

Diets of **High Salt** and **High Fat**: Metabolic and Pubertal Effects in the Rat

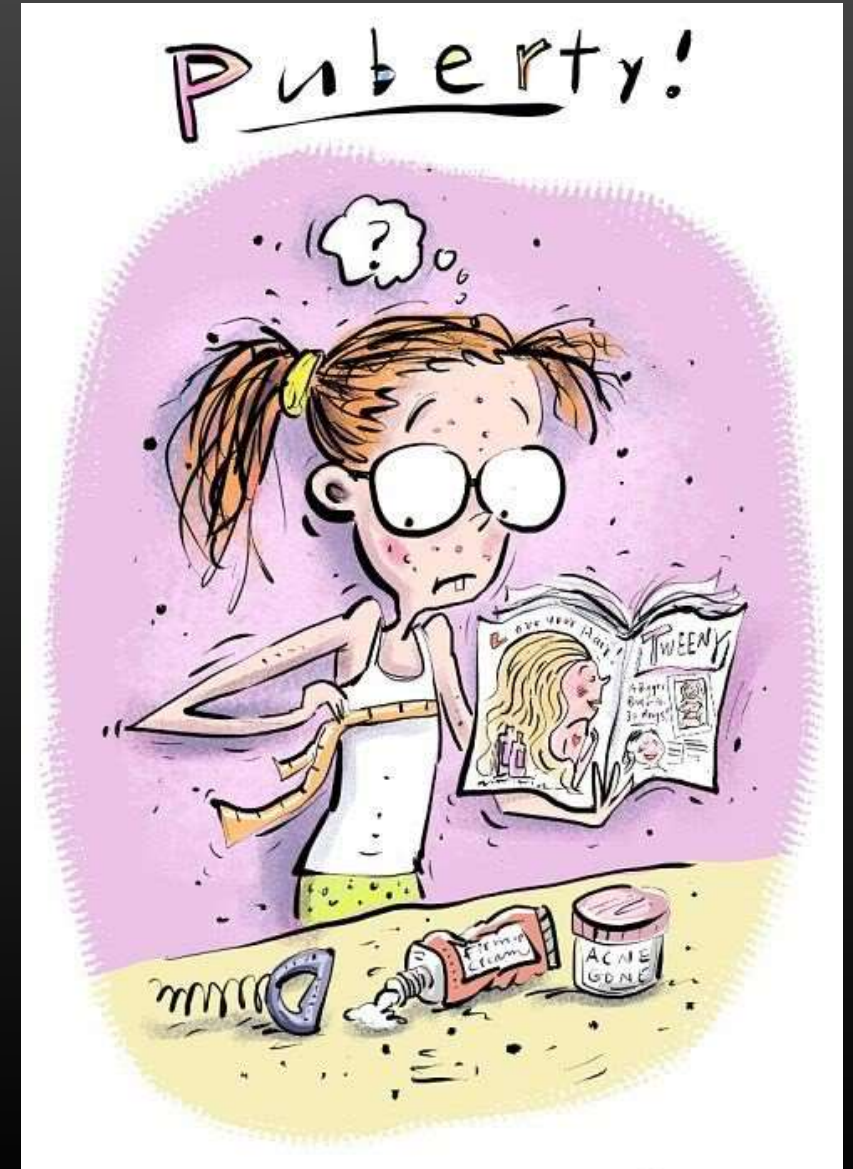
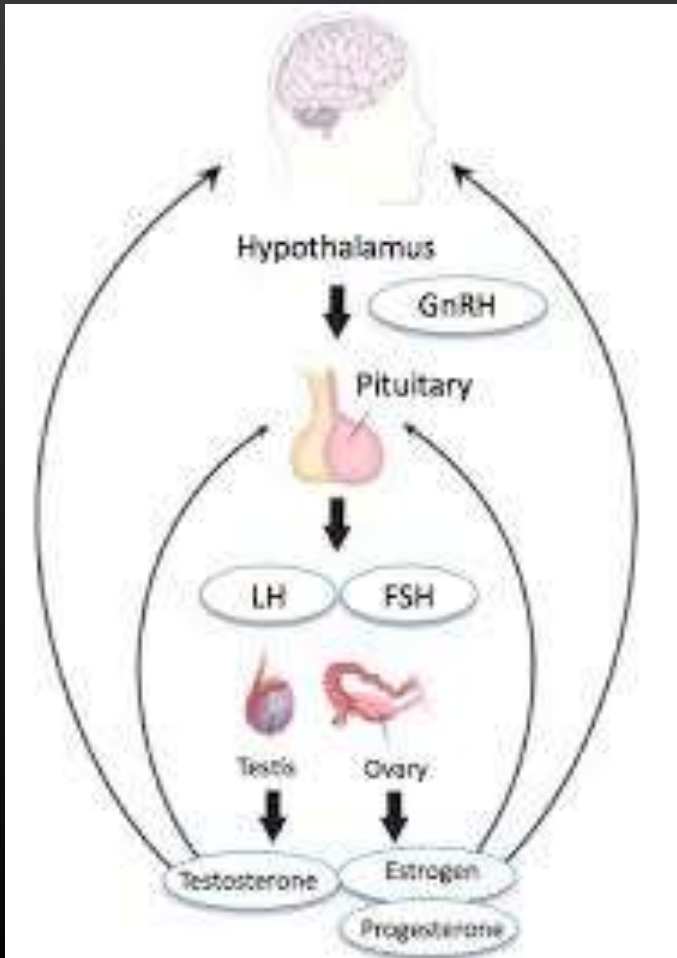
Margaret P. Schmill with Dori R. Pitynski and Dr. Donal C. Skinner

