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TITLE: The Effect of MEK Inhibitor Treatment on Metabolism in Neurofibromatosis T

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CONTRACTING ORGANIZATION: Children's National Hospital, Washington, DC

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5f. WORK UNIT NUMBER

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14. ABSTRACT

Patients with NF1 are predisposed to the development of plexiform neurofibromas (pNF) and also have other defining clinical features including those related to growth and energy metabolism, such as short stature, low weight, and decreased bone density--findings that are often more prominent in patients with high pNF burden. Interestingly, this phenotype changes in patients who are on Mek-inhibitor treatment. However, the mechanism for this metabolic phenotype in patients with NF1 and its association with pNF tumor burden and change with MEK- inhibitor treatment is not currently understood. In this proposal, we will perform metabolic and metabolomic analyses on NF1-deficient mice that develop plexiform neurofibromas and patients with NF1 who do or do not have plexiform neurofibromas. The effect of Mek-inhibitor treatment on metabolism in NF1 will also be explored. All regulatory approvals have been acquired, and the patient and mouse studies are ongoing.

15. SUBJECT TERMS

Neurofibromatosis Type 1, Metabolism, Plexiform Neurofibroma

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1. Introduction:

Neurofibromatosis Type 1 (NF1) is a neurocutaneous syndrome caused by genetic inactivation of one copy of the tumor suppressor *NF1,* resulting in activation of the Raf/MEK/ERK (MAPK) pathway. Patients with NF1 are predisposed to the development of plexiform neurofibromas (pNF) and also have other defining clinical features including those related to growth and energy metabolism, such as short stature, low weight, and decreased bone density--findings that are often more prominent in patients with high pNF burden. Indirect calorimetry (IC) of adults with NF1 reveals findings consistent with underfeeding (i.e. increased lipid oxidation) at baseline, despite having normal diets. The mechanism for this metabolic phenotype in patients with NF1 and its association with pNF tumor burden is not currently understood. We plan to address these gaps in this study. We hypothesize that germline loss of Nf1 promotes a pro-catabolic state resulting in increased lipid oxidation, and that MEKi treatment reverses this phenotype, promoting anabolic metabolism and weight gain. In addition, we hypothesize that MEKi treatment of pNF alters tumor metabolism, leading to specific tumor and plasma metabolomic signatures containing candidate pharmacodynamics markers of treatment response for future development. In order to test this hypothesis, we will have three Aims. **Aim 1: To establish that MEKi treatment alters clinical metabolic parameters in a pNF mouse model.** Using the *Nf1flox/flox*; *Postn*-cre pNF mouse model, we will examine acute (cohort 1; 4 weeks) and chronic (cohort 2; 8 weeks) effects of MEKi (compared to vehicle) treatment on clinical metabolic parameters in NF1-deficient (cre+) and wildtype (cre -) mice, using weight as a primary outcome and energy expenditure (IC) as an exploratory outcome. **Aim 2: To determine the effects of MEKi treatment on global and pNF metabolomic signatures in a pNF mouse model.** To examine the effect of MEKi on metabolites in NF1, global metabolomic profiling of plasma, nerve (pNF), and liver tissue will be performed on acute and chronic MEKi-treated mice compared to vehicle. Histologic analysis of pNF will be performed for Cohort 2 mice to correlate metabolomics signature with treatment response. We will also explore the effect of MEKi on microbiome-related metabolites by collecting stool from cohort 2 mice. **Aim 3**: **To identify the effects of MEKi treatment on metabolic and metabolomic profiles in patients with NF1.** Using a similar strategy as the mice, we will study the effects of standard of care MEKi treatment on metabolic (weight and IC), metabolomic (plasma) and microbiome (stool) profiles in patients with NF1 and clinically significant pNF (NF1 patients with low/no pNF as controls), and perform a global analysis of all mouse/patient data to detect shared metabolic signatures.

