Sep 30, 2019

Glucose "Clamp" for measurement of whole body insulin-stimulated glucose disposal V.2

Donald McClain[1]

[1]University of Utah

1 Works for me dx.doi.org/10.17504/protocols.io.7s9hnh6

Diabetic Complications Consortium
Tech. support email: rmcindoe@augusta.edu

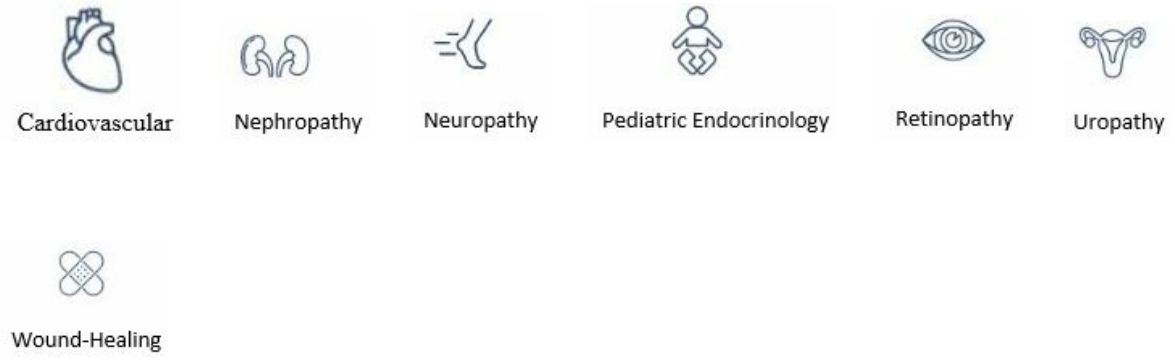
Lili Liang

ABSTRACT

Summary:

This is the standard protocol for measuring insulin action in terms of total body glucose disposal. It is the "gold standard" for quantifying insulin resistance/sensitivity.

Diabetic Complications:



EXTERNAL LINK

https://www.diacomp.org/shared/document.aspx?id=10&docType=Protocol

MATERIALS

NAME	CATALOG #	VENDOR
Avertin (12.5mg/ml)	See Reagent Preparation	
Ampicillin (15mg/ml)	A-0797	Sigma Aldrich
Betadine solution		
70% isopropyl alcohol pads		
Lacri-Lube NP	4240	Allergan
Deltaphase operating board model 39 OP with Isothermal pad model 39 DP		Braintree Scientific
Surgical scissor 10cm	FST 14078-10	Fine Science Tools

NAME	CATALOG #	VENDOR
Serrated fine forceps 10cm	FST 11052-10	Fine Science Tools
Suture tying forceps	FST 00272-13	Fine Science Tools
Small curved hemostats	FST 13003-10	Fine Science Tools
Micro scissors 8 cm	FST 15002-08	Fine Science Tools
Vessel cannulation forcep	FST 00574-11	Fine Science Tools
Dissecting microscope	Semi 2000	Zeiss Semi 2000
6-0 braided silk suture material		
Micro-Renathane tubing 45cm	MRE 025	Braintree Scientific
Blunt ended 25-gauge needle		
1cc syringe		BD Biosciences
Saline heparin mix (0.9% 20U/ml)		
2.5% Lidocaine ointment	Xylocaine	
Nexaband surgical glue	S/C 909004	J.A.Webster, Inc
INSTECH Solomon tether system		
Multi infusion pump	PHD 2000	Harvard Apparatus
Dual infusion pump	Pump 33	Harvard Apparatus
50% dextrose		
Saline insulin mix		
5cc syringe	309603	BD Biosciences
10cc syringe	309604	Bd
Glucometer Elite XL		

MATERIALS TEXT

Reagents Quantity Required

Reagent/Material	Quantity Required
Avertin (12.5mg/ml)	0.15ml/10g body weight, ip
Ampicillin (15mg/ml)	0.15 ml/g body weight .ip
Small curved hemostats	2
6-0 braided silk suture material	3 3cm pieces for each animal
INSTECH Solomon tether system	See protocol for details Number 1, subsections m,n,o
Saline insulin mix	(0.9%, 20mU/ml)

