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Project Page Link: https://review.jove.com/files_upload.php?src=21853603

Title: An Ex Vivo Mouse Model for High-Fat Diet-Like Adipose Tissue Inflammation

Authors and Affiliations:

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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**

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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. **No**

Current Protocol Length

Number of Steps: 16

Number of Shots: 31

Interviews

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

Authors: Please note that **the questions will not appear on screen**. Please answer in **stand-alone full sentence with sufficient context**

INTRODUCTION:

~~**REQUIRED:** Summarize your research focus and the main questions it aims to answer.~~

- 1.1. **Raphaela Wehr:** We are investigating inflammation in adipose tissue, focusing on the role of adipose tissue macrophages in this process.
 - 1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera *Suggested B roll: Figure 1*

~~**Describe the research contexts or systems in which this protocol can be applied.**~~

- 1.2. **Raphaela Wehr:** This model can be applied in a great variety of adipose tissue inflammation research where the focus is on the adipose tissue itself.
 - 1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

CONCLUSION:

~~**What does this protocol allow researchers to study or measure?**~~

- 1.3. **Raphaela Wehr:** This method can be used to study the intercellular functional processes and alterations that occur during adipose tissue inflammation.
 - 1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

~~**Describe the most important consideration or challenge when performing this protocol.**~~

- 1.4. **Raphaela Wehr:** The most important challenge when performing this protocol is to handle the tissue as gently as possible so that the tissue explants remain viable throughout the culture period.

- 1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 3.1.2*

~~How can future studies build on this work? Describe the questions or research directions that can be investigated next.~~

- 1.5. **Raphaela Wehr**: Future studies can use this method to dissect adipose inflammation signaling, evaluate cell-specific responses to therapies, and reduce animal use.
- 1.5.1. 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.

Ethics Title Card

This research has been approved by the local authorities of the state of Saxony, Landesdirektion in Leipzig, Germany. The ethics committee of the Faculty of Medicine at the University of Leipzig has also approved the research projects.

Protocol

***Videographer:** Please always record an audio slate while filming each shot mentioned in the shotlist.*

2. Isolation of Mouse Adipose Tissue

Demonstrator: Raphaela Wehr

- 2.1. To begin, prepare the culture medium under sterile conditions [1]. Warm the culture medium and sterile PBS to 37 degrees Celsius [2]. Clean all equipment and the work area inside the tissue culture hood with 70% ethanol [3].
 - 2.1.1. WIDE: Talent at the working bench with the prepared culture medium in the frame. ***Videographer:** Please obtain CU shot as well for this shot.*
 - 2.1.2. Talent placing the culture medium and sterile phosphate-buffered saline in a water bath.
 - 2.1.3. Talent wiping the equipment and the work area inside the tissue culture hood with 70% ethanol.
- 2.2. After placing the sacrificed animal in a glass beaker containing 70% ethanol [1-TXT], wipe the exterior of the glass beaker with ethanol [2] and transfer it into the tissue culture hood [3].
 - 2.2.1. Shot of the animal placed in a glass beaker containing 70% ethanol. **TXT: Mice Used: Csf1r-eGFP-Adipoq-ERT2-Cre-CAG-tdTomato**
 - 2.2.2. Talent wiping the exterior of the glass beaker with ethanol.
 - 2.2.3. Talent transferring the glass beaker into the tissue culture hood.
- 2.3. Using blunt forceps, place the mouse in a supine position and pin it to a dissection board [1].
 - 2.3.1. Talent positioning the mouse in a supine position and pinning it.
- 2.4. Then, add a thin layer of pre-warmed PBS to a Petri dish [1].
 - 2.4.1. Talent adding a thin layer of pre-warmed PBS to a Petri dish.
- 2.5. Change to a fresh pair of gloves [1]. Using forceps, lift the lower abdominal skin [2] and make a small horizontal incision using scissors to open the abdominal cavity [3].
 - 2.5.1. Talent putting on a fresh pair of gloves.
 - 2.5.2. Talent lifting the lower abdominal skin with forceps.
 - 2.5.3. Talent making a small horizontal incision with scissors to open the abdominal cavity.
- 2.6. Make a vertical incision, extending from the initial cut to expose the internal organs [1].

