# Candidate Information

## 1) Candidate's Background

### RESEARCH EXPERIENCE

1. Department of Internal Medicine-Division of Endocrinology and Metabolism, University of Cincinnati, Cincinnati, OH, September 2009-present
   a. *Research Assistant Professor, Div. of Endocrinology, Department of Medicine, University of Cincinnati, Metabolic Diseases Institute*
      i. *Ongoing research projects: 1) the beneficial metabolic effects of duodenal nutrient exclusion by barrier endolumenal sleeve, 2) FGF21 as a mediator of the chronic effects of glucagon receptor agonism, 3) Use of GLP-1R agonists to optimize bariatric surgery in a DIO rat model, and 4) Glucagon-receptor agonism stimulates insulin action in mice.*
   b. *Postdoctoral Fellow*, University of Cincinnati, Metabolic Diseases Institute
      i. Ongoing research projects: 1) the beneficial metabolic effects of duodenal nutrient exclusion by barrier endolumenal sleeve, 2) FGF21 as a mediator of the chronic effects of glucagon receptor agonism
      ii. Completed research projects: 1) the *in vivo* role of ghrelin in atherogenesis, 2) Leptin-FGF21 and exendin-4 (Ex4) combination therapies for metabolic defects, 3) metabolic reprogramming after caloric restriction, and 4) resistance of hepatic deficient *protein kinase C-lambda* (PKCλ) mice to diet induced obesity and glucose intolerance.
2. Department of Biochemistry and Molecular Biology, Indiana University School of Medicine, Indianapolis, IN, January 2005- July 2009
   a. *Doctoral dissertation research* with Dr. Jeffery Elmendorf: Examined the role of plasma membrane cholesterol in insulin-dependent and -independent glucose-transporter 4 trafficking.
      i. *Thesis title*: Plasma membrane cholesterol in exercise and insulin resistance.
3. Department of Medicine- Division of Endocrinology- Alcoholism Research Center, Indiana University School of Medicine, Indianapolis, IN, July 2002- January 2005
   a. *Research assistant* to Dr. Lucinda Carr: Congenic P/NP and NP/P line development.

### TEACHING EXPERIENCE

1. Mentored (with Dr. Matthias Tschöp) University of Cincinnati graduate student 2009-11
   a. *Metabolic reprogramming after caloric restriction- Diabetes 2012*
2. Mentored (with Dr. Matthias Tschöp) the project of visiting graduate student (German Institute of Human Nutrition, University of Potsdam) 2011-12
   a. *Comparison of hepatic and adipose deficient protein kinase C-lambda mice to diet induced obesity and glucose intolerance- Adipocyte 2012*
3. Mentored (with Dr. Matthias Tschöp) the summer research project of a University of Arizona undergraduate student 2010
   a. *Use of GLP-1R agonists to optimize bariatric surgery in a rat model of diet-induced obesity. (Diabetes 2013, in press)*

**3) Career Development/Training Activities During Award Period**

To ensure the success of this proposal, and more importantly a successful transition to independence, I will pursue several lines of development activities. During the course of this award I will pursue additional training in scientific presentation and writing, as well as experimental design and technique. To obtain this training I have identified Dr. Randy Seeley as my primary mentor for this award. Professor Seeley is a well-respected leader in the field of obesity research, and his group investigates the neuroendocrine regulation of energy and glucose homeostasis. Furthermore, Dr. Seeley is an expert in the field of bariatric surgery and as such his expertise makes him well suited as a mentor for this award. I will maintain one-on-one meetings with Dr. Seeley on a weekly basis to discuss the results and direction of this project. *I have also chosen Dr. Jim Herman as a co-mentor for this award. Dr. Herman will insure that during this training period I receive an equally thorough and balanced training in the neurological aspects of this project. A major focus in Dr. Herman's research involves the study of ascending hindbrain systems, many of which overlap with the circuitry described in this application. To facilitate this training I will attend Dr. Herman's weekly lab meetings, which will expose me to work being conducted in this diverse group. I will also meet with Dr. Herman on a bi-weekly basis to discuss project-related scientific inquires and to insure my career progression.*

Another key component of my training will be interactions with my newly formed Career Advisory Committee (see attached letters of support). This will consist of my co-mentors, Drs. Herman and Seeley, and 3 senior faculty members, Drs. D'Alessio, Obici, and Sandoval from the Departments of Medicine. I will maintain informal contact with these investigators, as well as, quarterly meetings with this committee. These members will insure that conceptual and technical training is obtained through the completion of the award.

