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GUIDANCE PROTOCOLS TO ORAL GLUCOSE TOLERANCE TEST, PANCREATIC ISLETS ISOLATION AND GLUCOSE STIMULATED INSULIN SECRETION IN RATS

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ABSTRACT

In diabetes research, glucose tolerance tests (GTT) and glucose-stimulated insulin secretion (GSIS) are both established methods to study glucose regulations and insulin release in response to test items. This Protocol will guide researchers in performing in vivo experiments using an oral GTT and ex vivo pancreatic islet isolation to proceed with GSIS procedures in rats. The blood glucose and insulin are measured in response to treatment and glucose administration for GTT. This is followed by an ex vivo experiment to study the GSIS involving Collagenase Digestion Method to obtain isolated pancreatic Islets. Using the overnight incubated pancreatic Islets, GSIS experiment can be performed to determine how well the Islets produce insulin at low and high glucose in response to test items. This Protocol applies to study antidiabetic properties and glucose-lowering effects using rodent models and cell lines. This procedure can also be applied in other rat models, including healthy non-diabetic, diabetic, and diabetic induced rodents.

KEYWORDS: Glucose Tolerance Test, Pancreatic Islets Isolation, Insulin Secretion, Glucose-Stimulated Insulin Secretion

INTRODUCTION

Diabetes is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion and/or insulin action. Decreased insulin action results from insufficient insulin secretion and/or diminished tissue responses to insulin at one or more points in the complex pathways of hormone action (American Diabetes Association 2014). Treatment and prevention of diabetes are important to prevent complications. Thus, analyzing blood glucose and insulin levels for the assessment of islet function is essential in diabetes research. This Protocol described in vivo (glucose tolerance test (GTT)) and ex vivo experiments involving pancreatic Islets digestion and glucose-stimulated insulin secretion (GSIS) procedures in response to test items that are known to be important for diabetes research. By analyzing insulin levels in response to glucose, it's possible to identify insulin resistance, a condition where the cells fail to respond properly to insulin. Previous literatures have described Protocols using GTT to study glucose homeostasis and insulin secretion in vivo, islet isolation and GSIS to determine islet function ex vivo (Al Rijjal and Wheeler 2022; Chen et al. 2023). However, the experiments mostly used mice models with different approaches, which might involve slight differences in the Protocol steps compared to rats. The detailed Protocols on how GTT and the GSIS procedures should be performed in rodents are still limited. In addition to this, the GTT and GSIS experiments described here are applicable to be used in both non-diabetic and diabetic rat models. For GTT, the in vivo experiment is important to understand glucose metabolism in diabetes, and steps on how oral GTT is being carried out are indicated accordingly. GTT is often considered a reliable method for diagnosing diabetes conditions since it comprehensively assesses how well your body processes glucose. For ex vivo study, steps on isolating Islets from pancreatic rat Islets are provided whereas the GSIS examines the effects of insulin secreted from isolated pancreatic Islets when challenged with low or high glucose and an addition of test item.As suggested here, the recommended Protocol may serve as a guideline, and further modifications can be made. For more information regarding the application and implementation of this Protocol, use (Lokman et al. 2023) as a guideline.

MATERIALS AND METHODS

List of reagent and materials

The reagents and materials used for OGTT, pancreatic islets isolation and GSIS procedures in rats are shown in Table 1.

Table 1. List of reagents and materials to perform experiments

REAGENTS/MATERIALS	ORIGIN	LABEL
Chemicals		
Glucose anhydrous (C6H12O6)	HmbG	CAS# 50-99-7
Collagenase from Clostridium histolyticum	Sigma-Aldrich	C9263
Hanks' balanced salt solution (HBSS)	Nacalai tesque	1745955
Histopaque 1077	Sigma-Aldrich	na
Histopaque 1119	Sigma-Aldrich	na
RPMI media	Sigma-Aldrich	R8758
Natrium chloride (NaCl)	HmbG	na
Kalium chloride (KCl)	HmbG	na
Magnesium sulphate-7-hydrate (MgSO4).7H2O	HmbG	CAS# 10034-99-8
Calcium chloride 2-hydrate (CaCl2.2H2O)	HmbG	na
Natrium bicarbonate (NaHCO3)	HmbG	na
Potassium dihydrogen phosphate (KH2PO4)	HmbG	CAS# 7778-77-0
HEPES	Sigma-Aldrich	na
Albumin	Sigma-Aldrich	A2153
L-glutamine	Sigma-Aldrich	CAS# 56-85-9
Penicilin/Streptomycin Solution (PEST)	Gibco by Life Technologies	15140122
Assays		

Enzyme-linked immunosorbent assay for rat/mouse kit (ELISA)	Millipore	EZRMI-13BK
Other		
Glucometer	Accu-check Performa	na
Glucose strips	Accu-check Performa	na
Mini Lithium Heparin tubes 0.5 mL	Vacutube	na
Gavage feeding tube	n/a	FTSS-18S-51
Dissecting scissors	Electron Microscopy Sciences	EMS 72951-15
21-23G needles	Terumo	na
Syringes (5mL,10 mL)	BD	na
Nylon syringe filter	na	na
Falcon conical tubes (50 mL)	BD	na
Polyethylene tube PE500. (ID: 0.58 mm. OD 0.965 mm. 30.5m (100')	Becton Dickinson and Company	Lot #: 7032557
Kelly Hemostat (straight) (61/2")	Electron Microscopy Sciences	na
Wax liner dissection 15"x12" tray	na	na
Disposable polystyrene petri dish (59.5 x 15.1 mm)	na	na
CO2 incubator	Memmert	na
Refrigerated centrifuge	Memmert	na
Digital block heater	Select Bioproducts	na
Inverted Microscope	Nikon	na
96-well plate	Eppendorf	na
Cold Block for tubes	Eppendorf	na
1.5 mL Centrifuge tubes	Eppendorf	na
Thread	na	na

na= not applicable

Reagent and Buffer Preparation

The preparation of reagent and buffer used for OGTT, pancreatic islets isolation and GSIS procedures in rats is shown in Table 2.

