotide Purification Cartridges (Applied Biosystems, Singapore). The cartridges are washed with distilled water, and then long-chain fatty acyl CoA esters (LCACoAs) are eluted slowly with 0.5ml of 60% acetonitrile. The eluent is dried, then reconstituted in 100µl of methanol/H₂O for ESI/MS/MS analysis. Analysis is performed on a bench top tandem mass spectrometer API3000 (Perkin-Elmer Sciex) interfaced with a TurboIonspray ionization source in flow injection mode. Using negative electrospray ionization mode, LCACoAs are ionized predominantly to doubly charged form and yields abundant specific product ions from CID (collision induced dissociation). LCACoAs are quantified by monitoring [M-2H]²⁻/ [M-H-80]⁻.

Y4059 ADP, ATP

Keywords: energetics, high-energy phosphates, mitochondria

Tissues (50 to 100 mg) are extracted with 0.9N ice-cold perchloric acid. The concentrations of nucleotides, ATP, ADP, and AMP, in the supernatant are then determined by HPLC using a Supelcosil SAX1 (25cm x 4.6 mm x 0.5 µm) column using a gradient of 5 mM ammonium phosphate, pH2.8 (buffer A) and 750 mM ammonium phosphate, pH 3.9 (buffer B) at a flow rate of 1 ml/min. A linear gradient is developed over 14 minutes at 0% buffer B to 9% buffer B, then from 14 to 32 minutes from 9% buffer B to 100% buffer B. A Rainin HPXL solvent delivery system (2 pumps) with a Rainin Dynamax UV-1 absorbance detector (254 nm) controlled by Rainin Dynamax HPLC Method Manager is used for solvent programming and data collection. Peak identification was assigned by comparison of retention times to known external standards (AMP: ~ 5.5 min, ADP: ~ 25.7 min, ATP: ~ 29.0 min). Nucleotide concentrations were calculated from the concentration standard curves of absorbance for the external standards

Y4070 Chem 7

Keywords: serum chemicals, serum metabolic panel

A complete panel consists of five analytical tests. The cost for a complete Chem 7 Panel is 5 times the price indicated for each analysis. Each of these tests can be individually chosen from the catalog.

The serum metabolic panel is obtained using the COBAS MIRA system (Roche Diagnostics, Indianapolis, IN).

The serum chemicals are as follows:

1) BUN (blood urea nitrogen)

- 2) Chloride
- 3) CO₂ (carbon dioxide)
- 4) Creatinine
- 5) Glucose
- 6) Potassium
- 7) Sodium

Y4071 Liver Function Tests

Keywords: serum chemicals, serum metabolic panel

A complete panel consists of seven analytical tests. The cost for a complete Liver Function Test panel is 7 times the price indicated for each analysis. For a subset of this panel, select each desired test individually from the catalog.

The serum metabolic panel is obtained using the COBAS MIRA system (Roche Diagnostics, Indianapolis, IN).

The serum chemicals, and enzyme activities, measured are as follows:

- 1) Albumin
- 2) ALT: Alanine Transferase
- 3) ALP: Alkaline Phosphatase
- 4) AST: Aspartate Aminotransferase
- 5) Total Bilirubin
- 6) Total Protein

Y4072 Lipid Panel

Keywords: serum chemicals, serum metabolic panel

A complete panel consists of six analytical tests. The cost for a complete Lipid Panel is 6 times the price indicated for each analysis. A subset of the complete panel can be chosen.

A serum metabolic panel is obtained using the COBAS MIRA system (Roche Diagnostics, Indianapolis, IN).

The serum chemicals, and enzyme activities, measured are as follows:

- 1) Non-esterified fatty acids
- 2) β -Hydroxybutyrate
- 3) Cholesterol
- 4) HDL-cholesterol direct
- 5) LDL-cholesterol direct
- 6) Triglycerides

Y4073 Divalent Ions

Keywords: serum chemicals

A complete panel consists of three analytical tests. The cost for a complete panel is 3 times the price indicated for each analysis. A subset of the complete panel can be chosen.

A serum metabolic panel is obtained using the COBAS MIRA system (Roche Diagnostics, Indianapolis, IN).

The serum divalent ions measured are as follows:

- 1) Calcium
- 2) Inorganic Phosphorus
- 3) Magnesium

Y4080 Insulin

Keywords: hormone

Plasma immunoreactive insulin is assayed using a double-antibody immunoassay kit and rat insulin standards (Linco Research, St. Louis, MO).

