the **Greene** Laboratory
at Auburn University

AN IMPROVED IN VITRO 3T3-L1 ADIPOCYTE MODEL OF INFLAMMATION AND INSULIN RESISTANCE

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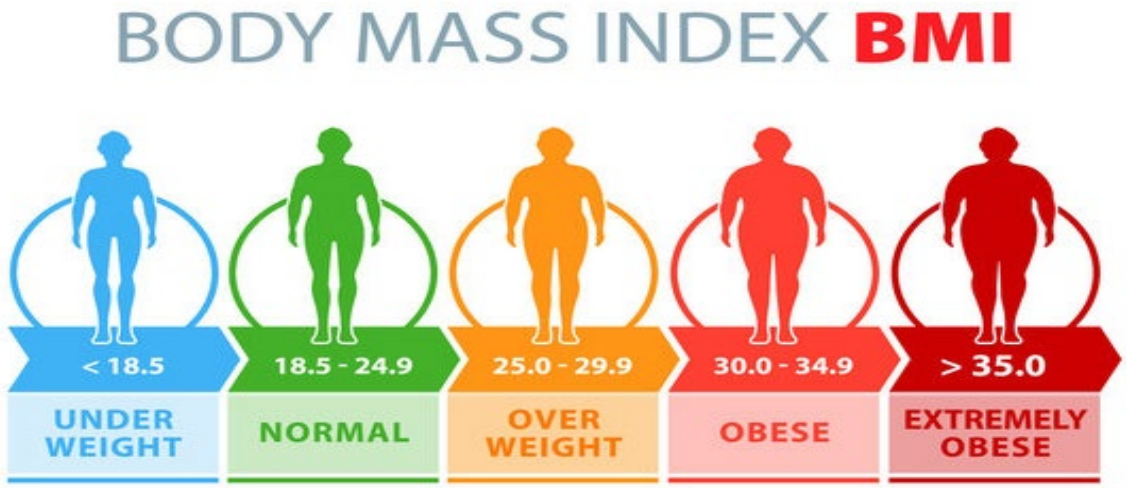
OUTLINE

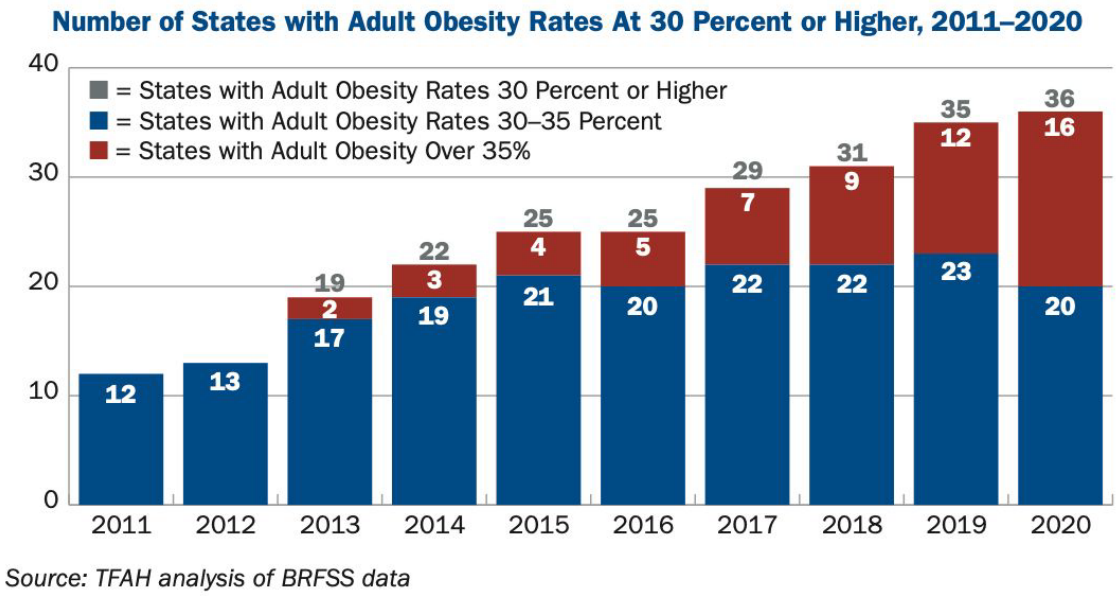
- State the public health problem
- Background
- Existing models and limitations
- Our newly developed model
- Future directions

THE PUBLIC HEALTH CONCERN

- Obesity is an increasing, global public health issue and over one third of U.S. adults have obesity.[1]
- Condition characterized by excess adipose tissue.[1]



BMI is a quantitative measure of obesity[3]

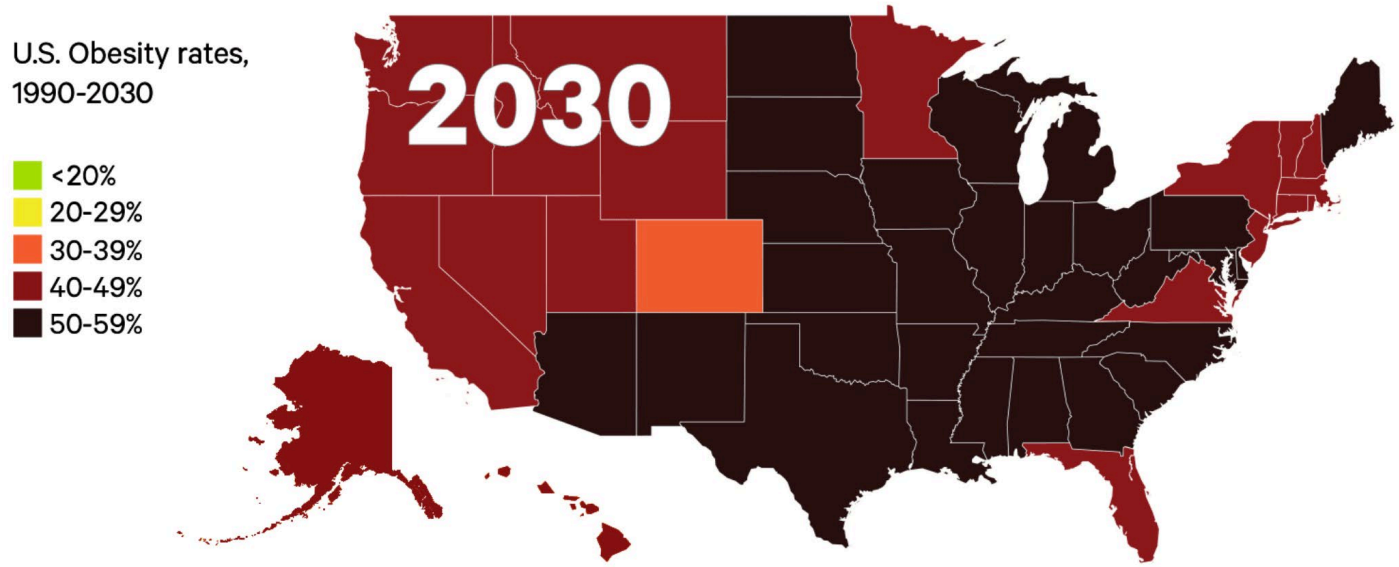


Source: TFAH analysis of BRFSS data

1. Fruh SM. Obesity: Risk faactors, complications, and strategies for sustainable long-term weight management. *J Am Assoc Nurse Pract*. 2017;29(S1):S3-S14. doi:10.1002/2327-6924.12510
2. https://www.cdc.gov/obesity/data/prevalence-maps.html
3. https://www.scientificworldinfo.com/2020/08/what-is-body-mass-index-bmi.html

