

## **Descriptions and Methods for Most Commonly Ordered Metabolic Core Services**

### **1. Hyperinsulinemic Euglycemic Clamp**

#### Description

The hyperinsulinemic-euglycemic clamp, or insulin clamp, is widely used as the "gold standard" method to measure insulin sensitivity. During an insulin clamp, hyperinsulinemia is achieved by providing a constant rate of insulin infusion. Euglycemia is maintained by adjusting the glucose infusion rate (GIR) throughout the procedure. The average GIR measured during the last 40 min of the procedure is used to calculate the insulin sensitivity.

#### Methods

Subjects will be asked to refrain from vigorous physical exercise for 48 hr before the clamp. At least 2 cannulae will be inserted, one for infusion of dextrose solution and insulin, and the second into a contralateral vein for blood sampling. A heat pad (temperature 30-40 deg C) will be applied on the phlebotomy arm to "arterialize" the venous blood. After baseline blood samples are taken, insulin will be infused at a fixed-rate of 40 mU/m<sup>2</sup> body surface area/minute for the duration of the clamp in order to achieve a steady-state insulin level of 70-100 U/ml above basal concentration. Blood glucose concentration will be measured every 5 minutes using the Yellow Spring 2300 StatPlus Glucose Analyzer (YSI Life Sciences, Yellow Spring, OH), and the infusion rate of the 20% dextrose will be adjusted to maintain a constant plasma glucose concentration [at approximately 90 mg/dL (5 mmol/L)] throughout the study. The average value of the glucose infusion rate (mg/min kg) measured during the final 40 min of the clamp is used to determine the insulin-mediated glucose uptake. The insulin sensitivity index (ISI) is expressed as the insulin-mediated glucose uptake divided by the steady-state serum insulin concentration.

### **2. Mixed Liquid Meal Tolerance Test (MMTT)**

#### Description

Mixed Meal Tolerance Test procedure involves consumption of a standardized meal which may contain measured composition of protein, fat and carbohydrates. The meal raises the subject's blood sugar and also stimulates the release of insulin from the pancreatic beta cells. The concentrations of glucose and insulin are measured at several time points and used to determine glucose tolerance and insulin sensitivity. Concentrations of fat and protein metabolites are also measured.

#### Methods

For MMTT, a standard liquid meal that consists of 30% of energy from fat (10% for saturated fat, 10% from polyunsaturated fats, and 10% from mono-unsaturated fats), 15% energy from protein, and 55% energy from carbohydrates will be used. This will be provided by a 502 g, 500 kcal chocolate flavoured shake (Ensure®, Abbott Nutrition, Abbott Laboratories, Columbus, OH). The shake contained 80 g carbohydrate, 12 g fat, and 18 g protein. Blood samples will be collected via an antecubital catheter immediately before meal ingestion, and at 15, 30, 60, 90, 120, 150 and 180 min after completion of the meal. The blood samples were collected in a pre-chilled EDTA tube containing aprotinin and dipeptidyl peptidase-IV inhibitor (or commercially available P800 tubes), and immediately centrifuged and stored at -80C until analysis. The stored blood samples will be used for analysis of glucose and insulin levels; gut hormones (ghrelin, glucagon, GLP-1, GIP, PYY); free fatty acids; acylcarnitines; and amino acids.

### **3. Indirect Calorimetry**

Indirect calorimetry procedure is to measure the resting energy expenditure of a person through a person's breath. An indirect calorimeter (Quark RMR, COSMED) is used to measure the oxygen and carbon dioxide levels which have accumulated inside the canopy hood or

mask of the machine. We can make measurements both during fasting and after meal consumption (eg, in conjunction with the MMTT).

#### **4. Intravenous Glucose Tolerance Test (IVGTT)**

##### Description

This test measures how well the body can break down glucose after an intravenous bolus dose of glucose. It provides a measure of the rapid insulin response and insulin sensitivity.

##### Methods

The subjects will be required to refrain from strenuous exercise for 48 hours, and to fast overnight (~10-12 hours) before coming for the study visit. A 3-hour frequently sampled intravenous glucose tolerance test (IVGTT) will be performed to estimate the acute insulin response to glucose (AIRg) and insulin sensitivity. A bolus of glucose (0.3 g/kg body weight in a glucose 50% solution) will be introduced within 60 seconds into the antecubital vein. Regular insulin will be administered as a bolus injection at 20 minutes (0.03 units/kg body weight Actrapid). Blood will be sampled from the intravenous cannula at -10, -5, 0, 2, 3, 4, 5, 6, 8, 10, 14, 19, 22, 25, 30, 40, 50, 70, 100, 140 and 180 min for assessment of the plasma glucose and insulin.

#### **5. Oral Glucose Tolerance Test (OGTT)**

##### Description

Oral glucose tolerance (OGTT) method is a simple method widely used in research and clinical settings to compare acute insulin response between normal people and diabetic patients. During OGTT, bolus dose of glucose will be given orally (liquid form) and value of glucose level in the blood will be tested at specific time points. This test can be used as an indicator of glucose tolerance.

##### Methods

A 75-g bolus dose of glucose will be given orally (liquid form) and the glucose and insulin levels in the blood will be measured at specific time points (0, 1-5, 30, 60, 90 and 120 min), depending on the research aims.

#### **6. Needle Muscle Biopsy**

A needle muscle biopsy involves the removal of a small (up to 50 mg) sample of muscle tissue for analysis. The procedure is typically performed on an outpatient basis or at the bedside. Under local anesthesia, a small incision is made and a co-axial introducer needle is inserted into the muscle in the thigh (*Vastus lateralis*) to guide the BioPince™ biopsy gun. Five to six bites of small muscle pieces are then extracted with the biopsy gun.

#### **7. Skinfold Measurement**

This is a simpler and less expensive method of estimating body composition than dual energy x-ray absorptiometry.

*Standard Operating Procedures (SOPs) are available for the above procedures. Please contact the core to ask about these.*