Department of Zoology and Physiology

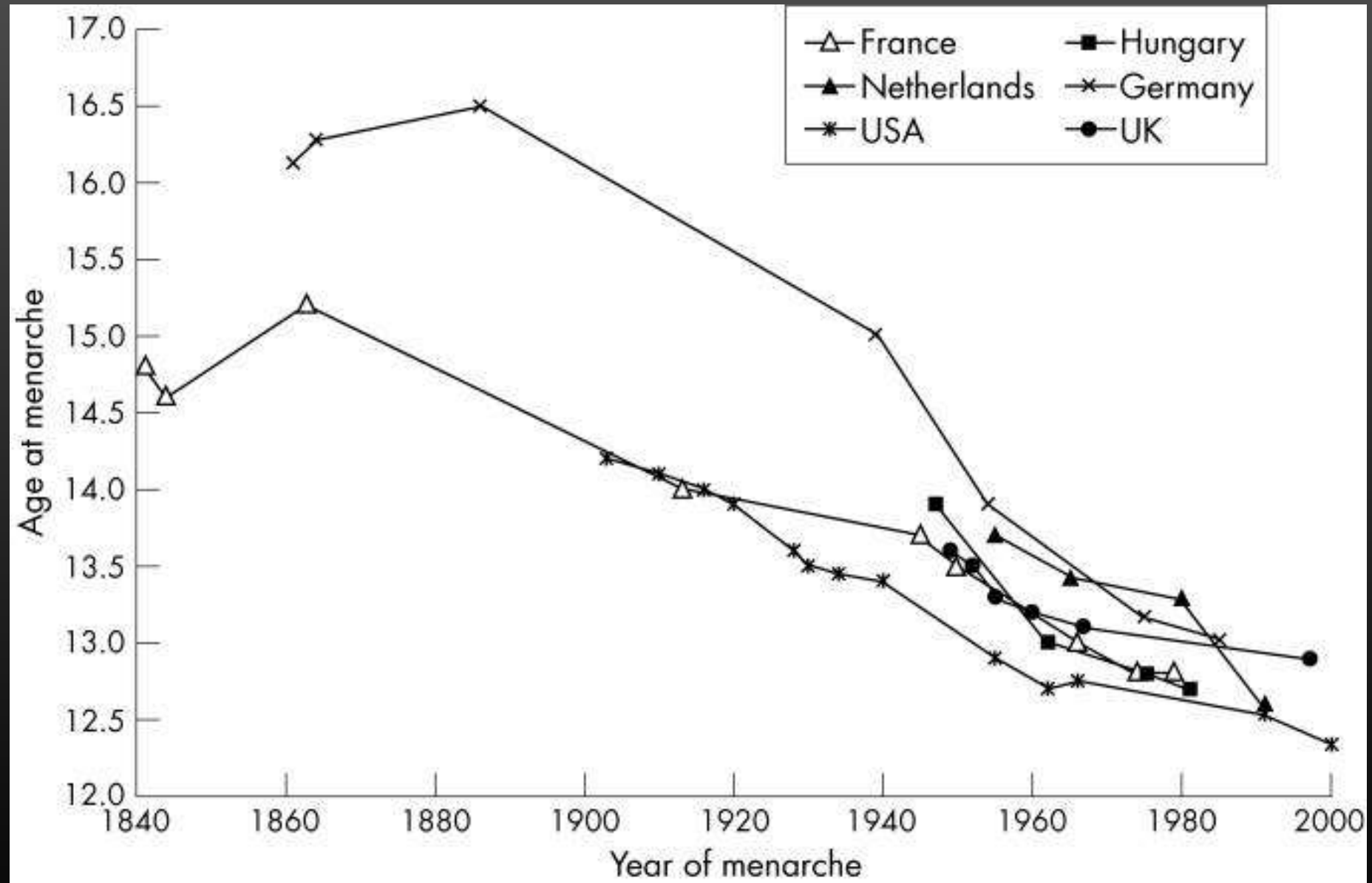
University of Wyoming

INBRE, Honors Program

An Introduction to the Skinner Lab



Advancement of Pubertal Onset



Bellis, M. A., Downing, J., & Ashton, J. R. (2006). Adults at 12? Trends in puberty and their public health consequences. *Journal of Epidemiology and Community Health*, 60(11), 910–911. <http://doi.org/10.1136/jech.2006.049379>

Skinner Lab Research

- Effects of different diets as an influence
 - Fat/Sugar/Salt
- Body Mass Index (BMI) linked, but not whole story
- Salt a contributing factor?
- To our surprise, high salt diet was shown to DELAY puberty



