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: Calcineurin-like Phosphoesterase Domain Containing 1 in Human Skeletal Muscle of Lean vs. Obese Individuals and its Relation to Insulin Resistance

NAME OF APPLICANT: Mackenzie Bridges
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DEPARTMENT: College of Arts and Sciences

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

CLASS RANK: ◯ Freshman ◯ Sophomore ◉ Junior ◯ Senior
GPA: 3.77
EXPECTED DATE OF GRADUATION: May 2022 *

* 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: Mackenzie Bridges Date: 09/23/2020
Faculty signature: Leslie Consitt Date: 09/23/2020
Faculty Advisor's Dept. Chair signature: Thomas J. Rosol Date: 09/30/2020

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 #: 16-F-3 Expiration Date: December 02, 2020, will be submitted for renewal in October 2020	19.052
☐	☐	Animal Species: Institutional Animal Care & Use Committee (IACUC) Approval #: Expiration Date:	19.049

☑ **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: Mackenzie Bridges Date: 09/23/2020

2. Abstract

Skeletal muscle insulin resistance causes type 2 diabetes. Unfortunately, the cause of insulin resistance remains unknown. I hope to provide novel evidence suggesting the protein, Calcineurin-Like Phosphoesterase Domain Containing 1 (CPPED1), plays a role in obesity-related insulin resistance. I will determine if skeletal muscle CPPED1 levels are higher in obese, insulin resistant individuals compared to lean, insulin sensitive individuals. I will also determine if skeletal muscle CPPED1 levels are related to whole-body insulin resistance and impaired skeletal muscle insulin signaling. This study will provide a novel mechanism for future research to help prevent or treat obesity-related insulin resistance.

3. Project Narrative

Goals and Scope

The overall goal of my research project will be to identify a novel protein in human skeletal muscle that is associated with obesity-related insulin resistance. In the United States, the prevalence of obesity was 42.4% in 2018 (CDC, 2020). Obesity is a major health risk factor, contributing to several comorbidities, and accounting for huge costs for affected individuals, families, healthcare systems, and overall society. Obesity contributes to the development of insulin resistance and type 2 diabetes (Barazzoni et al. 2018). Unfortunately, the cellular mechanisms that contribute to obesity-related insulin resistance remain unknown, making preventative and/or treatment programs ineffective.

Insulin resistance occurs when the human body resists the effects of insulin - a hormone that regulates the movement of sugar into cells. Skeletal muscle is responsible for about 80% of insulin-stimulated glucose uptake in healthy individuals through insulin signaling (see Figure 1A) (Esteves et al. 2018). Impairments in the ability to 'activate' the insulin signaling cascade with obesity results in insulin resistance (Esteves, et al. 2018). Unfortunately, little research information is available about the cause of skeletal muscle insulin resistance. Since it is not well understood, the shortfall of knowledge inhibits the ability to develop interventions for this major health condition. I plan to do research using human skeletal

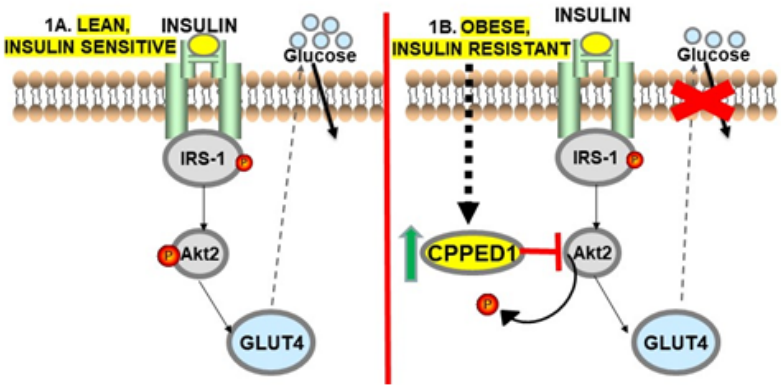

Figure 1A (left): *Skeletal* muscle insulin signaling cascade leading to *healthy, insulin* sensitivity (glucose uptake) in lean individuals. ***Figure 1B (right):*** *Proposed model of increased skeletal muscle CPPED1 levels in obese individuals (hypothesis 1) leading to reduced Akt phosphorylation and insulin resistance (impaired glucose uptake, hypothesis 2).*

muscle tissue samples from lean, insulin sensitive individuals and obese, insulin resistant individuals (previously collected) to propose a novel role for Calcineurin-Like Phosphoesterase Domain Containing 1 (CPPED1) in insulin resistance. My project proposes to demonstrate for the first time that skeletal muscle CPPED1 is **a)** ***elevated in obese, insulin resistant individuals*** and **b)** ***associated with insulin resistance (see Figure 1B)***. Such a discovery will provide a new mechanism that could contribute to insulin resistance and allow future research to focus on ways to reduce CPPED1 and ultimately prevent/treat obesity and obesity-related health complications.