Y4081 Glucagon

Keywords: hormone

Plasma immunoreactive glucagon is assayed using a double-antibody immunoassay kit (Linco Research, St. Louis, MO).

Y4082 Leptin

Keywords: hormone

Plasma immunoreactive leptin is assayed using a double-antibody immunoassay kit (Linco Research, St. Louis, MO)

Y4061 Lysophosphatidic Acid

Keywords: lipids

Y4083 Blood Glucose

Keywords: carbohydrate, diabetes, glucose, plasma, serum chemicals, serum metabolic panel

Measures serum or plasma glucose levels by the Hexokinase (HK) Glucose-6-phosphate dehydrogenase (G-6-P-DH) reactions. The test is performed on a Roche COBAS Mira Plus automated chemistry analyzer.

Y4084 Blood Urea Nitrogen

Keywords: renal, serum chemicals, serum metabolic panel

Determination of "blood" urea nitrogen (BUN) is used as a test for renal function, usually in conjunction with other tests such as creatinine. The test is performed on serum or plasma using the Roche COBAS Mira Plus automated chemistry analyzer.

Y4085 Blood Creatinine-HPLC

Keywords: kidney, muscle, renal, serum chemicals, serum metabolic panel

The quantitation of creatinine in serum or plasma can be useful in determining renal function, particularly in combination with the BUN assay. Creatinine is quantified by HPLC/MS/MS.

Y4086 Urine Creatinine-HPLC

Keywords: kidney, muscle, renal, serum chemicals, serum metabolic panel, urine

The quantitation of creatinine in urine can be useful in determining renal function, particularly in combination with the BUN assay. Creatinine is quantified by HPLC/MS/MS.

Y4087 Blood Electrolytes-Na/Cl/K

Keywords: electrolytes, metabolism, muscle, pH, plasma, potassium, serum chemicals, serum metabolic panel, sodium

For the determination of plasma or serum electrolyte levels (chloride, potassium, and sodium) using ion-selective electrodes on the Roche COBAS Mira Plus automated chemistry analyzer. All three analytes are measured simultaneously, and are therefore ordered and reported as a group.

Y4089 Blood Bicarbonate/CO2

Keywords: carbon dioxide, metabolism, serum chemicals, serum metabolic panel

Approximately 90% of carbon dioxide present in serum or plasma is in the form of bicarbonate. The remainder is in the form of dissolved gas and as carbamino-bound CO₂. The measurement of serum or plasma CO₂ content, when performed in conjunction with the determination of pH, is useful in the assessment of disturbances acid-base balance in respirator or metabolic acidosis and alkalosis. The enzymatic assay is performed on blood serum or plasma with a Roche COBAS Mira Plus automated chemistry analyzer.

Y4091 Blood Albumin

Keywords: liver, plasma, serum albumin, serum chemicals

Elevated serum albumin is seldom encountered, and it is usually a result of dehydration. Main causes are malnutrition, decreased synthesis in liver diseases, proteinuria in the nephrotic syndrome, losses or decreased absorption in gastrointestinal diseases, carcinomatosis, congestive heart failure, and/or losses from extensive skin lesions such as diffuse dermatitis and burns. Determinations of blood albumin levels are conducted on the Roche COBAS Mira Plus automated chemistry analyzer, using the albumin-bromocresol green reaction.

Y4092 Alanine Aminotransferase

Keywords: ALT, liver, plasma, serum chemicals

The ALT test can be used for the diagnosis of acute hepatic diseases. The determination of ALT activity in serum or plasma is conducted on the Roche COBAS Mira Plus automated chemistry analyzer.

Y4093 Aspartate Aminotransferase

Keywords: liver, plasma, serum chemicals

This test is used for the quantitative determination of aspartate aminotransferase activity in serum or plasma on the Roche COBAS Mira Plus automated chemistry analyzer.

Y4094 Alkaline Phosphatase

Keywords: liver, plasma, serum chemicals

Alkaline phosphatase is found in almost every tissue in the body. Most of the ALP in normal adult serum is from the liver or biliary tract. Elevation of alkaline phosphatase values occurs in liver diseases such as hepatitis, cirrhosis, malignancy, chemical toxicity, and in bone diseases such as metastatic carcinoma, rickets, Paget's disease, and osteomalacia. This test is for the quantitative determination of alkaline phosphatase (E.C. 3.1.3.1) activity in serum or plasma and uses the Roche COBAS Mira Plus automated chemistry analyzer.