Overview: My Research

1



Oxygen

CO₂

2



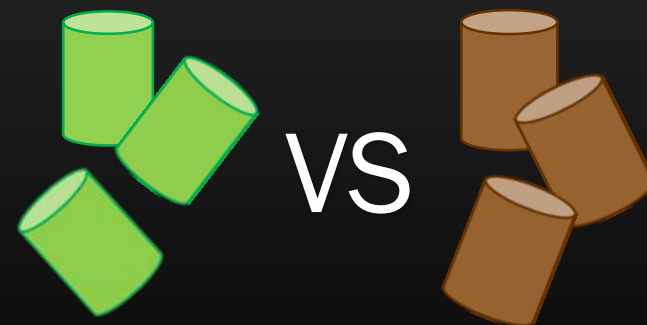
Kisspeptin



HPG
Axis

3

Control Diet
Study

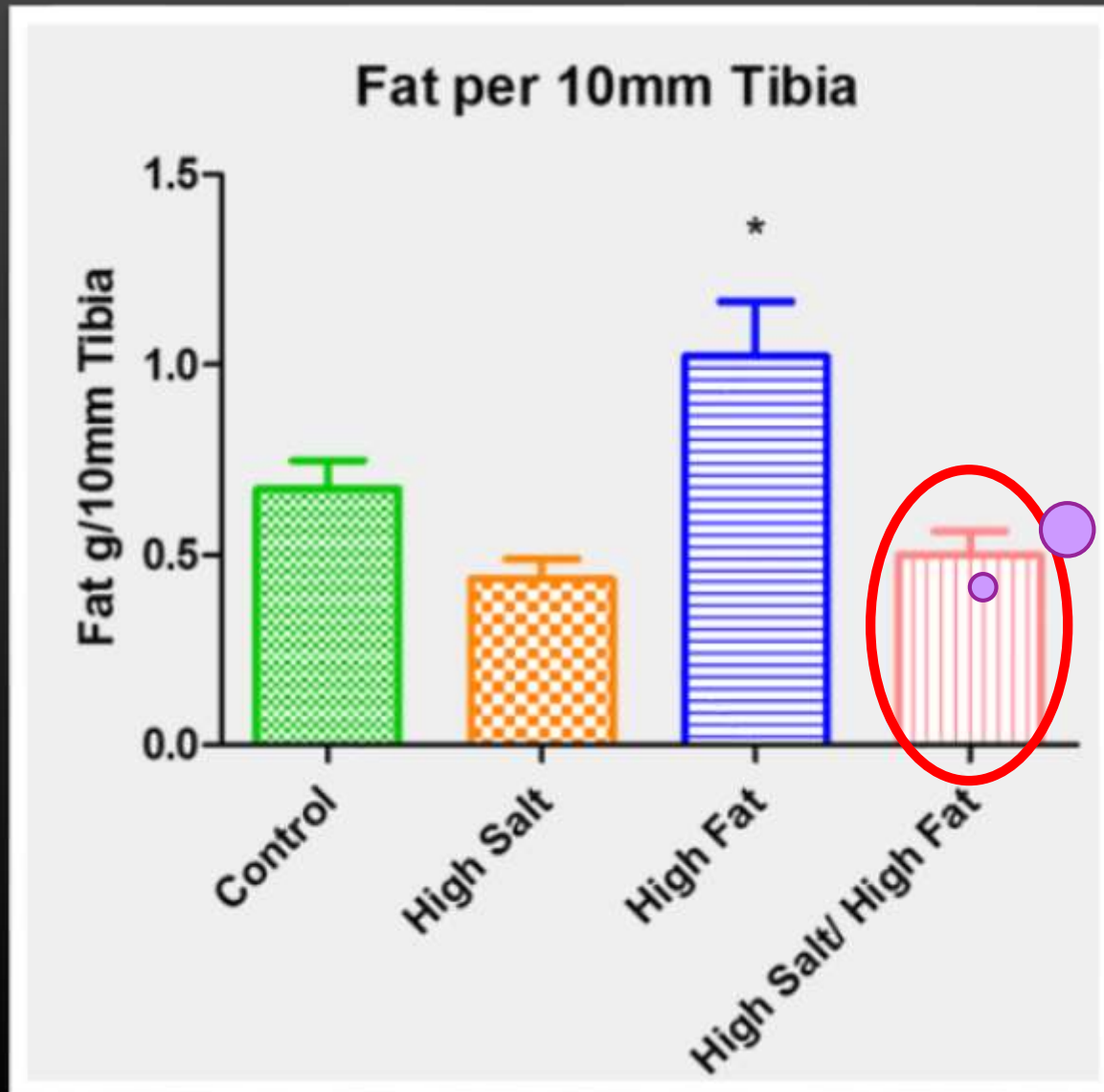


Metabolic Project – Rat Information

- Female Sprague-Dawley rats 21-45 days of age
- Fed 4 different diets:
 - Control
 - High Salt (8%)
 - High Fat (60%)
 - High Fat (60%) and High Salt (8%)
- Food and water intake measured daily



Metabolic Project - Background

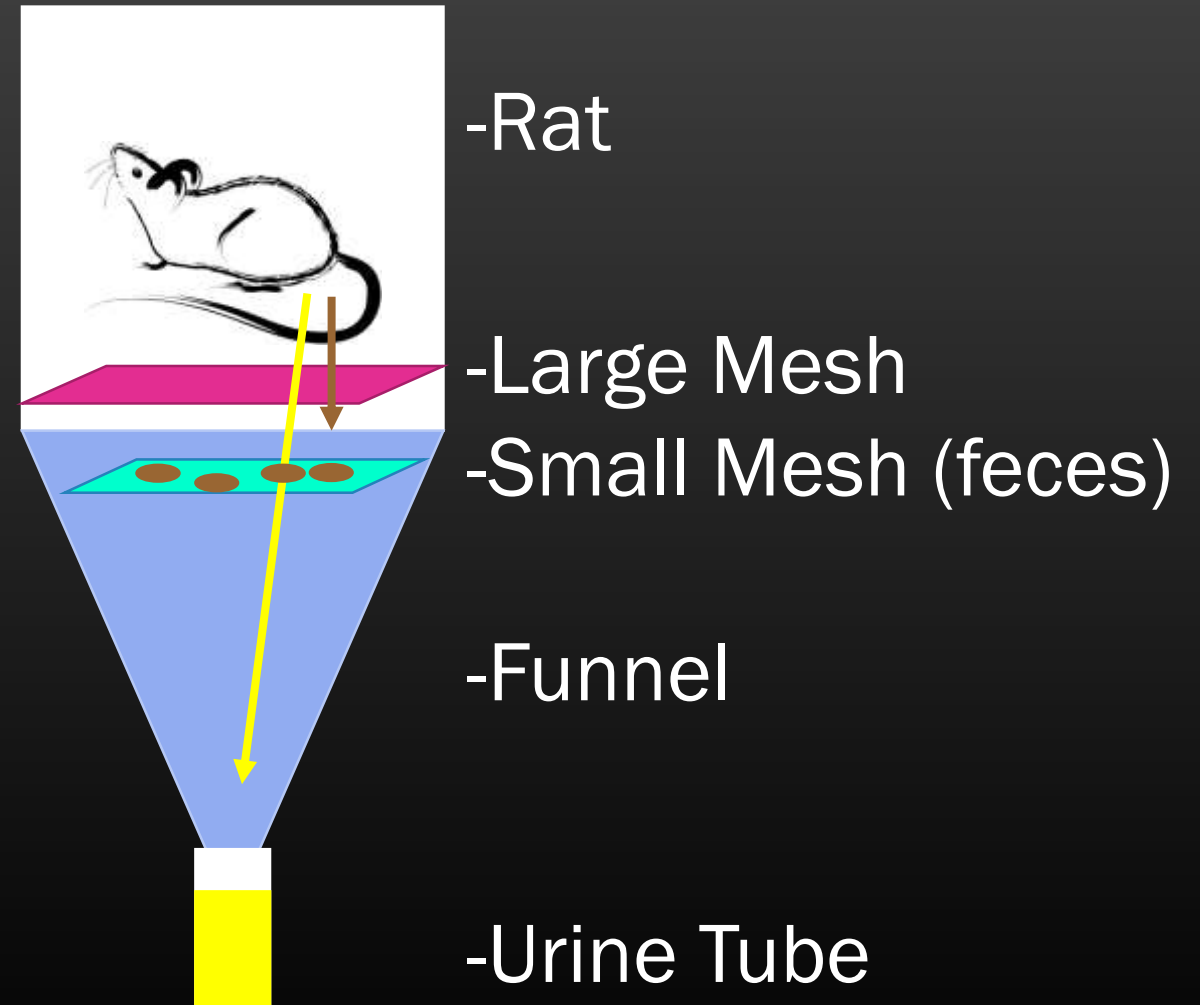


WHY?

