

This is provided as an example proposal.
It is important that you follow the current
guidelines.

The mentor letter has been removed.

**The John J. Kopchick Molecular and Cellular Biology/Translational Biomedical Sciences Undergraduate Student Support Fund
Cover Page**

Academic Year 2019-2020

NAME OF APPLICANT: Noah Powell

E-MAIL ADDRESS: np715016@ohio.edu

DEPARTMENT: Biological Sciences

EXPECTED GRADUATION DATE (Month and Year): May 2021

TITLE OF PROJECT: Brown Adipocyte Subpopulations

FACULTY MENTOR INFORMATION:

NAME: Kevin Lee, PhD

E-MAIL ADDRESS: leek2@ohio.edu

DEPARTMENT: Biomedical Sciences

NUMBER OF YEARS FACULTY IN THE MCB OR TBS PROGRAM: 4

BUDGET: Total Request \$1500
(May not exceed \$1,500)

STATEMENT OF HOW THE RESEARCH IS RELEVANT TO TRANSLATIONAL BIOMEDICAL SCIENCES* (500 character limit)

Brown adipose tissue plays an essential role in human metabolism and energy expenditure. Research in our lab has begun to uncover functionally distinct subpopulations of these cells. The proposed research will help to further characterize these differences in an effort to study their effects on obesity and metabolic disorders, possessing the potential for clinical application in the development of possible treatments for these diseases.

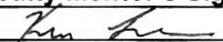
*For the purposes of this program, translational biomedical sciences is defined as the translation of basic research effectively into enhanced healthcare outcomes for the entire population in fields such as biomedical research, bioengineering, drug development, informatics, communications, health policy and planning.

IRB AND IACUC APPROVAL:

To ensure that the University is in compliance with all federal regulations, complete the checklist below. *Note: your proposal can be approved prior to IRB or IACUC approval (put "pending" or "to be submitted" instead of approval number), but funding will be withheld until notification of approval or exemption.*

Yes	No	Office of Research Compliance	Policy #
	✓	Human Subjects in Research (including surveys, interviews, educational interventions): Institutional Review Board (IRB) Approval #: Expiration Date:	19.052
✓		Animal Species: Mus musculus Institutional Animal Care & Use Committee (IACUC) Approval #: 15-H-024 Expiration Date: 6/3/21	19.049

SIGNATURES

Applicant's Signature		Faculty Mentor's Signature	
Signature		Signature	
Name	Noah Powell	Name	Kevin Lee

Optional: Yes No

If selected for funding, I give permission to the Office of the Vice President for Research and Creative Activity to use my proposal as an example during training and workshop exercises.

Biographical Sketch

Noah Powell

B.S. Biological Sciences (Anticipated May 2021)

Ohio University, Athens, OH

GPA: 3.85

Relevant Coursework

Fundamentals of Animal Cell Biology (BIOS 3200)

Cell and Microbiology Techniques (BIOS 3205)

Writing for the Science Researcher (PBIO 4180J)

Molecular Genetics (BIOS 4260)

Personal Statement

I am a third year undergraduate currently studying biological sciences with a projected graduation date of the Spring Semester, 2021. Following graduation, I plan to continue my education by pursuing a PhD in the field of molecular biology. I began conducting undergraduate research in Dr. Kevin Lee's lab in February of 2019, and since then I have been able to learn not only numerous laboratory techniques, but also the process and mindset that research requires. So far in the lab, I have worked to characterize the composition of white and brown adipocyte subpopulations in various adipocyte-containing tissues.