- 2.6.1. Talent making a vertical incision from the initial cut to expose the internal organs.
- ~~2.7. Using small sharp scissors and pointed forceps, locate the white adipose tissue [1] and excise it using a separate set of instruments to prevent contamination [2].~~
 - ~~2.7.1. Talent locating the white adipose tissue using small sharp scissors and pointed forceps.~~
 - ~~2.7.2. Talent excising the white adipose tissue with a separate set of instruments.~~
Author's NOTE: 2.7 is same as 2.8. We have not filmed 2.7 and decided to film only 2.8
- 2.8. For male mice, lift the gonadal fat pad carefully [1] and cut above the epididymis [2]. Alternatively, excise the epididymis and testis together with the gonadal fat [3] and remove the epididymis and testis afterward [4].
 - 2.8.1. Talent lifting the gonadal fat pad in a male mouse. **Author's NOTE: This may have been named as 2.7.1**
 - 2.8.2. Talent cutting above the epididymis.
 - 2.8.3. Talent excising the epididymis and testis together with the gonadal fat.
 - 2.8.4. Talent removing the epididymis and testis from the excised tissue. **Author's NOTE: 2.8.3 and 2.8.4 were combined**
- 2.9. For female mice, lift the gonadal fat pad carefully [1] and excise along the uterine horn and ovaries [2].
 - 2.9.1. Talent lifting the gonadal fat pad in a female mouse.
 - 2.9.2. Talent excising the fat pad along the uterine horn and ovaries.
- 2.10. Place the isolated fat pads into a Petri dish filled with PBS [1].
 - 2.10.1. Talent placing the isolated fat pads into the PBS-filled Petri dish.
- 2.11. Using a razor blade, remove any residual epididymis, testis, ovaries, or blood vessels from the fat pads [1].
 - 2.11.1. Talent removing residual epididymis, testis, ovaries, and blood vessels from the fat pads using a razor blade.
- 2.12. Transfer the cleaned fat pad into a second Petri dish containing pre-warmed PBS [1].
 - 2.12.1. Talent transferring the cleaned fat pad into a second Petri dish containing pre-warmed phosphate-buffered saline.

3. Explant Preparation and Culture

- 3.1. Using a razor blade, cut the fat pad into strips [1] and then cut the strips into cubes of approximately 10 cubic millimeters [2].
 - 3.1.1. Talent cutting the fat pad into strips using a razor blade.
 - 3.1.2. Talent cutting the strips into cubes of approximately 10 cubic millimeters using a razor blade.
- 3.2. Add 1 to 1.5 milliliters of culture medium to each well of a 6-well plate [1]. Using a sterile spoon or spatula, transfer five explants into each well [2]. Arrange the explants evenly in a circular pattern [3].
 - 3.2.1. Talent adding culture medium to each well of a 6-well plate.
 - 3.2.2. Talent transferring five explants into a well using a sterile spoon or spatula.
 - 3.2.3. Talent arranging the explants evenly in a circular pattern within the well.
- 3.3. Using sterile forceps, cover the explants with a cell culture insert [1]. Incubate them at 37 degrees Celsius, 5% carbon dioxide, and 21% oxygen for 7 to 10 days [1].
 - 3.3.1. Talent placing a cell culture insert over the explants using sterile forceps.
 - 3.3.2. Talent placing the explant culture plate into the incubator.
- 3.4. After a culture period of 7 to 10 days, collect explants or culture supernatants for downstream analyses as required for the experimental endpoint, followed by statistical analysis [1].
 - 3.4.1. Talent collecting explants, or culture supernatants for downstream analyses.

Results

4. Results

- 4.1. TdTomato (*T-D-Tomato*) expression remained stable throughout the 14-day culture period [1].
 - 4.1.1. LAB MEDIA: Figure 3A.
- 4.2. Lactate dehydrogenase levels remained low relative to maximal release following complete tissue lysis, indicating preserved tissue integrity [1].
 - 4.2.1. LAB MEDIA: Figure 3B. *Video editor: Highlight the bars labelled 'd 1', 'd 3', 'd 5', and 'd 7'.*
- 4.3. Perilipin-positive adipocytes remained intact for up to 10 days of culture [1]. The proportion of DAPI-positive cells remained stable throughout the culture period [2].
 - 4.3.1. LAB MEDIA: Figure 3C.
 - 4.3.2. LAB MEDIA: Figure 3D and E.
- 4.4. Expression of Pparg (*P-Parg*), Adipoq (*Adipo-Q*), Lep (*L-E-P*), and Lpl (*L-P-L*) remained stable throughout the culture period [1].
 - 4.4.1. LAB MEDIA: Figure 3F.
- 4.5. A key feature of adipose tissue inflammation is the formation of crown-like structures by adipose tissue macrophages around dying adipocytes [1], primarily occurring between days 5 and 7 of culture [2].
 - 4.5.1. LAB MEDIA: Figure 4A.
 - 4.5.2. LAB MEDIA: Figure 4A and B. *Video editor: Highlight the white arrows in the image labelled 'Day 5' and 'Day 7' in 4A and highlight the bars "d5" and "d7" in 4B.*
- 4.6. Expression of inflammatory genes and Tumor necrosis factor alpha increased over time [1].
 - 4.6.1. LAB MEDIA: Figure 4C and D.
- 4.7. Multinucleated giant cells developed and contributed to dead adipocyte clearance [1]
 - 4.7.1. LAB MEDIA: Figure 4F.
- 4.8. Although macrophages accumulated within crown-like structures and fused into multinucleated giant cells, the overall number of adipose tissue macrophages remained relatively stable, as a proliferation peak was observed on day 5 [1].