Context

Previous research shows that insulin-stimulated phosphorylation (activation) of the insulin signaling cascade is critical for insulin sensitivity (Consitt et al. 2018) (figure 1A). Research also shows that obese individuals have impaired skeletal muscle insulin signaling at the level of Akt phosphorylation which results in the inhibition of GLUT 4 moving to the plasma membrane, and reduced glucose uptake causing insulin resistance (Brozinick et al. 2003). Unfortunately, the mechanism for this impairment in skeletal muscle insulin resistance remains unknown. A recent study showed that individuals that became more insulin sensitive after weight loss also had significant decreases in adipose CPPED1 and that reducing CPPED1 expression in adipose cells (in cell culture) resulted in increased insulin-stimulated glucose uptake (Vaittinen et al. 2013). This suggests that CPPED1 may contribute to insulin resistance in adipose tissue. Subsequent studies suggested that CPPED1 caused impaired insulin signaling (specifically dephosphorylation of Akt) (Zhuo et al. 2013). Based on this previous huge find in adipose tissue, my research will focus on the main target of insulin, skeletal muscle. Large proteomic studies indicate CPPED1 is expressed in skeletal muscle (Murgia et al. 2017), but no research has determined its role in this important tissue. Therefore, my research will hypothesize that ***skeletal muscle CPPED1 will be higher in***

obese individuals compared to lean individuals (hypothesis 1, figure 1B) and skeletal muscle CPPED1 will be positively associated with markers of insulin resistance, including Akt dephosphorylation (hypothesis 2, figure 1B). I aim to prove a potential novel role of CPPED1 that contributes to insulin resistance. Such a discovery in CPPED1 provides an opportunity for the prevention and intervention of insulin resistance due to obesity.

Methods

My study will use human skeletal muscle samples that have been previously collected from lean, insulin sensitive individuals and obese, insulin resistant individuals during an oral-glucose tolerance test (OGTT), as part of a larger study.

Hypothesis 1: Skeletal muscle CPPED1 will be higher in obese individuals compared to lean individuals. To test this hypothesis, I will perform western blots and statistics.

Western Blot Procedure: Skeletal muscle samples from 12 lean (6 male, 6 female) and 12 obese (6 male, 6 female) age-matched individuals will be homogenized in lysis buffer containing appropriate protease and phosphatase inhibitors, sonicated, and centrifuged (15000g) for 20 minutes at 4˚C with supernatant protein content determined by bicinchoninic acid assay (BCA). Protein samples (25 μg) will be separated by SDS-PAGE, transferred onto polyvinylidene difluoride membranes (Millipore, Billerica, MA) and incubated overnight at 4˚C with primary antibody for CPPED1. The next day, membranes will be washed and incubated for 1 hour at room temperature with horseradish peroxidase (HRP) conjugated secondary antibody (Cell Signaling). Amersham ECL Prime Western Blotting Detection Reagent (GE Healthcare Life Sciences) will be used to view the protein on an X-ray film using a developer (Hope). The band density at 36 kDa (corresponding to the molecular weight for CPPED1) will be quantified using ImageJ Software. Samples will be normalized to a control sample on each gel so multiple gels can be compared, and

membranes will be stripped and re-probed for α-tubulin (Cell Signaling) as a loading control. **Statistics:** A 2 (lean, obese) x 2 (male, female) ANOVA will be used to compare CPPED1 levels in lean and obese individuals (also accounting for sex differences). Analyses will be performed using SPSS version 23 (Chicago, IL) with statistical significance set at $P<0.05$.

Hypothesis 2: Skeletal muscle CPPED1 will be positively associated with markers of insulin resistance. To test this hypothesis, I will use statistics to measure relationships between CPPED1 data from western blots described above (hypothesis 1) and measurements of insulin resistance in the subjects. **Statistics**: Pearson correlation coefficient will be run between CPPED1 levels and markers of insulin resistance with SPSS. Markers of insulin resistance were previously determined in these subjects with the OGTT procedure and include: a] Matsuda Index (whole body insulin sensitivity) and b] Skeletal muscle insulin signaling (Akt phosphorylation) 1 hour after glucose ingestion.

Timeline

See Table 1. On average, I will devote at least 10 hours per week on the project. Certain procedures (i.e. western blot procedure) may require longer time commitments.

Table 1: Timeline of PURF activities.

MONTH	RESEARCH
November	Order Supplies
December	Test Sample Optimize Antibodies
January	Run Samples
February	Run Samples
March	Quantify Data Run Statistics
April	Present Data Start manuscript

Student's Role

The proposed study began from the desire to investigate further the complex mechanisms of insulin resistance in the muscle, through insulin-signaling. I discovered in previous literature that reduced levels of CPPED1 lead to higher insulin-stimulated glucose uptake in adipose tissue (Vaittinen, et al. 2013). That study sparked my interest to identify a novel role for CPPED1 in human skeletal muscle. I will perform western blots on skeletal muscle tissue from lean and obese individuals to compare their CPPED1 levels. I will analyze the results by a statistical program (SPSS) to develop

conclusions from these western blots. I will prepare a poster and present the research at the spring Student Research and Creative Activity Expo 2021, and I will help prepare a manuscript of the data for publication. This project will also expand my research skills by providing me the opportunity to design the overall research layout, independently run western blot procedures, perform statistical analysis, present and defend data, and learn to write scientific manuscripts.