2. Keywords

Neurofibromatosis Type 1, Metabolism, Mek-inhibitor

3. Accomplishments

a. Major tasks of the project – completed year 1

Major Task 1: Collect weight data from pNF mice

Subtask 1: Submit documents for IACUC approval (CNH and JHU- completed)

Subtask 2: Submit IACUC approval and documentation to ACURO for approval (CNH-completed)

Milestone 1: IACUC and ACURO approval received (goal month 6)

- CNH IACUC approved 8/9/2021
- CNH ACURO approval 12/2/2021
- JHU (subcontract) IACUC approval 7/19/2019

- JHU ACURO approval 2/4/2022

Major Task 5: Obtain Metabolomic parameters and samples for metabolomic and microbiome studies

Subtask 1: Submit documents for local IRB review (Months 1-4) – Completed

Subtask 2: Submit IRB approval and necessary documents for HRPO review

Subtask 3: Establish data transfer agreement between CNH and GWU and CNH and JHU

- No human data will be exchanged between CNH and JHU, thus DTA was determined not to be necessary
- De-identified data transfer to GWU was approved by the IRB 6/27/2021

Milestone 7: HRPO approval received

- IRB approval 6/27/2021
- HRPO approval 11/3/2021

b. Major tasks of the project- completed year 2

Major Task 1: Collect weight data from pNF mice

Subtask 3 (Cohort 1- JHU): Collect weight data (Month 8-18)

- Task completed

Subtask 4 (Cohort 2- CNH): Collect weight data (Month 8-18)

- Task completed

Major Task 2: Collect Energy Expenditure Data from pNF mice (Month 12-18)

Subtask 1 (Cohort 1- JHU) (Month 12-18)

- Task completed

Major Task 3: Collect plasma and tissue samples from pNF mice to send for metabolomic analysis

Subtask 1 (Cohort 1- JHU): Collect plasma and tissue samples following acute tx (Month 14-18)

- Task completed

Subtask 2 (Cohort 2- CHMC): Collect plasma and tissue samples from untreated baseline and following chronic treatment (Month 14-18)

- Task completed

Major Task 4: Collect stool samples from pNF mice to send for microbiome analysis (Month 12-20)

Subtask 1 (Cohort 2- CNH): Collect samples for microbiome studies using the treated (MEKi or Veh) mice (Month 12-20)

- Task completed

b. Major tasks of the project - ongoing

Major Task 3: Collect plasma and tissue samples from pNF mice to send for metabolomic analysis

Subtask 3: Submit mouse plasma and tissue samples for metabolomics analysis (Month 20)

- Progress: Samples are collected, but due to increased costs associated with submitting samples to UT Southwestern as initially planned, there will not be enough funding to submit all the intended samples for metabolomic analysis. The PI will be moving to Lurie Children's (Northwestern University) starting in January 2024 where they have a metabolomics core that would be equivalent to UT Southwestern and more affordable with the onsite discount. Therefore, the plan is to wait to submit samples for the global metabolomic analysis until the move. Lipidomics were also proposed, and quotes from

Metabolon have been required for this analysis. Depending on costs, which have also gone up since the grant was submitted, samples from the JHU studies will be submitted, and the hope is to also have funding to submit the long term Veh and MEKi samples from the CNH samples to Metabolon for lipid analysis.

Milestone 2: Obtain metabolomic data from pNF tumor mice (Month 22)

- Progress: Pending

Subtask 4: Perform analysis of metabolomic data from pNF tumor mice (Month 22-24)

- Progress: Pending data (see above)

Major Task 4: Stool samples from pNF mice to send for microbiome analysis (Month 12-20)

Subtask 2: Submit mouse stool samples for microbiome analysis (Month 12-20)

- Progress: Samples are collected, but due to unexpected expenses from the mouse studies, there will not be enough funding to submit the stool samples for microbiome analysis at this time. The PI will be moving to Lurie Children's (Northwestern University) starting in January 2024 and will inquire about costs of microbiome analysis once there as the cost would be less than quoted through Children's National Hospital (George Washington University) where she currently has a position

Milestone 3: Obtain microbiome data from pNF tumor mice (Month 14-22)

- Progress: Pending submission of samples (see above)

Subtask 3: Perform Global analysis of initial metabolomic and microbiome pNF mouse studies (Month 22-26)

- Progress: Pending submission of samples (see above)

Milestone 4: Submit abstract to national meeting with preliminary mouse metabolomic/microbiome data (Month 22-26)

- Progress: Pending

Milestone 5: Co-author manuscript on paired omics functional integration analysis of microbiome and metabolomic data in mice (Month 24-26)

- Progress: Pending

Milestone 6: Submit collaborative grant with co-Is for additional funding to support analysis of additional tissue sources (i.e. muscle, heart, brain) (Month 22-28)

