This is provided as an example proposal.
It is important that you follow the
current guidelines.
The mentor letter has been removed.

A PROPOSAL TO STUDENT ENHANCEMENT AWARD REVIEW COMMITTEE

TITLE OF PROJECT: Effects of Early vs Late Life Weight Cycling on Oxidative Stress & Cellular Senescence

NAME OF APPLICANT: Maya Djalali-Gomez

STATUS: ☒ Undergraduate ○ Graduate ○ Medical

CAMPUS/LOCAL ADDRESS: 36 North Mckinley Avenue
E-MAIL ADDRESS: md169519@ohio.edu
DEPARTMENT: Applied Nutrition – College of Health Science & Professions

EXPECTED GRADUATION DATE (Month and Year): May 2024

RE-SUBMISSION: ○ YES (Original Submission Date_____) ☒ NO

PROPOSAL CATEGORY (select one):
☒ Life/Biomedical ○ Social/Behavioral
○ Arts/Humanities ○ Physical Sciences/Engineering

BUDGET: Total Request $6,000
(May not exceed $6,000)

FACULTY MENTOR INFORMATION:
NAME: Edward List
E-MAIL ADDRESS: list@ohio.edu
DEPARTMENT: Edison Biotechnology Institute
DEPT/COLLEGE ADMIN. NAME & E-MAIL: beha@ohio.edu

IRB AND IACUC APPROVAL:
To ensure the University's compliance with all federal regulations, complete the checklist below. *Note: if your IRB/IACUC is not approved prior to submission, put "pending" or "to be submitted" instead of approval number. Note: funding will be withheld until IRB/IACUC notification of approval or exemption.*

Yes	No	Office of Research Compliance	Policy #
○	☒	Human Subjects in Research: Institutional Review Board (IRB) Approval #: Expiration Date:	19.052
○	☒	Animal Species: Institutional Animal Care & Use Committee (IACUC) Approval #: Expiration Date:	19.049

SIGNATURES

Applicant's Signature		Faculty Mentor's Signature	
Signature	Maya Djalali-Gomez	Signature	
Name	Maya Djalali-Gomez	Name	Edward List
Dept/School	CHSP	Dept/School	Edison Biotechnology Institute
Date	1/18/2023	Date	1/18/2023

School or Dept Chair's/Director's Signature

Signature	
Name	Shiyong Wu
Dept/School & Date.	Edison Biotechnology Institute

☒ **Optional:**
If selected for funding, I give permission to the Research Division to use my proposal as an example during training and workshop exercises. (Sign below)
Signature: Maya Djalali-Gomez Date: 1/18/2023

STUDENT ENHANCEMENT AWARD
APPLICATION CHECKLIST

Applicants **must** complete and sign the checklist. The checklist should be included as the second page of the application (following the cover page).

☒ Cover page	use SEA form
☒ Checklist	use SEA form
☒ Abstract*	1 double-spaced page
☐ Resubmission Summary (**For Re-submissions Only**)*	1 double-spaced page
☒ Project Narrative	5 double-spaced pages
☐ Glossary/Definition of Terms* (**Not required**)	2 double-spaced pages
☒ Bibliography (**Not required**)	2 pages
☒ Presentation of Results	1 double-spaced page
☒ Mentor's Endorsement	1 page
☒ Biographical information (**Applicant(s) and key personnel**)	3 pages per person
☒ Budget and Justification	no limit specified
☐ Appended Materials/Multimedia Files	5 pages; and no more than 10 minutes of footage
☐ Electronic copy of proposal	Single Acrobat file, containing entire proposal and required signatures

Sections marked with a bullet (*) identify text sections that should be written in language understandable by an informed layperson to assist the Committee in its review.