THE PUBLIC HEALTH CONCERN

- Nearly half of Americans will be obese by 2030

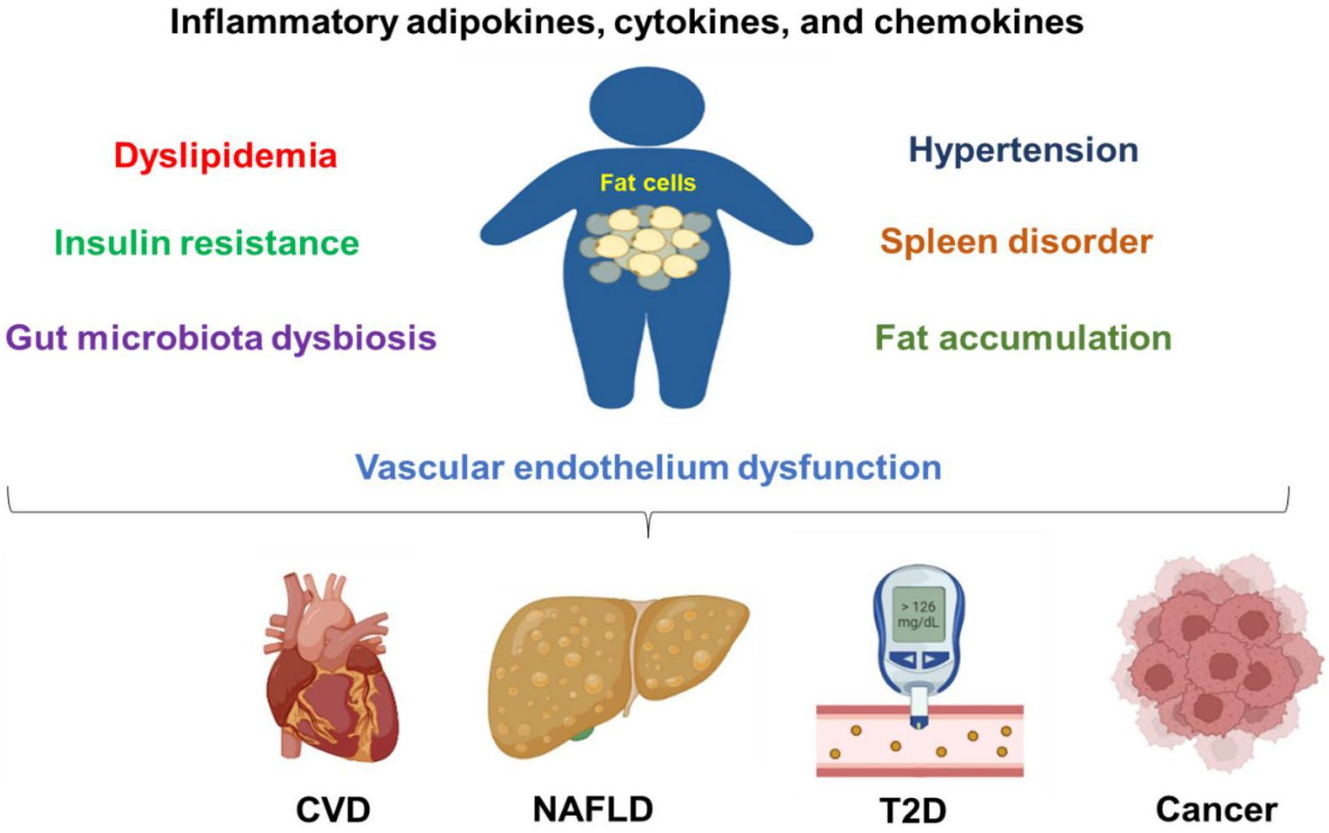


CHOICES | Childhood Obesity Intervention Cost-Effectiveness Study

Ward ZJ, Bleich SN, Cradock AL, Barrett JL, Giles CM, Flax CN, Long MW, Gortmaker SL. Projected U.S. State-Level Prevalence of Adult Obesity and Severe Obesity. N Engl J Med. 2019;381:2440-50. doi: 10.1056/NEJMsa1909301

THE PUBLIC HEALTH CONCERN

- Obesity contributes to chronic disease development and progression.[1]
- Obesity also contributes to other diseases, such as COVID-19 infection.[1]

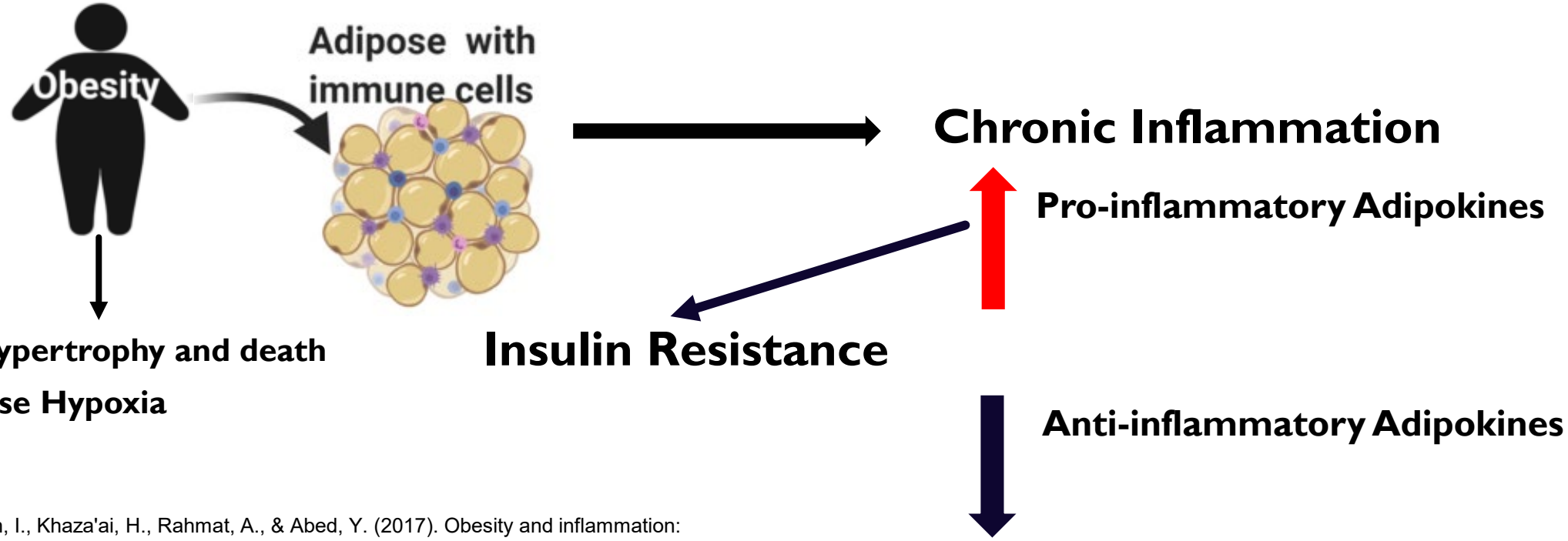


Co-Morbidities Associated with Obesity[1]

1. Yang, M.; Liu, S.; Zhang, C. The Related Metabolic Diseases and Treatments of Obesity. *Healthcare* **2022**, *10*, 1616

OBESITY-INFLAMMATION-INSULIN RESISTANCE LINK

- Obesity is associated with chronic inflammation in obese subjects.[1]
- The excess of macronutrients in the adipose tissues stimulates them to release inflammatory mediators.[1]



1. Ellulu, M. S., Patimah, I., Khaza'ai, H., Rahmat, A., & Abed, Y. (2017). Obesity and inflammation: the linking mechanism and the complications. *Archives of medical science : AMS*, *13*(4), 851–863.

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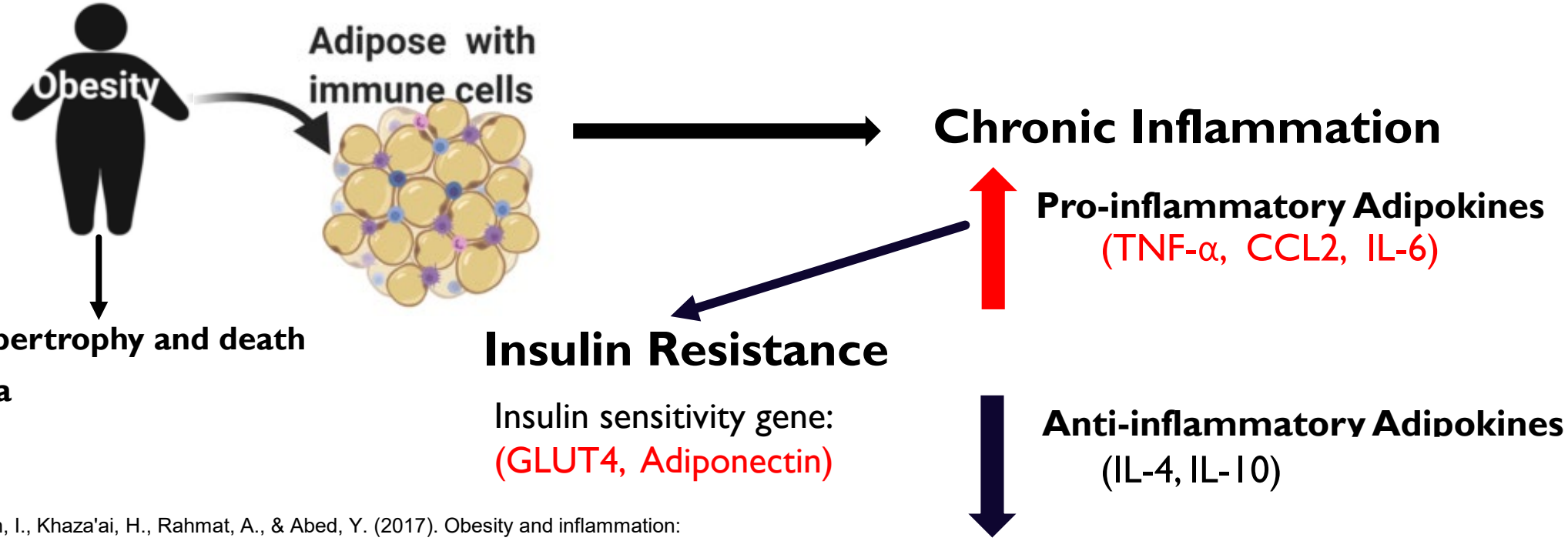