Table 2. List of Reagent and Buffer preparation according to the procedures

Procedure	Reagent	Final concentration	Amount	Notes
GTT (for glucose mixture)	Glucose anhydrous	30%	3g/10 mL of ddH2O	Glucose anhydrous (keep at room temperature)
	Water ddH2O	-	10 mL	
	Total	**-**	**10 mL**	
Pancreatic islet isolation	Collagenase enzyme	9-24 mg/10 mL	10 mL	Collagenase (keep at -20˚C for 6 months)
	HBBS			
	Total	**-**	**10 mL**	

Islet incubation	RPMI media- 1640 PEST L-glutamine Glucose Fetal bovine serum (FBS)	- 0.25% 30% 5.5 mM/11mM 10% from RPMI media	99.75 mL 0.25 mL 30 mg 99 mg/198 mg 10 mL	RPMI 1640 media, PEST, L-glutamine (keep at 4°C). FBS (keep at -20˚C).The solution should be freshly prepared and filtered before use.
	Total	**-**	**100 mL**	
GSIS – (Krebs-Ringer Bicarbonate (KRB) buffer solution preparation)	1)Preparation of Stockchlorin NaCl KCl MgSO4.7H2O 2) Preparation of NaHCO3 3)Preparation of CaCl2. 2H2O •CaCl2.2H2O 4)Preparation of KH2PO4 •KH2PO4 Mixure of KRB buffer • Stockchlorin •KH2PO4 •H2O •CaCl2. 2H2O NaHCO3 HEPES BSA	 39.6 g 2.0 g 1.67 g 1.29 g 1.62 g 0.925 g 138 mg 116 mg	 Mix NaCl+KCl+MgSO4.7 H_2O in 500 mL H_2O 100 mL 100 mL 250 mL 5 mL 2.5 mL 42.5 mL 1.3 mL 6.7 mL	Stock solutions and CaCl2. 2H2O (keep for 1 month (4˚C). BSA and HEPES can be mixed, stirred and added in freshly prepared KRB; 1) NaHCO3 should be kept for 4˚C for 1 week and exposed to 1 hour to 5% CO2, 95% O2; 2) Prepare stock solutions: stockchlorin, NaHCO3, CaCl2. 2H2O and KH2PO4. Then use the prepared stock solutions to prepare KRB buffer; 3) Adjust the Mixture of KRB to pH 7.4 before use.
	Total	**-**	-	
GSIS (Low and high glucose mixture preparation)	Glucose (Low) Glucose (High) KRB buffer	3.3 mM 16.7 mM	0.018 g/30 mL of KRB buffer 0.09 g/30 mL of KRB buffer)	Glucose should be freshly prepared.
	Total	**-**	**30 mL**	

Preparation, Procedure and Analysis

The techniques used for *in vivo* method OGTT and ex vivo methods consisting of pancreatic islets isolation and GSIS procedures in low and high glucose in rats are summarized in Figure 1.