- Progress: Pending

Major Task 5: Obtain Metabolomic parameters and samples for metabolomic and microbiome studies

Subtask 4: Recruit, consent and enroll 69 patients/human subjects to the pre-clinical study (Month 9-33)

- Progress: Enrollment is ongoing, but still slow as it has been difficult recruiting patients during the school year. A recruitment flyer was created and is being distributed to patients at Children's National Hospital, and the PI has full days set aside for testing patients in August. We had initially planned to recruit patients from JHU as well, but given changes in their NF1 program, this is currently on hold. A total of 12 patients have been recruited, which is below expected. However, it is important to note that a majority of these patients are individuals who have had both pre/post MEKi studies, which will be the most helpful for identifying changes in metabolism on MEKi treatment. Depending on our initial results with these patients, we may increase the number of pre/post MEKi studies, and decrease the number of patients coming for single timepoint analysis.

Subtask 5: Submit microbiome specimens for analysis (Month 9-33)

- Progress: Out of the 10 patients recruited thus far, only one has opted to submit a stool sample for the microbiome analysis (this was made optional). Therefore, it is possible that this subtask may need to be eliminated from the current study, and can be attempted again in the future pending results of the mouse microbiome studies if these can be done

Milestone 8: Obtain data for patient microbiome studies (Month 9-33)

- Progress: Pending (see above)

Subtask 6: Submit plasma samples from patients (stored in -80C) for metabolomic analysis (Month 33)

- Progress: Pending

Milestone 9: Obtain data for patient metabolomic studies (Month 35)

- Progress: Pending

Subtask 7: Begin global analysis of patient and mouse microbiome and metabolomic data (Month 33-36)

- Progress: Pending

Milestone 10: Submit abstract to national meeting with mouse/patient data incorporating metabolic, microbiome and metabolomic data (Month 32-36)

- Progress: Pending

Milestone 11: Begin manuscript on global analysis of metabolism in mouse/patient incorporating metabolic, microbiome and metabolomic data (Month 32-36)

- Progress: Pending

	Year 1				Year 2				Year 3			
Target Enrollment (per quarter)	**Q1**	**Q2**	**Q3**	**Q4**	**Q1**	**Q2**	**Q3**	**Q4**	**Q1**	**Q2**	**Q3**	**Q4**
Site 1 (CNH)				6	10	10	10	10	10	8	5	
Target Enrollment (cumulative)				**6**	**16**	**26**	**36**	**46**	**56**	**64**	**69**	

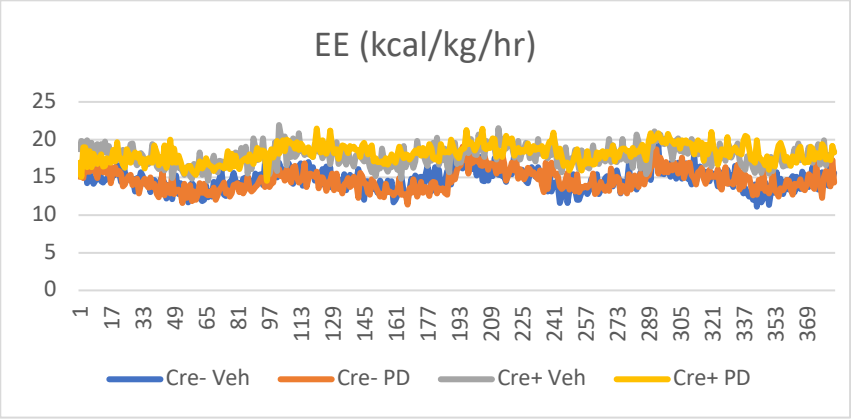
c. Accomplishments from goals

(1&2) Major activities and specific objectives: There were 2 major activities that were completed in the Year 2 reporting period. This included both mouse studies, one with short term MEKi treatment, and the other with long term MEKi treatment. The goal of these experiments was to study baseline metabolism in an NF1-/- mouse model ($NF1^{flox/flox}$; Periostin cre) that develops plexiform neurofibromas, and then study the impact of MEKi treatment on metabolism.