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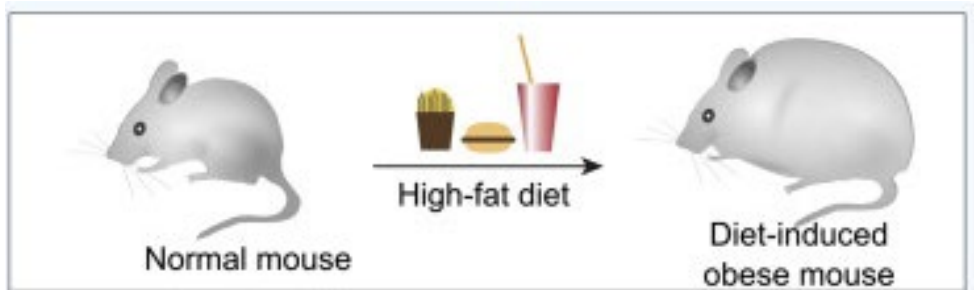
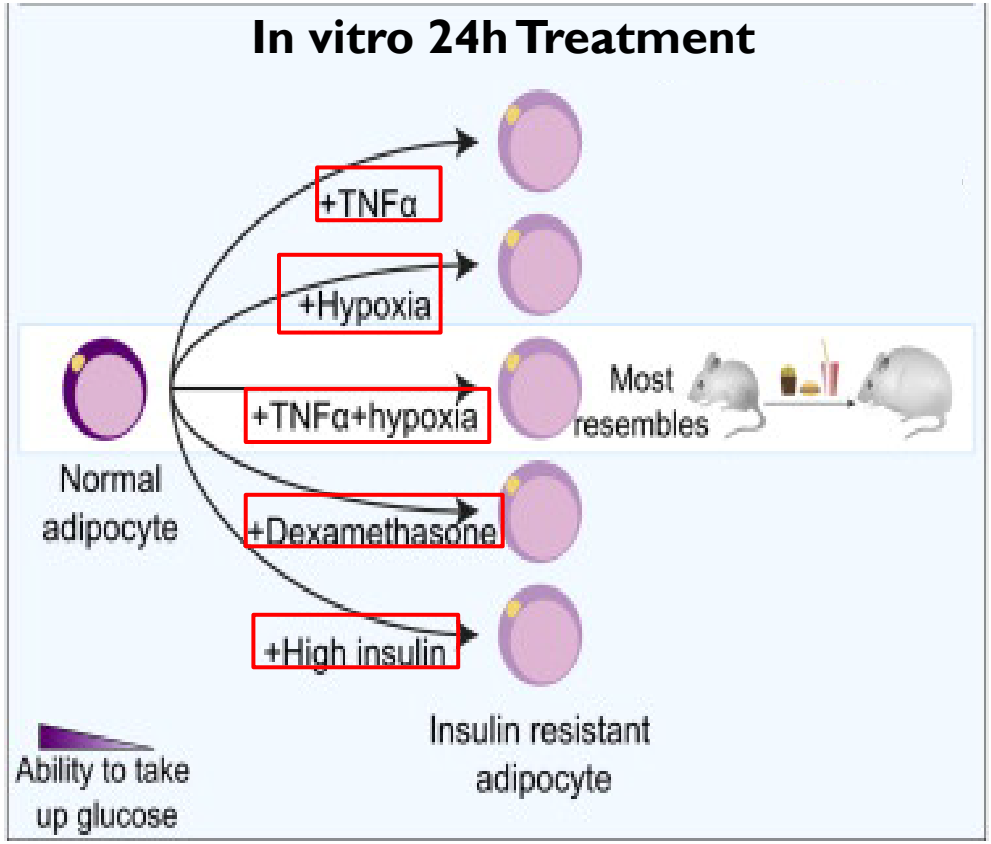
EXISTING OBESITY MODELS AND LIMITATIONS



Limitations:

- More difficult to manipulate
- Expensive (e.g knocking down/out of genes)



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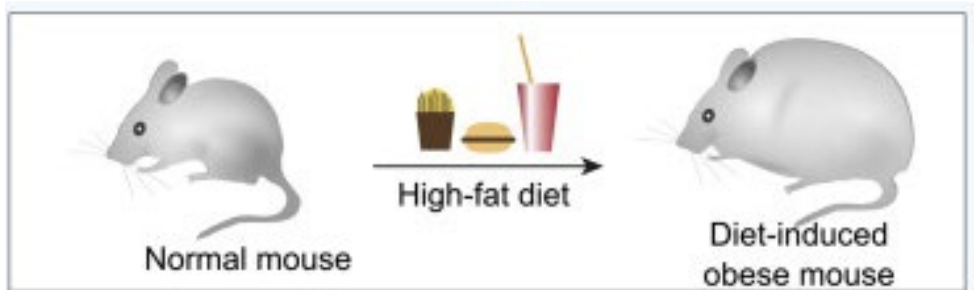

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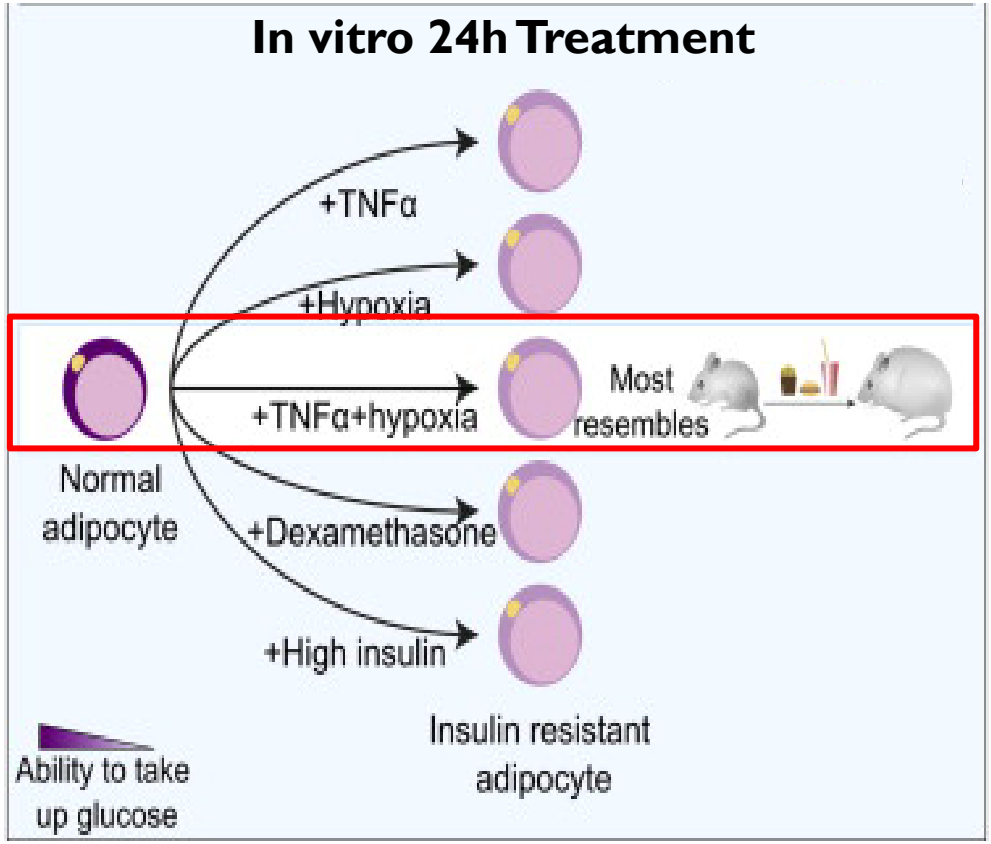

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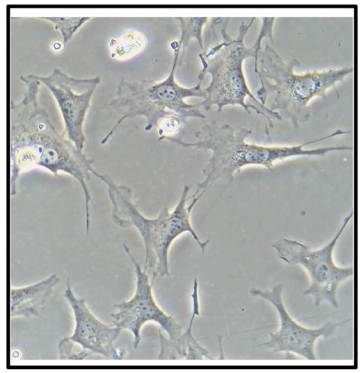
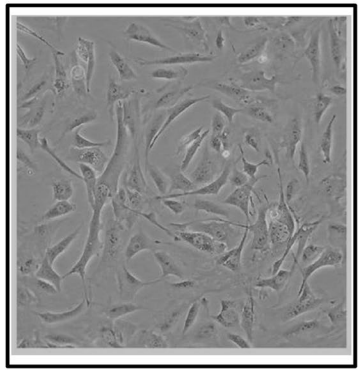
IN-VITRO ADIPOCYTE MODEL

- **3T3-L1** is a murine cell line
- 3T3-L1 cells have a fibroblast-like morphology[2]