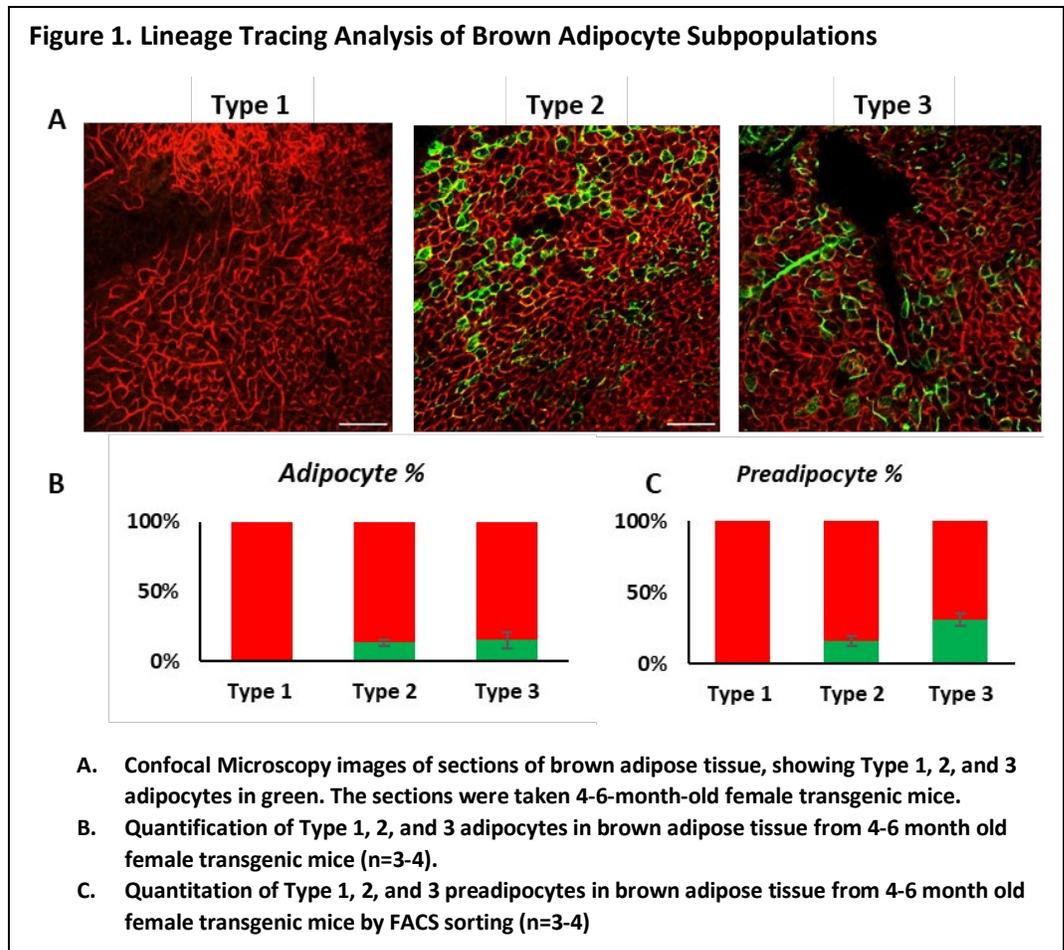
The proposed project will further enhance our understanding of the functional differences between these subpopulations, specifically in brown adipose tissue. Since brown adipose tissue is so important in metabolism, the project has the potential to be clinically significant for diseases such as diabetes. Personally, this project will increase my laboratory competence and allow me the opportunity to conduct an entire research project from start to finish. This experience will be invaluable in preparing me for both graduate school and my eventual career. This will also allow me the opportunity to present my research at a national conference, giving me valuable experience that I will undoubtedly utilize in the future.

Project Narrative

Objective and Scope: Two main types of adipose (Fat) tissue exist in humans: brown and white. These tissues both play essential roles in energy storage, metabolism, and endocrine signaling. While white adipose tissue's main function is to store energy and release hormones and cytokines, brown adipose tissue is more thermogenic in nature and contributes towards energy expenditure (1). Furthermore, extensive research has identified functional differences within white adipose tissue based on their location. An accumulation of visceral white adipose tissue was found to increase the risk of metabolic disorder, whereas subcutaneous white adipose tissue was found to be protective against metabolic disorders. These differences suggest the existence of further functionally distinct subpopulations of white adipocytes. This led our lab to identify three distinct subpopulations of white adipocytes, marked by the expression of three separate genes. Type 1 expresses Wilm's Tumor Suppressor 1 (Wt1), Type 2 expresses Transgelin (Tagln), and Type 3 expresses Myxovirus 1 (Mx1). These subpopulations are present in different ratios depending on the fat depot they are found in, possess different functional properties including differences in cellular metabolism, and respond differently to exogenous signals including insulin, growth hormone, and inflammatory cytokines (2). Recent evidence has also suggested the existence of distinct subpopulations of brown adipocytes that possess different metabolic properties and thermogenic capabilities (3-5).

