

SEPTEMBER 2023

Sun	Mon	Tue	Wed	Thu	Fri	Sat
27	28	29	30	31	1	2
3	4	5	6	7	8	9
10 *cancel Dr. Horowitz (check-up)	11 -Dr Garcia meet	12	13	14	15	16
17 *cancel Dr. Horowitz (check-up)	18	19	20	Meet Dr. Horowitz (5:15 pm)	22	-Send back waiver + UPL form
24	25 +meeting w Dr. Garcia	26	-meet w Mannah (12:30)	Sept. Logbook -meeting w Dr. Garcia → send back YPAL waiver	29	30

Wednesday, September 6, 2023

Today we learned and installed Paperpile (reference manager) and learned about the Biweekly Check-ups.

Paperpile - Reference Manager

↳ Org. Literature

↳ Cite

↳ References

Dr. Herzog is currently at a conference but is coming back this Thursday and will contact me after Thursday with an updated project idea. In the mean time, I will:

↳ Tasks:

① Learn/Practice using Paper pile

Friday, September 8, 2023

In the coming days I am expecting an email from Dr. Herzog regarding meeting times and project ideas. Although I have not heard from him, I am planning to:

Tasks:

Due dates?

① Look into recent papers published by Dr. Herzog to get a better understanding of the generalized topic area.

Email Dr. Herzog if he doesn't email me by the weekend

→ find article: "Reflections on obesity, exercise, musculoskeletal health"

Tuesday, September 12, 2023

- Meeting with Dr. Garcia (ask about formatting for logbook)

Tasks:

Due date?

① Notes on "Reflections on obesity, exercise, musculoskeletal health" (published by Dr. Herzog)

Notes from class:

→ Young Persons accessing U of C labs by U of C / Parents - yourself

→ Meet mentors during class time

1) email attend @ webber to excuse you

2) CC Dr. Garcia / email her

Tuesday, September 12 continued...

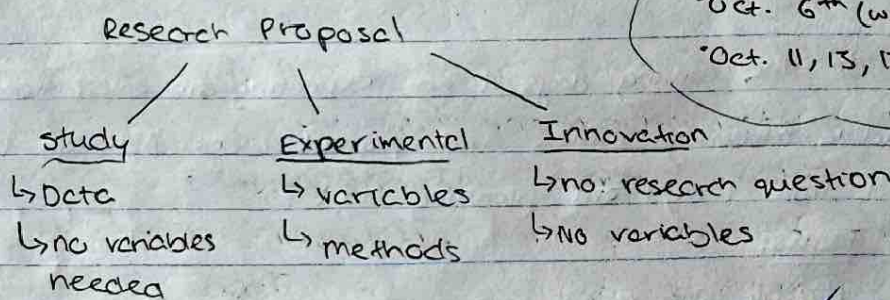
Notes from Meeting w/ Dr. Garcia

→ Important Tasks:

- ① Type of Project → writing for Dr. Herzog
- ② Experimental v.s. Study
- ③ Ethics for either type of project ✓
- ④ Watch videos/read book on basic structure
 - ↳ "Anatomy and Physiology: The Easy Way"
 - ↳ start on some notes on the book
- ⑤ Importance of booking next meeting each time ✖
 - ↳ any literature?
 - ↳ type meeting schedule?
 - ↳ offer Day 6 (starting at 2 ⇒ spare block)

Thursday, September 14, 2023

Class Notes:



Tentative Dates

- Oct. 6th (written)
- Oct. 11, 13, 17th (oral)

Outline:

- ↳ Background Research
 - ↳ imp. aspects of research
- ↳ Research-Question
- ↳ Goals (short-term/long-term)
- ↳ methods (variable)
- ⇒ ~~***~~ significance ~~***~~ ✓

Tasks Today:

- ① Read "Anatomy and Physiology" + take basic notes
- ② Update calendar w/ new dates
- ③ Wait for Dr. Herzog email?

where are these notes

Sunday, September 17, 2023

When are these due?

Tasks:

- research from "Anatomy and Physiology: The Easy Way"
- email Dr. Herzog
- update calendar

Any notes on this? ✓

Monday, September 18, 2023

↳ Notes from class (Research Proposal)

1 → Title Suggestion

- sentence

2 → Intro: Outline

• Broader Aspect Field

Variables } experimental project (study?)
Hypothesis

↓
Narrower Topic?

Tasks

Continue to

"Anatomy and Physiology"

3 - Research Question

4 - Goals | Research Question

ST LT

no word count

↳ 12 pt

↳ double spaced

↳ figures - legend

Wednesday, September 20, 2023

Today, I heard back from Dr. Herzog and have a meeting planned for tomorrow (Sept. 21) at 5:15 pm.

Meeting Goals:

↳ topic?

↳ meeting schedule?

↳ form / UCID?

Tasks:

Continue working on background research from "Anatomy and Physiology: The Easy Way"

Notes? ✓

Thursday, September 21, 2023

Today, I had my first meeting with Dr. Herzog:

Meeting #1: Notes

↳ Hannah Smith (day-to-day leader/mentor)

↳ Hannah will provide the literature

↳ she will contact me first to set meeting

↳ UCID (Barbara Herzog) ⇒ first talk to Hannah

Meeting #1 Notes continued:

↳ Image Analysis Project

- high fat/sucrose (HFS) diets induces obesity and low-level systemic/inflammation in male rats
- led to ↑ fat in vastus lateralis muscle but not soleus muscle
- New study → using female diet induced obesity + HFS had more intramuscular fat than thechow-fed control group rats
- fibrosis not diff. along diff. diet groups + muscles
- link b/w ↑ intramuscular fat infiltration + fibrosis ⇒ suggesting that intramuscular lipid content in skeletal muscles induces local inflammation ⇒ activates muscle repair process
- ↳ ∴ ↑ fibrosis

- Next step (my project):

↳ quantify the muscle specific inflammation using CD68+ immunohistochemistry

↳ macrophage marker indicating local inflammation to determine if

differences in local inflammation can explain why (female rats) muscles are protected from fibrosis in presence of HFS diet intervention.

Friday, September 22, 2023

→ Notes from Class

↳ Logbook mark:

- content (article summaries) / planning for future
- points
→ questions
→ discussing

↳ schedule

- sept. AND oct. calendars

↳ class dates, meeting dates, prop. deadlines

↳ Communication (email summaries)

Friday, Sept 22 cont...

↳ Try for weekly meetings

- In emails

- report progress / ask questions / set up meets / ideas for next steps / UCID

⇒ * Research prop. + oral presentation (end of October ⇒ delayed)

⇒ * Background submitted to google classroom for Logbook ✓

Tasks:

Find papers regarding obesity / HFS diets on musculoskeletal health

Try to find some of Hannah Smith (mentor) works

- Papers: "Skeletal Muscle: A review of molecular structure and function, in health and disease"

- Video (Hannah Smith): "The Effect of nutrition on musculoskeletal health: A diet-induced obesity model by Hannah Smith"

⇒ received email from Barbara Halash

↳ Fill-out YPAL waiver

Fill-out UCID request form

Monday, September 25, 2023

- Today, I received an email from Hannah Smith to set up meeting time.

↳ Possible dates to give her:

- Wed. (12:30 pm - 1:40 pm) (Day 4)

- Thur. (12:30 pm - 1:45 pm) (Day 5)

- Friday (2:10 pm - 3:30 pm) (Day 6)

- Any day after 4:15 pm ✓

⇒ received email from Barbara Halash ⇒ re-do YPAL waiver (* talk to Hannah before filling it out *)

Tasks:

Email back Hannah for meeting time

Tuesday, September 26, 2023

- Meeting w/ Hannah planned for tmr. at 12:30pm
↳ ask Dr. Garcia where I should do meeting

Things to mention during meeting

- ① Study V.S. experiment
- ② YPAL waiver
- ③ Tent. due date on October 6th
- ④ Guidelines of Research Proposal
- ⑤ Ethics??
- ⑥ Literature to review?
- ⑦ Lab safety courses?
- ⑧ Meeting-time schedule?

Tasks for Today:

- meeting in-class w/ Dr. Garcia
- notes on "Anatomy and Physiology: The Easy Way"
- figure out where meeting take place
- fill in what I can for YPAL waiver updated

moved to thursday

Wednesday, September 27, 2023

Meeting #2) with Hannah

- ↳ email her Guidelines for research
- ↳ get USB that has data
- ↳ timeline?
- ↳ she will send literature
- ↳ prob. no Lab safety but she will check
- ↳ she's going to create doc. w/ notes
- ↳ *send her schedule*
- ↳ *send her guidelines*

Tasks:

- send Hannah guidelines + schedule
- email Hannah about her question about proposal length
- make sure Logboon is good/ready to turn in ⇒ Due tmr
- start working on the YPAL waiver

Thursday, September 28, 2023

CLASS NOTES

↳ must be peer-reviewed sources

⇒ Textbook

⇒ PubMed / NHI

⇒ Google scholar

⇒ Health Canada

↳ Citations

↳ IEE

↳ AMA } numbered

→ order → 1 to 4



Notes From Meeting w/ Dr. Garcia

↳ email / establish new meeting schedule (weekly) w/ Hannah

↳ Literature Review

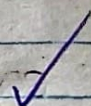
↳ R.Q.

↳ ST / LT Goals

↳ Find literature papers (immunohistochemistry)

↳ Oct 19th → R.P. goel due date

↳ create data table w/ proposed / organizing timeline



Tasks:

☑ Email Hannah (weekly meet? next meet? 4PAL waiver?)

↳ Upcoming Meeting poss. times (check agenda)

☑ Logbook ⇒ due tmr

☑ Look for papers on:

↳ CD68+ immunohistochemistry

↳ muscle inflammation (relation to HFS)

↳ fibrosis connection to HFS

↳ "CD 68 / macrophage: not just a histochemical marker"
(added to paper pile)

↳ "Acute and chronic changes in rat soleus muscle after high-fat high-sucrose diet"
(added to paper pile)

• Background research notes missing

Sept - Great work! { Good notes on meetings and class - Due dates on all tasks
* Citations need work → all classes have to show tasks

2023 OCTOBER

SUNDAY	MONDAY	TUESDAY	WEDNESDAY	THURSDAY	FRIDAY	SATURDAY
1	2 Tasks ↳ read/annotate reference #4	3 Tasks ↳ create notes on Hannah's doc outline	4 meet w Dr. Garcia ↳ read/annotate paper 5	5 meet w Hannah ↳ ref 5 notes finished	6 Research Proposal (final) ↳ title + research question + objectives ↳ outline in R.P.	7
8 ↳ hypothesize outline for R.P. ↳ variables outline for R.P. ↳ annotate ref 4	9 ↳ methodology outline for the R.P. ↳ start ref. 6	10 ↳ significance outline for the R.P. ↳ start intro outline for R.P. ↳ notes on ref 7	11 ↳ work on intro outline for R.P. ↳ work on ref 4 ↳ logbook check ↳ organize O's for meeting time	12 meet w Hannah (4:15pm) ↳ ref 6 finished	13 ↳ re-write significance ↳ add controlled variables ↳ finish ref 7	14 ↳ finish ref 4 ↳ finish ref 9
15 ↳ res 8 + 10 doc	16 ↳ re-organize outline intro ↳ email Hannah	17 meet w Dr. Garcia ↳ email about course ↳ intro outline ↳ finish ref 11 notes	18	19 ↳ homework / violence course 1 ↳ finish intro (edit + revise)	20	21
22 ↳ complete Hazard Assessment Training + occup health safety course	23 ↳ complete homework / violence course 3 ↳ meeting w Hannah (4:15pm)	24 meet w Hannah (4:15)	25 Research Prop. final finished ↳ homework + intro course #2 finish	26	27 meet w Dr. Garcia ↳ send Dr. Garcia R.P. ↳ finish intro course 3	28 Finish Training courses
29	30 ↳ alter R.P. w Dr. Garcia's suggestions ↳ start working on Outline for Submission	31 meet w Hannah (4:15) Research Proposal Due ↳ email Hannah ↳ work outline for oral P.R.S.				

