Dear Celebration Committee,

I am writing to support my students' proposals to attend The Endocrine Society's 2016 Annual Meeting (ENDO 2016) with me April 1-3 in Boston, MA. Our abstract, entitled "Endoplasmic Reticulum Stress Alters the Transcriptional Profile of GnRH-Producing GT1-7 Cells," was accepted for poster presentation in January. Noah Levi, Jared Santana, Graham Redweik, and Zack Campbell each made valuable contributions to the design and execution of the experiments we will present, as well as to data analysis and figure generation. While the hands-on laboratory work these students have done is a critical component of their science education, learning how scientists communicate their work is also essential to their understanding of the process of science. I also strongly believe that our students deserve exposure to research at multiple levels and that we as educators should encourage their engagement in the world outside of Wabash. By attending a national research meeting like ENDO 2016, my students will gain valuable experience with scientific communication while at the same time be exposed to cutting-edge research from top-tier institutions from all over the world.

ENDO 2016 is the 100th annual meeting of The Endocrine Society, an internationally recognized professional society that promotes basic, translational, and clinical research in endocrinology. I have attended this meeting multiple times as a graduate student and post-doctoral fellow, and have always had extremely positive experiences. This meeting is somewhat unique because it bridges the gap between basic research and clinical work. Since each of my students are considering careers either in biomedical research or medicine, I believe they too will leave feeling energized about how research like ours can make a larger impact. This meeting also has multiple opportunities for participants at all levels (including undergraduates) to network with other scientists; but, more importantly for Noah, Jared, Graham, and Zack, it will be a chance to hone the communication skills they have built by presenting their research on campus this academic year at the Hays Hall poster session and the Research Celebration. Furthermore, this meeting represents an opportunity for these students to interact with leaders in our sub-specialty of reproductive neuroendocrinology and get feedback on the scientific merit of our work.

The accepted abstract combines work from all four students to create a stronger scientific "story," and as their mentor I will work with the students as we design our poster to ensure they each understand how all of the parts come together. Furthermore, I will be on-hand during our poster session to help them field difficult questions, but based on their recent presentations at the Research Celebration, I am confident that these four students will represent Wabash wonderfully. Finally, this will be the first meeting I attend and present my work at since establishing my own research program. Thus, I am excited for the opportunity to provide exposure for Wabash College at a new national venue, and hope that this meeting will forge new academic collaborations for me and the College.

In closing, I am grateful for the committee's consideration of my students' proposal to attend ENDO 2016. Below you will find our accepted abstract, statements from all four students, and a

budget of estimated travel costs. Please feel free to contact me should you need any additional information.

Sincerely,

Heidi E. Walsh

ABSTRACT:

Endoplasmic Reticulum Stress Alters the Transcriptional Profile of GnRH-Producing GT1-7 Cells.

Noah J. Levi, Jared R. Santana, Graham Redweik, Zachery R. Campbell, Heidi E. Walsh

Obesity is linked to infertility, but the impact of obesity-induced metabolic changes on the reproductive axis is unclear. In mammals, reproductive function is regulated by hypothalamic gonadotropin-releasing hormone (GnRH), which controls pituitary gonadotropin release and subsequent gonadal function. Dysfunction of GnRH-producing neurons leads to infertility in both sexes. The excess of circulating nutrients generated from overnutrition interferes with the function of hypothalamic neurons that control food intake by disrupting normal protein folding in the endoplasmic reticulum (ER). When protein folding load exceeds cellular folding capacity, the cell initiates an ER stress response (the unfolded protein response, or UPR) through a defined set of signaling pathways and transcription factors. In obesity, ER stress can also promote inflammatory responses in hypothalamic neurons, creating a vicious cycle of cellular dysfunction. Using the immortalized mouse hypothalamic cell line GT1-7 as a model, we measured three important transcriptional targets (*Fos, Il6,* and *Gnrh1*) to determine how fertility may be impacted by ER stress. The transcription factor c-fos, encoded by the *Fos* gene, is implicated in protein kinase C (PKC)-induced downregulation of *Gnrh1* as well as upregulation of the pro-inflammatory cytokine *Il6* by inflammatory signals. In GT1-7 cells, ER stress (induced by tunicamycin or thapsigargin) increased *Fos* expression in a PKC-dependent manner, as pretreatment with the broad-spectrum inhibitor Gö6983 blocked *Fos* induction. ER stress induced a canonical UPR in GT1-7 cells, as measured by increased *Ddit3* and *CEBPB* mRNA levels, as well as an inflammatory response, as evidenced by increased *Il6* mRNA. The upregulation of *Il6* by ER stress was also PKC-dependent. Expression of *Gnrh1* was significantly repressed by ER stress, but because PKC inhibition did not fully rescue this repression, multiple ER stress-induced pathways could converge on *Gnrh1*. Our work demonstrates that obesity may impact fertility centrally by changing the transcriptional profile of GnRH neurons. Specifically, the reduction of *Gnrh1* mRNA by ER stress we observe in concert with upregulation of the pro-inflammatory cytokine *Il6* suggests that inflammatory signals may exacerbate ER stress-induced dysfunction of GnRH neurons.

