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Title: Development and Validation of a Methodology for Establishing Obese Rat Models with Typical Fatty Pancreas

Authors and Affiliations:

Yujue Wang^{1,*}, Youling Gao^{1,2,*}, Chenxi Deng¹, Yingxuan Tang¹, Yun Liu¹, Guanhu Yang^{3,4}, Bin Xu¹, Youbing Xia¹, Tiancheng Xu¹

¹Key Laboratory of Acupuncture and Medicine Research of Ministry of Education, Nanjing University of Chinese Medicine

²Department of Acupuncture and Moxibustion, Yangzhou TCM Hospital Affiliated to Nanjing University of Chinese Medicine

³Department of Specialty Medicine, Ohio University

⁴Research Department, Swiss University of Traditional Chinese Medicine

Corresponding Authors:

Tiancheng XU

xtc@njucm.edu.cn

Youbing XIA

xybd1968@njucm.edu.cn

Email Addresses for All Authors:

Yujue WANG

202410520@njucm.edu.cn

Youling Gao

lyndagao1218@gmail.com

Chenxi DENG

dcx09299@163.com

Yingxuan TANG

055122107@njucm.edu.cn

Yun LIU

20200413@njucm.edu.cn

Guanhu YANG

guanhuayang@gmail.com

Bin XU

xubin@njucm.edu.cn

Tiancheng XU

xtc@njucm.edu.cn

Youbing XIA

xybd1968@njucm.edu.cn

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? **No**
- 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**
- 4. Testimonials (optional):** Would you be open to filming two short testimonial statements **live during your JoVE shoot**? These will **not appear in your JoVE video** but may be used in JoVE's promotional materials. **Yes**

Current Protocol Length

Number of Steps: 06

Number of Shots: 11

Introduction

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

INTRODUCTION:

- 1.1. **Yujue Wang** : Our study aims to establish an obese rat model centered on fatty pancreas to explore obesity-related pathological changes.
 - 1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

CONCLUSION:

- 1.2. **Yujue Wang**: We re-examine simple obesity from a pancreatic perspective, aiming to offer a new viewpoint for the clinical management of metabolic diseases.
 - 1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera
- 1.3. **Yujue Wang**: The modeling method and IHC/WB protocols proposed in this study lays a solid foundation for subsequent basic research on the mechanisms of fatty pancreas.
 - 1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera
- 1.4. **Yujue Wang**: Our future studies will investigate the microscopic expression differences between normal and fatty pancreas at the molecular level.
 - 1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

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

Testimonial Questions (OPTIONAL):

Videographer: Please capture all testimonial shots in a wide-angle format with sufficient headspace, as the final videos will be rendered in a 1:1 aspect ratio. Testimonial statements will be presented live by the authors, sharing their spontaneous perspectives.

- Testimonial statements will **not appear in the video** but may be featured in the journal's promotional materials.
- **Provide the full name and position** (e.g., Director of [Institute Name], Senior Researcher [University Name], etc.) of the author delivering the testimonial.
- Please **answer the testimonial question live during the shoot**, speaking naturally and in your own words in **complete sentences**.

How do you think publishing with JoVE will enhance the visibility and impact of your research?

1.5. **Yujue Wang** : (authors will present their testimonial statements live)

1.5.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

Can you share a specific success story or benefit you've experienced—or expect to experience—after using or publishing with JoVE? (This could include increased collaborations, citations, funding opportunities, streamlined lab procedures, reduced training time, cost savings in the lab, or improved lab productivity.)

1.6. **Yujue Wang** : (authors will present their testimonial statements live)

1.6.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

Authors: Could you please also deliver the above statements in Chinese?

Videographer: Please film the testimonials in both English and Chinese

Ethics Title Card

This research has been approved by the Animal Ethics Committee at Nanjing University of Traditional Chinese Medicine

Protocol

2. Pancreatic Isolation from the Obese Rat

- 2.1. To begin, position the anesthetized animal on the surgical table [1-TXT].
 - 2.1.1. LAB MEDIA: 69553-1 00:00-00:03. TXT: Anesthesia: Isoflurane; Induction: 4%; Maintenance: 1.5 – 2%
- 2.2. Monitor the depth of anesthesia with a toe pinch test and continuously track the respiratory rate [1].
 - 2.2.1. LAB MEDIA: 69553-1 00:05-00:15.
- 2.3. Disinfect the surgical area using alcohol or iodophor [1] and ensure the entire abdominal surface is evenly treated before proceeding [2].
 - 2.3.1. LAB MEDIA: 69553-1 00:15-00:22.
 - 2.3.2. LAB MEDIA: 69553-1 00:55-01:00.
- 2.4. Using forceps in the left hand, grasp the abdominal skin [1]. With scissors in the right hand, incise the skin along the midline from the xiphoid to the pubis [2]. Then, bluntly dissect the subcutaneous fascia to separate the connective tissue [3].
 - 2.4.1. LAB MEDIA: 69553-1 01:03-01:06.
 - 2.4.2. LAB MEDIA: 69553-1 01:06-01:15.
 - 2.4.3. LAB MEDIA: 69553-1 01:32-01:38.
- 2.5. Now, gently elevate the spleen using forceps [1] and isolate the pancreas by progressively cutting its attachments to the spleen, stomach, and duodenal mesentery [2].
 - 2.5.1. LAB MEDIA: 69553-1 03:00-03:06.
 - 2.5.2. LAB MEDIA: 69553-1 03:35-03:45.
- 2.6. Finally, transfer the isolated pancreas into a 5-milliliter freezing tube [1]. Submerge the tube in liquid nitrogen within 30 seconds and store the sample long-term at minus

80 degrees Celsius [2].