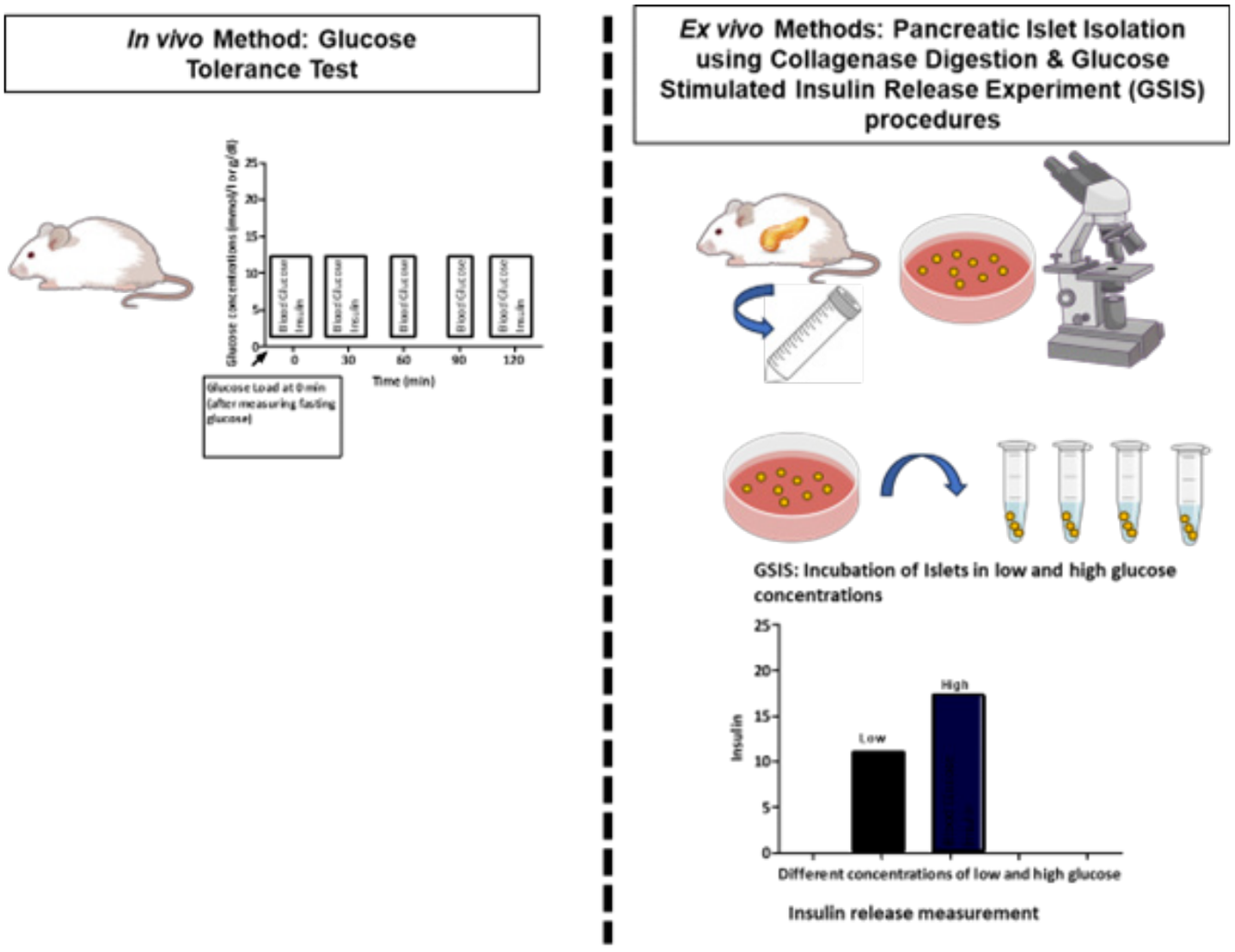


Figure 1. The summary of techniques used for OGTT (*in vivo*), pancreatic islets isolation and glucose stimulated insulin secretion (GSIS) (*ex vivo*) procedures in rats. OGTT measures the blood glucose and insulin at different time points. Pancreatic islets isolation using collagenase digestion step followed by GSIS experiment are done to measure insulin secretion in response to low and high glucose concentrations.

Oral GTT (OGTT)

Timing: 1-2 days. The steps here show how OGTT is being done with blood being taken at different time points to assess how well the beta cell Islets secrete insulin after receiving a glucose load. Previous Protocols have reported steps for performing OGTT (Lokman et al. 2023).

Preparation of OGTT

a) For OGTT procedure, prepare glucose solution (30%). Heat the glucose at 50˚C 30 min to dissolve the glucose. Keep the glucose at 4˚C and recommended to be kept not more than 2 days.
b) Prepare and decide the glucose dosage which is determined according to the body weight. Determine the glucose dosage at 2g/kg of glucose (30%) for each rat using the calculation (Al Rijjal and Wheeler 2022):

$$\frac{\text{Rat weight (kg) x (2g/kg)}}{\text{(30g/100mL)}}$$

For instance, the volume administered to a rat that weighs 150 g is 1 mL of glucose mixture.

a) Transfer the rats into new cages, do not provide food and make sure to supply drinking water only. Fast the rats overnight to perform OGTT on the next day.
b) Prepare gavage, glucometer, glucose strips, labelled 1.5 mL heparin tubes, labelled 1.5 mL centrifuge tubes, cold block for tubes, a centrifuge, blood glucose measurement record sheet with different time points and glucose load based on body weight.

Procedure:

a) For blood glucose, record measurements at 0, 30, 60, 90, and 120 minutes, and squeeze the rat tail gently from the tail to the tip back and forth. Blood will be measured using a glucometer. Wipe the tail with an alcohol swab prior to pricking with a 23G needle on the blood vessel. For glucose measurement, turn on the glucometer, insert the glucose strip and place the blood drop to the edge of the test strip and record the value at each time point.
b) Before starting the OGTT procedure, weigh the rats first prior to starting with the OGTT. Administer the glucose load at 0 min according to the rat's body weight. (Please refer to step 3.1(b) as stated above)
c) Insert gavage into a syringe and slowly aspirate the glucose solution based on the calculation and recommended volume (see materials and equipment).
d) After taking baseline blood for glucose and insulin at 0 min, administer glucose orally gently using a gavage according to the body weight. Repeat the step on each rat.
e) For insulin measurement, collect blood in heparin tubes by cutting the tip of the tail (1 mm) using a scalpel blade or a sharp surgical scissor or tail vein blood withdrawal (advisable to perform once or when necessary) with a 23G needle at 0, 15, 30 and 120 min. Squeeze the rat tail gently from the tailback and forth. Place the tubes in a cold block. Spin the blood at 2000 x g, 10 min, 4˚C. Aspirate the plasma and transfer into tubes and keep in the refrigerator (4˚C) for measurement later.
f) Once the experiment is done, provide food and drinking water to the rats and keep them rest for at least 2-3 days before performing any other procedures.
g) For insulin measurement, use a rat Insulin ELISA kit based on the recommended Protocol.