Y4095 Total Bilirubin

Keywords: bilirubin, liver, serum chemicals

This test is intended for the quantitative determination of total bilirubin in serum using the Roche COBAS Mira Plus automated chemistry analyzer.

Y4097 Total Protein

Keywords: liver, plasma, serum chemicals

This test is intended for the quantitative determination of total protein in serum or plasma using the Roche COBAS Mira Plus automated chemistry analyzer.

Y4098 HDL Cholesterol

Keywords: cholesterol, lipids, lipoproteins

This test is intended for the quantitative determination of HDL cholesterol in serum or plasma using the Roche COBAS Mira Plus automated chemistry analyzer.

Y4099 LDL Cholesterol

Keywords: cholesterol, lipids, plasma, serum chemicals

This test is intended for the quantitative determination of LDL cholesterol in serum or plasma using the Roche COBAS Mira Plus automated chemistry analyzer.

Y5000 Cholesterol

Keywords: cholesterol, lipids, plasma, serum chemicals

This test is intended for the quantitative in vitro measurement of total serum or plasma cholesterol concentrations, utilizing the Roche COBAS Mira Plus automated chemistry analyzer.

Y5001 Triglycerides

Keywords: lipids, plasma, serum chemicals

This test is intended for the quantitative determination of triglycerides in serum or plasma, utilizing the Roche COBAS Mira Plus automated chemistry analyzer.

Y5002 Non-Esterified Fatty Acids

Keywords: fatty acids, lipids, non-esterified fatty acid, serum chemicals

This test is an in vitro enzymatic colorimetric method for the quantitative determination of non-esterified fatty acids (NEFA) in serum, utilizing the Roche COBAS Mira Plus automated chemistry analyzer.

Specimens that are noticeably icteric, hemolyzed, or lipemic will require a sample blank in order to yield accurate results. A sample blank requires a minimum serum volume equal to that of the normal assay, and will be billed as a separate assay.

Y5003 Beta-Hydroxybutyrate (COBAS)

Keywords: diabetes, ketones, plasma, serum chemicals

This test is intended for the quantitative determination of beta-hydroxybutyrate in serum or plasma, utilizing the Roche COBAS Mira Plus automated chemistry analyzer.

Y5004 Blood Calcium

Keywords: serum chemicals

This is intended for the quantitative determination of calcium in serum, utilizing the Roche COBAS Mira Plus automated chemistry analyzer.

Y5005 Blood Inorganic Phosphorous

Keywords: inorganic phosphate, phosphate, serum chemicals

This test is intended for the quantitative determination of inorganic phosphorous in serum using the Roche COBAS Mira Plus automated chemistry analyzer.

Y5007 Magnesium

Keywords: serum chemicals

This test is intended for the quantitative determination of serum magnesium, utilizing the Roche COBAS Mira Plus automated chemistry analyzer.

Y5006 Urine Inorganic Phosphorous

Keywords: inorganic phosphate, phosphate, urine

This test is intended for the quantitative determination of inorganic phosphorous in urine, utilizing the Roche COBAS Mira Plus automated chemistry analyzer.

Y5008 Creatine Kinase

Keywords: creatine kinase, serum chemicals

This test is intended for the quantitative determination of creatine kinase in serum, utilizing the Roche COBAS Mira Plus automated chemistry analyzer.

Y5009 Lactate Dehydrogenase

Keywords: serum chemicals

This test is intended for the quantitative determination of lactate dehydrogenase activity in serum, utilizing the Roche COBAS Mira Plus automated chemistry analyzer.

Y5010 Apolipoprotein C3

Keywords: lipids, lipoproteins, serum chemicals

This test is intended for the quantitative determination of human apolipoprotein (ApoC3) in serum by immunoturbidimetric assay, utilizing the Roche COBAS Mira Plus automated chemistry analyzer.

Y4088 Urine Electrolytes-Na/K/Cl

Keywords: electrolytes, muscle, potassium, urine

For the determination of urine electrolyte levels (chloride, potassium, and sodium) using ion-selective electrodes on the Roche COBAS Mira Plus automated chemistry analyzer. All three analytes are measured simultaneously, and are therefore ordered and reported as a group.