2.6.1. LAB MEDIA: 69553-1 03:48-03:53.

2.6.2. LAB MEDIA: 69553-1 03:58-04:04.

Results

3. Results

- 3.1. Compared to normal pancreatic tissue [1], lipid-laden pancreatic tissue exhibited pancreatic parenchymal adipocyte infiltration as a characteristic histological alteration [2].

3.1.1. LAB MEDIA: Figure 4A

3.1.2. LAB MEDIA: Figure 4B. *Video editor: Zoom in on the pancreatic tissue image showing areas filled with large clear fat spaces compared to surrounding tissue.*

- 3.2. Normal pancreatic tissue showed a well-preserved structure without adipose infiltration [1].

3.2.1. LAB MEDIA: Figure 4A

- 3.3. Perfused rats exhibited pronounced limb rigidity with limbs maintained in an extended upright position [1], in contrast to the soft and pliable limbs of non-perfused controls [2].

3.3.1. LAB MEDIA: Figure 5A. *Video editor: Highlight the perfused rat*

3.3.2. LAB MEDIA: Figure 5A. *Video editor: Highlight the non-perfused rat*

- 3.4. The perfused pancreas displayed a distinct pale coloration [1], whereas the non-perfused pancreas retained a bright red appearance [2].

3.4.1. LAB MEDIA: Figure 5B. *Video editor: Zoom in on the perfused rat image.*

3.4.2. LAB MEDIA: Figure 5B. *Video editor: Zoom in on the non-perfused rat image.*

- 3.5. Western blotting showed clear Vinculin bands in pancreatic tissue from both Control and Model groups, indicating preserved tissue integrity [1].

3.5.1. LAB MEDIA: Figure 5C.

4. Anesthetized
Pronunciation link: <https://www.merriam-webster.com/dictionary/anesthetized>
IPA: /əˈniːsθəˈtaɪzd/
Phonetic Spelling: uh·nees·thuh·tyzd
5. Anesthesia
Pronunciation link: <https://www.merriam-webster.com/dictionary/anesthesia>
IPA: /ˌænəsˈθiːzə/
Phonetic Spelling: a·nuhs·thee·zhuh
6. Isoflurane
Pronunciation link: <https://www.merriam-webster.com/dictionary/isoflurane>
IPA: /ˌaɪsoʊˈfloʊrɪn/
Phonetic Spelling: eye·soh·floor·ayn
7. Iodophor
Pronunciation link: <https://www.merriam-webster.com/dictionary/iodophor>
IPA: /ˈaɪoʊdəˈfɔːr/
Phonetic Spelling: eye·oh·duh·for
8. Xiphoid
Pronunciation link: <https://www.merriam-webster.com/dictionary/xiphoid>
IPA: /ˈzɪˌfɔɪd/
Phonetic Spelling: zih·foyd
9. Pubis
Pronunciation link: <https://www.merriam-webster.com/dictionary/pubis>
IPA: /ˈpjuːbɪs/
Phonetic Spelling: pyoo·biss
10. Subcutaneous
Pronunciation link: <https://www.merriam-webster.com/dictionary/subcutaneous>
IPA: /ˌsʌbkjuːˈteɪniəs/
Phonetic Spelling: sub·kyoo·tay·nee·uhs
11. Fascia
Pronunciation link: <https://www.merriam-webster.com/dictionary/fascia>
IPA: /ˈfæʃə/
Phonetic Spelling: fash·uh
12. Duodenal
Pronunciation link: <https://www.merriam-webster.com/dictionary/duodenal>
IPA: /ˌduːəˈdiːnəl/
Phonetic Spelling: doo·uh·dee·nuhl
13. Mesentery
Pronunciation link: <https://www.merriam-webster.com/dictionary/mesentery>
IPA: /ˈmesənˌteri/
Phonetic Spelling: meh·suhn·ter·ee
14. Histological
Pronunciation link: <https://www.merriam-webster.com/dictionary/histological>

IPA: /ˌhɪstəˈlɑːdʒɪkəl/

Phonetic Spelling: hiss·tuh·laa·ji·kuhl

15. Parenchymal

Pronunciation link: <https://www.merriam-webster.com/dictionary/parenchymal>

IPA: /pəˈrɛŋkɪməl/

Phonetic Spelling: puh·ren·kih·muhl

16. Adipocyte

Pronunciation link: <https://www.merriam-webster.com/dictionary/adipocyte>

IPA: /ˈædəˌpoʊsaɪt/

Phonetic Spelling: a·duh·poh·site

17. Perfused

Pronunciation link: <https://www.merriam-webster.com/dictionary/perfused>

IPA: /pərˈfjuːzd/

Phonetic Spelling: per·fyoozd

18. Vinculin

Pronunciation link: <https://www.merriam-webster.com/medical/vinculin>

IPA: /ˈvɪŋkjʊlɪn/

Phonetic Spelling: vin·kyuh·lin

19. Celsius

Pronunciation link: <https://www.merriam-webster.com/dictionary/Celsius>

IPA: /ˈsɛlsiəs/

Phonetic Spelling: sel·see·uhs