1. Green H, Kehinde O (1975). "An established preadipose cell line and its differentiation in culture. II. Factors affecting the adipose conversion". *Cell*. **5** (1): 19–27.
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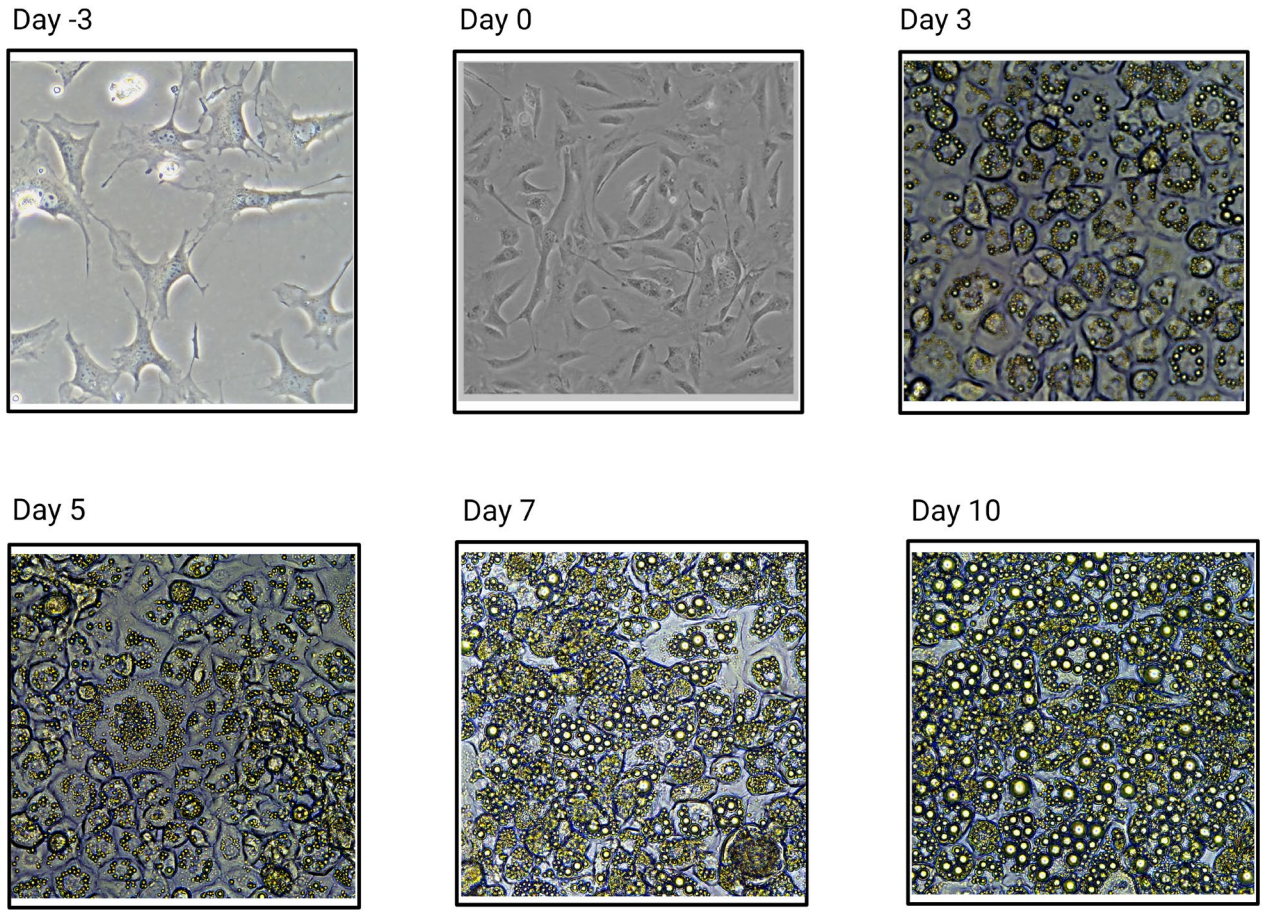
3T3-L1 CELLS

- **3T3-L1** is a murine cell line
- 3T3-L1 cells have a fibroblast-like morphology[2]
- The cells can be differentiated into an adipocyte-like phenotype[1]



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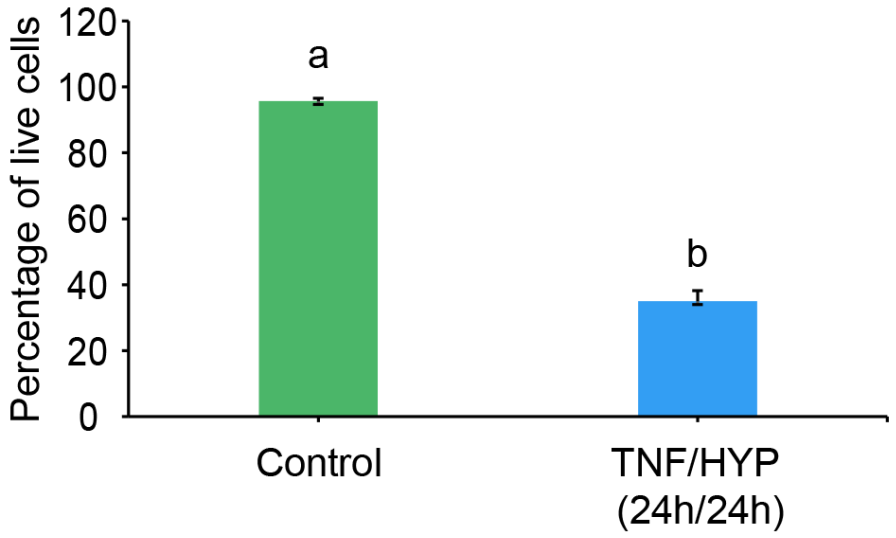
RATIONALE/PROBLEM

- We want to use this inflammatory, insulin resistance model of adipocytes in a co culture experiment to examine cancer cell growth. We hypothesized that secreted factors from the adipocytes are driving the cancer cell growth.

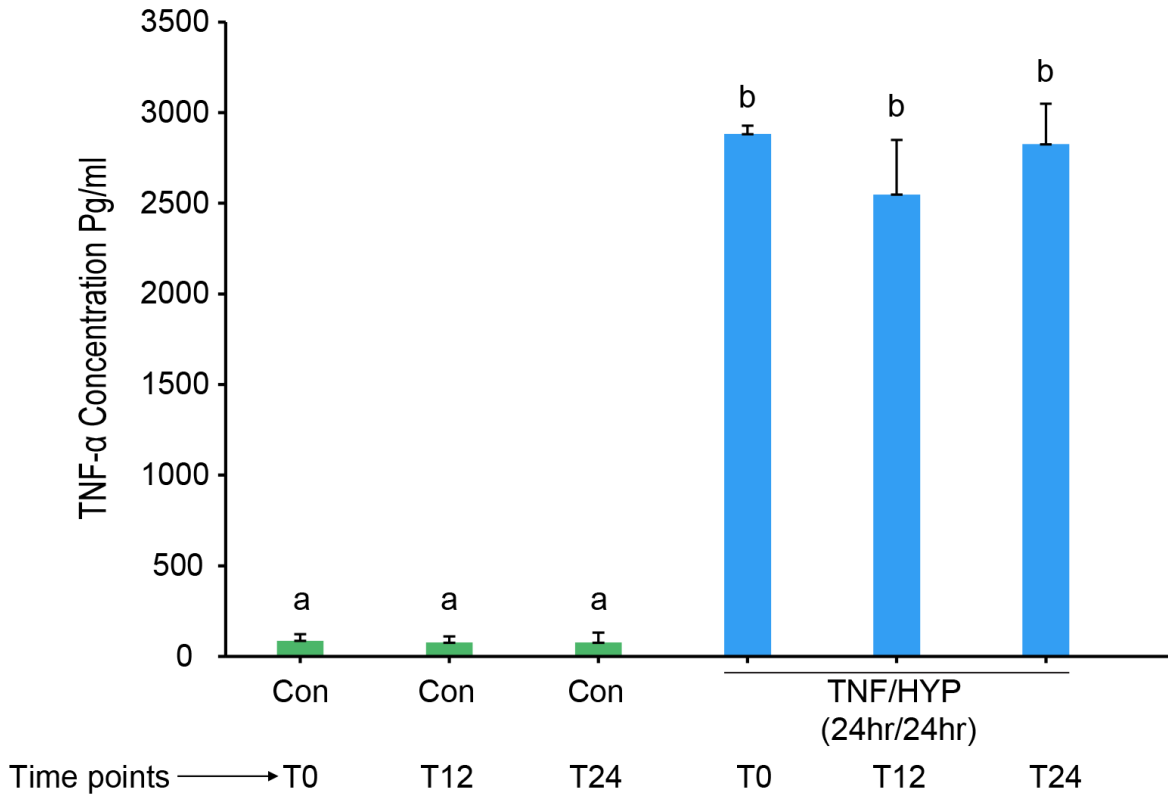
- TNF-α/Hypoxia are known to induce cell death.

- In addition, it's unclear whether the TNF-α remains in the culture media and therefore carrying over to the co-culture experiment and possibly cofounding our treatment.

TNF-α (24 HOURS) AND HYPOXIA (24 HOURS) INDUCE SIGNIFICANT CELL DEATH



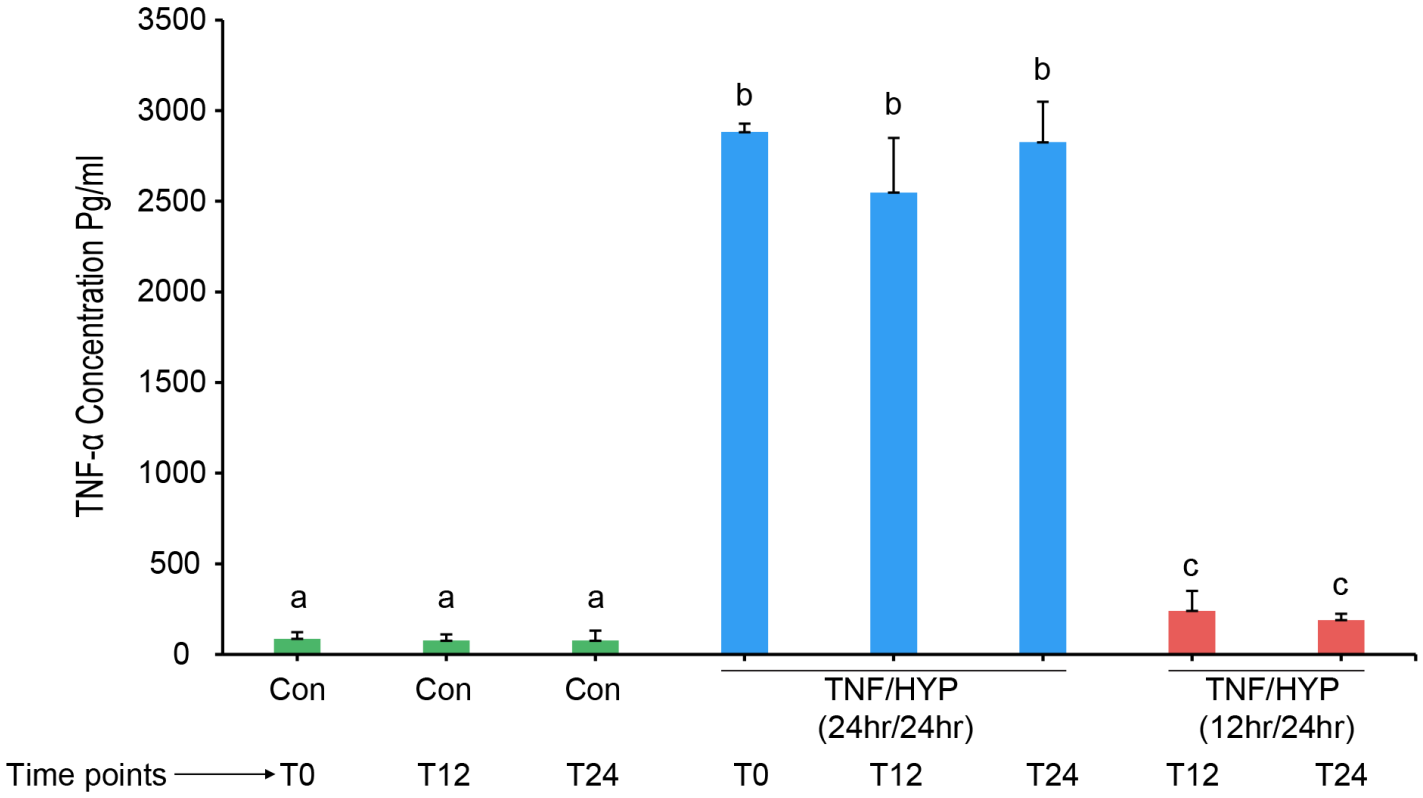
IS TNF-α MAINTAINED OVER THE COURSE OF THE 24HR TREATMENT IN THE ADIPOCYTE CULTURE MEDIA?