Noah Levi Statement

Attending the Endocrine Society's national conference in April offers both a tremendous educational and experiential opportunity. Not only would I have the chance to learn about and investigate the various research being done in the field of endocrinology first hand, but I also could experience the atmosphere and environment of a national scientific conference with all that it entails.

As a biology major, and more importantly as a scientist, it is imperative to maintain a basis of the research being conducted in your field of interest or study and have the skills to understand and appreciate the research that is being conducted and shared. It is also important to be able to share the research that you and your counterparts have conducted. I believe attending this conference would satisfy both of the aspects, and more importantly they are skills that I believe would benefit me down the line. I am interested in a career in scientific research in the field of medicine or biology with medical application. This exposure would give me a preview of the skills necessary for a career in which I am interested.

ENDO 2016 offers various sessions ranging from clinical to basic research which are both very interesting, but I think that learning more about translational research in endocrinology would be amazing. I love the idea of taking a concept of bridging the gap between the laboratory and clinical setting. Learning more about the translational projects at different poster sessions would be a top priority. Additionally this experience can help me decide specifically if endocrinology is a medical or scientific field in which I would want to continue researching. Some of the talks I am interested in attending are "Stress Signaling and Translational Control of Gonadotropin Synthesis by GnRH" as well as the "Sex, Drugs, and Rocky Road: Neuroendocrine Control of Behavior". This first talk has direct relatability to the research we performed over the summer because it deals with the aspects of GnRH synthesis and stress. The second talk incorporates more psychological and psychiatric perspectives, which piques my interest in human behavior as a psychology minor.

Attending the conference would be significant for me because this past summer I helped run experiments and collect data relevant to the abstract that she submitted to the Endocrine Society. Most of the research revolved around measuring transcriptional levels of mRNA for various genes including the main gene of interest Gonadotropin releasing hormone (GnRH). The experiments I carried out utilized qPCR to determine the up or down regulations of GNRH and c-Fos in GT1-7 cells exposed endoplasmic reticulum stress/ tunicamycin. This data was instrumental in formulating an abstract for poster presentation. I believe that this work is extremely relevant because it gives insight into the cellular pathway of obesity related infertility. It further supports the prevalence of obesity as comorbid with other medical conditions (i.e. thyroid problems and diabetes which are both relevant in endocrinology).

This conference sounds like a great opportunity and I would be very thankful if I had the chance to attend and experience it for myself.

Jared Santana Statement

My involvement with the project began this previous summer. Dr. Walsh took me into her lab for a summer internship; upon which, my colleagues and I were given individual projects in efforts to piece the interaction of ER-stress and GnRH levels in GnRH neurons together. Given my previous exposure to inflammatory pathways from my Endocrinology course, Dr. Walsh and I thought it would be fitting for me to take on this portion of the project. In other words, my role became investigating the inflammatory pathways ER-stress was inducing to increase inflammatory signals. During the summer, I ran multiple experiments analyzing signaling pathway activity and gene expression. This portion of the project was quite interesting as an unexpected conclusion was drawn regarding the inflammatory pathway. The initial protein of interest (NFkB) did not show significant signs of phosphorylation upon ER-stress induction. So, Dr. Walsh and I began to investigate a leading alternative by examining JNK phosphorylation. It was at this point that my summer internship came to an end; however, my time in lab did not.

After my summer internship, I was offered (and accepted) an independent study opportunity in Dr. Walsh's lab for the Fall 2015 semester. During this time, the investigation of JNK continued and was being fine-tuned. A conclusive figure was not achieved; however, preliminary results show increased activation of JNK upon ER stress induction. This aspect of the project is still under way and has been handed off to another student, although, my contribution to the project was nevertheless quite considerable. I was a research student in the lab for approximately 6 months and contributed to a novel idea pertaining the inflammatory pathway in these cells as a result of ER-stress. Having the opportunity to share these findings and learn about the discoveries in other laboratories at ENDO 2016 would be remarkable.

This national meeting of the Endocrine Society would contribute to my background substantially and offer insights into more avenues of endocrinology. I will have the opportunity to attend sessions discussing "Day-Night Rhythm in Skeletal Muscle Mitochondrial Function," "The Effects of Growth Hormone Treatment on Bone Mineral Density and Body Composition in Children with Idiopathic Short Stature," and a closer to home session on "Optogenetic Regulation of GnRH and Kisspeptin Neurons to Control Gonadotropin Secretion." Each of these topics is just a sample of the many that will allow me to learn about areas of endocrinology research that spur my interest. The initial topic sparked my interest quite sincerely as it proposes the concept of a circadian rhythm cycle of mitocho1009ET3u3(w)4(74.304 433.8792Ba008300)TJETT08hat se5T1 0 0 1 e1009ET3u3(w)4(

graduate programs after Wabash. Additionally, I feel as though my contribution to the research is sound, and that this Endocrine Society meeting would provide me with a unique ability to see what other areas and questions endocrinology research is investigating. I would be extremely grateful to be given the support and opportunity to make this trip to Boston with my Professor and colleagues.