- 4.8.1. LAB MEDIA: Figure 5A-D. *Video editor: Highlight the bar labeled 'd 5' in figure 5D*
- 4.9. The adipose tissue macrophages phenotype shifted from a predominantly M2-like state at day 1 [1] toward an increased proportion of M1-like macrophages at day 7 [2].
 - 4.9.1. LAB MEDIA: Figure 5F. *Video editor: Highlight the bar labelled 'M2-like' at 'd 1'.*
 - 4.9.2. LAB MEDIA: Figure 5F. *Video editor: Highlight the bar labelled 'M1-like' at 'd 7'.*
- 4.10. Metabolically activated macrophages increased during culture, with increased BODIPY (*Bodipy*) staining [1] and increased lysosomal content [2].
 - 4.10.1. LAB MEDIA: Figure 5I. *Video editor: Highlight the bars comparing 'd 1' and 'd 7'.*
 - 4.10.2. LAB MEDIA: Figure 5M. *Video editor: Highlight the bars comparing 'd 1' and 'd 7'.*
- 4.11. Multiple macrophage populations were identified, including pro-inflammatory [1], metabolically activated [2], proliferating [3], inflammation-resolving [4], and anti-inflammatory macrophages [5].
 - 4.11.1. LAB MEDIA: Figure 6A. *Video editor: Highlight the panel labelled 'Itgax'.*
 - 4.11.2. LAB MEDIA: Figure 6A. *Video editor: Highlight the panel labelled 'Alox15a2'.*
 - 4.11.3. LAB MEDIA: Figure 6A. *Video editor: Highlight the panels labelled 'Mki67' and 'Top2a'.*
 - 4.11.4. LAB MEDIA: Figure 6A. *Video editor: Highlight the panel labelled 'Spint1'.*
 - 4.11.5. LAB MEDIA: Figure 6A. *Video editor: Highlight the panels labelled 'Mrc1' and 'Lyve1'.*
- 4.12. Clodronate liposomes reduced macrophage numbers by approximately 60% by day 7 [1], and PLX5622 (*P-L-X-Five-Six-Two-Two*) reduced macrophage numbers by approximately 70% by day 7 [2].
 - 4.12.1. LAB MEDIA: Figure 7E. *Video editor: Highlight the 'Clod' bar at 'd 7'.*
 - 4.12.2. LAB MEDIA: Figure 7F. *Video editor: Highlight the 'PLX' bar at 'd 7'.*

1. Adipose
 Pronunciation link: <https://www.merriam-webster.com/dictionary/adipose>
 IPA: /'æd.ə.pouz/
 Phonetic Spelling: ad·uh·pohs
2. Sterile
 Pronunciation link: <https://www.merriam-webster.com/dictionary/sterile>
 IPA: /'ster.əl/, /'ster.aɪl/
 Phonetic Spelling: stair·uhl, stair·eyel

3. Phosphate-buffered
Pronunciation link: No confirmed link found
IPA: /'fɑ:s.feɪt 'bʌf.ə-d/
Phonetic Spelling: fos·fayt buf·erd
4. Ethanol
Pronunciation link: <https://www.merriam-webster.com/dictionary/ethanol>
IPA: /'eθ.ə.nɔ:l/
Phonetic Spelling: eth·uh·nawl
5. Supine
Pronunciation link: <https://www.merriam-webster.com/dictionary/supine>
IPA: /su:'paɪn/
Phonetic Spelling: soo·pyne
6. Dissection
Pronunciation link: <https://www.merriam-webster.com/dictionary/dissection>
IPA: /daɪ'sek.ʃən/
Phonetic Spelling: dye·sek·shuhn
7. Abdominal
Pronunciation link: <https://www.merriam-webster.com/dictionary/abdominal>
IPA: /æb'dɑ:m.ə.nəl/
Phonetic Spelling: ab·dom·uh·nuhl
8. Epididymis
Pronunciation link: <https://www.merriam-webster.com/dictionary/epididymis>
IPA: /,ep.ə'dɪd.ə.məs/
Phonetic Spelling: ep·uh·did·uh·muhs
9. Testis
Pronunciation link: <https://www.merriam-webster.com/dictionary/testis>
IPA: /'tes.tɪs/
Phonetic Spelling: tes·tiss
10. Uterine
Pronunciation link: <https://www.merriam-webster.com/dictionary/uterine>
IPA: /'ju:.tə'raɪn/
Phonetic Spelling: yoo·tuh·rine
11. Ovaries
Pronunciation link: <https://www.merriam-webster.com/dictionary/ovary>
IPA: /'oʊ.və'i:z/
Phonetic Spelling: oh·vuh·reez
12. Explant
Pronunciation link: <https://www.merriam-webster.com/dictionary/explant>
IPA: /'eks.plænt/
Phonetic Spelling: eks·plant
13. Incubate
Pronunciation link: <https://www.merriam-webster.com/dictionary/incubate>
IPA: /'ɪŋ.kjə'beɪt/
Phonetic Spelling: ing·kyuh·bayt