Metabolic Project Design

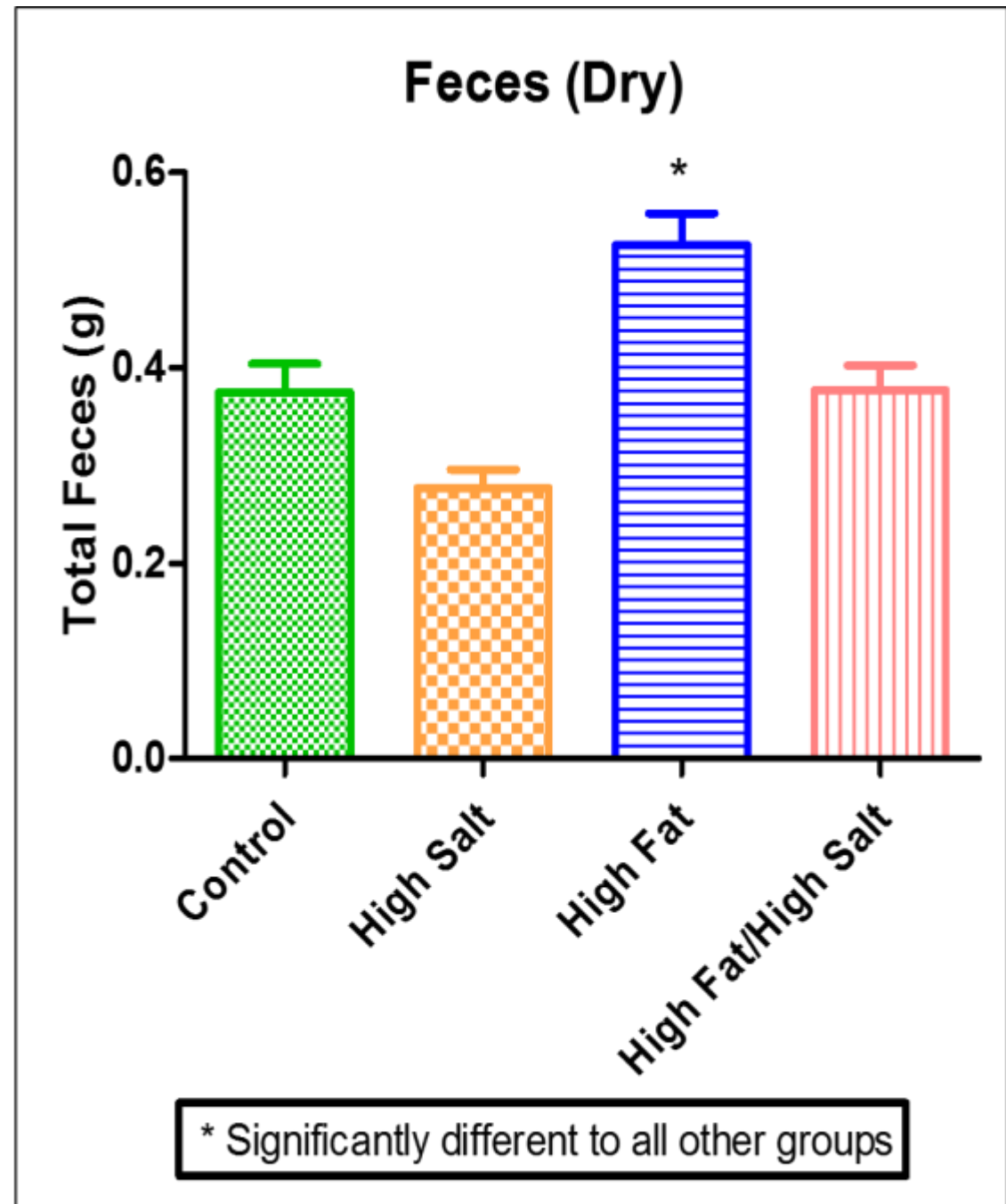
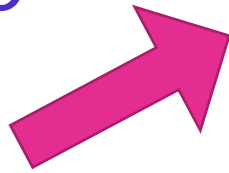
- Metabolic cages

- * Fecal collection



Metabolic Project Feces Data

What did we do
with all this
poop?!



Metabolic Project – Fecal Lipid Extraction

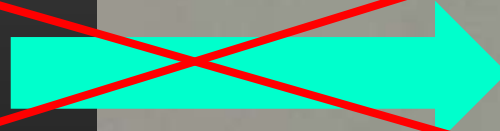
➤ Freeze dry



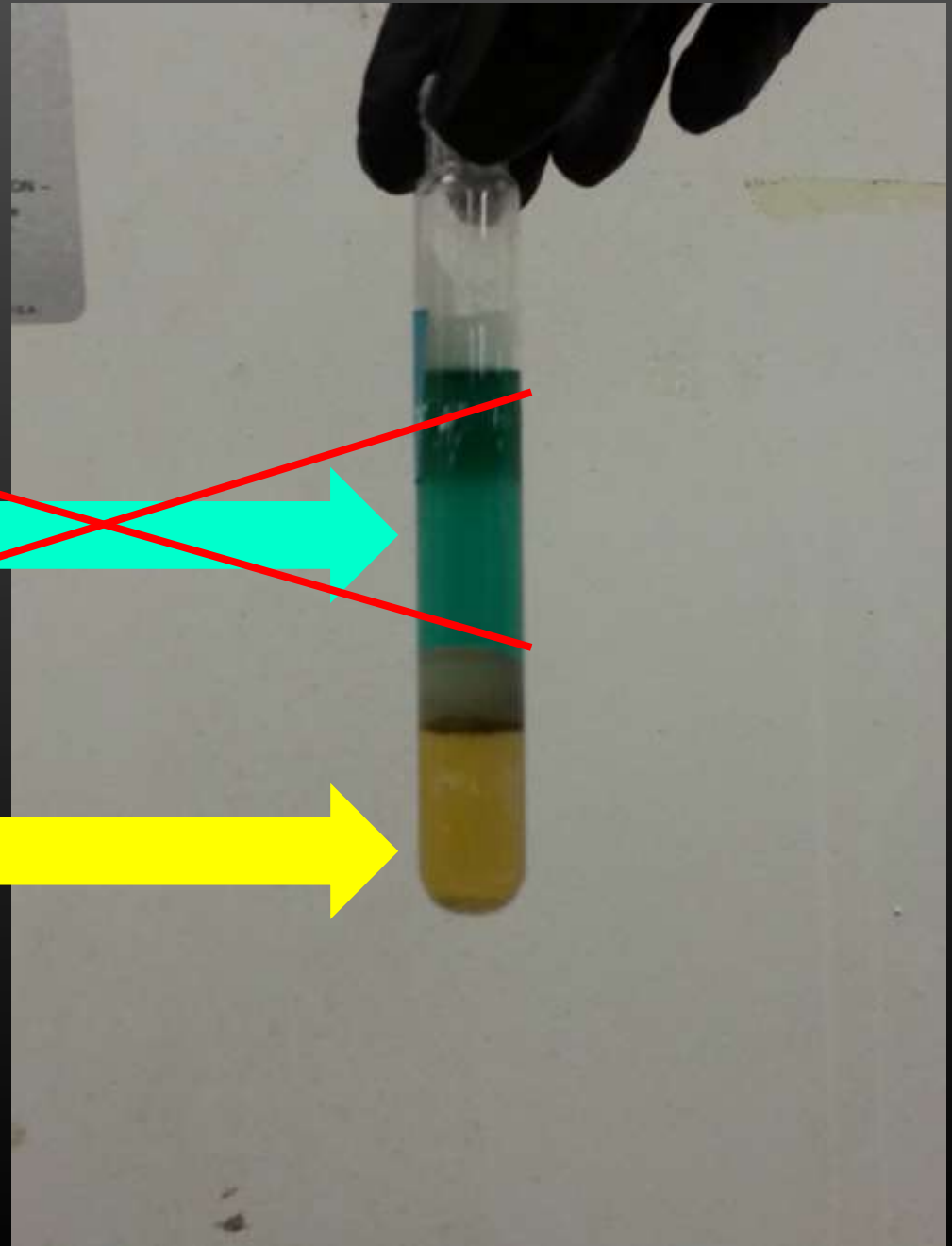
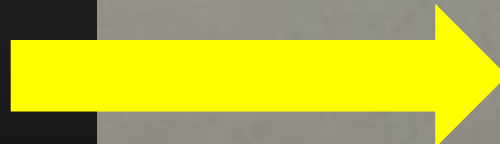
+Chloroform
+Methanol
+Water