Monday, October 2, 2023

Class Notes:

- Calendar / Schedule
- ↳ each class (list of things to do) → (meetings / readings)
- ↳ tasks in logbook need due date
- ↳ summarize or paste digitally
- ↳ Lots of notes ⇒ print out notes? ✓

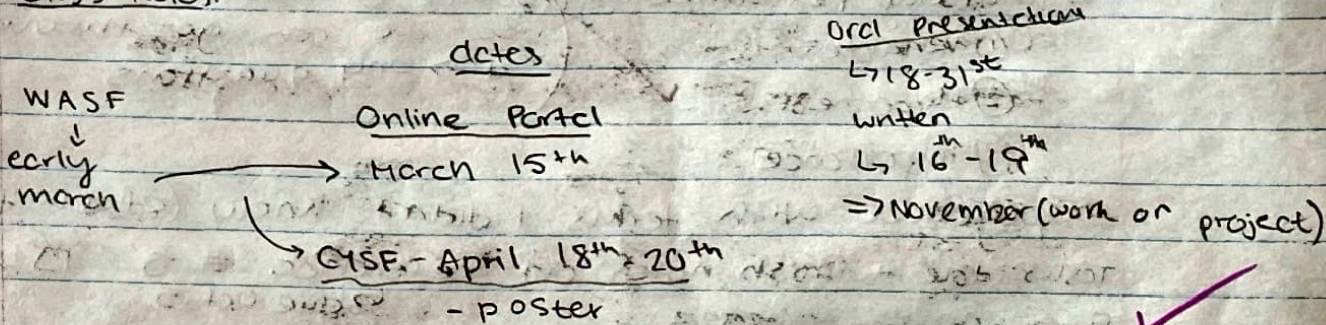
- Hannah sent 2 papers and document

Tasks:

- ↳ Update Calendar ✓
- ↳ Read + Annotate "A High-Fat Sucrose Diet Rapidly Alters Muscle Integrity, Inflammation and Gut Microbiota in Male Rats" (Reference #4) ⇒ in class (done by October 14) ✓
- ↳ meeting w Hannah tmr (read thro her document) ✓

Wednesday, October 4, 2023

Class Notes:



- meeting w Hannah moved to Thursday

Tasks:

□ Read ref 5 (due Oct. 5) ✓

✓ Work / Read / take notes →

✓ Meeting w Dr. Garcia

Notes From Dr. Garcia Meeting

- check logbook (calendar ⇒ check to make sure it's all organized, etc.)
- email Dr. Garcia + Hannah (after meeting w the meeting notes/etc)
- R.P. by 19th ⇒ push it later
- Oral pres. by 31 ⇒ push it later when? ✓

Things to mention in meeting w/ Henrich ✓

- due date (19)
- oral proposal (31)
- reading about macrophages (pro vs anti inflammatory?)
- reading literature (lots of words \Rightarrow created doc)
- notes on meeting after it's finished
 - ↳ when i'll have paper done etc. / lit. read
- confused about CD68 \Rightarrow found a paper explaining it ✓
- logbook
- APA or AMA \Rightarrow recommendation?
- Gut Microbiota \rightarrow HFS diet had \rightarrow bacteria types \rightarrow names
 - ↳ do I have to be familiar with these?

Thursday, October 5, 2023

Notes on Meeting w/ Henrich

- \rightarrow Reference style \rightarrow APA
- \rightarrow Ask about how in-depth the methodology has to be
- \rightarrow Thursday next meeting (outline of research proposal)
 - so we can go through it \rightarrow any questions
 - ① variables ✓
 - ② Hypothesis
 - ③ Importance
- \rightarrow google doc with terms I didn't know (attach that)

Tasks: take + finish notes on reference #. 5 ✓
↳ due Oct. 5

Friday, October 6, 2023

Class Notes: \rightarrow Wednesday (show improvement)

↳ improvement

↳ tasks - due dates calendar

Take 10-15 min \rightarrow EVERY ASP CLASS

↳ logbook (meet / email / resp. proposal) ✓

↳ communicate with mentor

↳ R.P. into presentation

Tasks for class:

↳ update calendar (add tasks) / etc to keep on track for Thursday's meeting? ✓

↳ write title / research question / objectives outline in the R.P. ✓

Sunday, October 8

Tasks:

- Complete Hypothesis outline in the Research Proposal
- Complete variables outline in the Research Proposal
- annotate + take notes on ref 4 (due Oct. 14)

Monday, October 9

Tasks:

- complete methodology outline in the Research Proposal
- notes on "High Fat With High Sucrose Diet Leads to Obesity and Induces Myoregeneration" (due Tues. Oct. 10)
↳ Ref # 6

Tuesday, October 10

Tasks:

- write + complete significance outline for the R.P.
- notes on "Macrophage Cytokines: Involvement in Immunity and Infectious Diseases" (due. Friday Oct. 13) (ref 7)
- work on outline of Intro for R.P. (outline due Oct. 12)

Wednesday, October 11

Class Notes:

written R.P. ⇒ turn it in + google classroom

↳ originality ↳ edits/feedback/grading

↳ deadline Oct. 31

Oral Presentation (10 min) ⇒ 5-10 min questions

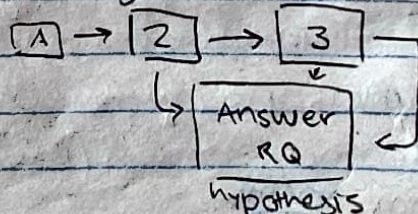
Sci Fair ⇒ early March 1-8

↳ GC (10-12 slides)

↳ Punch Line (elevator pitch)

↳ Background → RQ/Goals → Methodology (graphs)

↳ Flow Diagrams



Tasks: Dread ref 4 + notes

□ finish para 1 + 2 outline for R.P.

□ organize questions of meeting time

- ↳ Q's: ① specificity of controlled variables? ④ organization for intro correct?
② VL + soleus muscle importance? ⑤ hypothesis make sense?
③ long term goal → ideas?

Thursday, October 12

Meeting Notes

→ Introduction

↳ first para good

↳ 2nd → don't do physiology

↳ INSTEAD → ① obesity / effects / why

② less known → muscles (specific)

↳ "long term health ..."

③ Previous studies / Macrophages

→ RQ →

↳ should be \bar{v} specific (2nd sentence)

↳ add the macrophage count

→ Objectives

↳ ask about long term (females ⇒ therapy obesity)

→ Hypothesis

↳ get rid of the 2nd + 3rd (more of a conclusion)

→ Variables

↳ put in later

↳ indep + dep good

↳ controlled → good → (housed in pairs / consistent light / dark schedule / diff litters (↑ genetic diversity))

→ Methodology

↳ good (tell next time → Hannah bring CD68 images)

↳ next time → CD68 immunohistochemistry

↳ brackets are just where

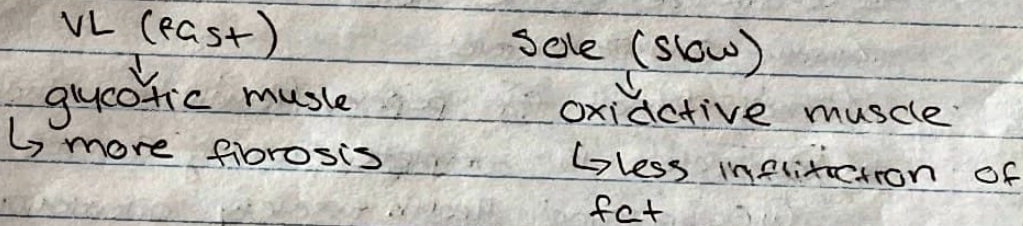
references suem/etc come from

Notes Meeting cont.

Significance → females + males (ADD THAT!)
 ↳ Long term goal → therapy women obesity → imp.
 ↳ what I have should go in intro

→ previous studies showed female rats some fibrosis?
 ↳ ↑ inflammation ⇒ * she will send papers explain better*
 ↳ female rats
 ↳ previous studies (for intro)

→ VL and Soleus Muscle Importance



* review VL + soleus for the introduction *
 Next Meeting: Wednesday, Oct. 18 (4:15 pm)

Tasks before next meeting: (all due Oct 18)
 ↳ finish/work intro for next time
 ↳ alter significance (long term → female)

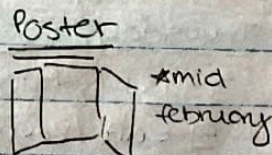
Today: → Amph ref 6

Friday, October 13

Class Notes: 13

WSE March 4th (1-8)
 ↓ 15 projects

March 14 - Online Portal CYSF
 closes



Mandatory for WSE

Basic Project / Ethics

Dr. _____ / code / phone / email

Task / deadlines	
<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>

Tasks today: ref 7 finish

re-write significance

add the controlled variables discussed in meeting yesterday

Saturday, October 14, 2023: finish ref 4+9

Sunday, October 15, 2023: finish ref 8+10

Tuesday, October 17, 2023

Tasks

email "ucsafty@ucalgary.ca" for help on Office Ergonomics Training course

Re-organize outline notes for Introduction

Meet w. Dr. Garcia

Email Hannah for papers (female vet studies)

Add dates to complete Training Courses

① Occupational Health + safety orientation

② Hazard Assessment Training (HACE form)

③ Office Ergonomics Training

④ Harassment and violence awareness training

finish ref 11

Meeting w. Dr. Garcia Notes:

→ Oct. 25 (finish)

→ Oct. 31 (finish + turn in)

→ Nov. 8 (oral present)

→ Oct. 28 (finish training courses)

* Hannah moved meeting to Monday *

Thursday, October 19, 2023

Tasks: finish Intro

Edit/Revise Intro + Significance (due Oct. 25)

APA citing (Oct 25)

Sunday, October 22, 2023

Today Hannah emailed me revisions for the Research proposal.

→ Oct 23 (tmw) → meeting @ 4:15pm w. Hannah

Questions to Ash Honnan:

- ① Go over R.P (changes/delets/etc.) ✓
- ② Office Ergonomics Training Course?
- ③ R.P => needs to be complete by Oct. 30
- ④ Oral Presentation => presenting on the Nov. 8
- ⑤ more meeting time?

Monday, October 23, 2023

Class Notes:

- Logbook
- Reading ✓
- Timetables - Calendar Tasks

Task Check List

Task	Date	Check
_____		✓
_____		✓
_____		✓

} have o.p. in here

Research Proposal → Oct. 31st, 2023

Style → Engagement to Audience
 ↳ graphics/visuals
 ↳ flows ↳ text (large font) ✓

Oral Presentation (10 minutes)

- ↳ Title
- ↳ Brief description (punchline)
- ↳ Background (2-3 slides)
- ↳ RA/Aims
- ↳ Methods | flow diagram → (2, 3 for each method)
 - ↳ variables
 - ↳ significance

Tasks:

- Review document Honnan returned (revise for meeting) ✓
- Do harassment 3 training Course ✓

Meeting to Honnan notes:

- Hypothesis → reason sentence (send back → revised)
 - M1 → relevance?
 - Tim Leonard / Wasser Herzog
 - ↳ UofC contact for Ergonomics Course
- (next meeting) Tuesday → 4:25 pm

Wednesday, October 25

Class Notes:

10-12 (10 mins)

- { BG
 - 1- Title/Punchline
 - 2- info
 - 3- info

- { RA - 1-2 slides
- { Goals

- { Method -
 - 1- Flow Diagram
 - 2- Method 1
 - 3- Method 2

{ Significance

* Logbook due Oct. 31st ✓

* RP due Oct. 31st

* Mentor Evaluation

* Ethics → CYSF portal

Tasks:

- Harassment Training Course #2
- Update Hypothesis (notes from Hannon)

Friday, October 27, 2023

Tasks:

- Meeting w Dr. Garcia
- Finish Training Course 3
- Send Dr. Garcia Research Proposal

Meeting w Dr. Garcia:

- Calendar (events/tasks/timeline for Data Analysis)
- Training Course (talk to Hannon)
- Start working on oral pres. → Nov. 8th
- Better idea on program will be using

Monday, October 30, 2023

Tasks:

- Alter/Change R.P. from edits from Dr. Garcia
- Start working on outline for Slideshow (Oral Pres.)
↳ due Nov. 1st

Tuesday, October 31, 2023

Notes on Jessica's Presentation

- ↳ Design + Construction of CRISPR/Cas-9-Mediated knockout Plasmids for Autism-Risk Genes
↳ method of knocking out genes
- social communications/restricted interests/behaviour
- gene knockout for autism genes ⇒ using CRISPR/Cas9
- 2-gRNA system incorporated to ↑ eff. of knockout of genes
- Using Snap Gene (online gene tool) ⇒ CRISPOR (online gene tool)
- ↳ m-chemy (Fluorescent protein)

Notes on Cooper's Presentation

- ↳ Changes in Complement Protein Deposition + Its Role in Microglia-Mediated Synaptic Pruning Following Repeated Mild

NOVEMBER 2023

Sun	Mon	Tue	Wed	Thu	Fri	Sat
29	30	Meeting w Hannah (4:15pm)	Outline for slides due	Sides 1+2 done organize questions meeting w Hannah	Slide 3+4 done	4 Email Hannah for slide photos finish slides 3/6/7
5	6 finish slides (8/9/10)	Meeting w Hannah (4:15pm) practice w Dr. Garcia (10:00)	Oral Presentation	9	10 Email Hannah (presentation + when to start data)	11
12	13 Meeting w Hannah @ UAC (1:00)	14 email Hannah about images	download image files download program images	16	17 start 3 images (H57 file)	18
19 work on H57 file (5 images)	20	21 meeting w Dr. Garcia work on CYSF images from H57-5015	22 email Hannah about the ethnics	23 send Hannah 3 images finish CYSF portal H58 file (2 images)	24 finish H57 file (10 images)	25 Intro Fina Paper H510 file (6 images)
26	27 finish H50 (4 images) ethnic code (CYSF portal) email Hannah	28 meeting w Hannah email files to Hannah	29 connect H57 file (add macrographs)	30 Logbook Nov. due H51501 (5 images)	1 H51501s file complete (5 images)	2

Need to have planned tasks for Nov!

Notes on Copper's Presentation cont...