****Please Note:** ***The committee has the right to return without review any proposals that do not conform to these format requirements******

Applicant signature: _____

Abstract

Along with rising obesity rates among adults and adolescents in the U.S, there has been an increase in weight control practices, many of which are not sustainable in the long term, and weight is gained back in a pattern of weight fluctuation cycles, known as weight cycling (WC). Weight cycling is not limited to adults. Increasing obesity rates in adolescents and media pressure on this vulnerable population influences body dissatisfaction and pushes them to weight loss even if not sustainable. Despite the prevalence of WC, little is known about its effects on health and overall mortality since survey studies in humans are conflicting. Because of this, controlled feeding studies from two separate laboratories were performed in mice to investigate how WC affects lifespan and both studies found that WC is healthier than remaining obese (i.e., WC mice outlived obese controls). Since both studies investigated WC over a lifetime, the impact of WC when isolated to early life or late life remained unexplored. To address this, our laboratory conducted a new study to evaluate 1) the impact of WC during the ~1st half of life vs 2) WC during the ~2nd half of life, vs 3) ever obese controls. While late life weight cycling (LLWC) extended lifespan vs high fat fed (HF) ever obese controls, mice that experience early life weight cycling (ELWC) were significantly shorter-lived vs HF controls. In light of this data, my project will look into two cellular mechanisms associated with aging in livers previously collected from a subset of ELWC, LLWC, and HF control mice. I will specifically evaluate a mechanism responsible for cellular detoxification by measuring the levels of five different xenobiotic metabolizing enzymes (XMEs). I will also measure the senescence-associated secretory phenotype (SASP) from serum samples previously collected from these mice by measuring protein markers that are released from senescent cells which accumulate with aging. Results obtained from this project are significant because they can be used to inform the public of the dangers of weight cycling in early life.

Project Narrative

Background. Obesity in the U.S has reached epidemic proportions and is affecting young and old alike with about two-thirds of the population either overweight or obese [1]. This is a pressing health concern since obesity leads to increased morbidity and mortality. To improve health outcomes, obese and overweight individuals are encouraged to lose weight. However, many individuals who lose weight will gain it back. In fact, only one in six overweight or obese individuals who achieve 10% weight reduction can keep the weight off over a 1-year period [2]. This process is referred to as weight cycling (WC). While weight cycling is common, little is known about its long-term effects on health. Conflicting studies in the current literature indicate that WC reduces all-cause mortality and is thus healthier than remaining obese while other studies suggest that WC increases all-cause mortality risk indicating it is worse to weight cycle than to remain obese. However, controlled feeding studies from two separate laboratories found that WC mice significantly outlive obese mice suggesting that WC is healthier than remaining obese [3,4]. While these studies are significant, they did not look at how the time of life when weight cycling occurs affects mortality. Either adult mice were used in this previous study [4] or the mice were weight cycled for their entire life [3]. Often, individuals go through periods of weight cycling where their weight fluctuates for a period and then remains stable. Weight cycling can affect adolescents as well as older adults. As rates of adult obesity increase so do the rates of adolescent obesity. With this comes an increase in the stigmatization of obesity in children who are susceptible to societal pressures to be thin [5]. Body dissatisfaction in young adolescents is increasing which is associated with greater intentions to change weight and diet and an increase in weight control behaviors including unhealthy behaviors such as fasting, diet pills, and excessive exercise which has a higher prevalence among overweight adolescents [6,7,

8]. These practices are not sustainable and can lead to early life weight cycling in adolescents. The desire to diet also extends to older adults. A 2003-2008 U.S National Health and Nutrition Examination Survey found that the prevalence of weight control behaviors in older women (>55 years) was less than in younger women (<55 years) but weight control behavior prevalence was similar between older and younger men [9]. Recognizing the limitations of the original WC study [3], a follow-up study was done to investigate how weight cycling at different points in the lifespan affects lifespan and various health parameters. This follow-up study weight-cycled mice for only part of their lives. The mice were either "early life" or "late life" weight cycled. In the original study, HF-fed obese mice lived about 88 weeks, so 44 weeks was set as the division between early and late life. Group 1 was fed a high-fat diet (HF), group 2 was early-life weight cycled (ELWC) and then fed a high-fat diet for the remainder of life, and group 3 was fed a high-fat diet for the first 44 weeks of life and then weight cycled till death (LLWC). The unpublished data from this study **(Figure 1)** shows that ELWC mice in a background of obesity had shorter lifespans than late life weight cycled mice from a high-fat background (*P*=<.0001) and even the high-fat controls (*P*=.0004). This result raises the question as to why ELWC mice have a dramatically shortened lifespan.