Notes: It is recommended that a maximum of up to 6 rats be tested in each experiment to allow sufficient time for glucose administration, blood taking for glucose and insulin steps. It is advisable to administer oral gavage once for each treatment to avoid injuries to rats. Refer Figure 2 for OGTT procedure.

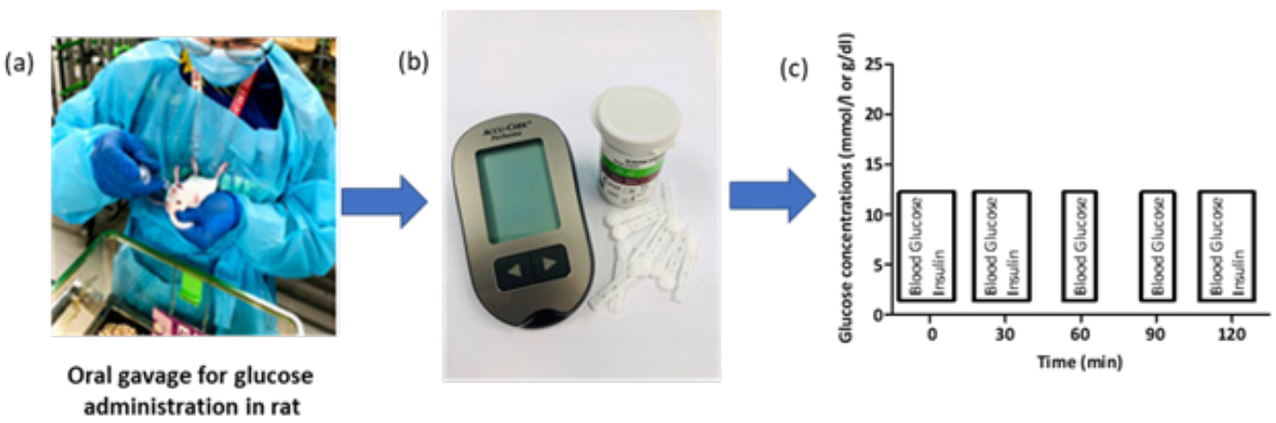


Figure 2. OGTT procedure
(a) Oral gavage for glucose administration in rat; (b) Glucometer and glucose strips to measure glucose level; (c) OGTT time point for blood and insulin measurement; Blood can also be collected for glucose and insulin at different time points depending on the research purposes.

Pancreatic Islet Isolation

Timing: [between 2-5 days]. Pancreatic Islets isolation and GSIS steps are indicated in this Protocol. The GSIS evaluates pancreatic beta cells functions. The experiment on Islets isolation by collagenase digestion is based on Lacy & Kostianovsky 1967. After isolation, the incubation of Islets is based on Ostenson et al. 1993 (Ostenson et al. 1993).

Procedure:

a) Euthanize the rats properly with either (i) inhalation anesthesia agent in a chamber using isoflurane (1.5-3%) supplied with oxygen (1-2L/min), followed by intracardiac puncture technique using 21-23G needles or (ii) place in a tank supplied with CO2 for 10 min. Observe the animal over a period of time. Lack of movement, absence of a heartbeat, respiration, or corneal reflex over an extended period of time indicates further confirmation of death.
b) Place the rat in dorsal recumbency on a flat surface on a wax-lined dissection tray, turn the rat upside down, cut the skin with a dissecting scissor and abdomen using a scissor, place the guts to the left and try to find the bile duct.
c) Close the duct where it enters the gut with a Kelly Hemostat. Free the bile duct near the liver with the forceps and pull the thread under.
d) Cut a small hole with a dissecting scissor in the bile duct and put the catheter (Portex PP25) 0.5 cm and tie the thread around. Inject collagenase (9 mg for non-diabetic and 24 mg for diabetic rats) dissolved in 10 ml HBSS (Sigma Aldrich, USA) slowly into the bile duct of pancreas to digest the pancreatic exocrine tissues.
e) The pancreas which is filled with HBSS containing collagenase is now swollen and carefully excises the pancreas by cutting the duct area with a sharp scissor. Ensure to carefully cut outside the swollen area to avoid leakage. Place the excised pancreas filled with HBSS containing collagenase into a falcon tube (50 ml).
f) The pancreas collected is then incubated without shaking for 25-30 min in a waterbath (37˚C) or digital block heater.
g) Put the tubes on ice or ice block and cut the pancreas into small pieces using the same tube. The Islets are resuspended with a syringe. Add 20 ml HBSS and centrifuge for 1 min at 1000 rpm.
h) Wash with HBSS (Sigma Aldrich, USA). Repeat washing with 20 ml HBSS and centrifuge for 1 min at 1000 rpm 2 times. Discard the supernatant. Filter the pellet through the restrainer and pour Hank's buffer gradually. Centrifuge for 1 min at 1000rpm.
i) Add 5 ml of Histopaque (1119) (Sigma Aldrich, USA) and Histopaque (1077) (Sigma Aldrich, USA) each. Then add 5 ml Histopaque (1077) (Sigma Aldrich, USA) to the mixture and 5 ml HBSS (Sigma Aldrich, USA) on the top layer of Histopaque 1077. Centrifuge for 20 min at 2000 rpm.
j) The Islets are available in the middle of the Histopaque (1077) (Sigma Aldrich, USA) and HBSS (Sigma Aldrich, USA) solutions. Transfer the media containing Islets into culture tubes, handpick and separate from acinar tissues under a microscope.
k) Transfer the Islets into HBSS. The selected Islets can be transferred into culture media.
l) The Islets are then incubated in glucose (11mM) in RPMI with 10% FBS in an incubator supplied with 5% CO2 overnight at 37°C.
m) The overnight islets can be used for batch incubation experiments. In this procedure, approximately 100-300 islets can be obtained from the rats. The amount of insulin can be measured using ELISA or Radioimmunoassay (RIA) based on recommended protocol. Refer to Figure 3a: Materials needed for islets isolation procedure and Figure 3b: Steps in pancreatic islets isolation procedures