- RmTBI in Adolescent (important development)
- Pruning? → ↑ or ↓
- Microglia → immune cell → destroy pathogens
- Complement system (immune response → O sponization)
↳ C3, C1q
- Studies → effect of RmTBI on adolescent
↳ microglia → brain → control injury
- What is motor cortex / Sham injury (objectives)
- speed of projectile (how fast) → controlled variable?
- Immunohistochemistry (methods) → fluorescence spectroscopy

Tasks:

- ☑ Email Hannah Back
- ☑ Work on Outline for oral presentation (due Nov. 1)

Oct calendar ✓

Daily Notes Oct ✓

Background Oct ✓

Nov. Calendar
not complete

14/15

Thursday, November 2, 2023


Tasks:

- ☑ Finish slides 1 + 2
- ↳ Slide 1: HFS/obesity / Muscle Types ✓
- ↳ Slide 2: Inflammatory system (macrophages / cytokines / MCP-1 / CPGs)
- ☑ Organize Meeting Questions for Hannah
↳ meeting today at 4:20 pm

Meeting Questions For Hannah:

- ① Organization for slideshow?
- ② What is significance of Sprague-Dawley Rats?
- ③ In significance → new therapies → any ideas (in case they ask)?
- ④ Program to look at CD68 cells (macrophage count)
↳ timeline? (start nov.) / schedule / plan?
- ⑤ Methodology → rat photos we talked about last time?
- ⑥ Citing for presentation
- ⑦ Ethics ???
- ⑧ Training courses → Office Ergonomics Course?

Meeting Notes w/ Hannah

- diff colour for each inflammatory molecules
- separate muscle types after
- previous studies → male
↳ females
- Objectives + Research Q → Csk about
- HSF → powder (39% fat, 45% sugar → butter + sugar)
- Sprague-Dawley → nutrition (moor in rats before)
↳ gain weight fat (easier to see)
- Rats vs. mice → too small (rats bigger) ✓
-  vs. hamburger/donut
- 24 rats → reduced animals
- Ethics approved
- Remind tmr afternoon (photos)
- U of C logs
- Slides on Sunday

Friday, November 3, 2023

Tasks:

- ✓ Finish slides 3 and 4 ✓

Saturday, November 4, 2023

- ✓ Email Hannah for slide photos
- ✓ Finish slides 5/6/7 ✓

Monday, November 6, 2023

Tasks:

- ☑ Finish slides 8/9/10

Tuesday, November 7, 2023

Q's for Hannah

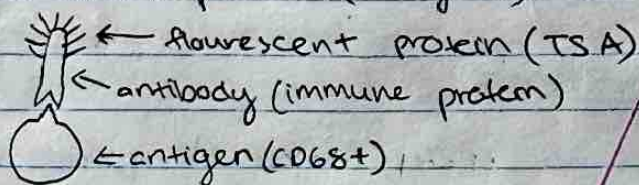
- Therapies/Treatments (significance) → possible treatments
 - ↳ physical treatments → weights/swimming/resistance bands
- Hormone/metabolic differences b/w males and females
- Why have most studies been conducted on male rats instead of female rats?
- Why Srege-Dowley Rats? → gain weight fast
- Timeline/starting of Data Analysis?

Notes From Meeting w/ Dr. George:

- Punch Line → what study is about
- IMF (infiltration → how/where)
- Previous studies citations
- Point to diagrams
- Cryostat in vehicles not necessary
- Explain Immunohistochemistry more
- Look at audience

Notes From Meeting w/ Hannah

- Antibodies w/ fluorescent proteins (TSA) will latch onto CD68 proteins (antigens)



- Add point about fat infiltration
- Yellow/Red points are CD68 cells/markers

Tasks:

- ☑ Edit/Practice Presentation

Wednesday, Nov. 8

Owen's Presentation

→ heart dissects/heart attacks

- electrocardiogram (PQST)

① ST-segment Elevation ② Non ST-Segment Elevation ③ Angina

→ Arrhythmia (irregular beating of heart)

Natalie's Presentation

- POTS (gravity pulls blood down)

- Effect females more ⇒ why?

- Methods (10 min HUT / 10 min Supine) → Why do you chose a 10 minute proto?

Elliot's Presentation

- Gut Microbiota

- ① Vagus Nerve ② Immune cells in gut ③ activate innate immunity

→ Why does it happen in males then females?

→ reflects of handling → confounding why?

→ why do you look at heat maps → see where rats are

→ ratio males to females 3:1 → why does it effect males more?

Tasks:

Present Presentation

Questions: → composition of HFS diet/why soleus has higher endurance/types of treatments/why females present differently

Monday, November 13, 2023

Notes on Meeting:

→ Images (blue → DAPI (nuclei)) (yellow → TSA (CD68))

→ Use ImageJ program (circle tool)

→ excel spreadsheet (image numbers)

→ Once finish muscle 7 → email them to her (make sure its right)

→ Meeting each Tuesday

→ share file with me

→ show around Lab

Wednesday, Nov. 15, 2023

Tosin and ZZ

- Effects of ketamine + Marijuana on neuronal viability + function through p2 rat Brain cell Cultures
- hippocampus (anesthetic → surgery) → ketamine
- Why do you chose them at 2 days old?

Brynn

- Genetic Diff. b/w Endangered and Non-endangered Pine species
- ↑ CO₂ → ↑ heat tolerance
- restricts gas exchange / soil moisture / earlier bud burst
- Synthetic Biology → engineering to give new function
- Changes in Habitat
- Changing diff genetic sequenes
- Loblolly Pine (control)
- Loblolly Pine VS Whitebark Pine (manipulated)

Tasks:

- Download Image File
- Download ImageJ Program / install

Friday, Nov. 17

Vincent Notes

- Determining effectiveness of Lytic Activity
- virus out of water → see if they will kill ecoli
- Bacterial (Phage) → latch onto Bacteria, phage DNA replicate
- 80% infections by E. coli ST131
- Phages types (diff)
- TSA / TSB

Tasks:

- Start 3 images in ImageJ (HST)

Sunday, Nov. 19

Tasks:

- Work on HST file (5 images)

Tuesday, November 21, 2023

Tasks:

- ☑ Work on Portal CYSF (due. Nov. 23)
- ☑ Meeting w Dr. Garcia
- ☑ Get 2 images of H 57501#15 file
 - ↳ finished file

Intro-Section!

- Lit. Review
- Reading
- Intro (Dec 15)

Notes from Meeting w Dr. Garcia

- ↳ need to organize/specify time
- ↳ organize calendar for tasks (calculate how long each image will take)
- ↳ Finish CYSF portal
- ↳ Intro for Final Paper (copy/paste \Rightarrow add RO at end, turn in sooner rather than later)
- ↳ Date Analysis done by Jenecry

Thursday, Nov. 23, 2023

Amy

- UCR (violent/other/Minor crimes)
- CSI (Crime Severity Index)
- 12/17, 18/24 \rightarrow higher violent crimes
- males more than females (why?)
- Psychopathy 18% - 40% \rightarrow crime offenders
- what could be common neurological traits?
- Gene \rightarrow violent/aggressive behavior
- young/old, males/females, psychopath/non-psychopath
- English/Dutch/Canadian \Rightarrow "2 test + wcs is that"?

Joel + Ashonk

- MIMotypes \rightarrow Type 1 Diabetes
- Metabolic Pathways (interact w environment)
- auto-immune disorder (no longer produce insulin \Rightarrow regulate blood sugar)
- Diab immune Study
- python script (diff metabolic pathways)
- \rightarrow why children in Europe develop more?
- \rightarrow why do females more likely to develop T1D?

Tasks:

- ☑ Email Hannah w File #5750115 answers
- ☑ Finish writing brief description for C4SF Portal
- ☑ Start HS8 file
 - ↳ 2 images → due Nov. 24

November 24, 2023

Tasks

- ☑ Finish HS8 file
 - ↳ 10 images

Questions For Hannah

- ↳ Image 1919-1920 (HS8 File) → Blue gradient in top left
- ↳ What is it?
- ↳ Answered: could be contamination (hair, etc.) or lack of editing (DAPI vs CD68+)

November 25, 2023

Tasks:

- ☑ Intro for Final Paper (read over) → due Dec. 15, 2023
- ☑ Work on HS10 file → due by Tuesday 28
 - ↳ 6 images completed

Nov. 27, 2023

Class Notes:

- * Logbook (30m) 1/week meeting
- * Intro. Final Paper (Dec. 15) 1 email/week

calendar { Dec-Reading
+ deeper understanding
+ theoretical aspects

Main Focus

- Data collection
- 2/3 week
- deep understanding methodology

Dec/Jan. → Feb (Poster/Prep.) WAF → March 4th

- winter break
- midterm period (Jan 8)
- *come up w calendar*

Tasks:

- ☑ Finish HS10 (4 images)
- ☑ Finish submitting ethics code (CYSF Portal)
- ☑ Email Hannah about image accuracy

Tuesday, Nov. 28, 2023

Meeting w Hannah notes:

- Triangle shapes (avoid → pooling b/w tissue)
- HS7 Sol #17 → (review add more in some files)
- only when circle (no lines)
- keep going w Soleus images first
 - ↳ finish soleus by Christmas
- send Hannah excel with numbers
- Next meeting: Tuesday, Dec. 5, 2023
- To delete circle: Alt + circle again (over-top)
- use mouse instead of trackpad
- by next meeting:
 - review HS7, HS8, HS10 (add macrophages)
 - finish HS11, HS12, HS19, NA51
- compare DAPI + CD68 stains while counting
 - ↳ useful/more accurate

Wednesday, Nov. 29, 2023

Tasks:

- review H57 File (take Homah's advice) ✓
↳ adding macrophages
- Logbook due tmr (make sure ready)

- Good Notes for Nov -

- It would be good to see pictures/
printouts of the data analysis done to
show how ~~the~~ your image analysis is done!

15/15

DECEMBER 2023

Sun	Mon	Tue	Wed	Thu	Fri	Sat
26	27	28	29	30	1	2
↳ HS12 sol 15 File completed (4 images)	↳ go over high-count (re-count)	↳ finish re-counting high-count images	↳ work on HS19 sol 15 (5 images)	meeting w Dr. Garcia ↳ HS25 sol 15 (5 images) <i>what else?</i>	↳ complete HS19 sol 15 (5 images) ✓	↳ NASH sol 15 completed
3	4	5	6	7	8	9
↳ email Hannah	↳ meeting w Hannah (4.15) ↳ NASH2 sol 15 completed (5 images)	↳ NASH3 sol 15 File complete → meeting w Dr. Garcia ✓	↳ NASH sol 15 (5 images)	↳ NASH sol 15 (5 images)	↳ NASH sol 15 (5 images)	↳ NASH sol 15 (5 images)
10	11	12	13	14	15	16
↳ NASH2 sol 15 (5 images) ✓ <i>class</i>	↳ NASH2 sol 15 (5 images) ✓	↳ NASH3 sol 15 File complete → meeting w Dr. Garcia ✓	↳ NASH3 sol 15 File complete → meeting w Dr. Garcia ✓	↳ NASH3 sol 15 File complete → meeting w Dr. Garcia ✓	↳ NASH3 sol 15 File complete → meeting w Dr. Garcia ✓	↳ NASH3 sol 15 File complete → meeting w Dr. Garcia ✓
17	18	19	20	21	22	23
↳ NASH6 sol 16 complete (5 images) ✓	↳ email Hannah	↳ NASH6 sol 16 complete (5 images) ✓	↳ NASH6 sol 16 complete (5 images) ✓	↳ NASH6 sol 16 complete (5 images) ✓	↳ NASH6 sol 16 complete (5 images) ✓	↳ NASH6 sol 16 complete (5 images) ✓
24	25	26	27	28	29	30
↳ NASH6 sol 16 complete (5 images) ✓	↳ NASH6 sol 16 complete (5 images) ✓	↳ NASH6 sol 16 complete (5 images) ✓	↳ NASH6 sol 16 complete (5 images) ✓	↳ NASH6 sol 16 complete (5 images) ✓	↳ NASH6 sol 16 complete (5 images) ✓	↳ NASH6 sol 16 complete (5 images) ✓

December 1, 2023

Tasks:

- ☑ Meeting w Dr. Garcia
- ☑ HSI2Soll5 File (5 images)

Notes From meeting w Dr. Garcia:

- 13-15 hrs/week
- keep in contact with mentor
- Finish by end of January
- Include Images of Data Analysis (Image J) in Logbook

December 3, 2023

Tasks:

- ☑ complete the HSI2Soll5 File (4 images)

December 4, 2023

- ☑ Email Hannah updated excel sheet/images for meeting tmr
↳ high numbers?

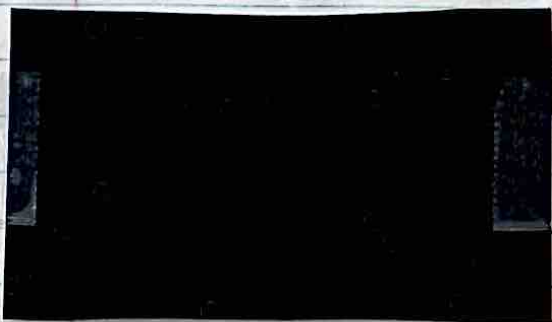
December 5, 2023

Tasks:

- ☑ Email Hannah Beck
- ☑ Go over too-high counts in Soleus Files (re-count/re-do)
- * Hannah moved meeting to Thursday instead *

Hannah's Advice From Email:

use original image



then compare original with overlaid ↓



Questions For Hannah (meeting Thurs.)

- High numbers → what am I doing wrong?
- How to differentiate? → (close together/overlapping)
- Could she send examples for me to go off (identify)?
- What is her winter break schedule?
 - ↳ 21- (midterm 1½ weeks after that)
- Tell her ethics got approved / working on Intro (Final Paper)

December 7, 2023

Class notes:

Plan for Sci fair (show next meeting)

- Dec →
- Jan →
- Feb → OP.
- March 4th Sci Fair Goal

Big task / Step deadline

⇒ } smaller tests / deadlines

* Planned into each week from now to end of Feb.

