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Title: Glucose-Stimulated Insulin Secretion via Perfusion Through the Mice Vasculature with an Intact Pancreas

Authors and Affiliations:

Jerice Banola, Gordon C. Weir, Cristina Aguayo-Mazzucato

Joslin Diabetes Center, Harvard Medical School

Corresponding Authors:

Jerice Banola (jerice.banola@joslin.harvard.edu)

Email Addresses for All Authors:

Jerice Banola (jerice.banola@joslin.harvard.edu)
Gordon C. Weir (gordon.weir@joslin.harvard.edu)
Cristina Aguayo-Mazzucato (cristina.aguayo-mazzucato@joslin.harvard.edu)

Author Questionnaire

1. **Microscopy**: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **Yes**

   If **Yes**, can you record movies/images using your own microscope camera?

   No, I do not have a microscope camera.

   Leica Stereozoom 7 or Leica Sterozoom S9E, understood that I will have to perform the procedure with one eye

   All steps involve the microscope

   Videographer: Please film all the SCOPE shots using the scope kit

2. **Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **No**

3. **Filming location:** Will the filming need to take place in multiple locations? **No**

Current Protocol Length
Number of Steps: 07
Number of Shots: 24

Introduction

Videographer: Obtain headshots for all authors available at the filming location.

1.1. **Jerice Banola:** We aim to better analyze the function of pancreatic islet cells at a level closer to physiological conditions than current research methods, and as an alternative approach for comparison.
   1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

What advantage does your protocol offer compared to other techniques?

1.2. **Jerice Banola:** Our protocol allows for preservation of the pancreas' natural microenvironment to better mimic a physiologic state for further comparison with other techniques for GSIS.
   1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B roll: 2.5*

What research questions will your laboratory focus on in the future?

1.3. **Jerice Banola:** Our laboratory hopes to focus further on implementing the different GSIS methods for the endocrine pancreas and its response to potential therapeutics like senolytics, which target dysfunctional senescent cells.
   1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B roll: Figure 3*

Videographer: Obtain headshots for all authors available at the filming location.

Ethics Title Card

This research has been performed in compliance with Joslin Diabetes Research Center guidelines

Protocol

2. Ligation of Major Abdominal Vessels and Dual Cannulation of the Mouse Aorta and Portal Vein

Demonstrator: Jerice Banola

2.1. To begin, identify the upper abdominal aorta by locating the bright red midline vessel **[1]** with the branching celiac artery **[2]**, superior mesenteric artery **[3]**. Ligate superior to the celiac artery and left renal artery **[4]**. Then identify the left renal artery **[5]**.
NOTE: VO changed to reflect order of moved shots

2.1.1. WIDE: Talent pointing out the upper abdominal aorta in the exposed abdominal cavity. **TXT: Anesthesia: Ketamine/xylazine mix (~75 mg/kg of ketamine HCl mixture), intramuscularly**

2.1.2. SCOPE: Talent pointing out the initial branching points of SMA and celiac artery.

2.1.3. SCOPE: Talent pointing out the superior mesenteric artery.

2.1.5: SCOPE: Talent using forceps to perform the ligation superior to the celiac artery

2.1.4. SCOPE: Talent pointing out the left renal vessels.
Author's Note: Shot moved below 2.1.5

2.2. Move the intestines to the animal's left side to gain access to the right kidney **[1]**. Using forceps, pinch the tissue or fat surrounding the right kidney **[2]** and identify the right renal vein as the dark red or purple vessel connecting the right kidney to the inferior vena cava **[3]**.

2.2.1. SCOPE: Talent gently moving intestines to the left side of the animal.

2.2.2. SCOPE: Talent pinching the tissue or fat surrounding the right kidney to lift and allow for easier isolation of the right renal vein.

2.2.3. SCOPE: Close-up of identified right renal vein.

2.3. Now, identify the abdominal portion of the inferior vena cava as a larger purple vessel with a branch connecting to the right renal vein **[1]** and perform the ligation **[2]**.

2.3.1. SCOPE: Talent pointing to the abdominal portion of the inferior vena cava with visible branching from the right renal vein.