Thus far, my work has been to utilize the lineage tracing mouse models available in the lab to examine if the three markers of white adipocyte heterogeneity that we established also marked distinct populations of brown adipocytes. Wt1-cre, Tagln-cre, and Mx1-cre mice were crossed

with ROSA26mT/mG mice that possess a two-color fluorescent Cre reporter allele, to label adipocyte Types 1-3, respectively. The read-out of this lineage tracing



analysis is as follows: In the absence of Cre expression, cells express Tomato (mT-red), and after Cre recombinase expression, GFP (mG-green) is observed in these cells and all future cell lineages. Utilizing whole-mount microscopy followed by confocal microscopy of brown adipose tissue, lineage positive (GFP+) Type 2 and Type 3 but not Type 1 brown adipocytes were observed (Figure 1A). Quantitation of these adipocytes revealed that 12±1.9% of brown adipocytes are Type 2, and 15±5.9 % are Type 3 adipocytes. Furthermore, we also used FACS (Fluorescent Activated Cell Sorting) to analyze the number of brown preadipocytes as defined by the expression of cell surface markers (CD31-, CD45-, Ter119-, CD29+,CD34+, Sca1+). We

found that approximately 16 ± 3.4 % and 31 ± 4.8 % of preadipocytes were Type 2 and 3 respectively, but similar to the adipocytes data, none were Type 1 (Figure 1C).

Materials/Methods: In order to determine if the Type 2 and Type 3 brown adipocytes represent molecularly and functionally distinct brown adipocyte subpopulations, we will isolate Tomato(+) and GFP(+) brown preadipocytes from TagIn-cre; ROSA26mT/mG mice, and Mx1-cre;ROSA26mT/mG mice to generate stable cell lines from preadipocytes. We will use a pBABE-Puromycin-based vector to generate retroviruses to infect these cells and stably express simian virus-40 large-T antigen (6). This will immortalize these preadipocyte populations and allow for their further study. A standard differentiation protocol which includes treating the cell lines with insulin, dexamethasone, 3-isobutyl-1-methylxanthine, and rosiglitazone, will be utilized to differentiate the pre-adipocytes into mature brown adipocytes. Importantly, since both Tomato(+) and GFP(+) preadipocytes are simultaneously isolated, each GFP(+) experimental sample, representing a defined preadipocyte subpopulation, will have a paired Tomato(+) control, representing the remaining preadipocytes. Thus, this experimental design should reduce experimental variability, yield more scientifically rigorous results, and allow us to more definitively characterize molecular and functional differences between these subpopulations. All mice and vectors necessary for this study are currently available in the laboratory. The expression of known markers of brown adipocyte heterogeneity including uncoupling protein 1 (Ucp1), fatty acid synthase (Fasn) and Medium-chain acyl-CoA dehydrogenase (Mcad1) will be measured by qPCR and Western Blot (3-5). Levels of TagIn and Mx1 will be utilized as a positive control. The metabolism and thermogenic capacity of these cells will be

tested by utilizing a Seahorse XF Analyzer, as previously described in prior experiments from our laboratory and others (7, 8).

Significance and Expected Results: RNA-seq analysis of FACS sorted white adipocytes suggests that Type 2 adipocytes have lower expression of peroxisome proliferator-activated receptor gamma (Ppar γ) regulated genes, $p < 10^{-58}$ (data not shown). Since Ucp1, the key regulator of thermogenesis in brown adipocytes, is a Ppar γ -regulated gene, we anticipate that thermogenesis will be decreased in these cells (9). Taken together, Type 2 and Type 3 adipocytes represent a sizable proportion (27%) of brown adipocytes. Since brown adipocytes expend energy through thermogenesis, understanding and controlling brown adipocyte action has great potential to combat the epidemic of obesity and its associated metabolic disorders.