↳ Presentation Class $\left\{ \begin{array}{l} \text{Feb 26} \\ \text{Feb 28} \\ \text{March 1} \end{array} \right.$

↳ Poster-Design / Creation (March 4th)

⇒ Hannah emailed and cancelled meeting

Tasks:

- Complete HSI9sol15 (5 images)
- Email Hannah Back → meeting moved to Dec. 12

December 8, 2023

Tasks

- Complete HSI9sol15 (5 images)

December 9, 2023

Tasks:

NA51Sol15 File (All images) completed

Are there
any notes
on how
the images
are analyzed?

December 11, 2023

NA52Sol15 File (5 images) completed

Go over Introduction / citations / edits

December 12, 2023

Tasks:

Meeting w Hannah (4:15 pm)

NA52Sol15 completed (5 images)

Notes From Meeting w Hannah

- ↳ went over schedule for before science fair
- How long will data analysis take? → one week
- send her dates for upcoming assignments (papers/pres.)
- Next Meeting: January 9th, 2023
- send Soleus for data analysis once finished

December 13, 2023

Tasks:

Meeting w Dr. Garcia

NA53Sol15 File complete (5 images)

Notes From meeting w Dr. Garcia

- Plan looks good
- keep pace w plan
- have to make trifold (Science Fair) and google slides (class presentation)
- ~~Biorender~~ good tool for diagrams

December 15, 2023

Tasks:

- Work on editing Final Paper Intro
- Turn in Final Paper

December 16, 2023

Tasks:

- NAS4Sol15 File completed (all images)

December 18, 2023

- NAS6Sol16 File completed (5 images)

December 19, 2023

↳ Hannah cancelled meeting

Tasks:

- Email Hannah Back

December 20, 2023

Tasks:

- NA6Sol16 File completed (5 images)
- * Last day of class before Winter Break *

December 22, 2023

Tasks:

- Complete NAS7Sol15 (all images)
- ↳ * now all the soleus images are completed *

December 23, 2023

Tasks:

- All Solves Files Complete → email to Hannah

December 28, 2023

HS7VL#15 File completed (all images) ✓

December 30, 2023

HS8VL#14 File completed (all images)

↳ Ask Hannah in next meeting (Jan 9) → Why are there many more images in VL files than the Soleus files? ✓

December 31, 2023

Tasks:

↳ Complete HS9VL#16 File

↳ Complete HS10VL#15 File ✓

January 1, 2023

Tasks:

↳ email Hannah about meeting time

↳ ask about diff. number of images in VL files ✓

↳ give her date for Methodology section

Dec 15/15

Lots of work covered!
Saw the analysis in data collection section ✓

JANUARY 2024

Sun	Mon	Tue	Wed	Thu	Fri	Sat
↳ complete HS9VL#16 / HS10VL#15	↳ email Hennon (meeting time)	2	3	↳ complete HS11VL#1 (5 images)	↳ complete HS11VL#1 (5 images)	↳ NAS1VL#2 File Complete 6
↳ complete NAS2VL#15 File	↳ complete NAS3VL#20 File	9	meeting w Hennon 10	11	↳ complete NAS4VL#16 File 12	13
14	15 mbtwnms	16	↳ complete NAS3VL#20 File 17	18	↳ complete NAS6VL#19 / NAS7VL#18 / NAS2VL#15 19	20
21	22	23	↳ complete NAS8VL#13 File 24	25	↳ NAS8VL#13 (completed) ↳ email Hennon (finished excel) ↳ ask abt. HS10VL#4 26	27
↳ complete HS12VL#4 File ↳ email Hennon	↳ work on Methodology section what part? 29	meeting w Hennon ↳ stats? meeting w Dr. Garcia 30	Logbook due 31	↳ work on Methodology section paper what part? 1	2	3

January 4th, 2024

Tasks:

↳ Complete HS11VL#1 (5 images)

January 5th, 2024

Tasks:

↳ complete HS 11VL#1 (5 images)

January 6th, 2024

Tasks:

↳ NAS1VL#2 File completed (all images)

↳ ask Hannah about such low numbers compared to soleus*

↳ if soleus are more oxidative shouldn't they have lower numbers?

↳ what could cause this?

January 7th, 2024

Tasks:

↳ Complete NAS2VL#15 File (all images)

↳ again there are lower numbers → what would this mean?

January 8, 2024

Tasks:

↳ complete NA53VL#20 File (all images)

Questions for Meeting w Hannah thur:

- HS12VL#4 File → doesn't have overlaped images but only the original images?
- differences in the number of images in each file → why?
- next meeting time?
- VL lower numbers but not oxidative → soleus should be more protective?
- last time talked about adding more images → is that happening?
- stats on soleus file update?

January 10, 2024

Meeting w Hannah notes:

- Next Meeting: January 24, 2023 (Wed.)
- HS VL #1 ⇒ she will send updated file
- graphs: bargraph / box plots w scatter plot
- stats next meeting (Jan. 24)

January 12, 2024

↳ complete NA56VL#16 File count

January 15, 2024

↳ complete NA53VL#20 File count

January 19, 2024

↳ Tasks:

↳ complete NA56VL#16 File

↳ complete NA57VL#13 File

↳ complete NA52VL#15 File

January 24, 2024

↳ Class Notes:

Important dates:

↳ January 31: December/January logbooks

↳ February 12: Procedures Section Deadline

↳ give 1 week before due

↳ should be:

↳ brief, succinct, dry, bunch of sections on each part of experiment

↳ done in past tense (as if it's finished)

↳ not why it was done only how

↳ usually short and has subsections

↳ citations (data sets from someone else → source, data sets, name, manufacturer)

↳ kits (TM, city, country) ↳ equipment (TM, city, country)

↳ software (excel,)/apps/libraries

Tasks:

↳ complete all images in NASBVL#13 File

January 26, 2024

Tasks:

↳ follow up on email to Hannah

↳ ask about HSVL#4 File

↳ clarify next meeting date

↳ NASBVL#13 File Completed (all images)

January 28, 2024

Tasks:

↳ complete HSVL#4 File

↳ email Hannah = completed File

January 29, 2024

↳ Things to mention in Hannah meeting tomorrow:

↳ statistics ⇒ what parts for paper?

⇒ how long they take?

⇒ what program/software to make graphs?

⇒ what is ED1 mouse anti-rc⁺ CD68⁺ stand for?

Tasks: start writing Methodology (finish rough by Feb 3)

January 30, 2024

Class Notes:

↳ Methodology section

Full description

~~~~~  
~~~~~  
~~~~~  
~~~~~  
(Fig 1)

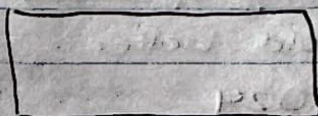
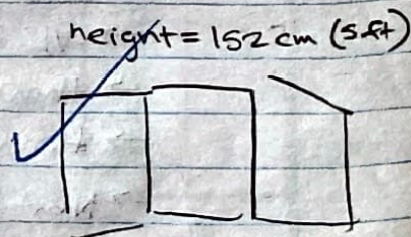


Fig 1. Title

Table 1

Table title	description



Tasks:

↳ Organize Methodology for meeting this afternoon

↳ Make sure logbook all good (due tmr)

↳ Organize Questions for Hannah

Questions for Meeting:

↳ Statistics tests ⇒ what's the plan?

↳ creating graphs ⇒ what program (excel)?

↳ methodology paper → should I add photo?

↳ ED1 mouse anti-rc⁺ CD68⁺ ?

↳ what's ED1 stand for?

↳ why does CD68⁺ have a plus?

none of antigen

Meeting w/ Hennich notes

- put in diagram (blue DAPI, red CD68, overlaid)
- as a figure 1 ↗
- ED1 mouse anti-rat CD68
 - ↳ rat ED1 antigen ⇒ expressed mainly cytoplasmic on most rat macrophages
- share methodology by Saturday Night
- she will send stats about MCP-1
 - ↳ possible explaining factor?
- brainstorm about soleus being higher for next meet
- read about T-tests
- fancy up the graphs
- she will send MCP-1 data
- VL and soleus graph (create)
- think abt presentation (coming up soon)
- Hennich thinks smaller tri-fold better
- Next Meeting: February 6, 2024

Tasks for me to complete by Feb 6 (Wed.)

- read/research T-tests
- send Methodology to Hennich (Sat. night (Feb) 3rd)
- Create new VL/soleus graphs
- Design HSP/Chow graphs

Jan 15
15

well done!

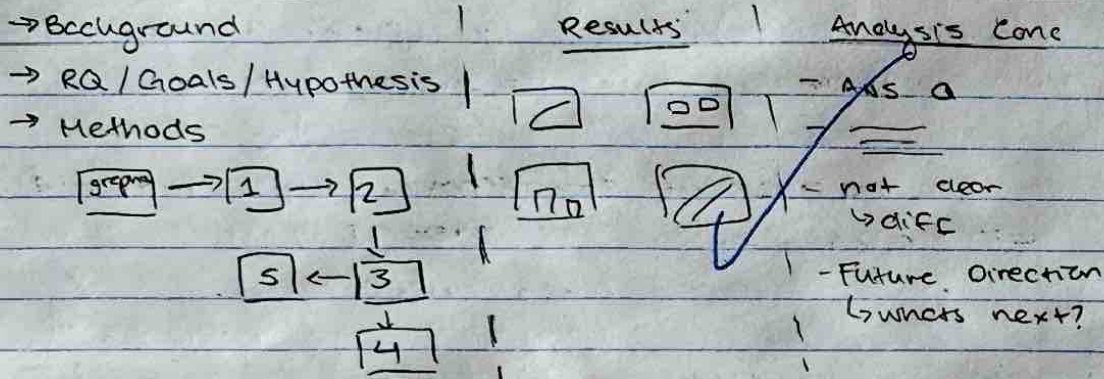
FEBRUARY 2024

Sun	Mon	Tue	Wed	Thu	Fri	Sat
28	29	30	31	1	2	3
<ul style="list-style-type: none"> → read about t-tests ↳ brain-storm ↳ finish graph 	<ul style="list-style-type: none"> ↳ create plan about possible explanations for Hennon discussion 	<ul style="list-style-type: none"> meeting w Dr. Garcia meeting w Hennon (4:15pm) 	<ul style="list-style-type: none"> ↳ work on graphs / explaining results (points) ↳ start on discussion points 	<ul style="list-style-type: none"> ↳ work on methodology (diagram) 	<ul style="list-style-type: none"> ↳ finish all graphs 	<ul style="list-style-type: none"> ↳ work on results / explanation (finish bullet points)
11	12	13	14	15	16	17
<ul style="list-style-type: none"> ↳ make final changes to methodology (Dr. Garcia edits) 	<ul style="list-style-type: none"> Methodology Section Due ↳ start google slide outline / plan 	<ul style="list-style-type: none"> meeting w Dr. Garcia ↳ trifold: background objectives, diagrams finish 	<ul style="list-style-type: none"> go over slideshow plan / outline meeting w Hennon (4:15 pm) 	<ul style="list-style-type: none"> ↳ make Hennon's edits to google slide (MCP-1) 	<ul style="list-style-type: none"> ↳ start trifold planning doc. 	<ul style="list-style-type: none"> ↳ start trifold planning doc.
18	19	20	21	22	23	24
<ul style="list-style-type: none"> ↳ slideshow (results all slides) ↳ finish trifold plan (doc) 	<ul style="list-style-type: none"> ↳ email CYSF ethics ↳ find citation ↳ email Hennon (VL vs chow) 	<ul style="list-style-type: none"> meeting w Dr. Garcia meeting w Hennon (4:15pm) ↳ send in trifold for printing 	<ul style="list-style-type: none"> work on graphs / explaining results (points) start on discussion points 	<ul style="list-style-type: none"> meeting w Hennon ↳ run through presentation / edits + reviews 	<ul style="list-style-type: none"> ↳ results panel / methods panel 	<ul style="list-style-type: none"> ↳ finish trifold ↳ send to Dr. Garcia + Hannah
25	26	27	28	29	1	2
		<ul style="list-style-type: none"> meeting w Hennon (4:15pm) ↳ send in trifold for printing 	<ul style="list-style-type: none"> Oral presentation 		<ul style="list-style-type: none"> ↳ get trifold from printing 	

February 4, 2024

Notes From Class:

Oral Presentation - Recommended Design



Presentation → 10-15 mins

Questions → 5 min

Participate 3/4 2/5

Tasks:

- ↳ read about t-tests (find vids/articles)
- ↳ finish HSF vs Chow graphs
- ↳ brainstorm results explanation

↳ Notes from T-test:

- ↳ tool evaluating the means using hypothesis testing
- ↳ define hypothesis, calculate test statistic ^{from} data and compare to theoretical from t-distribution
- ↳ depending on result → either accept or deny

↳ T-test assumptions:

- ↳ data continuous
- ↳ randomly sampled
- ↳ variability

↳ 3 types:

- ① one-sample t-test
- ② two-sample t-test → im doing this one
- ③ paired t-test

↳ purpose: determine if pop. mean from two diff groups are equal or not

February 5, 2024

Notes from t-test cont.:

Null: no diff between groups

Alternative: diff. exists

p-value: value resulting from t-test

↳ tells us how likely the difference b/w two groups could have happened by accident

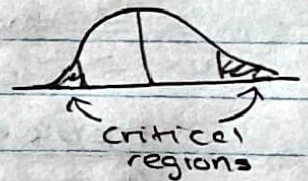
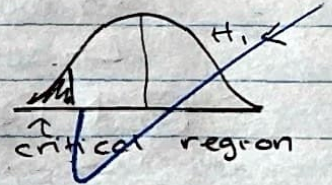
↳ if $p < 0.05$ → statistically significant diff. b/w

↳ if $p > 0.05$ two groups

↳ no sig. diff.