Reagent Preparation:

Avertin:

Reagents and Materials:

Reagent/Material	Quantity Required	Vendor	Stock Number
2,2,2-Tribromoethanol	0.625g	Aldrich Chemical Company, Inc	T4,840-2
Tertiary amyl alcohol	1.25ml		
Sterile Water	50 ml		

Procedure:

Mix 2,2,2-Tribromoethanolwith tertiary amyl alcohol and bring to 50 ml with Sterile Water. Store at 4°C in the Dark.

Note:

Sigma-Aldrich (RRID:SCR_008988)

BD Biosciences (RRID:SCR_013311)

1 **Jugular Vein Catheterization:**

a. Anesthetize mouse by injection of 12.5mg/ml Avertin (0.15ml/10 grams body weight, ip).
Recipe for Avertin: dissolve 0.625g of 2,2,2-Tribromoethanol (Aldrich Chemical Company, Inc. T4,840-2) in 1.25ml tertiary amyl alcohol, bring solution up to 50ml with sterile water, store at 4C in the dark.

b. Give a prophylactic injection of 15mg/ml Ampicillin (Sigma A-0797, 0.15 ml/g b.w.ip).

c. Wait 10 minutes and check pedal reflex by extending hind limb and firmly pinching the hind paw. If the mouse attempts to withdraw limb, give an additional 50ul of Avertin. Periodically (about every 10 min) check for pedal reflex throughout surgery.

d. After anesthesia has been achieved shave area over right external jugular vein and nape of neck with electric razor. Clean shaved areas with Betadine solution and wipe off excess with 70% isopropyl alcohol pad.

e. Protect from dry eye with a small amount of ophthalmic lubricant (Lacri-Lube NP, Allergan No. 4240) in each eye.

f. Place the mouse on a pre-warmed surgical surface (Braintree Scientific Inc., Deltaphase operating board model 39 OP with Isothermal pad model 39 DP) covered with sterile drape. Wear sterile gloves and use aseptic technique throughout the surgery.

g. Make a small incision through the skin on shaved area of neck. Feed blunt end scissors (surgical scissor 10cm, Fine Science Tools, FST 14078-10) subcutaneousley toward the right shoulder to create a path to exteriorize catheter later on.

h. Place the mouse on its back with head oriented towards surgeon. Loosely tape down chin and all four limbs in a spread-eagled fashion. With the aid of a dissecting microscope (Zeiss Semi 2000), make a horizontal incision (~1 cm) above the jugular vein anterior to the clavicle centered in the shaved area. Using small curved forceps (serrated fine forceps 10cm, FST 11052-10) to create a subcutaneous pocket on anterior side of incision. Gently tease fat tissue away from the incision site until the jugular vein can be visualized.

i. Clean a 1 cm section of the jugular vein anterior to the pectoral muscle of fat and other tissue by blunt dissection. Isolate the jugular vein by placing small curved forceps under vein. Feed three pieces (approximately 3cm each) of 6-0 braided silk suture material under the vein. Tie with suture tying forceps (FST 00272-13) the anterior ligature to occlude vein. Use two small curved hemostats (FST 13003-10) and attach to each end of the anterior ligature. Adjust hemostats to place light tension on vein. Make a tie to the posterior ligature but leave loose. The center ligature is left loose with no tie.

j. Prepare catheter by attaching a 45 cm length of Micro-Renathane tubing (Braintree Scientific, Inc., MRE 025) to a blunt ended 25-gauge needle. Attach needle to a 1cc syringe filled with a saline heparin mix (0.9%, 20U/ml). Fill catheter with saline heparin mix making sure there are no air bubbles present. The end of the catheter should be blunt. Using micro scissors (8 cm, FST 15002-08) make a small incision in the vein between the anterior and posterior ligatures making sure not to cut through vessel. Using catheter forceps (vessel cannulation forcep, FST 00574-11) to hold the catheter, feed the blunt end into the lumen of the vessel. Feed the catheter into the vessel until it just disappears past the pectoral muscle. Check for occlusions by aspirating the syringe catheter assembly. Blood from the vein should flow freely in and out of the catheter.