The isolated pancreas should be placed cold after collection. Please refer to the previously published Protocol on the amount of collagenase used for diabetic (24 mg) and non-diabetic (9 mg) rats (Zambrana et al. 2018; Seed Ahmed et al. 2012). Diabetes diagnosis is made based on American Diabetes Association (American Diabetes Association 2014).

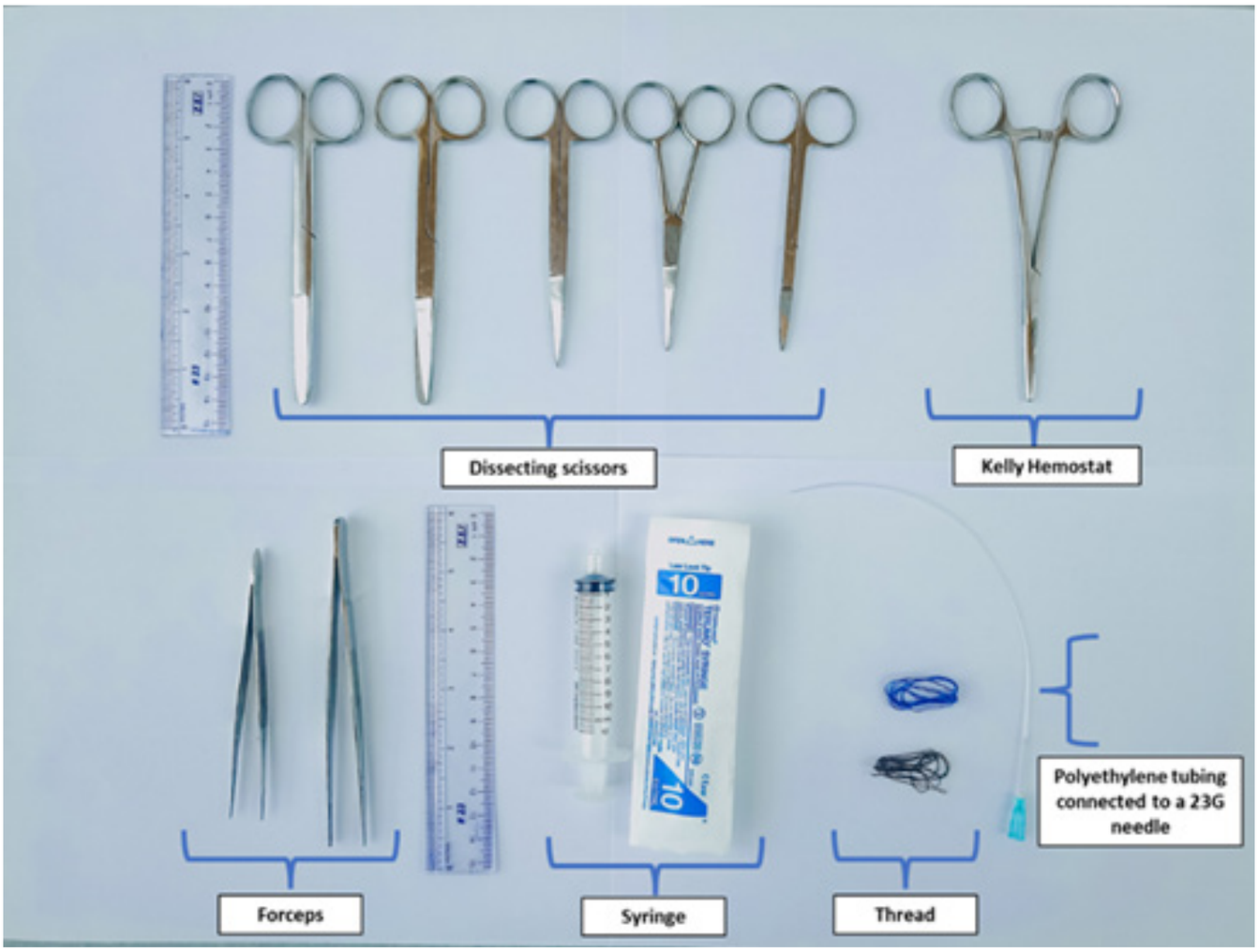


Figure 3a. Materials needed for pancreatic islet isolation procedure, including dissecting scissors, Kelly Hemostat, forceps, syringe, thread and polyethylene tubing connected to a 23G needle. It is recommended that the materials are sterile before performin the procedures.