↳ one-tailed test ⇒ "increase" / "decrease"

↳ two-tailed test ⇒ "change / some/diff"



Possible reasons for soleus results (brainstorm)

↳ Problem 1: Soleus macrophage > VL macrophage (both diets)

① ↳ technical problem ⇒ ASK ABOUT FIELD OF VIEW

② ↳ ↑ passive delivery of M. to soleus vs VL through blood supply

soleus

↳ slow oxidative

↳ richer capillary supply (↑ capillary density, more tortuous)

↳ ∴ ↑ vesicular capillaries ⇒ result ↑ delivery

↳ idon't know if relevant ⇒ ↑ mitochondria / myoglobin

↳ ↑ vesicular endothelial growth factor

VL

↳ glycolytic / larger muscle diameter

↳ ↓ capillary (less delivery?)

↳ ↓ mitochondria / ↓ myoglobin

∴ ↑ vesicular bed in soleus means ↑ delivery of blood and cellular elements (macrophage)

③ Diff. in muscle activity?

- ↳ soleus \Rightarrow used more chronic stimulated muscle \Rightarrow \uparrow chronic activity \therefore \uparrow delivery macrophage
- ↳ VL (short/sudden) vs soleus (more regularly)
- ↳ soleus used more \Rightarrow higher demand \rightarrow more macrophages?

④ \uparrow active recruitment of macrophages

- ↳ more MCP-1 \Rightarrow calling more macrophages
- ↳ some other unknown cellular mediator?

⑤ \uparrow lifespan of macrophage in soleus

- ↳ since it's oxidative, maybe cells have more access to oxygen and can \therefore live longer \Rightarrow \therefore \uparrow macrophage count?

Problem 2: No diff. in diet

① paper saying \rightarrow male rats gained weight more rapidly

- ↳ took females 3 weeks to reach that same weight

↳ male rats overconsumed (then compared to female)

\therefore some hormone/metabolic diff sep. female from male?

Things to bring up in meeting:

↳ poster (template? / printing out)

↳ How to change colour on graphs?

↳ go over one-tail / two-tail stats?

↳ methodology?

↳ go over reasons for results?

↳ oral presentation \Rightarrow next time?

Feb 6, 2024

Notes from meeting w Hannah:

- went over methodology
 - ↳ sent new DAPI image (brighter blue)
- Hannah found paper
 - ↳ slow-twitch muscles \Rightarrow higher inflammatory response
 - ↳ capillary density in soleus
 - ↳ VL: fast-twitch (don't need oxygen \Rightarrow less capillaries)
- she will send poster trifold template
- Deadline for poster: February 26, 2024
- MCP-1 no diff \Rightarrow no diff in HSE vs Chow
- the MCP-1 collected through blood serum (systemic)
 - ↳ not specific to one muscle
 - ↳ possible future expense \Rightarrow MCP-1 on 1 muscle
- oral presentation \Rightarrow next Thursday (powerpoint completed)
- work on poster / trifold \Rightarrow following weeks
- diff colours not needed for graphs
- bold graphs (see more)
- overlaid images \Rightarrow command in Image J

Feb. 7, 2024

Tasks:

- Work on graphs (comparing muscles) \rightarrow finish by 9th
- Work on creating points explaining results

Feb. 8, 2024

- Finish Drzt Methodology

Feb. 9, 2024

- Finish (comparing muscle graph)
- All graphs now completed

Feb. 10, 2024

- ☑ Finish creating bullet points exploring the results + graphs

Feb. 12, 2024

- ☑ Make final edits to methodology
 - ↳ changes from Dr. Garcia

Feb. 13, 2024

- ↳ Turn in Methodology ☑
- ☑ Start Outline for slide show
 - ↳ plan organization
 - ↳ add citations in the plan
- ☑ email Hannah about paper talked about before (slow-twitch vs fast-twitch)

Feb. 15, 2024

Things to mention in meeting:

- ↳ go over slideshow outline / plan
- ↳ trifold template
- ↳ template on power point?
- ↳ paper: Hannah discussed before
 - ↳ slow-twitch having higher inflammatory responses

Notes from meetings:

→ make the title shorter (slide 1)

→ make photo less blurry

→ Hannah will think about graph

↳ VL vs chow

↳ need to add error bars

→ slide 1: lack of fibrosis

→ slide 2: MCP-1 levels (systemic)

→ cite capillary paper

↳ APA

↳ Meeting next Thursday (Feb. 22)

↳ Complete before next meeting:

• make changes to slideshow for presenting next meeting

• start on outline trifold (powerpoint)

↳ she will send the trifold template

Feb. 16, 2024

Tasks:

☑ Make Hannah's changes to the slideshow

↳ ask her about the MCP-1 slide vs the fibrosis slide?

Feb. 17, 2024

Tasks:

☑ start outline / organizing points / picking diagrams for the trifold → due. 24th

February 18, 2024

Tasks:

- ☑ Practice presentation (google slides)
- ☑ Finish outline for the trifold
 - ↳ have all points organized on google doc
 - ↳ have all diagrams picked out
 - ↳ have all citations completed + organized

February 19, 2024

Tasks:

- ☑ Email Hennch to ask about poster organization
- ☑ Email ethics CYSF portal
 - ↳ ask if there is a problem w form?
- ☑ Email Hennch about stats on (VL vs chow)

Feb. 20, 2024

Tasks:

- ☑ Work on trifold organization → due 24th
 - ☑ Meeting w Dr. Garcia
- background/objectives/diagrams

Notes from Meetings:

- ↳ went over graphs
- ↳ meeting to go over google slides → on Tues. 27, 2024
- ↳ make sure trifold on time
- ↳ okay to have Hennch graph as long as cite/give her recognition

Feb. 22, 2024

Things to mention in meeting:

- ↳ cite capillary paper
- ↳ slide on fibrosis / slide on MCP-1 \Rightarrow limit on slide number so might keep together
- ↳ trifold \rightarrow not ready but working on outline
- ↳ 10-15 minute presentation
- ↳ Do I have to add error bars on VL vs Chow?
- ↳ Cite paper on slow-twitch having higher inflammatory response?
- ↳ Will presentation for trifold be similar to google slides?
- ↳ Can I send trifold sometime on Saturday? \rightarrow also send to Dr. Garcia
- ↳ I have to put variables \rightarrow do I have to put confounding on poster?
- ↳ what should I put for p-values?

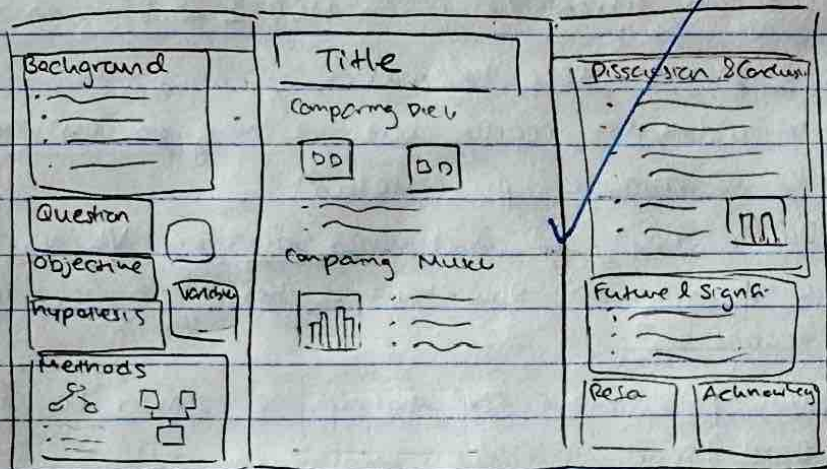
Notes From Meeting:

↳ Advice for Slideshow:

- ↳ first-part good
- ↳ she will re-send DAPI image
- ↳ change confocal to fluorescent microscope
- ↳ put ~~curve~~ over graph VL vs chow
- ↳ trifold say the exact same thing
- ↳ send trifold as soon as done
- ↳ make sure to put variables on trifold
- ↳ put CD68 + DAPI \rightarrow with arrow to overlaid
- ↳ image with macrophages actually circled
- ↳ Next meeting: Tuesday (4:15 pm)
- ↳ email Honnch Sunday night if hasn't sent yet (DAPI + paper)

Feb. 23, 2024

→ Outline / Plan Trifold



Tasks:

- ☐ Continue organizing/editing trifold
- ↳ results panel / methods

Feb. 24, 2023

Tasks:

- ☐ Finish trifold → significance panel
- ☐ Send to Hannah + Dr. Garcia

Feb. 25, 2023

Brynn Presentation

- ↳ Genetic Diff B/W Endangered + Non-Endangered Pine Species
- ↳ ↑ CO₂, ↑ Heat, ↓ H₂O
- ↳ Synthetic Biology
- ↳ why temp only white-bark
- ↳ Lobby for control

Jessica Presentation

- ↳ ☐ In future investigation side, behavioral study → what would that look like
- ↳ CRISPR/Cas-9 → knock out plasmids on autism risk genes

Owen's Presentation

- ↳ Electrocardio reading
- ↳ Time Domain / frequency domain

Cooper's Presentation

- ↳ RmTBI's effect on brain proteins
- ↳ Synaptic Pruning → destruction of weak synapses
- ↳ ↑ Alzheimers + other NDDs (neurodegenerative diseases)
- ↳ causes inflammatory immune response (RmTBI's)

Feb. 27, 2024

Things to mention:

- ↳ how would local muscle MCP-1 be measured?
- ↳ extraction
- ↳ should eat HSF → no effect → why IMF but no fibrosis or inflammation or time?
- ↳ has systemic been measured in males?
- ↳ time → IMF affecting with not a macrophage?
- ↳ diet longer

Notes from meeting w Hannah:

- ↳ Intro might be too wordy
- ↳ put an asterix on top of comparing muscle bars
- ↳ Local-muscle MCP-1 levels ⇒ MRNA analysis
 - ↳ MRNA → in between PNA + protein
 - ↳ look at tissue and MRNA → tell if there's proteins there
- ↳ IMF ⇒ her hypothesis is because rats not on diet long enough ⇒ more fibrosis if longer b/cuz
- ↳ Fibrosis limiting contractile → decreasing volume of healthy muscles
- ↳ Overall → asterix, numbers bigger on graphs

Tasks:

- ↳ Make Final Edits to Poster
- ↳ Send Poster in for Printing

Feb. 28

↳ Oral presentation



Elliot's Presentation

DQ: Why use the BRBT Ret model? Is there a significance?

15 / 15 Feb

MARCH 2024

Sun	Mon	Tue	Wed	Thu	Fri	Sat
25	26	27	28	29	1	2
3	4	5	6	7	8	9
↳ write date/ conclusions/ citations / acknowledg	science Fair	↳ write out 5 SF notes (recommendations) ↳ email Hannah	meeting w Hannah ↳ edit rough draft of results	↳ start results section (diet results)	↳ pick up trifold	↳ practice presentation ↳ read papers
10	11	12	13	14	15	16
17	18	19	20	21	22	23
24	25	26	27	28	29	30
31						

Portals due ifc + sk

Spring breed starts →

March 1, 2024

Tasks

☑ Print / Pick up tri-fold and stick it together

March 2, 2024

Tasks:

☑ Practice presentation

☑ Review old papers / notes

March 5, 2024

Tasks:

☑ Email Hannah (SF + meeting + results)

☑ Write out SF notes (recommendations)

Science Fair Notes / Reflection / Recommendations

↳ Recommendation about adding scales on immunofluorescence images

↳ Spelt levels wrong in MCP-1 graph

↳ emphasize what is known + compare w/ data to strengthen conclusions

↳ look into stats \Rightarrow error bars

\Rightarrow why bars diff length

\Rightarrow emphasize type of stat used (t-test)

↳ know all acronyms (MCP-1)

↳ think more about female hormones + inflammation

↳ do females have an M1 skew?