ANOVA Post Hoc Tukey test. Significant difference ($p<0.05$) in groups with different letters. (n=3)

Manuscript in preparation

TNF-α MAINTAINED OVER THE COURSE OF THE 24HR TREATMENT IN THE ADIPOCYTE CULTURE MEDIA



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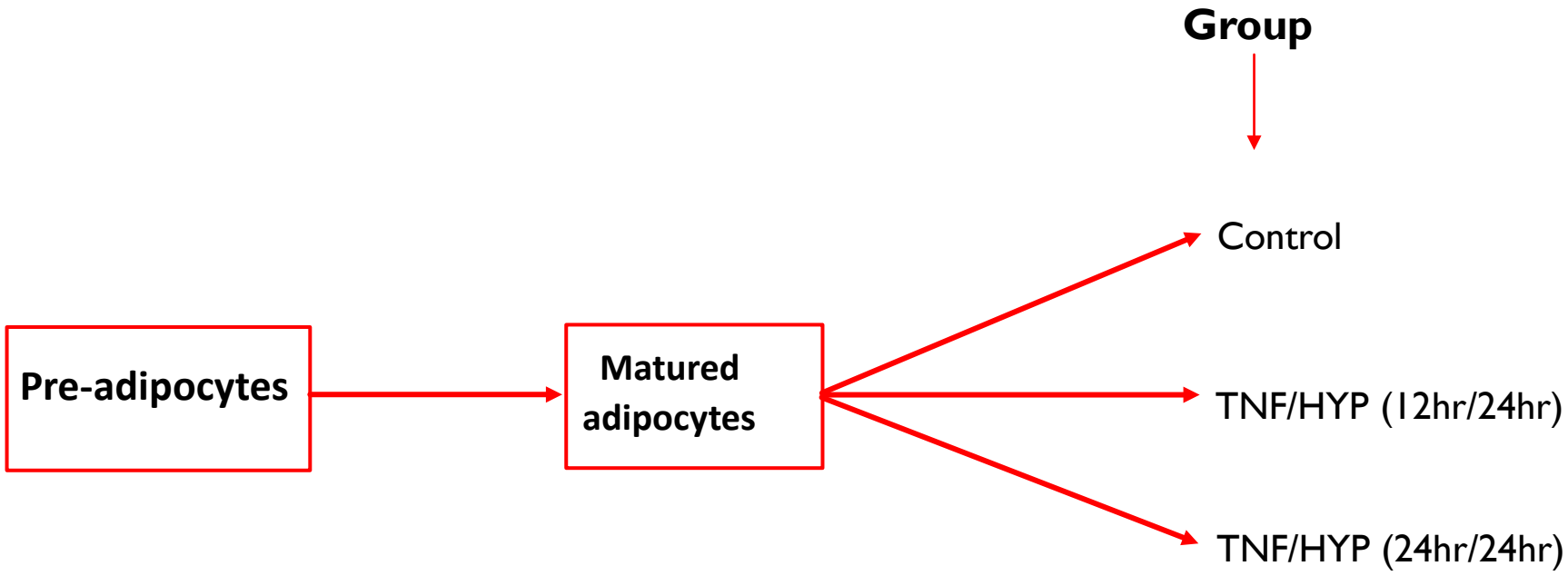
Manuscript in preparation

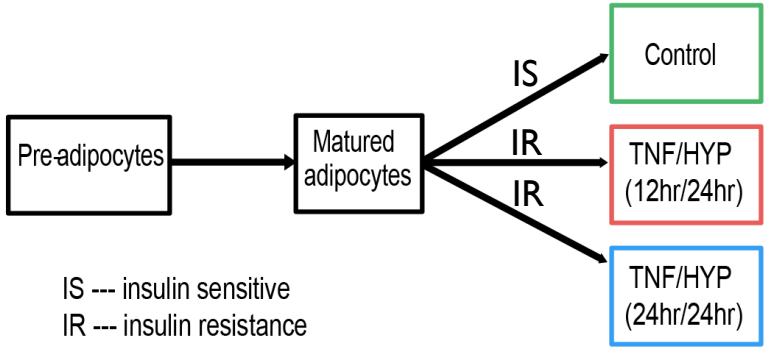
PROJECT QUESTION 2

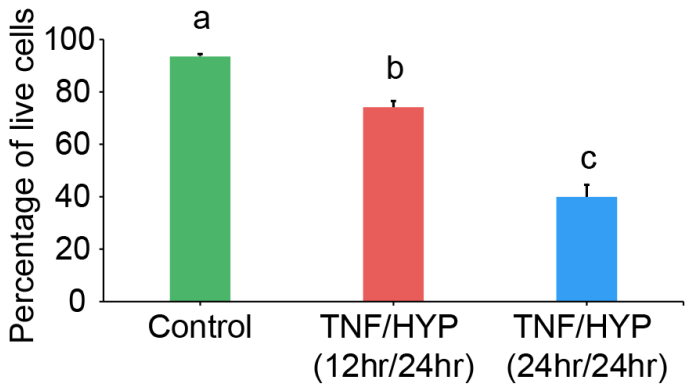
- Because the TNF-α is maintained in the treatment media, we questioned whether changing the media to remove the TNF-α can improve the cell viability in 3T3-L1 adipocytes?