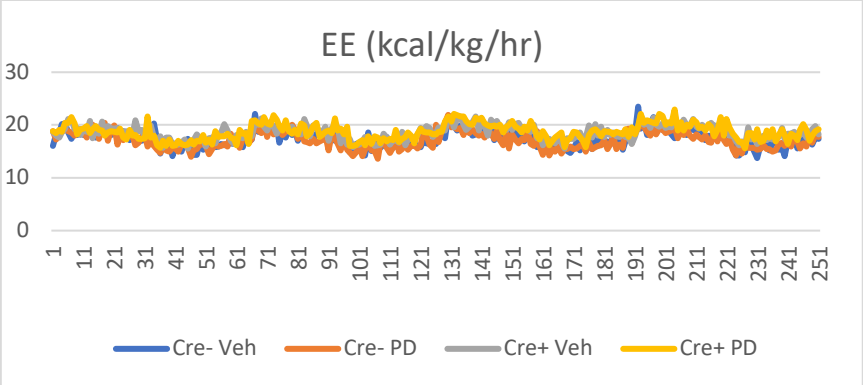
(3) Significant results:

a. *Short term MEKi study*

This study included measurement of indirect calorimetry, glucose tolerance test, insulin tolerance test, and collection of basic anthropometric measurements to study the short-term impact of MEKi



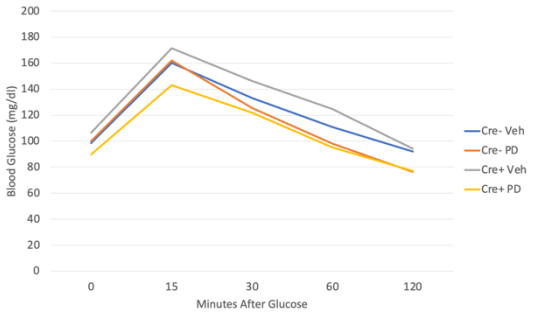
Energy expenditure of Male mice over time (hours)



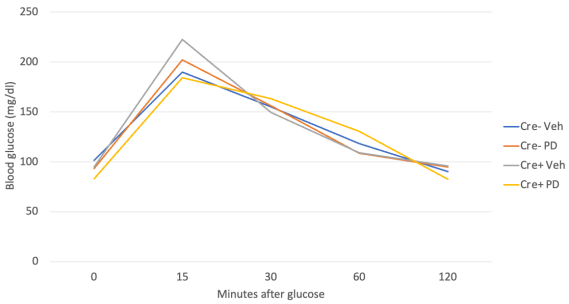
Energy expenditure of the Female mice over time (hrs)

treatment on the NF1$^{flox/flox}$; Periostin cre mouse model (NF1$^{-/-}$; MUT and NF1$^{+/+}$; CTR) Full data analysis is still ongoing. The preliminary analysis showed increased energy expenditure in the NF1$^{-/-}$ male mice both before and after MEKi treatment, but not the female mice. When comparing the weights of the mice in each group (MEKi treated and Vehicle treated) there was not a significant difference between the cohorts. Our preliminary studies, and long-term MEKi treatment studies (see below) did show a difference in weights after treatment with MEKi, so it is possible that the treatment was not long enough to show a significant change.

Glucose tolerance test:



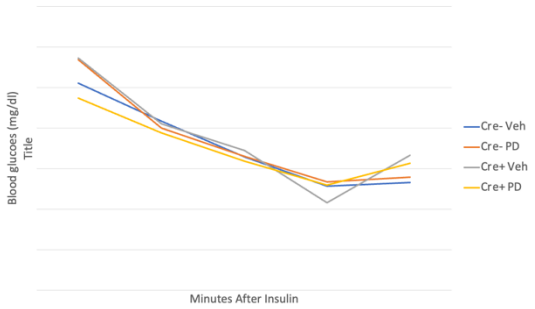
Female mice glucose tolerance test



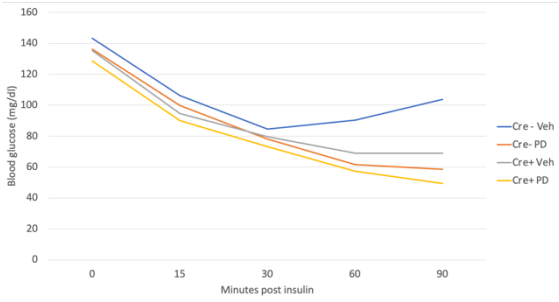
Male mice glucose tolerance test

The glucose tolerance test showed several interesting findings. In the female and male cohorts, the NF1$^{-/-}$ mice that were treated with vehicle (Cre+ Veh) had a significant glucose spike at 15 minutes after giving the glucose load, while the MEKi (Cre+ PD) treated NF1$^{-/-}$ mice had less of a spike. This suggests that Cre+ status impairs glucose tolerance, at least in the acute phase, and MEKi treatment seems to have a glucose lowering effect.