↳ spend more time explaining the formation of the problem + how can have broad impact on society beyond obesity

March 7, 2024

Tasks:

- start results section
↳ diet results + graphs

March 9, 2024

Tasks:

- write problem/method/research for CYSF portal +
save it on portal

March 10, 2024

Tasks:

- write out data/conclusions/citations/acknowledgements
for the CYSF portal

March 11, 2024

Notes:

Term 3 Grades

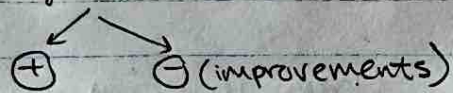
Org/Com. (20%)	Logbook (20%)	O.P (30%)	W.A. (30%)
BWR → 6 MEval → 3 10% 15- MEval 15- BWR	→ 15 → 10% → 3 left	SF / 2 left / 30 ↓ 100 → 20% (50%) June 4th	R/D/E 10% / 20% / 50%

OP (30%)

- SF (20%)
- gold → 100
- silver → 95
- bronze → 90

Logbook

reflection → Judges comments



Tasks:

☑ Work on results paper (muscle graphs / results)

☑ Meet w Dr. Garcia

Meet w Dr. Garcia notes

↳ Create presentation

↳ upload things in C4SF

↳ know more stats

↳ know more about physiology of muscles

↳

Reference #1) *The Anatomy and Physiology: The Easy Way* ✓

- Chapter 8: Muscle Physiology
- Muscle tissue is one of the four basic tissues (distinguished by contraction and mechanical work)
- Structural Unit: the muscle cell/muscle fiber (elongated shape)
- Skeletal muscle connected to bones/ components of organs in digestive/respiratory tracks/ make up blood vessels
- 3 types of muscle: smooth muscle, cardiac muscle, skeletal muscle
- Smooth (unstriated) muscle → mass of individual spindle-shaped contractile cells.
 - Found in walls of digestive tract/uterus/linings of blood vessels/certain ducts
- Cardiac muscle → composed of cells but has much more organization of the contracting proteins than in smooth muscle cells
 - Special linkages in the walls of the heart

*Great notes
Just add
dates!*

Characteristic	Skeletal Muscle	Smooth Muscle	Cardiac Muscle
Location	Attached to skeleton	Walls of intestines, blood vessels, etc.	Walls of heart
Type of Control	Voluntary	Involuntary	Involuntary
Shape of Fibers	Elongated cylindrical/ blunt ends	Elongated, spindle-shaped, pointed ends	Elongated, cylindrical fibers that branch
Striations	Present	Absent	Present
Number of nuclei/fiber	Many	One ✓	One or two
Position of nuclei in cell	Prepheral	Central	Central
Speed of contraction	Most rapid	Slowest	Intermediate
Ability to remain contracted	Least	Greatest	Intermediate

Skeletal Muscle

- Ability to exert force, but only in one direction.
- Muscle cells contract by an active mechanism and relax by a passive mechanism: contractions only occur when stimulation is provided.
- The complex patterns of movement for locomotion require that two sets of muscles move body parts in opposite directions. Muscles then work against each other and are said to be

antagonistic. Example) leg joint at the patella (small bone at the front of the knee) is bent by flexor muscles in the posterior (back side) and straightened out by extensor muscles in the anterior (front) aspect. → the muscle contraction moves parts of the skeleton to which they are attached.

Skeletal Muscle Tissue Structure

- Muscle cells are separated and wrapped in layers of connective tissues.
- Endomysium wraps individual muscle fibers, perimysium wraps a bundle of fibers (or fascicles), and then the epimysium and fascia enclose the entire muscle.
- Outer layer of the fascia (the superficial fascia) contains much fat in obese individuals. The portion of the muscle with fibers is called the gaster (belly).
- The endomysium, perimysium, epimysium, and face all extend beyond the gaster of the muscle to form the tendon, which attaches the muscle to bone.

Muscle Cell Structure

- The skeletal muscle of the body is under voluntary control: usually it contracts only when stimulated by neurons that deliver nerve impulses to it.
- It is composed of bundles of muscle fibers (each fiber has a set of 4 to 20 rodlike filaments known as myofibrils)
 - each myofibril is about one or two micrometers wide
 - up to 100 micrometers in length
- The myofibrils are bathed in cytoplasm → sarcoplasm in muscle fibers
- Numerous mitochondria scattered throughout the sarcoplasm and they provide ATP as an energy source
- Myofibrils are organized along their length into smaller units called sarcomeres (about 2 micrometers in length)
- Sarcomeres → “functional unit” of skeletal muscle (the repetition of sarcomeres within the muscle fiber gives the muscle its characteristic striated pattern).
- Two types of myofilaments: thin filaments and thick filaments (run parallel to each other)
- Thick filaments are composed of the protein myosin, while the thin filaments contain the protein actin.
- The point where actin filaments from adjacent sarcomeres interweave is a line called the z-line.
- Z-line bisects a broad stripe called the I band.
- The large dense stripe in the center of the sarcomere formed by the overlapping myosin filaments in the A band.
- A band and I bands account for the striation pattern in the myofibrils of striated muscle
- Thin filaments (actin filaments) anchored to the z-line (opposing actin filaments are pulled along myosin).
- Movement Main Two Rules:
 - 1) Proteins tend to change shape when molecules bind to them
 - 2) Changing shapes can allow proteins to bind or unbind with other molecules

- The Sliding Filament Model
 - Research indicates that the contraction of muscle fibers occurs when thin filaments are pulled towards one another in the muscle fiber.
 - Molecules of myosin are composed of two polypeptide chain (single linear chain of amino acids)
 - Each myosin filament is surrounded by thin actin filaments so that the protruding myosin head and the actin filaments come into contact with each other when the muscle contracts.
- Filament Model Steps:
 - Actin wants to move towards myosin but is prohibited due to tropomyosin and troponin
 - Sarcoplasmic reticulum loaded with calcium pumps (move ATP and calcium)
 - Receptors on the muscle cells cause sodium to go into the cell, opening the sodium channels in the muscle open
 - Actin potential goes to transverse tubules
 - Calcium stores in the transverse tubules is then released
 - Troponin binds with the calcium, causing the protein to shift and change shape, pulling the tropomyosin away
 - ADP attached to the myosin → stretched position
 - Myosin binds to the actin
 - Myosin releases stored energy and retracts the muscle
 - ADP and phosphate then unbind with the myosin and ATP binds with myosin
 - Myosin with the ATP release from actin
 - Myosin breaks down the ATP into ADP/phosphate
 - Tropomyosin goes back into place

Reference #2) "Reflections on obesity, exercise, and musculoskeletal health" (Walter Herzog, Published 2020)

- Experiment → (HFS (high fat and sucrose) diet on mice to induce obesity, added wheel running (aerobic exercise) in order to study the effects of diet and exercise on knee arthritis
- They found little diet and exercise effects on knee osteoarthritis but did find distinct differences in metabolic and inflammation of mice exposed to normal diet and HFS diet as well as mice exposed to aerobic exercise and not exposed to exercise
- Argued that there might be metabolic and inflammation profiles that can be used as bio-markers for risks such as knee osteoarthritis
- Age of exposure rather than the duration of exposure was crucial factor
- Rats exposed to HFS diet in childhood seemed able to cope better than rats raised on normal diet

Reference #3) "Skeletal Muscle: A review of molecular structure and function, in health and diseases" (Kavitha Mukund, Shankar Subramaniam, July 3 2019)

- Skeletal muscle → biomechanical device → interacting components:
 - 1) the autonomic nerves for impulse transmission
 - 2) vasculature for efficient oxygenation
 - 3) embedded regulatory and metabolic machinery
 - 4) maintaining cellular homeostasis.
- A new type of muscle research allows researchers to discern tiny details of molecular cross-talk required for effective coordination between the myriad interacting components for efficient muscle function.

Reference #4) "A High-Fat High-Sucrose Diet Rapidly Alters Muscle Integrity, Inflammation, and Gut Microbiota in Male Rats" (Walter Herzog, 17 November 2016)

- Obesity is also associated with Muscle wasting which is a process that includes
- intramuscular fat deposition and fibrosis.
- Low-level inflammation associated with obesity is proven to harmfully affect muscle composition.
- How obesity leads to muscle loss has not been explored in detail
- The goal of the paper: evaluate the relationships between muscle integrity, diet, systematic inflammatory mediators, adipose tissue, and gut microbiota in male Sprague-Dawley rats.
- Systematic inflammatory mediators → Central to the formation of inflammation
 - messenger that acts on blood vessels and/or cells to promote an inflammatory response
- Intramuscular fat, fibrosis, number of pro-inflammatory cells increased by 3 days and was sustained across 28 days of high fat high sugar feeding compared to control diet animals
- Understand systemic contributors to muscle damage → dynamic changes in gut microbiota and serum inflammatory markers were evaluated
- Gut microbiota: is the system of microorganisms in a person's gastrointestinal system
- Serum inflammatory: markers of inflammation in serum form**
- Data linked metabolic challenge
- Data will provide framework linking changes in obesity and muscle integrity
- Animals fed HFS (7 days) had more body fat
- Animals fed HFS (14 and 28) had more body fat and body mass
- Dynamic alterations in visceral adipose tissue levels were clear
- Similar levels of blood glucose and insulin were detected between all groups
- TNF - alpha : an anti-inflammatory cytokine protein (major regulator of inflammatory response)
- IL - 6 : an anti-inflammatory cytokine protein
- TNF - alpha and IL - 6 both increased with the HFS diet as early as 3 days

- Leptin levels increased in HFS animals
- Leptin: hormone in your adipose tissue → used to maintain normal weight (regulates hunger by producing satiety)
- Associations between outcomes suggest integrated and dynamic biological shift leading to compromised muscle integrity
- Discussion:
- Muscle integrity is important in obesity, but development of compromised muscle integrity is poorly understood
- Link exists between HFS metabolic challenge and increases in intramuscular fat, fibrosis, and CD68+ inflammatory cell number in the VL muscle
- Perturbation: disturbance in motion that increases the chance of muscle degeneration
- Competition of perturbations → leads to failure
- This failure initiates the process that leads to obesity and compromised muscle integrity
- Abnormal muscle repair = sustained muscle fibrosis (interferes with healing)
- Myocellular → relating to the muscle cells
- Suggests that inflammation resulting from increased IL-6 and TNF- α (due to diet induced changes in gut microbial composition) recruit pro-inflammatory macrophages to muscle tissue → promotes infiltration of macrophages in the tissue
- Increased CD68 cells (macrophages) and MCP-1 in affected muscle at 28 days in male rats fed HSF diet. These findings correlated with muscle inflammation and fibrosis

Reference #5) "CD68/macrosialin: not just a histochemical marker" (Dimitry A Chistiakov, January 2019)

- Is heavily glycosylated glycoprotein that is highly expressed in macrophages and other mononuclear phagocytes
- Glycosylated glycoprotein: the process by which carbohydrate components are covalently added to proteins to form glycoproteins
- mononuclear phagocytes → a family of cells comprising bone marrow progenitors, blood monocytes and tissue macrophages. Macrophages are a major cell population in most of the tissues in the body, and their numbers increase further in inflammation, wounding and malignancy.
- CD68 is exploited as a valuable cytochemical marker to immunostain macrophages in the histochemical analysis of inflamed tissues or tumor tissues (other too)
- Cytochemical = the study of the chemical constituents of cells by selective staining of cell parts
- Histochemical = identification and distribution of the chemical constituents of tissues
- CD68 is mainly located in the endosomal/lysosomal compartment but can rapidly shuttle to the cell surface
- CD68 not involved in binding bacterial/viral pathogens

- Pathogen = bacteria or virus

Reference #6: "High Fat With High Sucrose Diet Leads to Obesity and Induces Myodegeneration" (Suhail Rasool, September 5, 2018)

- with HFS feeding is detrimental because high rates of FA oxidation in skeletal muscle can lead to the buildup of toxic metabolites of fat metabolism and the accumulation of pro-inflammatory cytokines, which further exacerbate the insulin resistance

Reference #7: "Macrophage Cytokines: Involvement in Immunity and Infectious Diseases" (Guillermo Arango Duque, October 7, 2014)

- the activity of macrophages can be increased by cytokines secreted by helper T cells, with interferon-gamma (IFN- γ) being one of the most potent macrophage activators
- evolution of macrophages has made them primordial for both development and immunity
- cytokines bias the fate of macrophages into a spectrum of inflammation-promoting "classically activated," to anti-inflammatory or "alternatively activated" macrophages
- Macrophages release cytokines
- Macrophages are phagocytic cells of the innate immune system that are located in various tissues.
- The main function of macrophages is to engulf foreign agents that enter the body.
- they eliminate apoptotic cells and recycle nutrients by digesting waste products from tissues
- Macrophages are hence essential not only for immunity but also for development and tissue homeostasis
- macrophages play a central role in inflammation.
- Cytokines and chemokines are potent signaling molecules
- molecules orchestrate a variety of processes ranging from the regulation of local and systemic inflammation to cellular proliferation, metabolism, chemotaxis, and tissue repair.
- the primary function of cytokines is to regulate inflammation, and as such, play a vital role in regulating the immune response in health and disease. There are proinflammatory and anti-inflammatory cytokines.

Reference #8: "Intramuscular fat infiltration, but not fibrosis, increases in rat vastus lateralis and soleus muscle in a diet-induced obesity model" (Hannah Smith, September 2023)

- Obesity and metabolic syndrome are risk factors in the development of sarcopenia or muscle wasting
- As muscle mass is a key indicator of long-term health, it is important to understand the relationship between diet and muscle integrity

- In previous work → demonstrated that HFS diet induces obesity + low-level systemic inflammation in ale Sprague-Dawley rats → led to increases in fat infiltration and fibrosis in more glycolytic vastus lateralis muscle
- However, the oxidative capacity of the soleus muscle seemed to project against intramuscular fat infiltration and fibrosis
- Objective: determine whether a 3-week delayed prebiotic fiber intervention activities of modified fat accumulation and fibrosis in the VL and soleus muscles of female Sprague-Dawley rats exposed to an HFS diet
- Hypothesize that there will be increased intramuscular fat infiltration and collagen content in the VL muscle but not the soleus muscle
- The HFS group was found to have more IMF (intramuscular fat) than the chow-fed control group for both the VL and soleus → however the collagen content had no difference across groups in both muscles
- Based on these results → concluded that female Sprague-Dawley rats were susceptible to IMF infiltration in both VL and soleus muscles
- However, neither VL nor the soleus muscle increased in fibrosis.
- Previous studies have shown a link between increases in IMF and fibrosis in that intramuscular lipid content in skeletal muscles induces local inflammation which signals the muscle repair process → leading to increased fibrosis and compromised structural integrity
- Next step → to quantify the inflammation in the local environment to see if this can explain the lack of increased fibrosis in the female muscles

Reference #9: "Skeletal muscle inflammation and insulin resistance in obesity" (Huaizhu Wu, January 3, 2017)

- Obesity has many health effects. This leads to insulin resistance and Type II Diabetes,
- The Link between HSF diet and muscle wasting may relate to chronic inflammation. A similar process has been studied in adipose tissue which exhibits chronic inflammation in response to HSF diet and obesity.
- For example, studies looking at insulin resistance and obesity show inflammation in fat cells may contribute to whole-body insulin resistance due to the release of inflammatory molecules. These same inflammatory molecules can affect skeletal muscle (SM)
- Since SM is one of the most important organs for glucose uptake and disposal, the normal functioning of muscle is crucial to maintain glucose metabolism. Chronic inflammation and fibrosis in muscle secondary to an HSF diet and obesity results in impaired muscle glucose metabolism and reduced insulin sensitivity.
- Understanding the process of muscle inflammation and fibrosis may be better understood in the context of similar changes in adipose tissue, which has been more extensively studied.