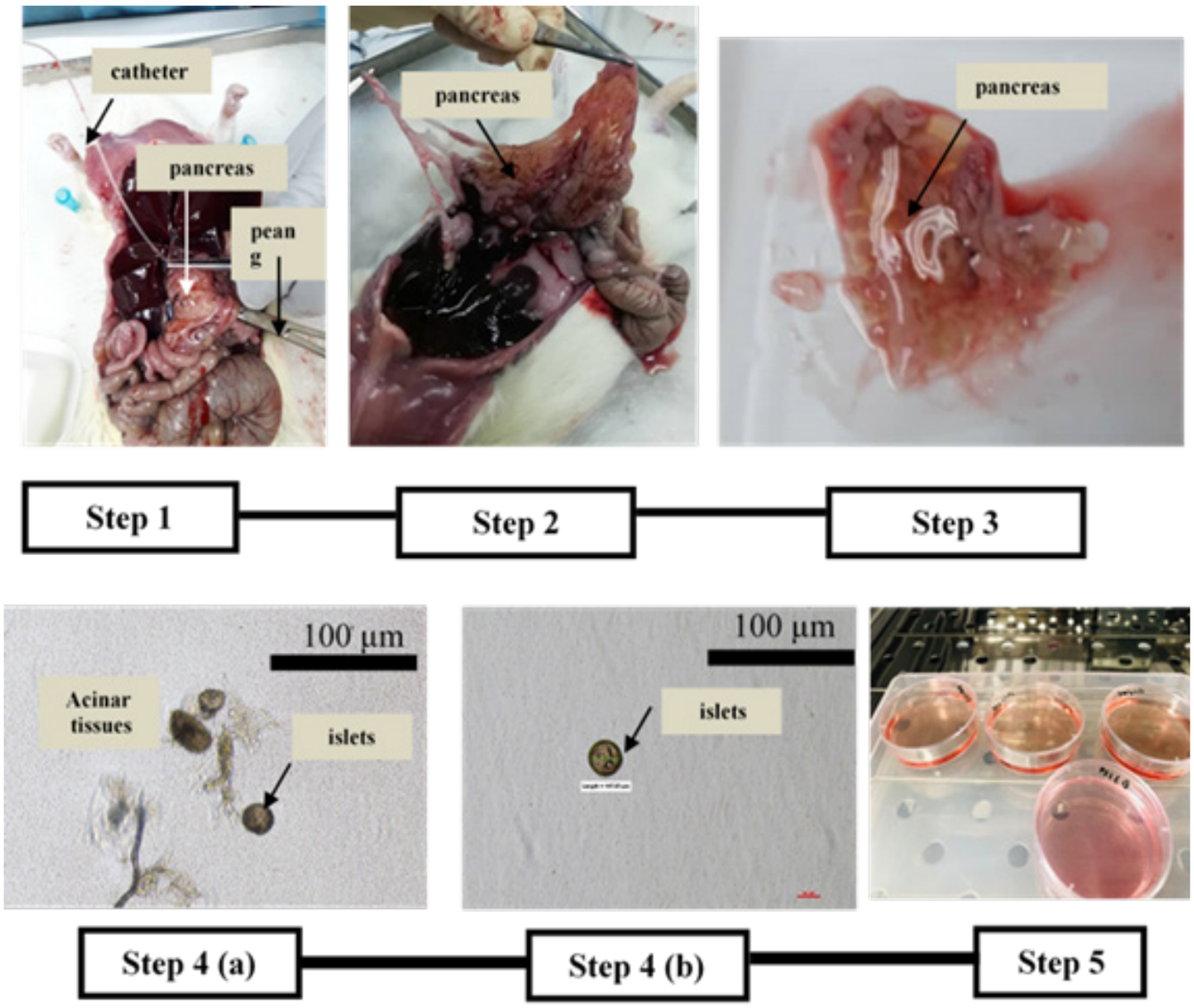


Figure 3b. Steps for pancreatic islets isolation using collagenase digestion method
Step 1: Image of collagenase in HBSS (10ml) is injected via duct into pancreatic Islets using a tube connected with a syringe under the naked eye.
Step 2: Image of swollen pancreatic Islets filled with collagenase in HBSS.
Step 3: Image of pancreatic islet containing collagenase in HBSS separated from rat and ready to be processed accordingly under the naked eye.
Step 4: (a) Microscopic image of a freshly isolated islet shown among pancreatic acinar tissue (Nikon Inverted Microscope TS2-Ds-Fi3, Japan)
Step 4: (b) Microscopic image of Islets purified from acinar tissue (Nikon Inverted Microscope TS2-Ds-Fi3, Japan)
Step 5: Image of Islets incubation overnight in an incubator supplied with CO2 at 37˚C.

GSIS

After performing the isolation procedure, the GSIS steps are done to evaluate the Islets functions and the insulin response (Lacy and Kostianovsky 1967; Ostenson and Efendic 2007). The incubated overnight cultured Islets in media containing glucose were used. Pick the Islets and preincubate for 30-45 min in culture plates (5 ml) at 37˚C and transfer into KRB solution at low sugar with 10% of Fetal bovine serum (Hoa et al. 2007; Muller et al. 2016).