Intellectual Property/ Innovation: In a previous manuscript from the laboratories, we described conditionally immortalized clonal preadipocyte cell lines that represent white preadipocyte Types 1-3. The Technology Transfer Office is currently in negotiations with Applied Biological Materials, Inc. (Richmond, BC, Canada) to license these cell lines. Since understanding brown adipocyte thermogenesis is of great interest, the cell lines generated in this proposal potentially have great commercial possibilities.

References

1. A. M. Cypess, C. R. Kahn, *Current opinion in pediatrics* **22**, 478-484 (2010).
2. K. Y. Lee *et al.*, *EMBO J* **38**, (2019).
3. R. Xue *et al.*, *Nat Med* **21**, 760-768 (2015).
4. A. Song, Min Jee Jang, Xiwei Wu, Lei Jiang, Philipp E. Scherer, and Qiong Wang., in *Diabetes* **68**. (2019).
5. Y. H. Lee, S. N. Kim, H. J. Kwon, J. G. Granneman, *Sci Rep* **7**, 39794 (2017).
6. J. J. Zhao *et al.*, *Cancer Cell* **3**, 483-495 (2003).
7. K. Mahdavian, I. Benador, O. Shirihai, *Bio Protoc* **5**, (2015).
8. K. Y. Lee *et al.*, *Diabetes* **66**, 2822-2829 (2017).
9. I. B. Sears, M. A. MacGinnitie, L. G. Kovacs, R. A. Graves, *Mol Cell Biol* **16**, 3410-3419 (1996).

Budget and Justification

<u>Item</u>	<u>Justification</u>	<u>Cost</u>
Cell Culture	Supplies: Culture plates, centrifuge tubes, Seahorse, media and supplements (DMEM, FBS, glutaMAX, normacin penicillin, streptomycin, tetracycline)	\$600
Conference	American Diabetes Association 2020 Meeting – Chicago, IL (June 12-16) Flight: \$205 (see page 9) Lodging: \$227: Splitting price of the room (see page 10) Meals: \$68 (Chicago per diem \$76 x 5 days) <i>*PI will defray any additional costs for travel, abstract submission fees, and meals</i>	\$500
qPCR	Supplies: 96-well plates, SYBR green, primers	\$200
Western Blot	Supplies: Protein gels, molecular size markers, stains, buffers	\$200
	Total Requested	\$1500

Trip & Price Details

Flight total estimate since fares are not available for the actual date this far in advance

✈ Flight <small>Modify Remove</small>		\$205.96	
Fri 2/7	CMH → MDW	1hr 30min Nonstop	<u>Wanna Get Away</u>
6:35AM	7:05AM		
Tue 2/11	MDW → CMH	1hr 10min Nonstop	<u>Wanna Get Away</u>
3:45PM	5:55PM		
		Price per passenger	\$164.98
		Taxes and fees per passenger	\$40.98
		Price per passenger	\$205.96
		Passenger	x1
		Flight total	\$205.96



 Need Help? Ask our [virtual assistant](#) | [Best Price Guarantee](#)

Let's reserve it.



Holiday Inn : Chicago-Elk Grove
1000 Busse Road, Elk Grove Village IL 60007 United States
Check in: 03:00 PM | Check out: 12:00 PM

6/12/2020 - 6/16/2020 | 4 Nights | 2 Guests | 1 Room

KING EXECUTIVE NONSMOKING
Book Early & Save - Advance Purchase



Room cost will be split in half (\$227)

Total Price for Stay: **454.71 USD**

[Hide rate details](#) ^

Guest Information

Already an IHG® Rewards Club member? [Sign in](#) to earn your points and save time with automatic form completion.

*Required

First Name*	Last Name*
<input type="text"/>	<input type="text"/>

Rate Description

Looking to stretch your travel dollars? Lock in special savings just by booking early and purchasing your stay in advance. Reservation requires full prepayment for the entire stay at the time of booking and is non-refundable. Payment is charged to your credit card between the time of booking and day of arrival.

Rate Information per Stay for 1 Room