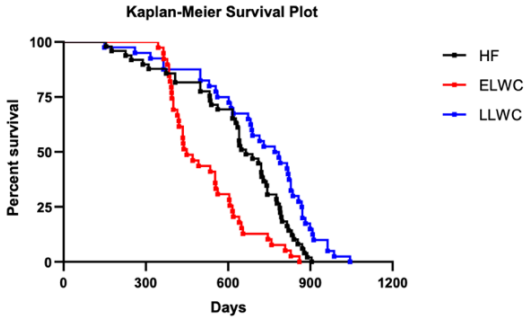

Figure 1: Survival Plot comparing lifespan of ELWC mice (red) vs Control (black) and LLWC mice (blue) (n=33-46/group).

Specific aims. To answer this question, I will evaluate how two cellular mechanisms are altered during stable obesity vs ELWC vs LLWC. This project will be part of my HTC thesis and build off work for which I received a Kopchick award to investigate how chronic weight-cycling an entire lifetime alters the mRNA expression of selected XMEs in the liver. The aim of this

proposal is to build off my initial proposal and look at xenobiotic metabolizing enzymes (XMEs) as well as markers of cellular senescence in mice that were weight cycled at different points in their lifespan. I am specifically choosing to look at XMEs to assess the effects of ELWC vs LLWC because XME levels are good indicators of cellular stress because these enzymes have low expression in low-stress cellular environments but in the presence of xenobiotics or oxidative stress, enzymatic expression is induced [10]. It had also been reported that obesity alters the expression of XMEs [11]. Unfortunately, little if any data is available to demonstrate how weight cycling affects XMEs in general let alone how ELWC vs LLWC affects XMEs. In addition to analyzing XME expression, I will be looking at cellular senescence, a mechanism of aging that is defined by irreversible cell arrest that occurs in response to cellular stressors [12, 13, 14]. Senescent cell accumulation can be a result of several factors including oxidative stress [13]. Cellular senescence can effectively be measured by the presence of bioactive molecules that are indicative of senescence-associated secretory phenotype (SASP). SASP is a secretory profile that is released from senescent cells. Markers in the secretory profile of SASP include inflammatory cytokines and chemokines [15, 16]. I will also be looking at activin A, an inflammatory mediator found to be a reliable marker of cellular senescence in mice and humans [17].

Methods & Data Analysis. To evaluate the cellular mechanisms that contribute to the difference in lifespan, I will determine the level of gene expression of specific XMEs in the liver of ELWC and LLWC mice. The liver samples to be used will be from ELWC mice, LLWC mice, and obese control mice from the C57BL/6J mice strain. C57BL/6J mice tissue is used because the C57BL/6J mouse strain is the most sensitive to diet-induced obesity as well as weight cycling. These liver samples have already been collected from the previously mentioned unpublished

study and are currently being stored at -80 degrees C for analysis. The details on these groups are as follows: Group 1 (HF) - obese control mice were fed a high-fat diet from 4 weeks of age until death. Group 2 (ELWC) - mice subjected to early life weight cycling using an established WC diet regimen [3] from 4 weeks until 44 weeks of age and then switched to a high-fat diet until death. Group 3 (LLWC) - mice subjected to LLWC by placing mice on a high-fat diet from 4 weeks until 44 weeks of age and then switched to the WC diet. Using liver samples, I will quantify the level of expression of CYP2a4, CYP2a5, ABCC4, NQO1, and CYP3a4 genes using real-time quantitative polymerase chain reaction (PT-qPCR). CYP2a5 was chosen because it has been shown to play a role in protection against thioacetamide-induced liver injury and liver fibrosis [18]. Thioacetamide is a hepatotoxin that is often used to model liver injury and cirrhosis [19]. Enzymes CYP2a4, CYP2a5, and CYP3a4 are important in cellular metabolism, homeostasis, and detoxification of drugs [20]. Enzyme NQO1 detoxifies reactive xenobiotics, and its activity is linked to reductions in oxidative stress [21]. I will isolate RNA from the liver samples of each mouse per instructions of ThermoFischer Scientific [22]. Precipitated RNA will be cleaned and suspended in molecular-grade water then the quantity and quality of the RNA will be analyzed in a NanoDrop spectrophotometer. If the concentration of RNA is less than 300 ng/μL then isolation will be repeated. The isolated RNA will then be used to synthesize complementary DNA (cDNA) using a reverse transcription reaction following cDNA synthesis protocol [23]. This single-stranded DNA will be the template to form the double-stranded cDNA, which will be used for the RT-qPCR reaction to determine the level of gene expression of each XME. Primers specific to the gene of interest will be used to replicate and amplify these genes. SyberGreen dye will be used to monitor this amplification. The five genes of interest from the high-fat control mice will be used to normalize the data. qRT-PCR will be performed in a