- Studies show that Fat cells become more numerous and swell with intracellular lipids in HSF diets/obesity. This can result in cellular hypoxia and death and result in the release in inflammatory molecules (cytokines). These cytokines are numerous but generally cause inflammation, angiogenesis, and recruitment of additional immune cells.
- This can result in inflammation fibrosis and metabolic dysfunction/insulin resistance in fat cells. HFS diet may similarly affect muscle cells. Depends first on the type of muscle.
- How does inflammation occur? Evidence shows that, like adipose tissue, muscle cells (myocytes) also secrete cytokines (myokines). Normally this is in response to exercise and activity and the molecules that are released generally have a positive effect on glucose metabolism and muscle repair/healing.
 - IL6 for example – is acutely released 100X by exercise improves glucose uptake and insulin sensitivity; increases lipolysis (fat breakdown), and reduces inflammation (by increasing anti-inflammatory IL10 and reducing inflammatory TNF-). However, if chronically increased can result in increased inflammation
 - There are a large number of cytokines with various inflammatory and anti-inflammatory effects, all of which contribute to the state of inflammation of the muscle
- List additional myokines: IL6, IL8, IL15, TNF-alpha, GRO, MCP-1, RANTES
- These may be released by myocytes depending upon stimulation from other inflammatory cytokines (that may be released from muscle or fat tissue) and by free fatty acids (FFA)
- Complex process
- These molecules help to regulate the inflammatory process, but they are only one part of the inflammatory cascade.
- Studies of adipose tissue suggest more important factor in inflammation is immune cell infiltration. Growing evidence suggests immune cell infiltration is also the primary factor in muscle inflammation and fibrosis.
- Increased macrophages found in muscle cells of obese subjects with insulin resistance, and in short-term studies in which subjects were fed an HFS diet
- In obesity and HSF diets, excess calories above metabolic requirements results in fat deposition and hypertrophy, including in and around muscle as described above
 - Adipose tissue cells may die and start a cascade of cytokines and molecules. One important molecule is MCP-1 which recruits macrophages into the damaged Tissue.
 - Affected muscles act similarly - releasing cytokines that increase inflammation and recruit macrophages
 - As a result, there is an increase in the NUMBER of Macrophages within the muscle tissue. Additionally, the TYPE of macrophages shifts into proinflammatory phenotype

Reference #10: "Adipose Tissue Macrophage in obesity-associated metabolic diseases"
(Jingfei Yao, September 2, 2022)

- Macrophages are heterogeneous populations of immune cells that function in tissue-specific immune responses. Specific types include Kupfer cells in the liver, alveolar macrophages in the lungs, and adipose macrophages in fat.
- Important to understand that there are 2 types of activated macrophages identified in fat and muscle tissue (named M1 and M2). Depending on local environmental cues, macrophages polarize into one of two types:
 - In normal situations (lean mice) – the main population is anti-inflammatory M2 cells which produce anti-inflammatory cytokines (IL10 and TGF-beta). These resolve tissue inflammation promote tissue homeostasis and repair
 - In mice fed the HSF diet and obese mice, increased recruitment of proinflammatory M1 cells. This shift to M1 cells occurs as fat cells and activated macrophages release various cytokines (IFN gamma and TNF alpha) and Monocyte Chemoattractant Protein 1 (MCP-1).
 - MCP 1 attracts monocytes and macrophages and induces them to proinflammatory M1 type. M1 cells demonstrate increased phagocytic activity to remove dead or damaged cells and also generate additional inflammatory cytokines. These cells thus recruit additional macrophages which polarize to M1 features, significantly increasing the M1 population
 - Cytokines activate fibroblasts and alter collagen dynamics. This process of healing and repair can become deranged and lead to fibrosis and abnormal function
- This leads to a vicious spiral of inflammation that interferes with normal homeostasis and tissue repair
- In a laboratory model, these M1 cells show enhanced expression of certain cell markers including CD68+

Reference #11: "Turning Macrophage Phenotype to Mitigate Skeletal Muscle Fibrosis"
(David M Stepien, April 15, 2020)

- Macrophages Have a major role in both muscle regeneration/healing and fibrosis. Their job is to clear necrotic (dead) muscle cells and promote stromal remodeling and regeneration of myocytes (muscle cells).

Muscle Extraction:

Twenty-four female Sprague-Dawley rats at 12 weeks of age were randomly assigned into two different experimental groups: 1) animals fed a chow diet (n = 12) and 2) animals fed a high fat/high sucrose (HFS) diet (n = 12). Each experiential group contained 12 rats. The rats' diets were maintained for a period of twelve weeks. At twenty-four weeks of age, the rats were sacrificed utilizing ethical techniques by qualified lab members, and their vastus lateralis (VL) and soleus muscles were harvested and flash-frozen in liquid nitrogen. A mid-belly muscle section was cut, mounted in an Optimal Cutting Temperature (OCT) compound, and sectioned at 10 μ m using a Lecia cryostat at -20°C. The muscle sections were then dried at room temperature and stored at -20°C until used. CD68⁺ immunohistochemistry was completed on the muscle sections.

Immunohistochemistry:

Muscle sections were mounted on slides. Slides were first fixed in 100% cold acetone. These slides containing muscle sections were briefly blocked, first with 3% hydrogen peroxide, and then with 2.5% normal horse serum (Vector Laboratories, Burlingame, CA). The sections were then incubated overnight at 4°C with the primary antibody, ED1 mouse anti-rat CD68⁺ (AbD Serotec, Raleigh, NC), a marker for inflammatory macrophages at a dilution ratio of 1:100. The secondary antibody, anti-mouse IgG peroxidase (IMPRESS kit, Vector Laboratories) was applied the next morning for 30 minutes at room temperature. Slides containing muscle sections were then incubated for 20 minutes in a Cyanine 3 fluorophore (cy3) (PerkinElmer, Waltham MA) which was prepared in a Tyramide Signal Amplification (TSA) buffer. To visualize nuclei, DAPI (4',6-diamidino-2-phenylindole fluorescent stain)(Vector Laboratories) was applied, and sections were mounted in Vectashield mounting media (Vector Laboratories). Ten images per

soleus cross-section and 20 images per VL cross-section were obtained using an Olympus BX51 fluorescent microscope (Olympus, Japan), with appropriate filters for cy3 and DAPI using an Olympus DP28 camera and Cell Sens software at 200x magnification.

Image Analysis:

To fulfill the objectives of this project, the macrophages identified by the CD68 marker were counted per image frame using the program ImageJ (ImageJ, U.S. National Institutes of Health 2018). The first step is to take images marked with the DAPI (4',6-diamidino-2-phenylindole fluorescent stain) marker indicating nuclei (Figure 1A), and overlap them with the CD68 marker indicating inflammatory macrophages (Figure 1B). These overlapped images were then evaluated with the ImageJ program (ImageJ, U.S. National Institutes of Health 2018) to assess for points where the CD68 marker (red/yellow) lines up with the DAPI marker (blue) (Figure 1C). These coinciding points indicate a nucleus of an inflammatory macrophage. The inflammatory macrophage counts for each image were stored in an Excel (Microsoft Excel, 2021) sheet sorted by image number. Once all images were counted, a non-parametric statistical test (t-test) to compare group means was used to determine any difference in macrophage infiltration between the chow and HFS experimental groups for both the VL and soleus muscles. If this t-test resulted in a p-value greater than 0.05 ($p > 0.05$), then there was no significant difference between the compared groups' means. If the t-test resulted in a p-value less than 0.05 ($p < 0.05$), then there was a significant difference between the compared groups' means.

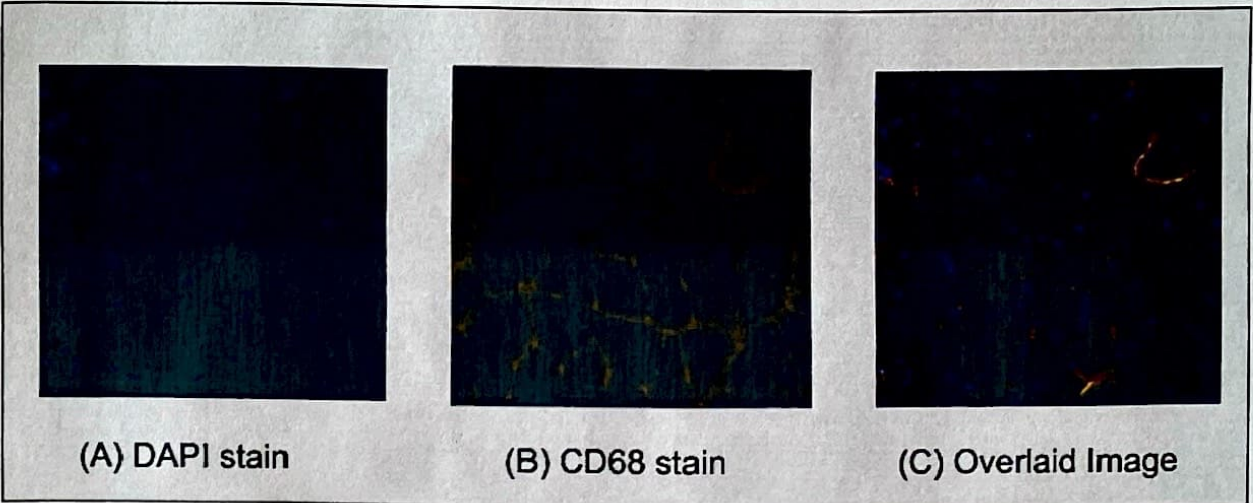


Figure 1. Comparison of Stained Immunohistochemistry Images of Soleus Muscle. (A) image using DAPI stain which indicates viable nuclei with royal blue fluorescence. (B) image using CD68 stain which indicates inflammatory M1 macrophages with yellow/red fluorescence. (C) overlaid image of (A) and (B) showing matching points of DAPI and CD68 fluorescence. These matching points indicate the localization of inflammatory M1 macrophages.

lots of work!

HS7Sol#15	Macrophage Count
Image1798_1799	58
Image1800_1801	59
Image1802_1803	42
Image1804_1805	38
Image1806_1807	26
Image1808_1809	47
Image1810_1811	47
Image1812_1813	46
Image1814_1815	49

HS8Sol#15	Macrophage Count
Image1913_1914	38
Image1915_1916	40
Image1917_1918	27
Image1919_1920	36
Image1921_1922	39
Image1932_1924	46
Image1925_1926	37
Image1927_1928	63
Image1929_1930	64
Image1931_1932	46
Image1933_1934	52

HS10Sol#16	Macrophage Count
Image1889_1890	62
Image1891_1892	62
Image1893_1894	41
Image1895_1896	56
Image1897_1898	37
Image1899_1900	53
Image1901_1902	47
Image1903_1904	49
Image1905_1906	53
Image1907_1908	63
Image1909_1910	41
Image1911_1912	51

HS11Sol#15	Macrophage Count
Image1370_1371	34
Image1372_1373	41
Image1374_1375	46
Image1376_1377	44
Image1378_1379	44

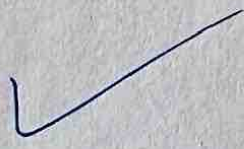


Image1380_1381	53
Image1382_1383	32
Image1384_1385	42
Image1386_1387	55
Image1388_1389	52

HS12Sol#15	Macrophage Count
Image1499_1500	51
Image1501_1502	59
Image1504_1505	21
Image1506_1507	27
Image1508_1509	54
Image1510_1511	48
Image1512_1513	55
Image1514_1515	66
Image1516_1517	51

HS19Sol#15	Macrophage Count
Image1762_1736	50
Image1764_1765	42
Image1766_1767	43
Image1768_17869	42
Image1770_1771	50
Image1772_1773	51
Image1774_1775	52
Image1776_1777	46
Image1778_1779	52
Image 1780_1781	42

NA51Sol#15	Macrophage Count
Image1728_1729	32
Image1730_1731	54
Image1732_1733	58
Image1734_1735	41
Image1736_1737	51
Image1738_1739	45
Image1740_1741	44


NA52Sol#15	Macrophage Count
Image1518_1519	34
Image1520_1521	34
Image1522_1523	39
Image1524_1525	40
Image1526_1527	34
Image1528_1529	33