➤ Mix overnight

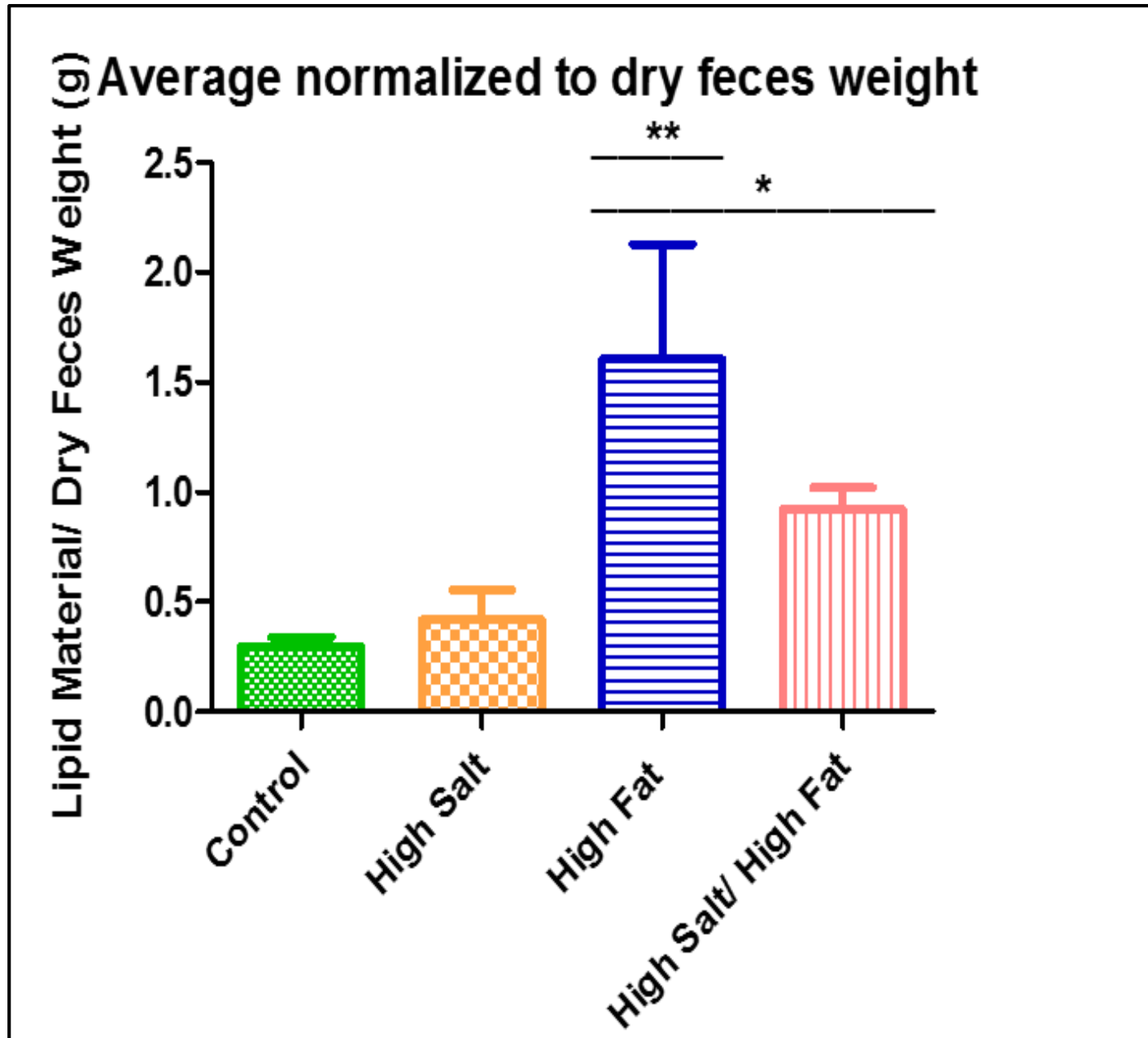
~~Water
Soluble
Phase~~



Lipid
Soluble
Phase

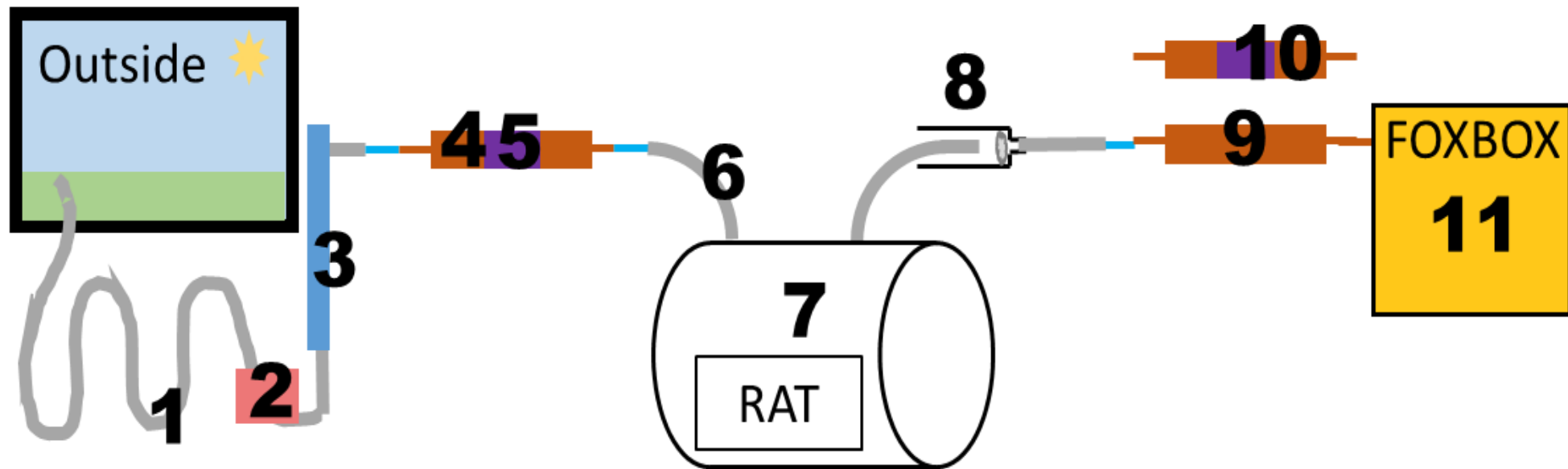


Metabolic Project – Lipid Data