2.3.2. SCOPE: Talent using surgical thread to ligate the abdominal portion of the inferior vena cava.

2.4. Identify the lower abdominal aorta just before its bifurcation **[1]**. Perform the ligation at this point **[2]**. Then, place a suture thread 0.5 centimeters above the first knot **[3]**. ~~Insert the tube for cannulation between the suture thread and ligated suture to ensure the perfusate flows up toward the celiac artery **[4]**.~~

2.4.1. SCOPE: Talent locating and pointing to the lower abdominal aorta just before bifurcation.

2.4.2. SCOPE: Talent tying the ligature on the lower abdominal aorta before the bifurcation.

2.4.3. SCOPE: Talent threading a second suture 0.5 centimeters above the first knot.

~~2.4.4. SCOPE: Talent inserting the cannulation tube into the space between the two sutures.~~

NOTE: Shot deleted by authors

2.5. Move the intestines to the animal's left to expose the portal vein **[1]**. After making an incision in the diaphragm and ribs, ligate the vessel as superior as possible to ensure space for cannulation later **[2-TXT]**. Place a second suture inferior to the first but above the pancreas to ensure collection of the perfusate and avoid backflow **[3]**.

2.5.1. SCOPE: Talent repositioning intestines to the left for clear view of portal vein.

2.5.2. SCOPE: Talent tying the ligature as close to the liver as possible on the portal vein. **TXT: Making an incision in the diaphragm and ribs results in the complete euthanasia of the mouse**

2.5.3. SCOPE: Talent placing a second suture below the first, just above the pancreas.

~~3. Cannulation of the Lower Abdominal Aorta and the Portal Vein~~

3.1. Create a 0.5 to 1.5-millimeter incision using scissors on the lower abdominal aorta, between the bottom knot and the superior suture **[1]**. Using the lower knot for leverage, insert the tube into the incision via the nick until it reaches the superior suture, being careful not to tear the vessel **[2]**. Tie the superior suture into a knot to secure the tube in place **[3]**.

AUTHOR'S NOTE: Move 3.1 below 2.4.3

3.1.1. SCOPE: Talent using scissors to make a small incision into the lower abdominal aorta between the bottom knot and the superior thread.

3.1.2. SCOPE: Talent inserting the tube carefully into the incision while holding the lower knot or directly the lower abdominal aorta for support.

3.1.3. SCOPE: Talent tying the superior suture into a surgeon's knot to fix the tube in

position.

3.2. Next, create a 0.5 to 1.5-millimeter incision in the portal vein just after it exits the pancreas **[1]**. Hold the top knot with blunt forceps in one hand for leverage and insert the tube into the portal vein using forceps in the other hand to guide it in **[2]**. Tie the second suture to secure the tube **[3-TXT]**.
AUTHOR'S NOTE: Move shot 3.2 directly below shot 2.5

3.2.1. SCOPE: Talent making a small incision in the portal vein just after it exits the pancreas.

3.2.2. SCOPE: Talent gripping the upper suture with forceps and using the other hand with forceps to insert the tube.

3.2.3. SCOPE: Talent tying the lower suture around the tube to lock it in place. **TXT: Optionally, secure it in place with a microclip**

Results

4. Results

4.1. This figure illustrates the insulin secretion dynamics in perfused mouse pancreas in response to varying glucose concentrations and 3-isobutyl-1-methylxanthine or IBMX *(I-B-M-X)* stimulation **[1]**.

4.1.1. LAB MEDIA: Figure 3.

4.2. Insulin levels showed a steady low secretion at 2.6 millimolar glucose for the initial 20 minutes **[1]**, followed by a marked first-phase increase upon switching to 16.8 millimolar glucose between 20 and 60 minutes **[2]**.

4.2.1. LAB MEDIA: Figure 3. *Video editor: Highlight the part of the graph under the label "2.6 mM Glc" (only the left 2.6 mM Glc)*

4.2.2. LAB MEDIA: Figure 3. *Video editor: Highlight the part of the graph under the label "16.8 mM Glc".*

4.3. When the glucose concentration was returned to 2.6 millimolar, insulin secretion sharply declined to baseline levels **[1]**.