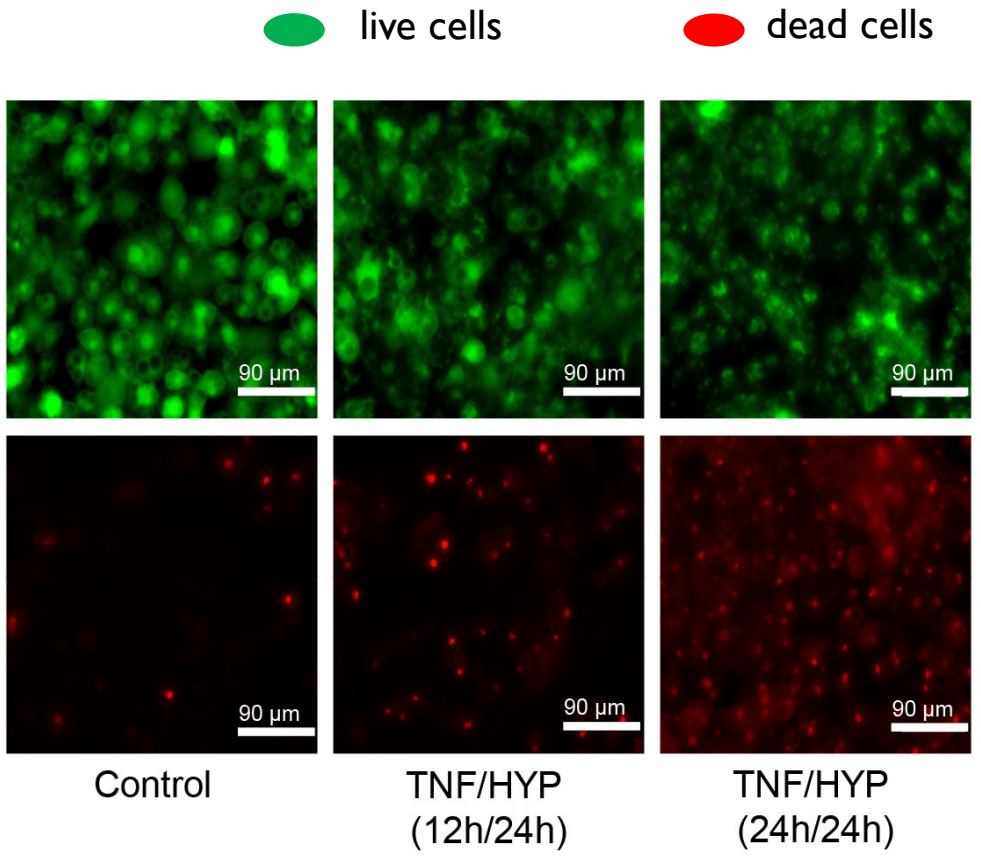
PROJECT FLOW



ALTERING TNF- α TREATMENT TIME IMPROVES CELL VIABILITY IN THE ADIPOCYTE INSULIN RESISTANCE MODEL







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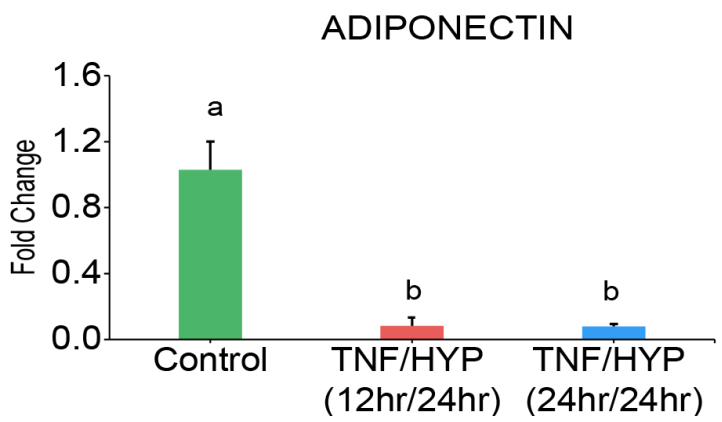
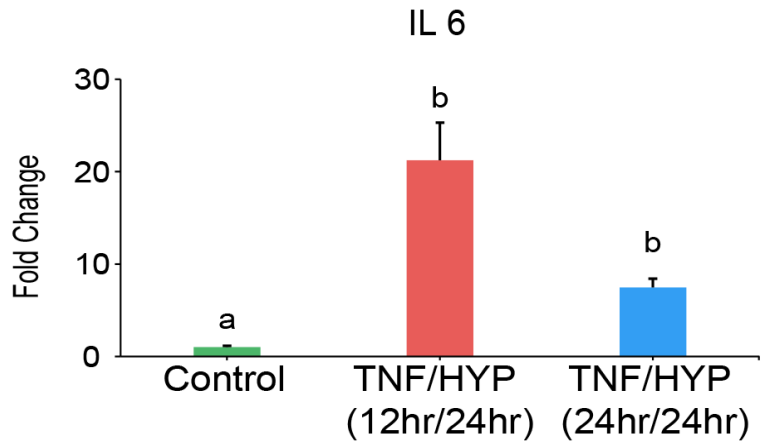
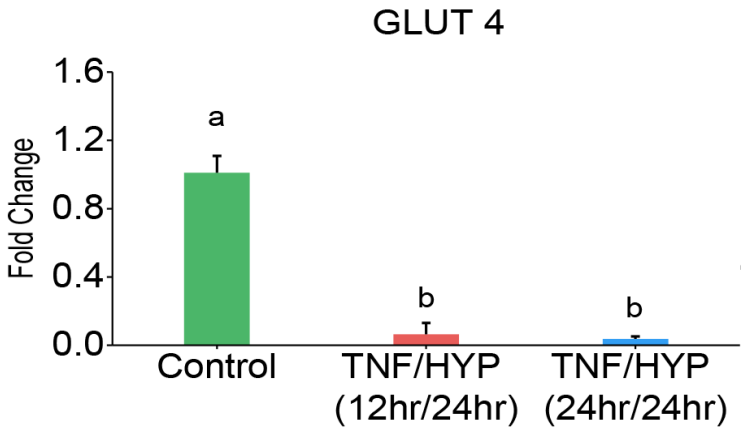
PROJECT QUESTION 3

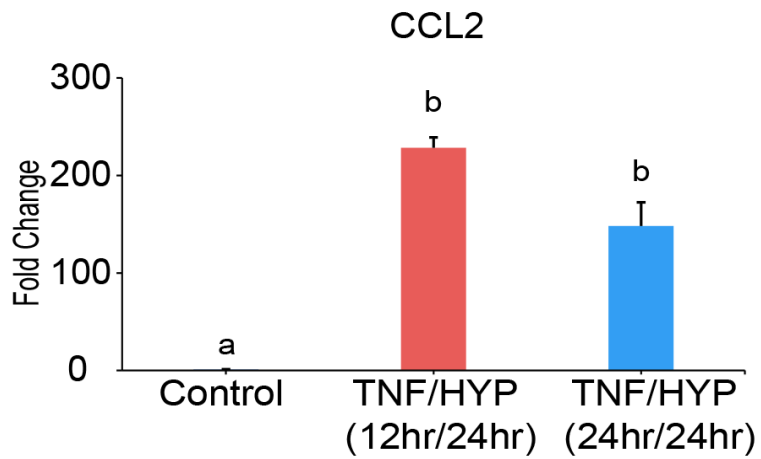
- Does the12h TNF-α treatment with hypoxia still maintains insulin resistant and inflammation?

12H TNF-α TREATMENT WITH HYPOXIA MAINTAINS DOWNREGULATION OF INSULIN SENSITIVE MARKERS AND UPREGULATE INFLAMMATORY MARKERS