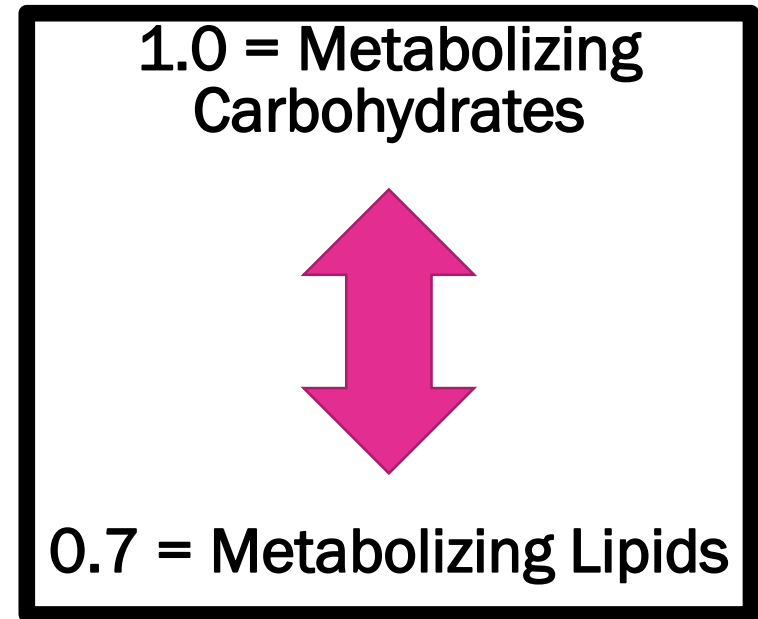
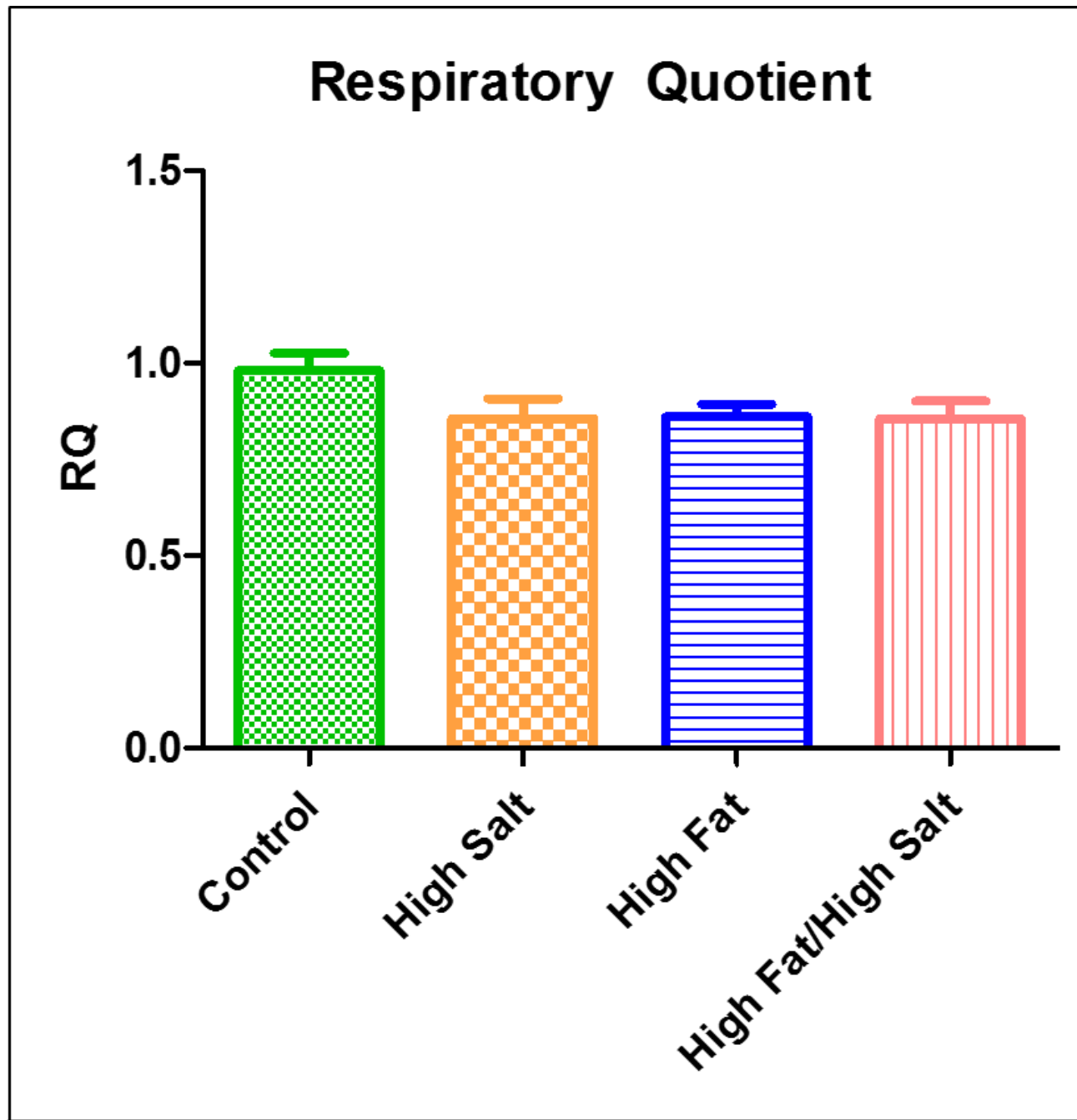
$$\frac{\text{CO}_2 \text{ output}}{\text{O}_2 \text{ Intake}} = \text{RQ}$$