Bio-Rad iCycleriQ RealTime PCR machine and SyberGreen concentration will be monitored throughout amplification on QuantStudio Design & Analysis software. The data from the XME genes and the control genes will be transferred from QuantStudio to qBase+ qPCR software to be analyzed using univariate analysis of variance (ANOVA). This will show if mRNA expression of XMEs is altered in weight-cycled mice. Since these enzymes play a large role in cellular metabolism and homeostasis, this data will provide insight into the cellular mechanisms that contribute to the difference in lifespan between ELWC mice and LLWC mice. To analyze the effects of weight cycling on cellular senescence I will perform a mouse Luminex Discovery Assay on previously collected plasma from the mice per manufacturer instructions. Each assay will test for 11 markers of SASP (R&D Systems). Activin A levels in plasma will also be measured using Activin A ELISA kits (DAC00B, R&D Systems) per manufacturer instructions [24]. Analysis of results will be performed using SPSS version 17.0 (Chicago, IL, USA). Comparisons will be made using a univariate ANOVA with Tukey's honestly significant difference post hoc test. Differences will be considered significant at $P < .05$.

Significance & Impact. Data looking into the cellular mechanism associated with ELWC vs LLWC would be significant because it would allow for insight into why ELWC is more detrimental to lifespan than LLWC or lifelong obesity. Considering the growing obesity epidemic in younger individuals and the concomitant increased prevalence of weight cycling coupled with the lack of research on this topic, this study will provide needed data. We hope that data will help shed light on the dangers of WC in early life and lead to additional studies on this topic that will ultimately be utilized by healthcare professionals to revise current protocols and therapies for dealing with childhood obesity.

Bibliography

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Presentation of Results

I plan to submit an abstract of this proposed study to the Endocrine Society Conference to take place in Chicago, Illinois from June 15-18, 2023. This presentation is appropriate and valuable for my proposed project because I believe the results of this study will prove to have impacts on the advice given to patients experiencing obesity. This conference will allow my research to reach the scientific community outside of Ohio University as well as allow me to meet researchers doing similar work and gain exposure to other areas of research that may influence my future career choices. Aside from this conference, the completion of this proposed study will serve as my senior thesis, a requirement for my graduation from the Honors Tutorial College at Ohio University. Finally, I will also present the results of this study in poster format at the 2024 Ohio University Student Research and Creative Activity Expo to share my work and gain exposure to more current research at my university.

Biographical Information

<table>
<tr><th colspan="4">Biographical Sketch</th></tr>
<tr><td colspan="4">NAME: Maya Djalali-Gomez</td></tr>
<tr><td>INSTITUTION</td><td>DEGREE</td><td>COMPLETION DATE</td><td>FIELD</td></tr>
<tr><td>Ohio University, Athens, OH</td><td>B.S.</td><td>May 2024</td><td>Translational Health-Applied Nutrition (GPA: 3.85)</td></tr>
<tr><td>MINOR/ CERTIFICATES</td><td colspan="3">COMPLETION DATE</td></tr>
<tr><td>Chemistry</td><td colspan="3">December 2022</td></tr>
<tr><td>Biological Sciences</td><td colspan="3">May 2024</td></tr>
<tr><td>Global Health</td><td colspan="3">December 2022</td></tr>
<tr><td colspan="4">PUBLICATION and PRESENTATION</td></tr>
<tr><td colspan="4">Howe, C.A., Corrigan, R.J., Djalali, M.., McManaway, C., Grbcich, A. & Aidoo, G.S. (2021). Feasibility of using BIA for assessing Youth Weight and Health Status: Preliminary Findings. International Journal of Environmental Research and Public Health, 18(19), 10094