Graham Redweik Statement

During my research in Dr. Heidi Walsh's lab last semester, I assisted in adding another piece to the puzzle in our attempt to understand how obesity is related to infertility. We knew prior to the research that obesity increases levels of reactive oxygen species in gonadotropin-releasing hormone (GnRH) neurons, which induces endoplasmic reticulum (ER) stress in cells. This ER stress, or unfolded protein response, causes a cellular cascade that inevitably prevents normal GnRH neurons from functioning properly, leading to infertility. However, the exact steps in this process are unclear, and my research helped provide additional insight into the mechanism of this pathway. I found that protein kinase C (PKC), a regulatory protein found in several tissue types, mediated ER stress by increasing levels of IL-6 (pro-inflammatory cytokine) and c-fos (transcription factor) gene expression in GnRH neurons. I am continuing work on this project in the lab this semester, and thus attending ENDO 2016 would be extremely valuable because I will get feedback and new ideas about our work.

The opportunity to present our research in Boston would be extremely beneficial to both my academic and aspirational development. Seeing how other people have gone about researching neuroendocrine pathways (even different from ours) would provide valuable insight and could potentially create new ways for us to more efficiently conduct our research. Additionally, observing findings from other researchers would benefit my development as a scientist, enabling me to become more knowledgeable in how the human body works. This is very important to me because I want to conduct research that could be utilized in a clinical setting, and having a greater base of knowledge would only enhance my ability to relate my research to ways to help people facing disease. Being in a setting with many graduate students as well as experts in their respective fields would provide a great opportunity for me to make connections with people I will not have had the opportunity to do so without this experience. This is especially important for me as I have currently applied to Ph.D. programs in Biology. Specifically, some of the talks I am interested in attending are "New Insights in Gonadotrope Biology" (Friday, April 1st), "Neuroendocrine Control: Is It All in Your Brain?" (Saturday, April 2nd), and "GnRH & Gonadotroph Biology & Signalling" (Saturday, April 2nd); all three of these talks tie into what we are researching, and these would give me a better understanding of how the brain mediates the endocrine system. Hopefully, I will have the pleasure of going to such an acclaimed research event, further reaffirming my interest to be involved in biological research as a career.

Zachery Campbell Statement

Obesity is a health concern that often leads to increased risk of other ailments such as infertility. Gonadotropin- Releasing Hormone (GnRH) is a protein that is expressed in specific neurons in the hypothalamus and is a key regulator of fertility. GnRH expression is stimulated by the neuropeptide Kisspeptin. Obesity causes stress on the Endoplasmic Reticulum (ER). When stress is placed on the system, the Unfolded Protein Response (UPR) is activated to counteract the stress.

We focused on understanding how GnRH neurons and their response to Kisspeptin is impacted by ER stress. I treated cultured GT1-7 mouse hypothalamus cells with Kisspeptin and the ER stress inducer, Tunicamycin, to determine the respective interactions between these signals, the UPR, and Gnrh1 gene expression. Quantitative RT-PCR studies confirmed that tunicamycin adversely affected the production of GnRH, while Kisspeptin increased GnRH production. There was also a trend seen between tunicamycin and the up regulation of expression of the Slc12a2 gene, which encodes a chloride transporter essential for GnRH neuron function. Future work will determine if effects on Slc12a2 are significant and whether ER stress can block the induction of Gnrh1 by Kisspeptin.

The ENDO 2016 conference in Boston provides a unique experience for those of us who worked in Dr. Walsh's lab this summer as well as a valuable gateway to represent Wabash College's Biology department. Over the three day period we would have substantial exposure to other researchers, physicians, and students, granting us the ability to expand our network of contacts. The opportunities for networking with a wide variety of people in top of the line science fields would be invaluable for our career paths. As students of science, effective communication with others in the scientific community is extremely important. The ENDO 2016 conference gives us a prime setting to refine the craft of presenting in a professional atmosphere, helping to give us a competitive edge in our post-graduation plans. We would gain insightful feedback about our research by others in the field while also showing the top notch research that we conduct at Wabash. We would be able to attend other events such as debates, interview sessions, and poster sessions on various topics related to our own line of work which would widen our own scope of knowledge on endocrinology.

The opportunity to present at ENDO 2016 would be an invaluable experience for me and the others who worked in Dr. Walsh's lab. As someone who wants to go into medicine after Wabash, many of the concepts that will be presented upon have implications in the medical field and are critical to my career aspirations. The chance to network, develop professional skill sets, and spend a weekend learning about Endocrinology from premier sources would be a cornerstone in my research experience and career at Wabash.

Proposed Budget:

ENDO 2016 – Boston, MA

April 1-3, 2016

Student Meeting Registration: $339 x 4 students* = $1017

Estimated Roundtrip Airfare (IND → BOS, April 1 - April 3): $400 x 4 students = $1600

Hotel (1 room for 4 students): $247 x 2 nights = $494

Food (per diem, 1 full day, 2 travel days): $172.50 x 4 students = $690

Total estimated cost for 4 students = $3801

Minus 50% contribution from Biology Department = $1900.50

Total requested funds = $1900.50

ENDO 2016 offers 3 student registrations for the price of 2