Metabolic Project – RQ Design





Metabolic Project – RQ Data



Control	0.982 ± 0.029
HS	0.857 ± 0.033
HF	0.865 ± 0.021
HF/HS	0.857 ± 0.029



Metabolic Project - Conclusions



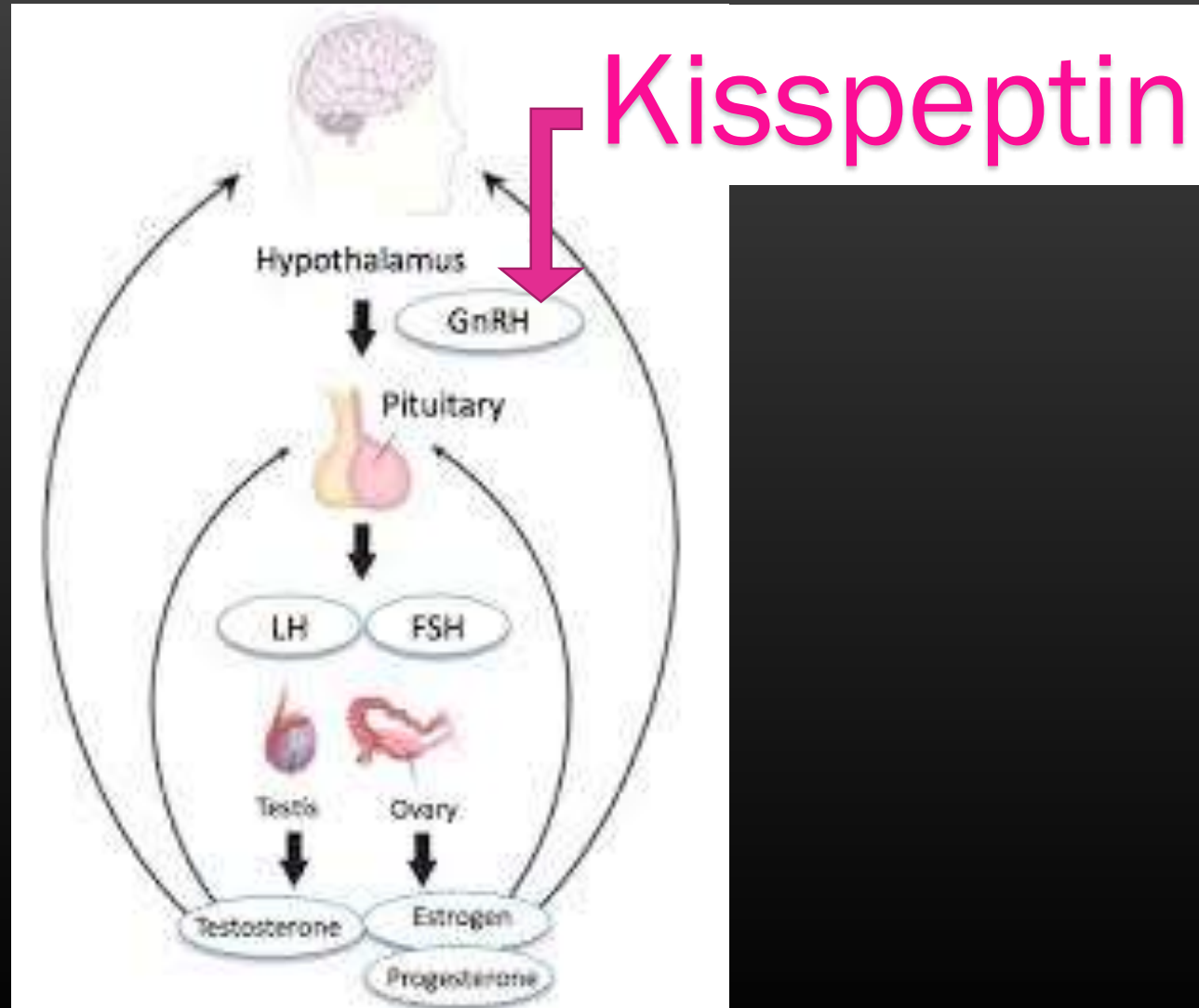
1. The HF/HS diet group did NOT excrete significantly more lipids than HF

2. Based on the CO₂/O₂ trend:

- Control rats → carbohydrates
- HS, HF, HF/HS → carbohydrates and lipids
- Groups with HS had the lowest average RQs



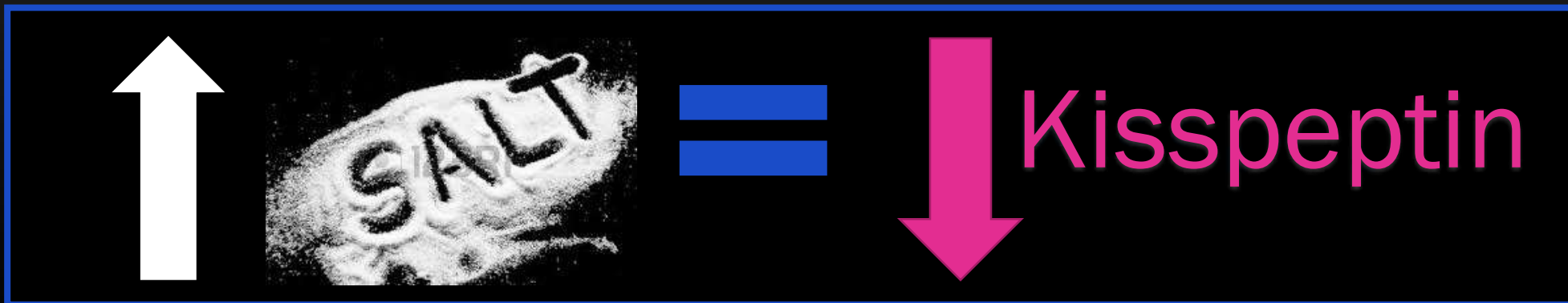
Kisspeptin Levels Project - Background



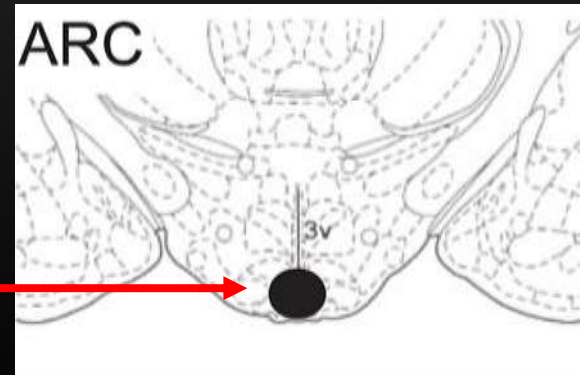
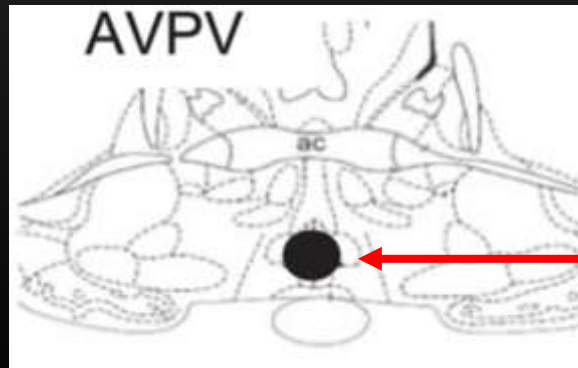
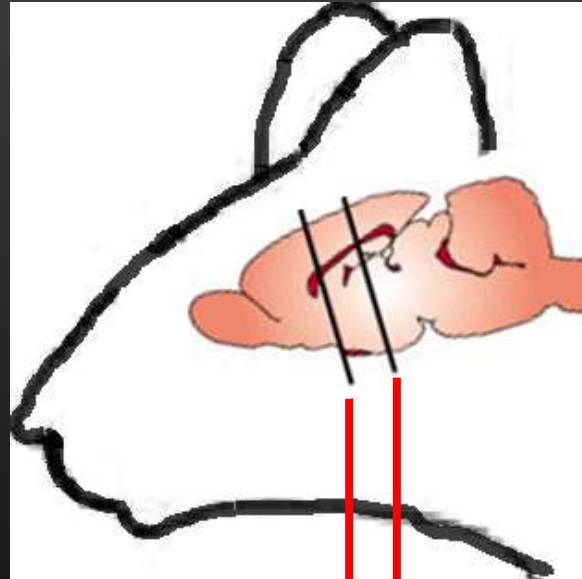
Kisspeptin Levels Project - Hypothesis

→ Investigate delay in pubertal onset with high salt

Rats fed **HS** or **HF/HS** diets will have lower kisspeptin in the Arcuate Nucleus and AVPV hypothalamic brain areas