Procedure

a) KRB buffer with low and high glucose are prepared. Prepare the substances that are to be tested and add in low and high glucose KRB Buffer. Each sample should be prepared in triplicates consisting of control group (KRB buffer only at low and high glucose) and different concentrations of test items. The GSIS Protocol has been previously described (Zambrana et al. 2018; Hoa et al. 2007; Muller et al. 2016).
b) Three Islets with almost similar sizes are added in 300 µl KRB in tubes (Zambrana et al. 2018; Seed Ahmed et al. 2012; Hoa et al. 2007; Muller et al. 2016).
c) Incubate Islets for an hour, in a water bath (shaking) (37˚C). After incubations, aliquots can be kept in the -20˚C for measurement later.
d) The amount of insulin from the aliquots can be measured using ELISA or RIA based on recommended Protocol.

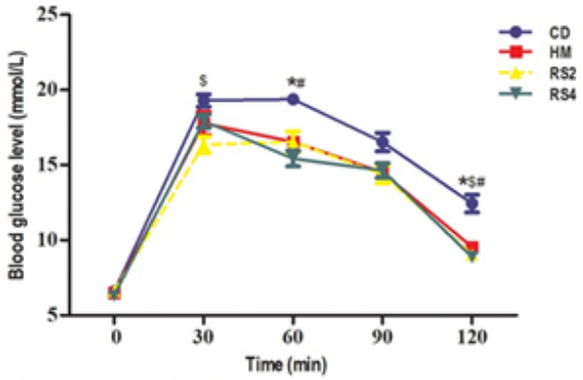
Quantification and statistical analysis

Data will be described as mean ± SD and the p-values (<0.05) are considered significant. The analysis will be done using SPSS 21.0 or GraphPad prism.

EXPECTED RESULTS

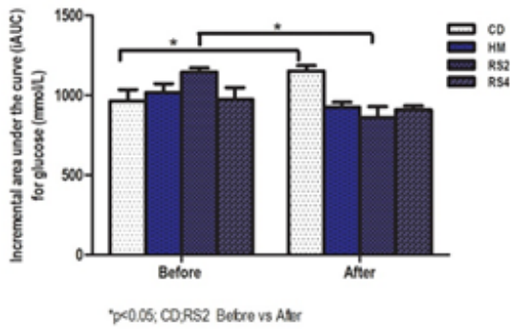
GTT and GSIS

GTT examines administration of exogenous glucose effects on the systemic clearance of glucose in the bloodstream as well as insulin response within specific time. Glucose can be administered orally or intra-peritoneally. The fasting glucose and insulin recorded at specific time point can presented as a graph. Glucose and Insulin measurement results from GTT can be presented as glucose/insulin (Y-axis) versus time (X-axis). The results can also be presented as Total Area Under the Curve (AUC) or incremental Area Under the Curve (iAUC). The AUC which is obtained from the GTT is usually applied to identify Glucose Tolerance impairment. In addition to this, the iAUC is used -since there are differences in fasting glucose. AUC and iAUC can be evaluated using GraphPad Prism Software.



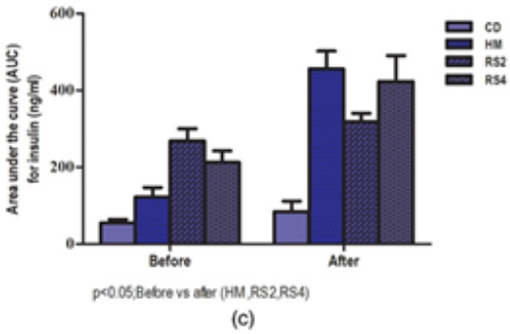
Min 30: $p<0.01: CD vs RS2
Min 60: *p<0.05: CD vs HM;RS2: #p<0.001:CD vs RS4
Min 120: *p<0.05: CD vs HM: $p<0.01:CD vs RS2; #CD vs RS4

(a)



*p<0.05; CD;RS2 Before vs After

(b)



p<0.05;Before vs after (HM,RS2,RS4)

(c)

Figure 4. (a) OGTT results after treatment (b) iAUC (glucose) after treatment and (c) iAUC (insulin) after treatment. Results: mean ± SEM. Legends: CD: Control diabetic; HM: Hi-Maze RS2: Resistant starch 2; RS: Resistant starch 4. Reference (Lokman et al. 2023)

Figure 4 illustrates the OGTT analysis after treatment with a test item as previously described (Lokman et al. 2023). The OGTT results were plotted as blood glucose versus time. Figure 4a shows the OGTT done in the diabetic rats. The OGTT was done to determine the antidiabetic effect of a plant extract on blood and insulin measurement. GK rats are glucose intolerant and the results showed that the fasting glucose level in the diabetic rats was elevated with higher glucose level at each time point. GK rats showed low insulin response to glucose due to an impaired glucose signaling in the β-cell islets. In the study, the treated groups showed lower glucose results vs placebo. Figures 4b showed iAUC for glucose levels in GK rats, which is calculated based on the OGTT time point results using GraphPad Prism Software. Lower iAUC values indicate greater glucose tolerance in rats. The study showed the iAUC was reduced in the treated GK rats vs placebo. Figure 4c indicated serum iAUC for insulin in GK rats, which was determined by Radioimmunoassay (RIA). Insulin secretion level in the OGTT is presented as insulin level versus time.