4.3.1. LAB MEDIA: Figure 3. *Video editor: Highlight the part of the graph under the "2.6 mM Glc" section (only the right 2.6 mM Glc).*

4.4. Administration of IBMX at 2.6 millimolar glucose caused a strong secondary rise in insulin secretion between 90 and 110 minutes **[1]**.

4.4.1. LAB MEDIA: Figure 3. *Video editor: Highlight the part of the graph under the section labeled "2.6 mM Glc + IBMX".*

4.5. The perfused mouse pancreas exhibits a biphasic insulin response to elevated glucose and retains responsiveness to secretagogues like IBMX even under low glucose conditions **[1]**.

4.5.1. LAB MEDIA: Figure 3.

Pronunciation Guide:

1. **Perfusion**
   Pronunciation link: https://www.merriam-webster.com/dictionary/perfusion Merriam-Webster
   IPA: /pəˈfjuːʒən/ Cambridge Dictionary+1
   Phonetic Spelling: per-FYOOSH-uhn

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2. **Senolytics**
   Pronunciation link: https://www.howtopronounce.com/senolytics How To Pronounce
   IPA: /ˌsɛnəˈlɪtɪks/ How To Pronounce
   Phonetic Spelling: sen-oh-LIT-iks

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3. **Anatomical**
   Pronunciation link:
   https://www.oxfordlearnersdictionaries.com/us/definition/english/anatomical Oxford Learner's Dictionaries
   IPA: /ˌænəˈtɑmɪkəl/ Oxford Learner's Dictionaries
   Phonetic Spelling: an-uh-TOM-ih-kuhl

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4. **Paracrine**
   Pronunciation link: Oxford, “paracrine” – (Cambridge also works) Cambridge Dictionary+1
   IPA: /ˈpærəˌkraɪn/ Wikipedia+1
   Phonetic Spelling: PAIR-uh-krine

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5. **Endocrine**
   Pronunciation link: Cambridge / Merriam-Webster Cambridge Dictionary+1
   IPA: /ˈɛndəˌkraɪn/ Cambridge Dictionary+1
   Phonetic Spelling: EN-duh-krine

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6. **Superior (as in Superior Mesenteric Artery)**
   Pronunciation link: Merriam-Webster etc. (“superior”) Cambridge Dictionary
   IPA: /səˈpɪriər/ Cambridge Dictionary
   Phonetic Spelling: suh-PEER-ee-er

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7. **Mesenteric**
   Pronunciation link: Cambridge / medical dictionaries (“mesenteric”) Cleveland Clinic+1
   IPA: /ˌmɛzənˈtɛrɪk/ Cleveland Clinic
   Phonetic Spelling: mez-un-TER-ik

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8. **Vasculature**
   Pronunciation link: Merriam-Webster (“vasculature”) Oxford Learner's Dictionaries

IPA: /ˈvæskjələtʃər/ Oxford Learner's Dictionaries
Phonetic Spelling: VAS-kyuh-luh-chur

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9. **Celiac** (as in celiac artery)
   Pronunciation link: Cambridge / Merriam-Webster ("celiac") Cambridge Dictionary
   IPA: /ˈsiːliæk/ or /ˈsiːliək/ Cambridge Dictionary
   Phonetic Spelling: SEE-lee-ak

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10. **Insulin**
    Pronunciation link: Merriam-Webster ("insulin") Cambridge Dictionary
    IPA: /ˈɪnsəlɪn/ Cambridge Dictionary
    Phonetic Spelling: IN-suh-lin

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11. **Glucose**
    Pronunciation link: Cambridge / Merriam-Webster ("glucose") Cambridge Dictionary
    IPA: /ˈgluːkoʊs/ Cambridge Dictionary
    Phonetic Spelling: GLOO-kose

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12. **Cannulation**
    Pronunciation link: Merriam-Webster ("cannulation") Cambridge Dictionary
    IPA: /ˌkænjuˈleɪʃən/ Cambridge Dictionary
    Phonetic Spelling: can-u-LAY-shuhn