Significance

Research in this discipline typically investigates kinases that phosphorylate proteins, but my research will show that there is another way to approach this issue by instead looking at a phosphatase (a protein that dephosphorylates). My project potentially provides a break-through for identifying a mechanism that contributes to insulin resistance. Impaired skeletal muscle insulin signaling causes insulin resistance and can lead to the development of type 2 diabetes. Preliminary data suggests that CPPED1 could play a role in insulin resistance in adipose tissue; however, no information is available about the role of CPPED1 in skeletal muscle. I believe that this project will fill a gap in needed research and the data will provide critical information and evidence of the potential novel role for CPPED1 in insulin resistance and specifically the dephosphorylation of skeletal muscle Akt. These findings could have a significant impact on the general public by providing a mechanism that could be targeted to treat or prevent type 2 diabetes. If my hypotheses are proven correct, future research could identify ways to reduce CPPED1 in skeletal muscle either by medicine or lifestyle changes (diet or exercise). It will also provide myself opportunities to expand on these results and learn additional lab techniques to answer more research questions in the future (i.e., immunoprecipitation experiments to determine if CPPED1 physically interacts with Akt and cell culture experiments to measure insulin signaling after manipulating CPPED1 expression).

4. Bibliography

1. **Barazzoni R, Gortan Cappellari G, Ragni M, and Nisoli E**. Insulin resistance in obesity: an overview of fundamental alterations. *Eat Weight Disord* 23: 149-157, 2018.

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8. **Zhuo DX, Zhang XW, Jin B, Zhang Z, Xie BS, Wu CL, Gong K, and Mao ZB**. CSTP1, a novel protein phosphatase, blocks cell cycle, promotes cell apoptosis, and suppresses tumor growth of bladder cancer by directly dephosphorylating Akt at Ser473 site. *PLoS One* 8: e65679, 2013.

5. Biographical Information

I am a junior undergraduate at Ohio University with a Biological Sciences major and Women, Gender, and Sexuality Studies certificate. I joined Dr. Consitt's lab my sophomore year, and this is my second year as a PACE researcher. I have gained much experience and knowledge for how to collect samples, run western blots and gather/organize data. Last year, I assisted with western blots on mitochondrial pyruvate carrier 1 (MPC-1) in lean and obese individuals which will prepare me for completing the westerns in this PURF and presented virtually at the Ohio University research expo. I have the experience and knowledge to conduct the research, and I understand the commitment and responsibility of the project.

6. *Budget*

Table 2. Itemized PURF Budget

Item	Amount	Source	Justification
CPPED1 Antibody	$390.00	Invitrogen	Used to identify CPPED1 protein in muscle samples
Alpha-tubulin Antibody	$114.00	Cell Signaling	Used as a loading control to normalize CPPED1 on gel.
4-12% Bis-Tris Acetate Gels, 1.0 mm, 12 well	$148.00	Invitrogen	Provide separation of proteins in a precast polyacrylamide concentration of 4-12% gradient
MOPS SDS Running Buffer (20X)	$87.00	Invitrogen	Used to run proteins on Bis-Tris gels only
Transfer Buffer (20X)	$179.00	Invitrogen	Used to transfer proteins from gel to membrane for western blots
LDS Sample Buffer (4X)	$18.00	Invitrogen	Prepare samples for gel electrophoresis
Sample Reducing Agent (10X)	$65.00	Invitrogen	Used to reduce protein samples for gel electrophoresis
Antioxidant	$31.62	Invitrogen	Maintains proteins in a reduced state for gel electrophoresis and protein transfer
Sharp Pre-stained Protein	$184.00	Invitrogen	Designed for accurate molecular weight estimation of proteins during SDS-PAGE and western blots
Anti-rabbit IgG, HRP-linked Antibody	$146.00	Cell Signaling	Targets antibody of interest based on animal used. CPPED1 is rabbit so we need this secondary. We have a mouse secondary in the lab that will be used for tubulin
Classic X-ray Film	$93.25	Research Products International Corp.	Necessary for western blot analysis
BSA	$106.00	Sigma	Used to protect antibody solution during incubation
10% Tween 20 Solution	$47.00	Bio-Rad	Used as a washing agent for western blots
Amersham ECL Prime Western Blotting Detection Reagent	$306.00	Fisher	Used on membrane to detect HRP enzyme activity.
Gel Tips	$25.00	USA Scientific	Loading sample into gel

Phosphatase Inhibitor Cocktail 2	$294.00	Sigma	Protects phosphorylated proteins from degradation
Phosphatase Inhibitor Cocktail 3	$273.00	Sigma	Protects phosphorylated proteins from degradation
Protease Inhibitor Cocktail	$302.00	Sigma	Protects proteins from degradation

Total: $2507.18 (see note below)

Amount Requested: $1500*****

*****If funded, the remaining $1007.18 will be covered by my research advisor Dr. Consitt through her American Heart Association Award and her Diabetes Action Foundation Award.