k. Secure the catheter by first tying off the posterior ligature. Next, tie the middle ligature around the vessel and catheter. Tie ends of posterior and middle ligature together to firmly secure catheter. Remove hemostats and tie off anterior ligature around catheter.

l. Trim off any excess thread. To exteriorize the catheter, use hemostats to break off needle from hub. Remove tape from chin and limbs. Gently turn mouse on its stomach. Feed blunt forceps subcutaneous through the hole in the nape of the neck to the jugular incision site. Grasp end of needle with forceps and pull catheter through leaving slack in the pocket created in the ventral incision site.

m. Return mouse to its back and place a small amount of 2.5% Lidocaine ointment (Xylocaine) on the surgical site. Using forceps, gather the skin together. Gently remove any moisture with gauze. Holding the skin, place surgical glue (Nexaband S/C 909004, J.A.Webster, Inc.) along incision. Return mouse to stomach. Place a small amount of Xylocaine on neck incision.

n. To attach mouse to the INSTECH Solomon tether system (Plymouth Meeting, PA) feed the catheter through the tether (CIH62) being careful not to put tension on the catheter itself. Place the mouse in the harness (CIH62) attached to the tether. Adjust the fit of the harness. Cut off any excess catheter to leave a small loop to connect to the end of the swivel (375/25).

o. Place the mouse in the Instech designed animal enclosure (STANK) and attach the swivel to the arm (SMCLA). Connect the line from a multi infusion pump (Harvard Apparatus, PHD 2000) to the swivel. The pump will infuse saline (0.9%) at a rate of 40ul/hr during the recovery period (48 hrs). The mouse should have free access to food and water throughout this period. Attach the lid to cage.

2 **Clamp procedure:**

a. After 48 h of surgical recovery the mouse is fasted 6 hours and transferred to a standard mouse housing cage with a tether arm attached to hold the tether system. A dual infusion pump (Harvard Apparatus, Pump 33) will be used to infuse 50% dextrose and insulin to the mouse. A constant flow rate of insulin and a variable rate of 50% dextrose will be infused to maintain a constant blood glucose level.

b. A saline insulin mix (0.9%, 20mU/ml) is loaded in a Becton Dickinson (BD) 5 ml syringe (309603) and placed in the syringe position #2. Preset diameter and flow rate settings to 12.06mm and 2.5ul/hr respectively. The 50% dextrose is loaded in a 10 ml BD syringe (309604) and the diameter preset to 14.5mm with a flow rate of 13.2ul/hr/gbw. Attach blunt ended 25g needles to both syringes and run 25g tubing from the syringes to a Y connector. From the connector run enough 25g tubing to reach the Instech swivel. Start pump at ~100x the programmed flow rate for 10 minutes to void air in the lines and equilibrate the insulin 50% dextrose mix. Turn off the pump and set insulin and 50% dextrose flows to infusion rates, connect tubing to swivel.

c. Clip a small portion of the mouse tail and measure blood glucose level with a glucometer (Glucometer Elite XL) before starting pump.

d. Start pump and timer. Measure blood glucose after the first 15 minutes and at 10 minute intervals for a total of 60 minutes. Adjust the 50% dextrosse flow rate during this period to stabilize blood glucose levels to 100-150 mg/dl (6.0-9.0 mmol).

e. Glucose disposal rate calculation=(flow rate(ul/hr)/60/kgb.w.)/2 This assumes glucose production rate (hepatic and renal gluconeogenesis and glycogenolysis) will be zero which is effectively the case at these levels of hyperinsulinemia. However, at lower insuin infusion rates (e.g. when generating an insulin dose-response curve for a particular action of insulin) the glucose production rate will need to be determined by isotope dilution (see the literature for protocols.) Glucose uptake into specific tissues can also be measured by the infusion of [14C]2-deoxy-D-glucose at the end of the procedure (see literature).