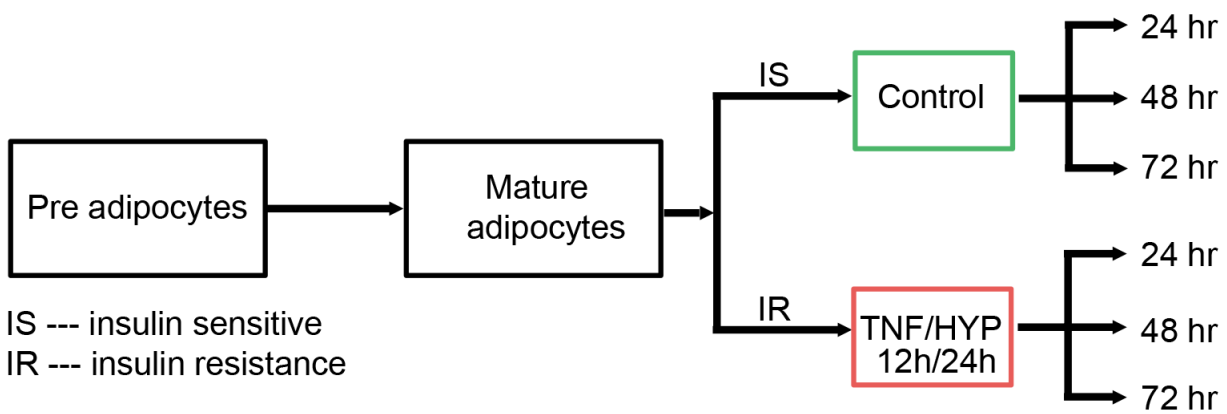


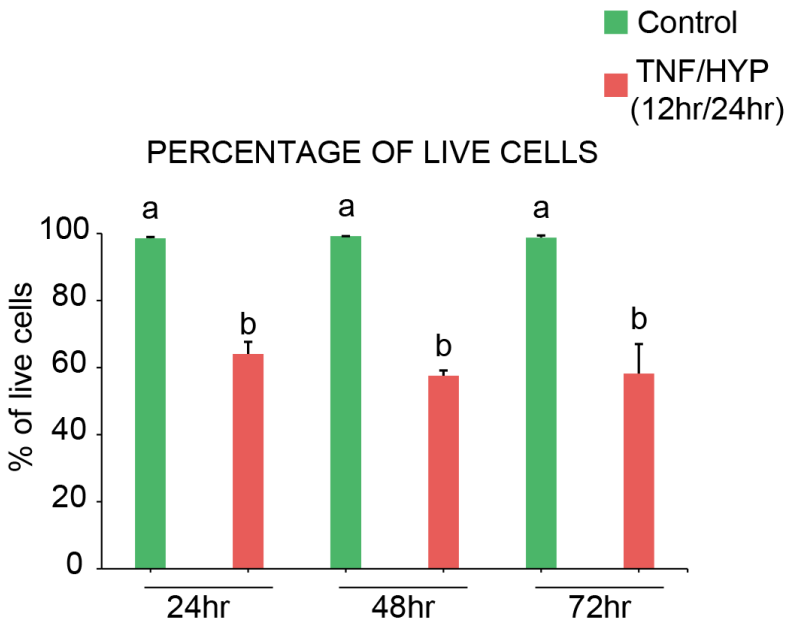


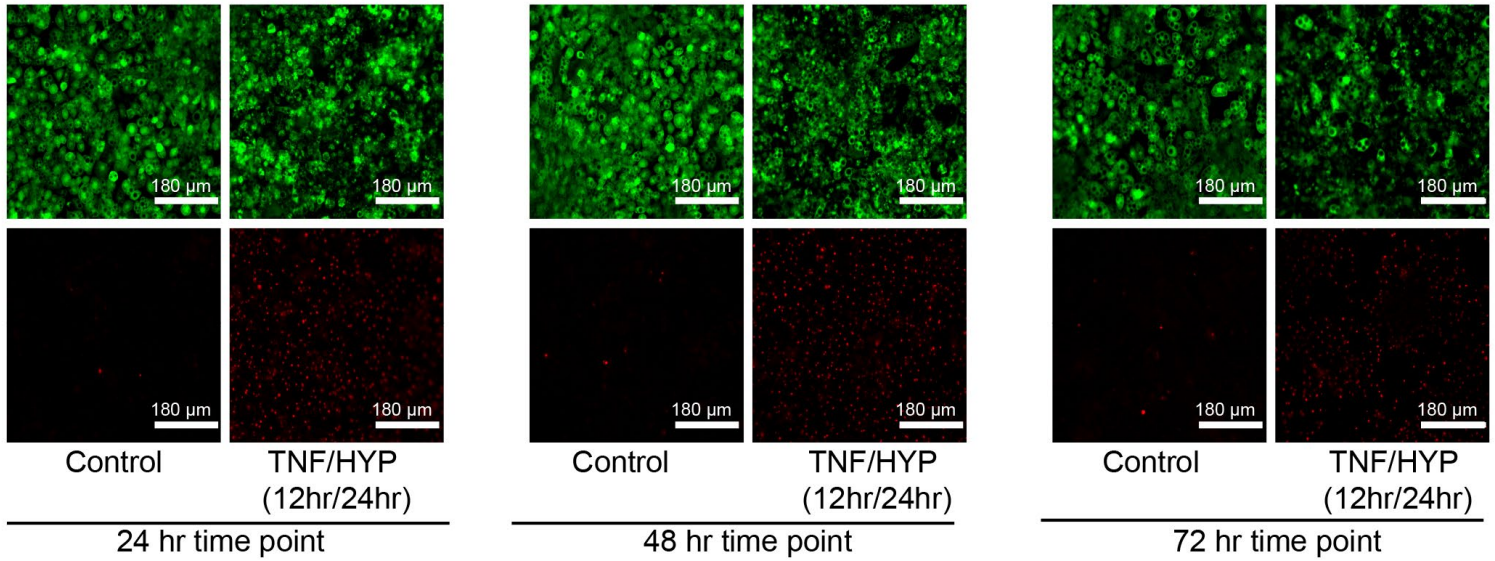
PROJECT QUESTION 4

- We next question how long the cells maintain their viability over time

TNF-α (12 HOURS) AND HYPOXIA (24 HOURS) INDUCE LONG-TERM INSULIN RESISTANCE IN VITRO ADIPOCYTE INSULIN RESISTANCE MODEL







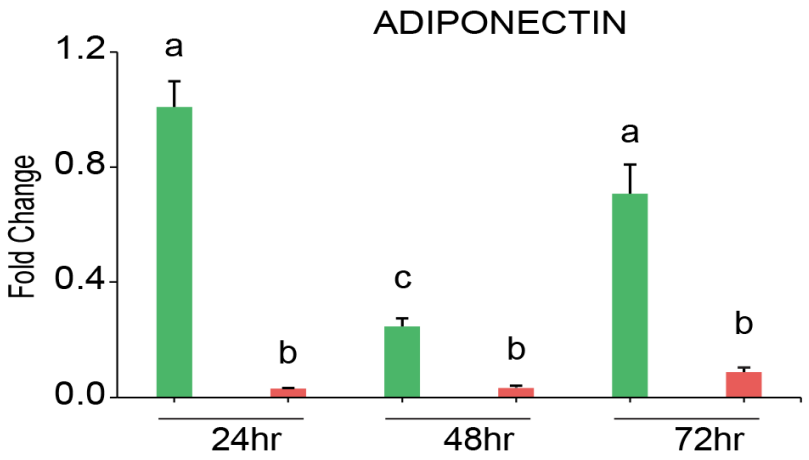
live cells

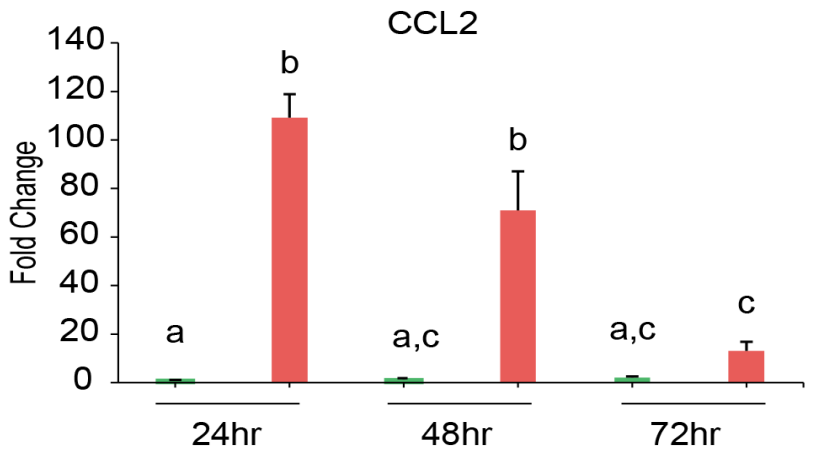
dead cells

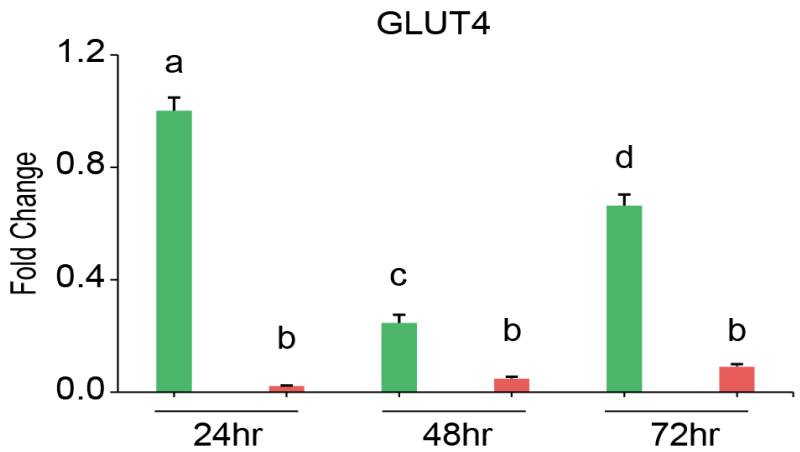
PROJECT QUESTION

- We next question how long the cells maintain their insulin resistance and inflammation state

TNF-α (12 HOURS) AND HYPOXIA (24 HOURS) INDUCE LONG-TERM INSULIN RESISTANCE IN VITRO ADIPOCYTE INSULIN RESISTANCE MODEL.



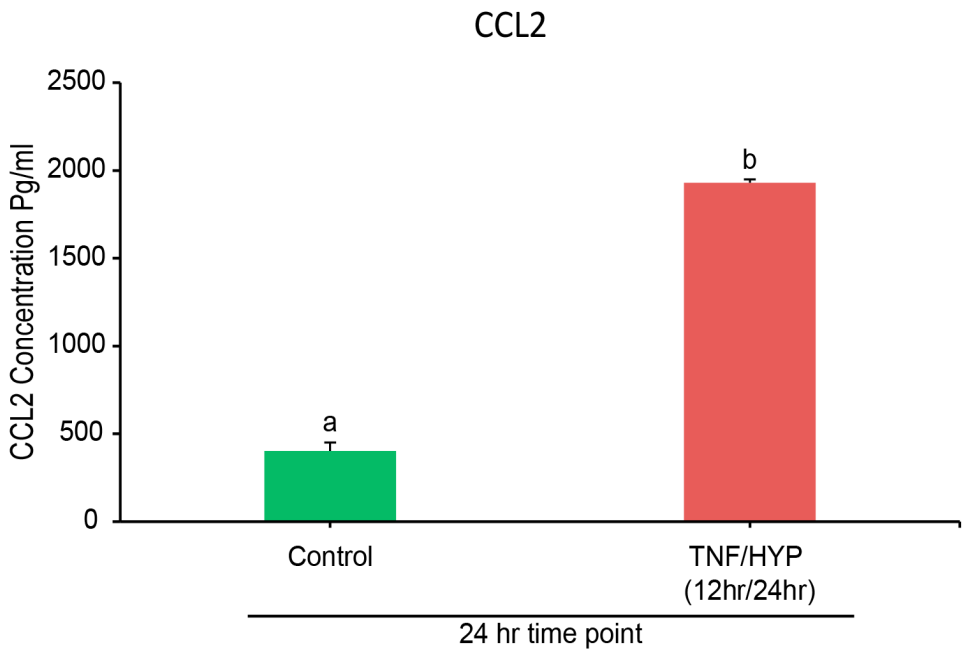


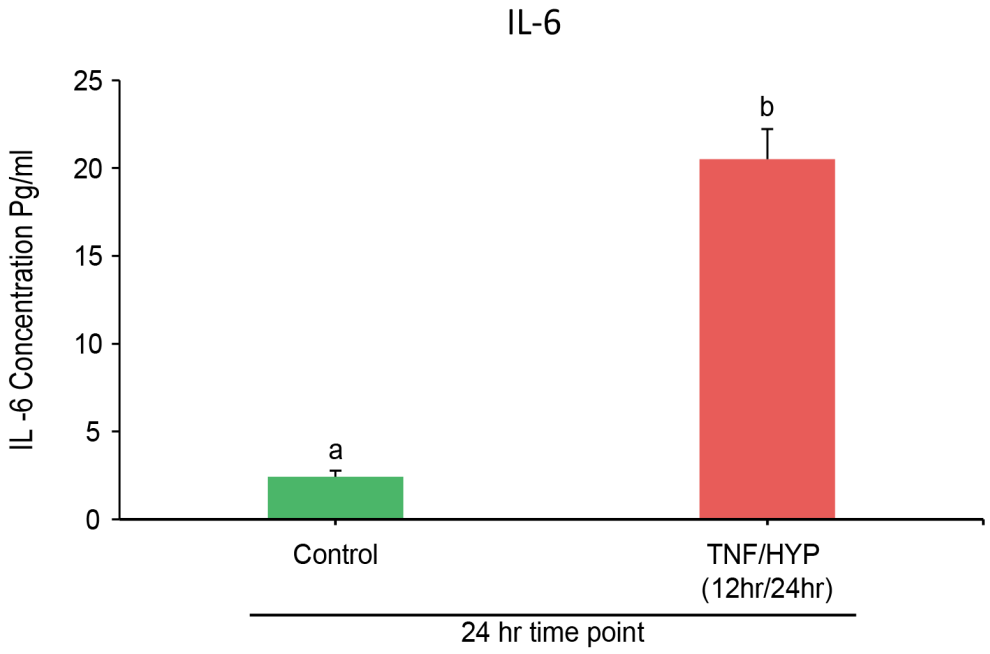


PROJECT QUESTION 5

- Next, we verify the cells were secreting inflammatory markers

CONDITION MEDIA FROM INFLAMED AND IR 3T3-L1 CELLS SECRETE MARKERS OF INFLAMMATION





SUMMARY

- By modulating the treatment time with TNF-α in the presence of hypoxia, we have solved the problem of low viability in the adipocyte insulin resistance model and importantly, the cells remain inflammatory