The islets in the non diabetic Wistar (W) rats are known to be intact with normal insulin production whereas the morphology and function of diabetic GK rats are varied between strains. Impaired insulin secretion found to be similar to T2D human islets due to a reduction in beta-cell mass and beta-cell impairment (Ostenson and Efendic 2007). For GSIS, the results are shown as a bar graph which can be plotted as insulin (Y-axis) vs test items in low and high glucose (X-axis) or can be presented in a table form. Table 3 illustrates the GSIS results as previously described by (Lokman et al. 2023). Please refer to the manuscript for details. This study showed that the C1 compound stimulated insulin secretion from the non diabetic and diabetic rat islets. In the non diabetic islets, C1 significantly improved insulin secretion higher than the diabetic GK rats at low and high glucose. Diabetic pancreatic islets possess both disordered insulin action and abnormalities. Thus, the diabetic rat islets showed an irregular insulin response when challenged with glucose. The experiment for GSIS protocol has also been described by (Lokman et al. 2023).

Table 3. Insulin secretion from rat islets at different glucose concentrations

Addition to incubation medium		Insulin release (μU/islet/h)	
Glucose (mM)	**T.crispa C1 (μg/ml)**	**W islets**	**GK islets**
33	None	2.4±0.1	1.2±0.2
	0.1	15.1±2.2**	4.7±.3*
	1	19.5±4*	7.5±.9*
	10	21.9±2***	10.5±.8*
16.7	None	32.5±1.8	1.2±0.2
	0.1	48.8±6#	20.6±.1#
	1	63.1±.9#	30.9±.8#
	10	164.5±1.3###	57.3±6.3#

*Note: Data are presented as means ± SEM. W: *P < 0.05 versus 3.3 mM glucose; **P < 0.01 versus 3.3 mM glucose; ***P < 0.001 versus 3.3 mM glucose; # P < 0.05 versus 16.7 mM glucose; ### P < 0.001 versus 16.7 mM glucose GK: *P < 0.05 versus 3.3 mM glucose; # P < 0.05 versus 16.7 mM glucose*

DISCUSSION

In overall, this Protocol guide researchers on how to perform GTT and pancreatic islet isolation as well as GSIS experiments in stages to understand the glucose regulations and insulinotropic properties of test items. Animal models are being used to elucidate the mechanisms underlying diabetes and to study glucose homeostasis. Several techniques are available for measuring different aspects of glucose tolerance and each of these methods has distinct advantages and disadvantages. Therefore, the suitable procedure may vary from one study to another depending on the animal model, the anticipated end-points and other practical considerations (Bowe et al. 2014). Glucose homeostasis is mainly influenced by two key factors: the rate at which insulin is secreted from the pancreatic Islets in response to blood glucose levels and the insulin sensitivity of target tissues to. These interactions between these factors determines the overall glucose tolerance and its ability to maintain glucose homeostasis. Therefore, a thorough evaluation of glucose homeostasis should include three primary components: measuring islet hormone secretion in response to changes in plasma glucose, assessing the sensitivity of target tissues especially to insulin and evaluating overall glucose tolerance, which reflects the combined effects of these factors (Bowe et al. 2014).

In order to achieve this, here are some troubleshooting steps and suggestions that researchers may follow if problems are encountered. Firstly, for OGTT, spillage might occur from the rat's mouth during oral gavage, as improper handling will cause the rat to struggle, and the force-feeding during solution loading may cause rat injuries. Spillage will give inaccurate blood glucose and insulin measurements. Improper handling may also cause the solution to enter the trachea and lead to death. Secondly, during blood collection for insulin, the tail vein might be invisible, which may result in difficulties in to collect blood at specific time point during the OGTT procedure, causing pain, stress, and trauma to rats. Hence several possible solutions include, if the vein is invisible, dip it into warm water to increase the size of blood vessels (37 – 39°C) for (from 5 to 15 min). Alternatively, for the tail snip procedure using surgical blade/scissors, the local anesthetic agent can be used. For tail prick/small incision/temporary cannulation, apply a local anesthetic cream on the specific surface of the tail at least 30 min prior to starting the procedure (Parasuraman et al. 2010). In addition, blood samples should only be taken from the base of the tail if no vein is visible elsewhere.Thirdly, for pancreatic islet isolation, collagenase solution leakage might occur during injection into the bile duct. The critical part involves the insertion of the catheter into the bile duct, causing ruptures and collagenase solution leakage during injection. Hence to address this, ensure the blood is being wiped properly using gauze and tie the catheter tightly with a thread. Hold the location with forceps while injecting the collagenase solution to avoid leakage. Fourthly, for the GSIS experiment, the number, size and quality of islets obtained to perform batch incubations may be varied or lower than the expected range. Ideally, approximately 100 Islets can be obtained from the collagenase digestion procedure depending on how well the procedures are being followed, technical skills and also the type of animal model used. Diabetic rats may have a lower number of Islets as compared to the non-diabetic models due to impairment.

In addition to the methods described, further tests such as HbA1c, Homeostasis model assessment (HOMA), insulin tolerance test and CLAMP (euglycemic-hyperinsulinemic, hyperglycemic-hypoglycemic), somatostatin can be explored to quantitate insulin secretion and resistance in vivo. Insulin sensitivity tests in the skeletal muscle, liver and adipocytes can be done to understand the glucose regulations pathways involved.

CONCLUSION

Assessment of glucose regulations and islet function in rat models are essential in diabetes research. We present a protocol that utilizes glucose tolerance test to investigate glucose homeostasis and insulin secretion in vivo. This is followed by islet isolation and glucose-stimulated insulin secretion assessments to evaluate islet function ex vivo. This protocol enables evaluation of glucose homeostasis and islets in rats which can also be applied to mice and cell lines

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