Image1530_1531	28
Image1532_1533	37
Image1534_1535	48
Image1536_1537	47

NA53Sol#15	Macrophage Count
Image1628_1629	37
Image1630_1631	33
Image1632_1633	46
Image1634_1635	66
Image1636_1637	42
Image1638_1639	58
Image1640_1641	63
Image1642_1643	64
Image1644_1645	65

NA54Sol#15	Macrophage Count
Image1684_1685	50
Image1686_1687	37
Image1688_1689	32
Image1690_1691	31
Image1692_1693	52
Image1694_1695	37
Image1696_1697	52
Image1698_1699	54
Image1700_1701	66
Image1702_1703	47
Image1704_1705	43
Image1706_1707	54



NA56Sol#16	Macrophage Count
Image1646_1647	36
Image1648_1649	48
Image1650_1651	42
Image1652_1653	43
Image1654_1655	62
Image1656_1657	42
Image1658_1659	30
Image1660_1661	36
Image1662_1663	41



NA57Sol#15	Macrophage Count
Image1431_1432	54
Image1433_1434	51
Image1435_1436	66

Image1437_1438	69
Image1439_1440	64
Image1441_1442	63
Image1443_1444	63
Image1445_1446	64
Image1447_1448	62
Image1449_1450	53
Image1451_1452	64

HS7VL#15	Macrophage Count
Image2320_2321	26
Image2322_2323	8
Image2324_2325	26
Image2326_2327	28
Image2328_2329	14
Image2330_2331	30
Image2332_2333	29
Image2334_2335	30
Image2336_2337	31
Image2338_2339	29
Image2340_2341	13
Image2342_2343	29
Image2344_2345	17
Image2346_2347	24
Image2348_2349	33

HS8VL#14	Macrophage Count
Image2422_2423	44
Image2424_2425	40
Image2426_2427	43
Image2428_2429	47
Image2430_2431	42
Image2432_2433	43
Image2434_2435	36
Image2436_2437	34
Image2438_2439	44
Image2440_2441	42
Image2442_2443	35
Image2444_2445	37
Image2446_2447	42
Image2448_2449	10
Image2450_2451	31

HS9VL#16	Macrophage Count
Image2567_2568	32

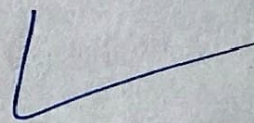


Image2569_2570	47
Image2571_2572	35
Image2573_2574	23
Image2575_2576	9
Image2577_2578	29
Image2579_2580	36
Image2581_2582	37
Image2583_2584	27
Image2585_2586	41
Image2587_2588	37
Image2589_2590	59
Image2591_2592	41
Image2593_2594	43
Image2595_2596	64
Image2597_2598	60

HS10VL#15	Macrophage Count
Image2453_2454	11
Image2455_2456	16
Image2457_2458	25
Image2459_2460	36
Image2461_2462	42
Image2463_2464	48
Image2465_2466	12
Image2467_2468	32
Image2469_2470	22
Image2471_2472	21
Image2473_2474	20
Image2475_2476	19
Image2477_2478	29
Image2479_2480	24
Image2481_2482	27

HS11VL#1	Macrophage Count
Image2487_2488	21
Image2489_2490	31
Image2491_2492	25
Image2493_2494	39
Image2495_2496	33
Image2497_2498	48
Image2499_2500	47
Image2501_2502	48
Image2503_2504	59
Image2505_2506	37
Image2507_2508	47

Image2509_2510	53
Image2511_2512	56

NA51VL#2	Macrophage Count
Image2717_2718	14
Image2719_2720	10
Image2721_2722	23
Image2723_2724	17
Image2725_2726	4
Image2727_2728	23
Image2729_2730	29
Image2731_2732	16
Image2733_2734	30

NA52VL#15	Macrophage Count
Image2765_2766	24
Image2767_2768	42
Image2769_2770	38
Image2771_2772	20
Image2773_2774	41
Image2775_2776	39
Image2777_2778	19

NA53VL#20	Macrophage Count
Image2823_2824	54
Image2825_2826	11
Image2827_2828	35
Image2829_2830	35
Image2831_2832	38
Image2833_2834	38
Image2835_2836	46
Image2837_2838	36
Image2839_2840	31
Image2841_2842	40

NA56VL#16	Macrophage Count
Image2227_2228	21
Image2229_2230	57
Image2231_2232	43
Image2233_2234	21
Image2235_2236	29
Image2237_2238	54
Image2239_2240	57
Image2241_2242	46
Image2243_2244	16

Image2245_2246	35
Image2247_2248	34
Image2249_2250	25
Image2251_2252	34
Image2253_2254	20

NA57VL#13	Macrophage Count
Image2643_2644	28
Image2645_2646	19
Image2647_2648	21
Image2649_2650	37
Image2651_2652	16
Image2653_2654	17
Image2655_2656	29
Image2657_2658	39

NA58VL#13	Macrophage Count
Image2735_2736	63
Image2737_2738	43
Image2739_2740	36
Image2741_2742	54
Image2743_2744	43
Image2745_2746	42
Image2747_2748	27
Image2749_2750	62
Image2751_2752	56
Image2753_2754	49
Image2755_2756	59
Image2757_2758	30
Image2759_2760	30
Image2761_2762	37
Image2763_2764	21

HS12VL#4	Macrophage Count
Image1985_1986	36
Image1987_1988	28
Image1989_1990	37
Image1991_1992	30
Image1993_1994	45
Image1995_1996	44
Image1997_1998	38
Image1999_2000	41
Image2001_2002	30
Image2003_2004	24
Image2005_2006	34

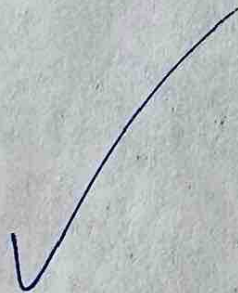
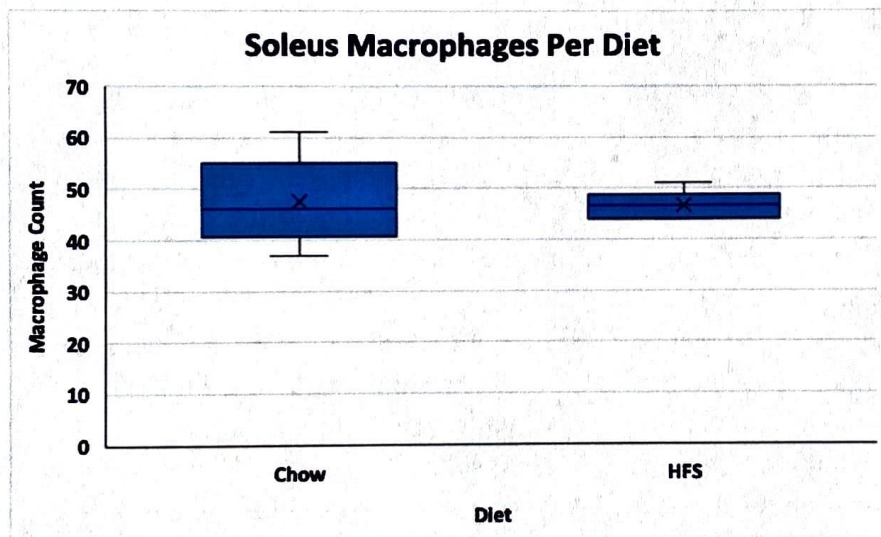
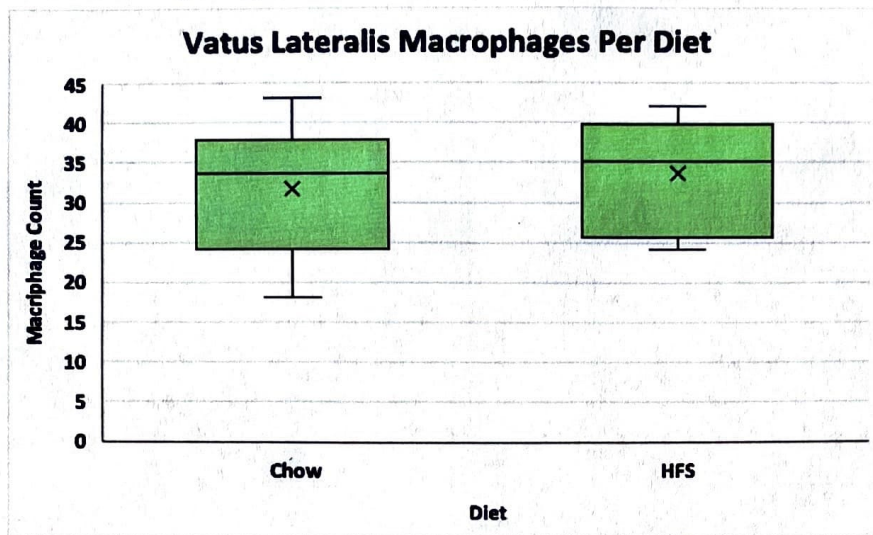


Image2007_2008
Image2009_2010
Image2011_2012

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15
19

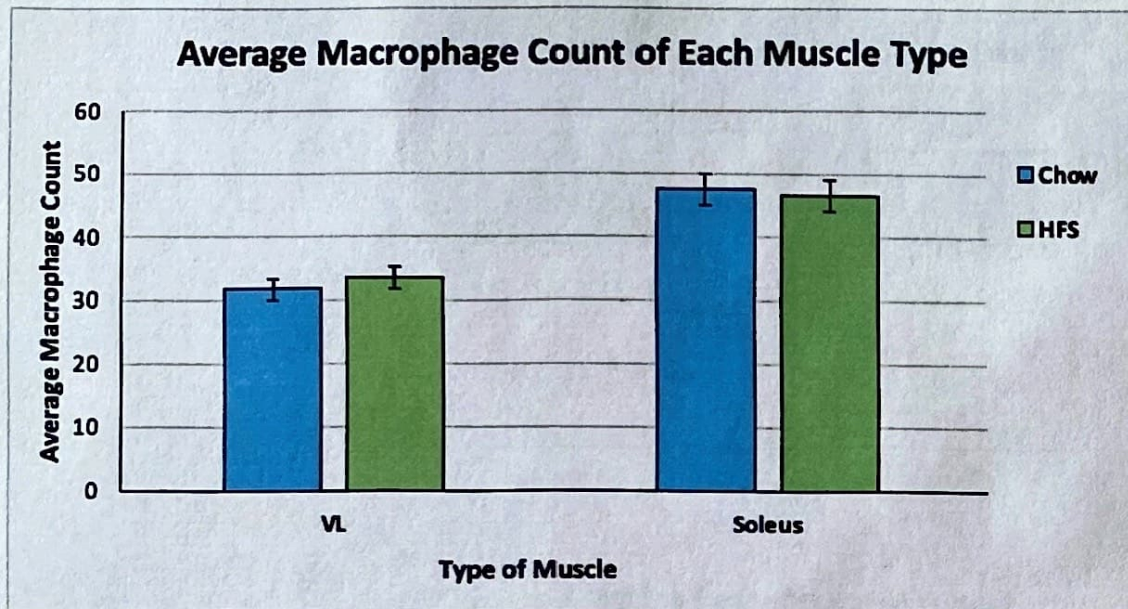
Results: Comparing Diets



Discussion: Comparing Diets

- No significant difference between chow and HFS diets for VL ($p > 0.05$)
- No significant difference between chow and HFS diets for soleus ($p > 0.05$)
- Therefore we know that diet does not affect muscle inflammation in either muscle. This supports my hypothesis which was based on a lack of fibrosis seen in a previous study.
- A possible explanation for these similar inflammatory responses between the diets could be similar systemic MCP-1 levels. In a study that looked at systemic female MCP-1 levels (Smith et al., 2023), they found no difference in MCP-1 levels between the two diets. Since MCP-1 is the cytokine that directly calls macrophages into damaged tissue, it makes sense to see similar macrophage levels.
- It is also important to note that MCP-1 can be impacted by hormones which could explain the females differing response from males, however, there needs to be further investigation into hormones.

Results: Comparing Muscles



- There is a higher macrophage count in the soleus muscle. Therefore, the soleus muscle had a higher inflammatory response.
- There is a lower macrophage count in the VL muscle. Therefore, the VL muscle had a lower inflammatory response.
- One possible explanation for the soleus's higher inflammatory response could be muscle usage differences. The soleus muscle is more chronically stimulated because it is a slow-twitch muscle. On the other hand, the VL muscles are used in short outbursts because they are fast-twitch muscles. Possibly, the rats were doing an activity that required them to use their soleus muscle more, which would explain the higher inflammatory response, therefore supporting these results.
- Another possible explanation for the soleus's higher inflammatory response could be increased passive delivery of macrophages into the soleus through the blood supply. Since the soleus muscle has a higher capillary density, it will receive more blood than the VL muscle. Since blood delivers macrophages into soft tissue, this could explain the higher macrophage count in the soleus muscle.

Future Investigations and Pathways:

- There could be further investigation into muscle-specific MCP-1 levels instead of systemic MCP-1 levels. This is because systemic MCP-1 is not specific to a local environment. If there was a difference found in regional muscle MCP-1 levels, this could account for the soleus and VL muscles differing inflammatory responses.
- This study could also be repeated over a longer time period (longer than 12 weeks) because a longer diet exposure may have a stronger effect on muscle responses.
- Females react differently than males which has been seen in their fibrosis and inflammatory response. Therefore, there should be further investigation into female hormones to determine their potential role in affecting muscle inflammation and dietary response.