- We have refined the in vitro model of insulin resistance and inflammation in 3T3-L1 cells.

ACKNOWLEDGMENTS

Auburn University, College of Human Sciences

Department of Nutritional Sciences

- **Michael Greene**, PhD*
- Ann Marie O'Neill, PhD
- Bulbul Ahmed, PhD
- Hadeel Aldhowayan
- Serhat Yildiz
- Stanley Wijaya
- Michael Wayne

Auburn University, Samuel Ginn College of Engineering

Department of Chemical Engineering

- Elizabeth Lipke, PhD
- Iman Hassani, PhD
- Yuan Tian, PhD
- Nicole Habbit, PhD
- Grace Hester
- Peter Abraham
- Benny Solomon

University of South Alabama, School of Medicine

Mitchell Cancer Center — Marty Heslin, MD

Auburn University Research Initiative in Cancer (AURIC) Major Grant (M.W.G., E.A.L.)

National Center for Advancing Translational Research of the National Institutes of Health (NIH). UL1TR003096-01 (M.W.G., E.A.L.)

United States Department of Agriculture, National Institute of Food and Agriculture (NIFA) Hatch Grant. ALA044-1-18037 (M.W.G.)

QUESTIONS?

SUPPLEMENTARY SLIDE - OBESITY-INSULIN RESISTANCE LINK

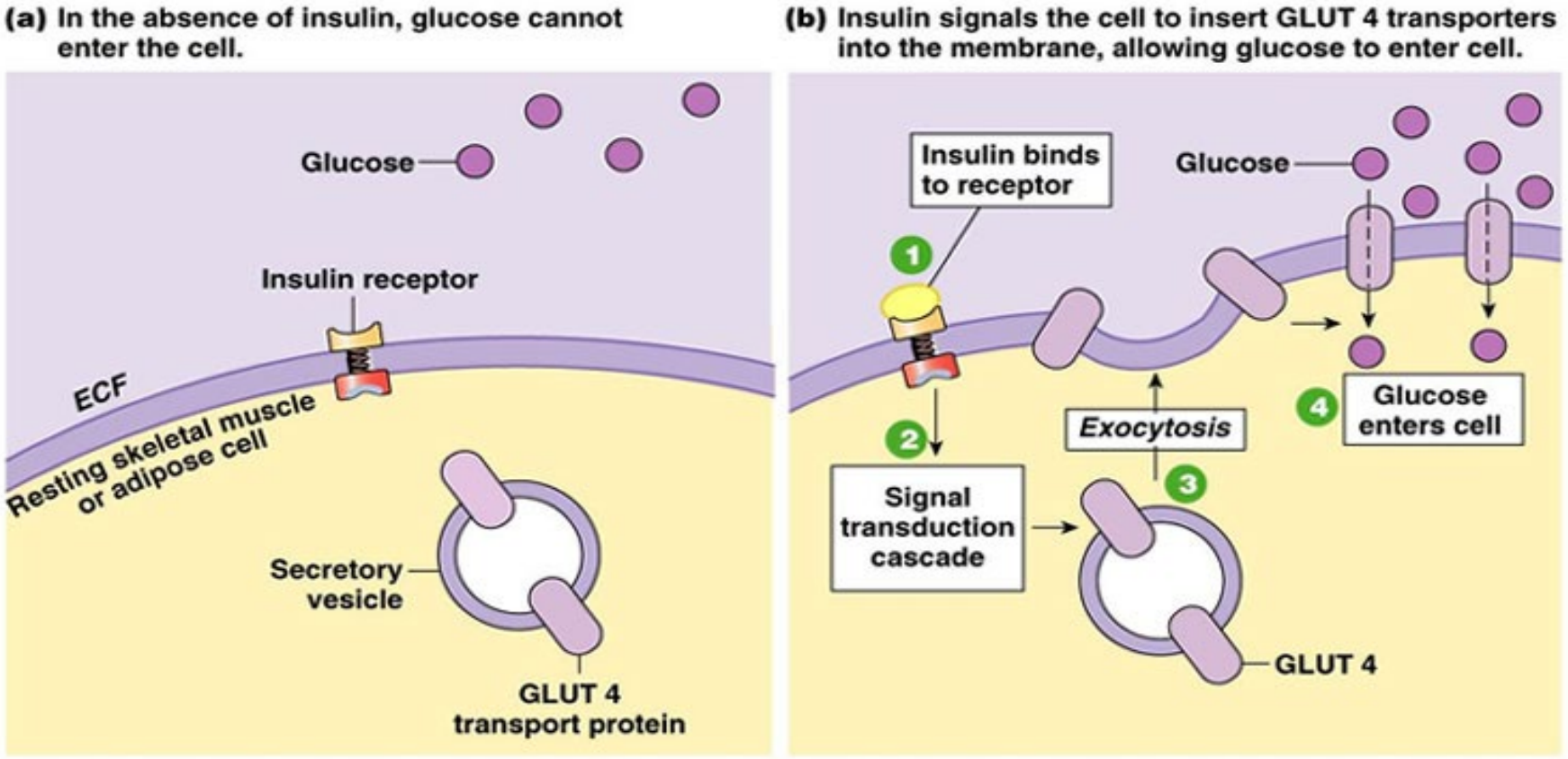
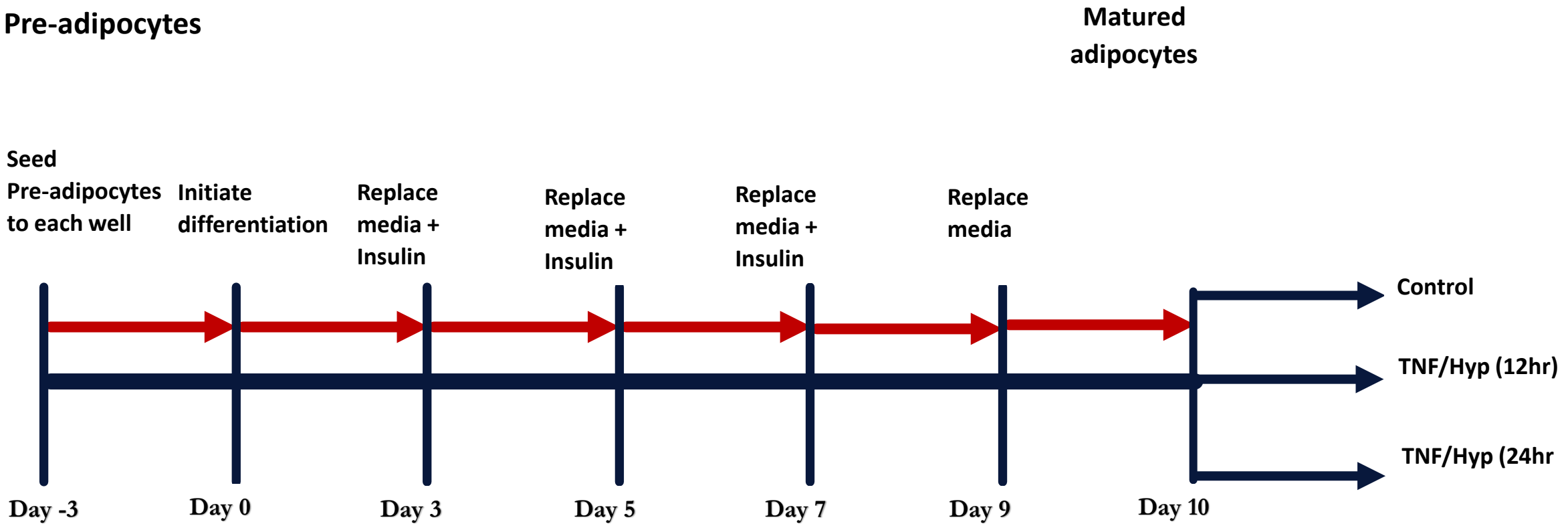


Fig. 22-12

https://fanaticcook.files.wordpress.com/2014/06/glut4.jpg

SUPPLEMENTARY SLIDE - ADIPOCYTE DIFFERENTIATION SCHEMATIC



SUPPLEMENTARY SLIDE- RT-QPCR STEP BY STEP