Insulin tolerance test:



Female mice insulin tolerance test



Male mice insulin tolerance test

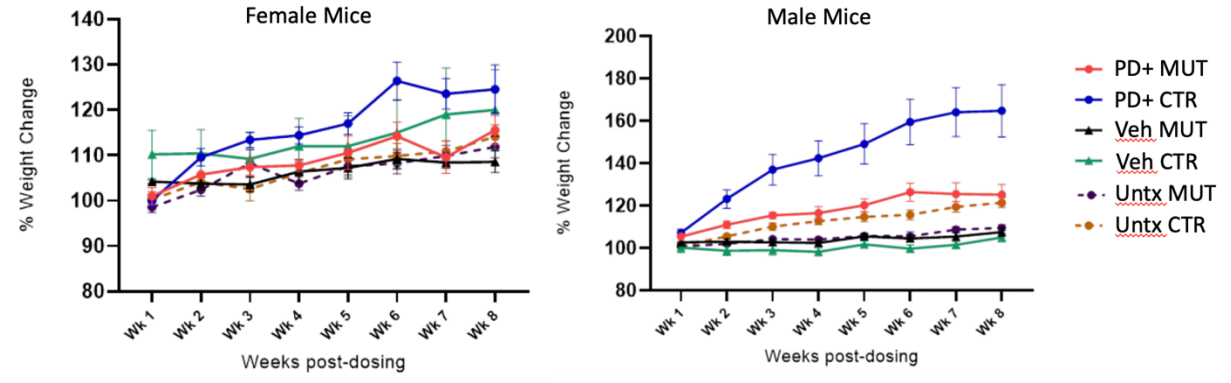
The male $NF1^{+/+}$ vehicle treated mouse had a normal glucose tolerance test. The female mice did not have a major difference amongst the groups, while the male MEKi treated and $NF1^{-/-}$ mice had delayed glucose rebound. This suggests that there is either prolonged insulin sensitivity or poorer insulin tolerance in these cohorts.

b. *Long term MEKi study*
This study looked at the impact of prolonged MEKi treatment (from 12 weeks to 20 weeks) on the $NF1^{-/-}$ and $NF1^{+/+}$ mice. During Mek-inhibitor or vehicle treatment, the mice were weighed 3 times per week, and the food bins in each cage were measured to get an idea of whether MEKi treatment influenced the amount of food eaten. We also had a cohort of untreated mice, but only weights were obtained for this cohort.

Mouse cohort	Average food/week
NF1 MEKi Female	42 gm/week
NF1 Vehicle Female	37 gm/week
NF1 MEKi Male	50 gm/week
NF1 Vehicle Male	40 gm/week

The MEKi treated mice ate, on average, 5-10 gm/week more food than the Vehicle treated mice.

When comparing weight change over the course of the 8 week experiment (with starting weight as 100% and any % change documented from this weight), the MEKi treated mice (PD+MUT, PD+ CTR) overall had higher weights than the Vehicle treated and untreated mice, with the exception of the Vehicle treated females that had similar weight gain as the PD+ $NF1^{-/-}$ mice. But this difference was not significant until around week 6 of treatment, and was more significant in the MEKi treated controls than the MEKi treated mutants, which may in part explain why we did not see a major difference in the weights of the mice that were treated with short (acute) therapy and underwent studies at JHU. Although it will not be possible to do this with the funding



provided by this award, we plan to repeat the JHU experiment (Indirect calorimetry, GTT, ITT) in mice treated long term with MEKi with an alternative funding source to be determined.

Although we did not have a mechanism to measure this for the current study, we noted significantly more fat in the MEKi treated mice on dissection than the Veh treated or Untreated mice, particularly in the male mice. All of the combined studies thus far supports our theory that MEKi treatment results in fat anabolism vs fat catabolism

d. Opportunities for training and professional development
During year 2 of the grant period, I attended the Children's Tumor Foundation annual meeting and had an opportunity to meet with Dr. Vincent Riccardi, MD who has a longstanding interested in understanding metabolic abnormalities in patients with NF1. We reviewed some of my study findings together, and he provided mentorship and guidance on interpretation of the results and additional studies/analyses that can be done.

