This is provided as an example proposal.

It is important that you follow the
current guidelines.
The mentor letter has been removed.

PURF COVER PAGE

TITLE OF PROJECT: The Effect of Weight Cycling on Lipid Peroxidation

NAME OF APPLICANT: Julie Slyby
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DEPARTMENT: Honors Tutorial College: Biological Sciences

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

CLASS RANK: (circle one) Freshman Sophomore Junior (Senior)

GPA: 3.8

EXPECTED DATE OF GRADUATION: May 2019 *

* Note: Students must be enrolled and maintain undergraduate student status during the proposed project period.

FACULTY MENTOR INFORMATION:

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We the undersigned have read the PURF Guidelines and understand the responsibilities we undertake should funding be granted.
We certify that the application has been conceived, written and completed by the student.

Student signature: Julie Slyby Date: 10/3/18
Faculty signature: Date: 10/3/18
Faculty Advisor's Dept. Chair signature: Date: 10/3/18

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 #***
	X	Human Subjects in Research (including surveys, interviews, educational interventions): Institutional Review Board (IRB) Approval #: Expiration Date:	19.052
	X	Animal Species: Institutional Animal Care & Use Committee (IACUC) Approval #: Expiration Date:	19.049

X **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: Julie Slyby Date: 10/3/18

2. Abstract

Weight cycling, the process of losing and regaining weight, remains a controversial topic with some suggesting the practice is more detrimental than remaining obese. Due to this controversy, our laboratory conducted a study and found weight cycled animals were significantly healthier and longer lived than obese controls. However, the mechanisms behind these health benefits remain unknown. Thus, in this study we propose to investigate the effects of weight cycling on oxidative stress, a theory of aging, in comparison to remaining obese.

3. Project Narrative

Project Description: Of the 323.1 million people living in the United States, 226 million people are either overweight or obese representing an astonishing two-thirds of the population [1]. This alarming statistic highlights the need for research to be done to help the 70% of our population affected by this epidemic. Unfortunately, numerous adverse health outcomes are associated with being obese including pathological conditions such as diabetes, heart disease and cancer [2]. With increasing research and education, many individuals are becoming aware of the negative effects of obesity and because of this, alter their diet and lose weight. Unfortunately, the vast majority of individuals (an estimated 80%) who lose weight will regain the weight within twelve months [3, 4]. The practice of weight loss and regain is referred to as weight cycling.

It has been reported that half of individuals classified as overweight or obese have attempted to lose weight, with 80% of these individuals failing, and two-thirds of the US population being overweight or obese, close to one-third of the US population has weight cycled [5]. Despite the prevalence of weight cycling, research is limited in both human and animal models. For example, several clinical studies using surveys to evaluate mortality in weight cyclers have concluded that weight cycling is more detrimental to health than remaining obese [6–12]. In contrast, other clinical studies have concluded the opposite and suggest that weight cycling is healthier than remaining obese [13–18]. Since there is no clear consensus as to the long-term health effects of weight cycling, my advisor (Dr. Edward List) and a former HTC student (Jacob Wright-Piekarski) used mice to publish a controlled feeding study to evaluate the effects of weight cycling on lifespan. Their results showed that weight cycled mice lived significantly longer compared to obese controls, suggesting that remaining obese was not heathier [19]. Taken together, this finding suggests that periodic weight loss in obese mice is

healthier than remaining obese and may slow the effects of accelerated aging associated with obesity. However, the cellular mechanisms behind this effect remain poorly understood. Thus, the goal of this project is to determine how weight cycling affects oxidative damage compared to remaining obese.

While there are many factors that make us age, one of the mechanisms proposed to promote aging is oxidative stress. This theory states that free radicals, which are produced in all living organisms, cause damage to all parts of the cell which accelerates aging and other disease processes [20]. Common examples of free radicals include OH- (hydroxyl radical) and O2- (superoxide anion), which are produced in the body as a result of cellular respiration in the mitochondria. As electrons travel through the mitochondrial electron transport chain, some leak from the inner membrane interacting with oxygen to form harmful ROS (reactive oxygen species). ROS can have negative effects by damaging DNA, proteins and lipids. To combat the effects of these free radicals, antioxidant defenses are present such as superoxide dismutase, catalase and Vitamin E. These enzymes catalyze the formation of non-radical species such as H2O2 (hydrogen peroxide) in place of ROS [21]. In a normal state, the antioxidant systems in place prevent the negative effects of ROS.

The term oxidative stress is used to describe the imbalance of excessive formation of ROS and limited amounts of antioxidants resulting in oxidative damage to DNA, lipids, and proteins [22]. In several long lived animal lines, increased life span has been correlated with a resistance to oxidative stress and decreased levels of oxidative damage [23]. In addition, increased ROS have been found to be correlated to degenerative diseases such as diabetes and cancer [24]. In relation to obesity, increased levels of oxidative stress and cellular damage have been reported and implicated in exaggerating the diseases associated with obesity. When an

individual has excess adipose tissue, the adipocytes (fat cells) secrete excess adipokines leading to a state of chronic inflammation. This imbalance in the adipocytes leads to excess production of ROS resulting in oxidative stress throughout the body [25]. In a study by Furukawa *et al.*, oxidative stress in obese individuals was associated with the development of diabetes, hypertension and arteriosclerosis. These results were also seen in three mouse models of obesity [26]. The relationship between oxidative stress and obesity has been clearly established in the literature but the literature regarding weight loss and oxidative stress is sparse.