14. Supernatants
Pronunciation link: <https://www.merriam-webster.com/dictionary/supernatant>
IPA: /ˌsuː.pəˈnei.tənt/
Phonetic Spelling: soo·per·nay·tuhnt
15. TdTomato
Pronunciation link: No confirmed link found
IPA: /ˌtiː diː təˈmeɪ.tou/
Phonetic Spelling: tee·dee tuh·may·toh
16. Lactate
Pronunciation link: <https://www.merriam-webster.com/dictionary/lactate>
IPA: /ˈlæk.tet/
Phonetic Spelling: lak·tayt
17. Dehydrogenase
Pronunciation link: <https://www.merriam-webster.com/dictionary/dehydrogenase>
IPA: /diːˌhaɪ.drəˈdʒen.eɪs/
Phonetic Spelling: dee·hy·druh·jen·ays
18. Lysis
Pronunciation link: <https://www.merriam-webster.com/dictionary/lysis>
IPA: /ˈlaɪ.sɪs/
Phonetic Spelling: lye·siss
19. Perilipin
Pronunciation link: <https://www.howtopronounce.com/perilipin>
IPA: /ˌper.əˈlɪp.ɪn/
Phonetic Spelling: pehr·uh·lip·in
20. Adipocytes
Pronunciation link: <https://www.merriam-webster.com/dictionary/adipocyte>
IPA: /ˈæd.əˌpoʊ.sarts/
Phonetic Spelling: ad·uh·poh·sytes
21. DAPI
Pronunciation link: <https://www.howtopronounce.com/dapi>
IPA: /ˈdæpi/
Phonetic Spelling: dap·ee
22. Pparg
Pronunciation link: No confirmed link found
IPA: /ˌpiː piː ɛr ˈɑːr dʒiː/
Phonetic Spelling: pee·pee·ay·ar·jee
23. Adipoq
Pronunciation link: No confirmed link found
IPA: /əˈdiː.poʊ kjuː/
Phonetic Spelling: uh·dee·poh·cue
24. Perilipin
Pronunciation link: <https://www.howtopronounce.com/perilipin>
IPA: /ˌper.əˈlɪp.ɪn/
Phonetic Spelling: pehr·uh·lip·in

25. Macrophages
Pronunciation link: <https://www.merriam-webster.com/dictionary/macrophage>
IPA: /'mæk.rəˌfeɪdʒ/, /'mæk.roʊˌfeɪdʒ/
Phonetic Spelling: mak·ruh·fayj, mak·roh·fayj
26. Multinucleated
Pronunciation link: <https://www.merriam-webster.com/dictionary/multinucleated>
IPA: /ˌmʌl.tiˈnuː.kli.ɛt.ɪd/
Phonetic Spelling: mul·tee·noo·klee·ay·tid
27. Phenotype
Pronunciation link: <https://www.merriam-webster.com/dictionary/phenotype>
IPA: /'fiː.nəˌtaɪp/
Phonetic Spelling: fee·nuh·type
28. Metabolically
Pronunciation link: <https://www.merriam-webster.com/dictionary/metabolically>
IPA: /ˌmeɪ.əˈbɑː.li.kli/
Phonetic Spelling: met·uh·bol·ih·klee
29. BODIPY
Pronunciation link: <https://www.howtopronounce.com/bodipy>
IPA: /'boʊ.dɪ.piː/
Phonetic Spelling: boh·dih·pee
30. Lysosomal
Pronunciation link: <https://www.merriam-webster.com/dictionary/lysosome>
IPA: /ˌlaɪ.səˈsoʊ.məl/
Phonetic Spelling: lye·suh·soh·muhl
31. Clodronate
Pronunciation link: <https://www.howtopronounce.com/clodronate>
IPA: /'kloʊ.drəˌneɪt/
Phonetic Spelling: kloh·druh·nayt
32. Liposomes
Pronunciation link: <https://www.merriam-webster.com/dictionary/liposome>
IPA: /'lɪp.əˌsoʊm/
Phonetic Spelling: lip·uh·sohm
33. PLX5622
Pronunciation link: No confirmed link found
IPA: /ˌpiː ɛl ˈeks faɪv sɪks tuː tuː/
Phonetic Spelling: pee·el·eks five·six·two·two