I also established a metabolism working group within CTF, and we had our first meeting at CTF and will have a follow up meeting in the Winter 2023. This working group will allow for sharing of ideas and collaborations with a goal to submit multiple abstracts and have a metabolism in NF1 session during the CTF meeting in 2025.

e. Dissemination to communities of interest
Information about the Metabolism study was shared during the NF Forum meeting in Chicago 2022 and NF family day at Children's National Hospital in 2023.

f. Plan for the next reporting period
During the next reporting period we will plan to submit all the collected samples for metabolomics and initiate analysis of the data. Patient enrollment will continue with a goal to enroll as many of the 69 proposed subjects as possible by the end of year 3 of the award. We will continue to advertise in clinic and at NF events (i.e. NF family day, NF walk) to recruit patients.

4. Impact
a. Impact on development of the principal disciplines of the project
Nothing to report

b. Impact on other disciplines
Nothing to report

c. Impact on technology transfer
Nothing to report

d. Impact on society beyond science and technology
Nothing to report

5. Changes and Problems
a. Changes in approach and reasons for change
There are several changes in approach that are being made prior to year 3 of the study. These are listed as follows:

- The initial plan to submit the metabolomic samples to UT Southwestern will be changed so samples can be sent to Northwestern University, once the PI changes institutions and becomes faculty there, due to insufficient funds to send this to UT Southwestern. Some of the samples may not be able to have lipidomic analysis due to funding constraints as well. We will ensure that all the MEKi treated and Veh treated samples are analyzed, but will decrease the number of Untreated samples analyzed

- Despite being collected, the mouse microbiome studies that were initially proposed will likely not occur due to lack of funding. This will be planned only if there is additional funding available at the end of the 3-year funding period to perform the studies
- Only 1 patient microbiome sample has been returned thus far, most likely because this was approved as a voluntary study by the IRB. Thus, like the mouse studies, although we will plan to collect as many samples as possible, this study will likely not be performed with the funds provided by this award

b. Changes that have had a significant impact on expenditures
The PI received funding from an anonymous donor to help cover salary, which included salary support for research such as the metabolism studies in this award. Therefore, the PI support was decreased from 30% to 17% following approval from the DOD.

The metabolomic studies have not been completed yet, and therefore the funding for these studies allocated for year 2 have not yet been used. These will be used in funding period year 3. All samples will be submitted following the PI move to Northwestern University/Lurie Children's Hospital in January 2024.

c. Significant changes in the use or care of human subjects
Nothing to report

d. Significant changes in the use or care of vertebrate animals
Nothing to report

e. Significant changes in the use of biohazards and/or select agents
Nothing to report

6. Products
a. Publications, conference papers, and presentations
Nothing to report

b. Website or other Internet sites
Nothing to report

c. Technologies or techniques
Nothing to report

d. Inventions, patent applications, and/or licenses
Nothing to report

e. Other products
Nothing to report

7. Participants and Other Collaborating Organizations

a. Individuals working on the project

Individuals with unchanged information from prior report:

Miriam Bornhorst, PI
Kathyrn Lemberg, Co-I

Additional individuals working on the project

Name	Gholamali (Ali) Rahnavard
Project Role	Co-Investigator
Researcher Identifier	0000-0002-9710-0248
Nearest person month worked	0.5
Contribution to Project	Assist with analysis of metabolism data acquired during year 2 of the study, as well as continued analysis of data generated prior to this study as a preliminary study

Name	Steven Cerna
Project Role	Technician
Researcher Identifier	NA
Nearest person month worked	6
Contribution to Project	Mouse studies – metabolic/metabolomic including setting up cages, generating mice with genotype for studies, weighing mice, and collection of samples at appropriate timepoints

b. Change in active other support of the PD/PI or senior/key personnel since last reporting period
PI received additional salary support from an anonymous donor to cover 13% of the PI salary dedicated to this project, so the PI could continue to have 30% effort on the study but use this money initially dedicated for PI salary for the technician salary instead

c. Organizations involved as partners
Nothing to report

8. Special Reporting Requirements: None

9. Appendices: None