"Strategies and Tools for Evaluating Pediatrics & Play (STEP)" at the Knee Injury Prevention Webinar (9/20/21)</td></tr>
<tr><td colspan="4">RELEVANT COURSEWORK</td></tr>
<tr><td colspan="2">Biochemistry 1 (CHEM 4901)

Lifespan Nutrition (NUTR 2100)

Biological Sciences 1: Molecules & Cells (BIOS 1700)

Statistics (PSY 2110)</td><td colspan="2">The Effect of Growth Hormone on Adipose Tissue Mass- Tutorial with Darlene Berryman (AHSW 2970T)

Diabetes, Beta-cell Dysfunction & Drug Development - Tutorial with Craig Nunemaker (AHSW 2971T)

Tutorial with Edward List (AHSW 3970T)</td></tr>
<tr><td colspan="4">LANGUAGE SKILLS</td></tr>
<tr><td colspan="4">English: Fluent
Spanish: Conversational
French: Basic</td></tr>
<tr><td colspan="4">HONORS & AWARDS</td></tr>
<tr><td colspan="4">Templeton Scholar (2020-2024)

Guy L. Harris Memorial Award (2020-2021)</td></tr>
<tr><td colspan="4">CURRENT, PENDING AND PREVIOUS FUNDING:</td></tr>
<tr><td colspan="4">Kopchick Award (2022)
Provost Undergraduate Research Fund (2022)</td></tr>
<tr><td colspan="4">OTHER FUNDING SOURCES FOR PROJECT</td></tr>
<tr><td colspan="4">Kopchick Award (2022) -- $1,500</td></tr>
</table>

Budget and Justification:

	Item	BRAND	JUSTIFICATION	PRICE
Consumable Supplies	#FERK0732, GeneJET RNA Purification Kit for 250 preps	Thermo Scientific™	Gives us the RNA we need to make our cDNA.	**$818**
	#FERK1642, Maxima™ First Strand cDNA Synthesis Kit for RT-qPCR, 200 reactions	Thermo Scientific™	This kit contains the reverse transcriptase that will allow us to make the cDNA.	**$854**
	#FERK0243, Maxima SYBR Green/Fluorescein qPCR Master Mix (2X), 4x12.5 mL	Thermo Scientific™	Reagent needed to perform the qPCR experiments to evaluate the expression level of the XME genes in each of the liver tissue samples	**$1,837**
	#LXSAMSM-11, Mouse Luminex Discovery Assay (11-Plex); CCL3/MIP-1 alpha (BR46), CXCL1/GRO, alpha/KC/CINC-1 (BR13), CXCL2/GRO beta/MIP-2/CINC-3 (BR20), FGF-21 (BR61), GDF-15 (BR15), IL-1 beta/IL-1F2 (BR19), IL-6 (BR27), IL-10 (BR28), Leptin/OB (BR35), M-CSF (BR45), TNF-alpha (BR14)	R&D Systems™	Assay needed to test the 11 markers of senescence-associated secretory phenotype	**$2,389**
	#DAC00B, Human/Mouse/Rat Activin A Quantikine ELISA Kits (3X)	R&D Systems™	Activin A ELISA needed to measure activin A levels	**$1,845**
Travel	ENDO 2023 conference Registration fee			**$400**
	Hotel in Chicago		Estimated lodging cost $100/night plus Chicago hotel tax	**$425**
	Roundtrip Columbus (CMH) to Chicago (ORD)		Estimated airfare cost according to Expedia	**$180**
			TOTAL	**$7,141**
			REQUESTED	**$6,000**
			Remaining $1,076 to be provided by advisor if funded	