Kisspeptin Levels Project - Methods



Kisspeptin Levels Project - Methods

- Goal → quantify gene expression levels of Kisspeptin (*Kiss1*) using **RT-qPCR**
- Gene expression can be measured by a cell's corresponding amount of *messenger RNA*
- RT-qPCR amplifies this genetic material

Kisspeptin Levels Project - Conclusions

- PCR is a delicate procedure!
- The semester was spent troubleshooting using practice tissue
- The lab is nearly ready to run the experimental tissue



Control Diet Project - Background

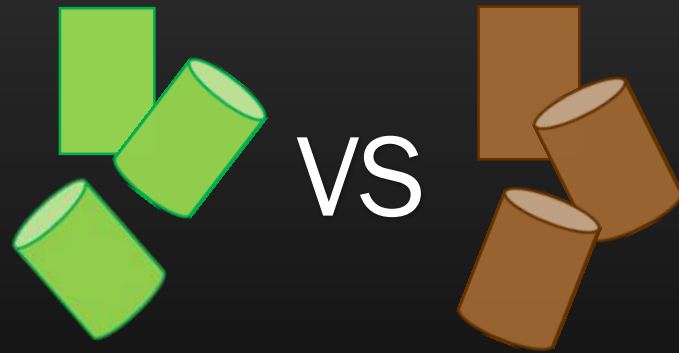
- Partner Lab of Dr. Kevin O'Byrne at King's College, London
- Our control rats vs. their rats show different dates of pubertal onset

Day 36 vs. 40

- 1 rat day = 110 human days (Sengupta, 2013)

Control Diet Project - Hypothesis

We hypothesize that the difference in day of pubertal onset is due to diet differences:

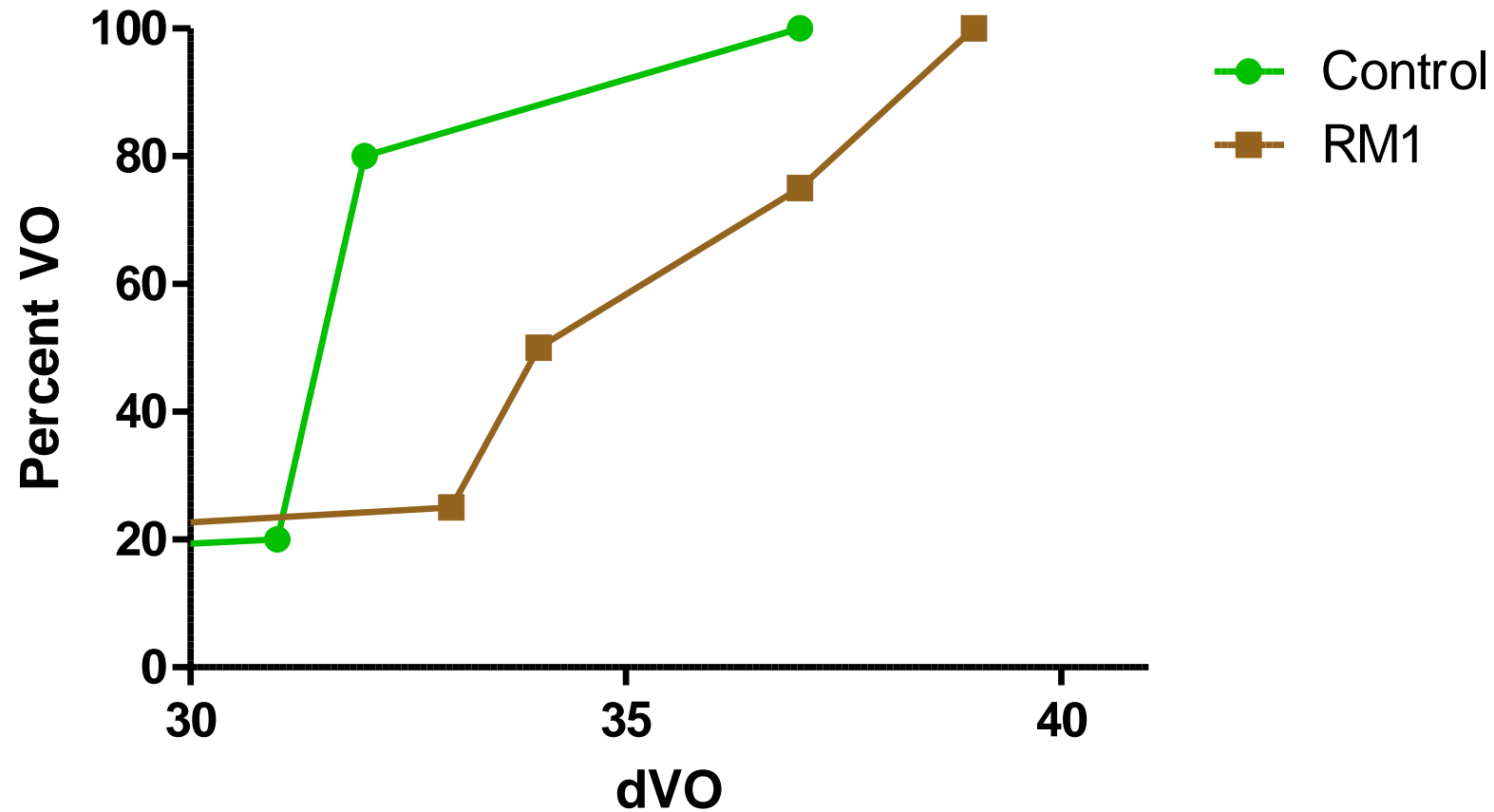


Skinner
Control

O'Byrne
Control

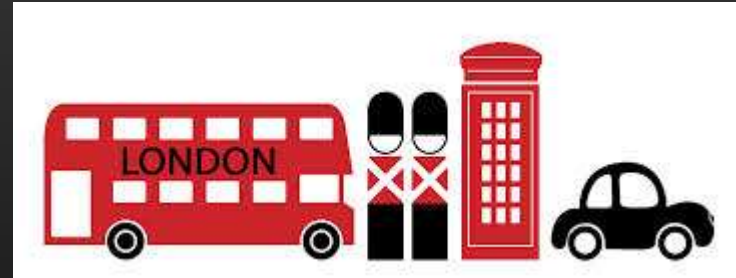
Control Diet Project – Preliminary Data

Control vs. RM1 Pubertal Onset



Control Diet Project - Conclusions

- Ran out of the control diet from London...



- Essential to figure out because a control in any situation is an important baseline for comparison

My Personal Experience

- Research is awesome!
- Science is all about messing up sometimes
- Though it may be difficult, the end product is worth the work

**"I have not failed. I've
just found 10,000 ways
that won't work."**

Thomas A. Edison



Acknowledgements

I would like to thank:

- **The INBRE Program** for funding this research
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- **Dori Pitynski** for her exceptional mentorship and allowing me to be a member of the Skinner Lab
- **Micah Ross** for her tireless assistance and contribution to these research projects
- **The Rats** for their contribution to scientific discovery



Questions?