In 2017, Himbert *et al.* conducted a systematic review of the literature involving intentional weight loss in relation to oxidative stress. They found that weight loss was specifically associated with decreased levels of 8-isoprostane, a marker of lipid oxidation. Based on these findings, Himbert concluded that initial weight loss was found to be associated with reducing obesity related oxidative stress [27]. Unfortunately, no studies, in human or animal models, have evaluated how weight cycling affects oxidative stress, which highlights the necessity of this proposed study.

As previously mentioned, oxidative stress can cause damage to DNA, proteins and lipids. The proposed study aims to determine the effects of oxidative stress on lipids (lipid peroxidation). Molecules that are produced as a result of this damage are able to be measured to determine levels of oxidation. The biomarker that will be measured in this study is 4HNE (4-hydroxynonenal) which is produced as a result of oxidative damage to lipids. When lipids are oxidized they undergo a process called fragmentation where the original lipid is broken down through a series of chemical reactions into several new molecules. During this process, the fragment 4-HNE is formed [28]. There are several biomarkers available to measure oxidative

damage to lipids, but 4HNE is considered the most reliable and "gold standard" in animal models [29].

Methods: I hypothesize that weight cycled mice will have decreased oxidative damage compared to obese controls. This study will utilize adipose tissue, liver and muscle previously collected from mice in four different diet groups with 10 mice per group. Group 1 was fed a low fat diet (LF) and serve as lean controls. Group 2 was fed a high fat diet (HF) and serve as the obese group. Groups 3 and 4 were placed on weight cycled diets in which mice were fed a HF diet for four weeks then moved to a LF diet for four weeks and repeated until dissection. The difference between groups 3 and 4 is that they are out of phase to allow tissues to be collected from weight cycled mice at the end of a HF cycle (group 3) and at the end of a LF cycle (group 4). In order to determine levels of oxidative damage to lipids, an ELISA kit from Cell biolabs will be utilized. To use this kit, 25-30 mg of previously collected flash frozen liver, muscle, liver and adipose tissue will be homogenized in RIPA buffer to isolate protein. Protein concentrations will be determined using a Bradford assay. Once the samples are prepared, manufacturer's instructions will be followed. To determine if the experimental groups are statistically different from one-another, one-way ANOVA will be used as the statistical test. Values will be considered significant at $p \leq 0.05$. SPSS software will be used to analyze the data.

Timeline: Tissue for this study has already been collected. If funded in November, we will place the order for the kits while I prepare the tissue for the remainder of November-December. As this is an integral part to my senior thesis, I will be staying over winter break to run the ELISA kits. I will then analyze the data and create my poster for the student expo.

Student's Role: I had to opportunity to choose this specific project. My advisor (Dr. List) allowed for me to choose a mechanism of aging I wished to study. After searching the

literature, I came to the conclusion that investigating oxidative stress was the area where we could contribute to the literature. I will be taking responsibility for completion of this project because it is a part of my senior thesis project. As I have already been training on how to run ELISAs, I will be able to run the kits and analyze the data based on my past experience. However, I will consult Dr. List when interpreting the results and deciding the next step in this project.

Significance: Obesity increases the risk of numerous diseases including diabetes, cancer and heart disease. While losing weight one time and remaining lean is ideal, this seldom occurs, as 80% of individuals that lose weight gain it back. Thus, a large portion of our population has weight cycled. Unfortunately, very little is known about the long term health effects of weight cycling and understanding how weight cycling affects our long term health is critical. This project is important because it will contribute to the limited amount of research that has been conducted on weight cycling and more importantly, may help determine if the burden of increased oxidative stress associated with obesity is alleviated with periodic weight loss. Also, this study will serve as my HTC thesis, allowing me to contribute to the field of obesity research. Publishing this data will bring notoriety to Ohio University and provide much needed information that hopefully results in development of therapies and/or therapeutics for obesity. In light of the obesity epidemic, these results are important to a large portion of the US population. Furthermore, Southern Ohio is especially vulnerable to the obesity epidemic.

4. Bibliography

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5. Biographical Information

As a senior in the Honors Tutorial College, I am currently in the process of completing my thesis project with Dr. List. I have been in Dr. List's lab since May 2017 and I have learned multiple laboratory techniques, scientific writing on grants and publications and how to present research. These skills I have acquired will allow for me to complete the project, analyze the results and present them in my thesis and at the EXPO. Before my work with Dr. List, I worked with Dr. De Lacalle beginning my freshman year. During this time, I was awarded a fellowship that required me to plan and complete a project. This was another experience taught me how to formulate a project from start to finish, which is a beneficial skill that will aide me with the proposed project.

6. Budget

Item	Amount	Source	Justification
OxiSelect HNE Adduct Competitive ELISA 96 well Kit (STA-838)	$638 x 3 = $1,914	Cell Biolabs	This ELISA kit is needed to test for the ubiquitous marker of oxidative stress in lipids. Each ELISA kit contains 96 wells. There are 40 mice and 3 tissues (adipose, liver and muscle) to be tested in duplicate. This will require a minimum of 240 wells. In addition, wells are needed to prepare standards to get accurate measurements. Three kits will provide sufficient space to test the samples and standards.

Subtotal = **$1914**

Amount requested = **$1500***

*If funded, the remaining **$414** will be covered by my research advisor Dr. List.