*This informal professional development will be supplemented with several lines of more formalized instruction. First, I will attend the Write Winning Grants seminar and the Intensive Grant-Writing workshop to hone and enhance my grant-writing ability. The University of Cincinnati Office of Research offers both of these workshops on an annual basis. Next, I will attend the Ready, Set, Go! Workshops offered by the University of Cincinnati Office of Research. This is a 10-workshop series designed to provide new faculty members with the competencies and skills to set up and maintain a successful research laboratory or clinical research program. The sessions address common problems and issues and allow faculty to better "hit the ground running" with his/her research program. The workshops cover the art of hiring skilled technical support, proper management techniques including conflict management and other personnel issues, time management, managing a dual career household, getting the most out of a mentoring relationship and navigating the thornier issues of independence and manuscript authorship. Finally, I will complete the Preparing Future Faculty (PFF) program offered by the University of Cincinnati Graduate Program. The UC PFF program provides **instruction in modern teaching** and learning and offers **mentoring experiences** that strengthen these career skills. This program consists of a Effective Teaching Colloquium, a Job Search Colloquium, five workshops, and completion of a 40-hour mentoring experience. This program prepares attendees for academic careers at postsecondary institutions and helps them conduct productive job searches and achieve early career success.*

Work from this proposal will be submitted for presentation at the annual meetings of the American Diabetes Association and the Society for Neuroscience, facilitating important interactions with investigators outside my institution. In addition, I have been and will continue to be a regular speaker in the Metabolic Diseases Institute's Neuroendocrinology of Obesity and Stress (NOS) seminar. The NOS seminar is used as venue for practice presentations for job interviews or talks given at international meetings and is also a forum to discuss strategy for grant applications.

The experiments described in this proposal will span both peripheral and central regulation of metabolic processes. As such, this proposal will provide expansive experience in neuroanatomical manipulations and clamping techniques. Specifically, I will be trained in the *in vivo* use of lentiviral vectors to manipulate gene expression in specific regions of the CNS, immunohistochemistry, hyperinsulinemic-euglycemic and hyperglycemic clamps. *Dr. Herman will be responsible for training me in use of lentiviral vectors and c-fos immunohistochemistry, while Dr. Sandoval will oversee my training with regard to hyperinsulinemic-euglycemic and hyperglycemic clamps.* When these techniques are integrated with my prior training, they comprise a powerful set of tools that will be invaluable in metabolic investigation throughout my career. In addition to this experimental training, I will also attend the workshop on "Glucose Clamping the Conscious Mouse: A Laboratory Course" at Vanderbilt-NIDDK Mouse Metabolic Phenotyping Center (MMPC), Nashville, Tennessee. This workshop will compliment and enhance the hands-on training provided by my career development committee as well as my prior training in metabolic tracers. I will also audit "Fundamentals of Neuroscience II" and "Advanced Topics in Neuroscience" offered by UC College of Medicine. Finally, to further develop my teaching skills, I will guest lecture in the graduate seminar "Neuroendocrinology of Homeostasis".

**2) Career Goals and Objectives**

My goal is to become an independent, NIH-funded investigator and involved mentor at a major research institution in the field of neuroendocrinology. My immediate scientific goal is to combine my graduate and post-doctoral training, as well as the training described in this proposal, into novel and highly relevant metabolic research. This synthesis of prior training and proposed future work will allow for substantial scientific growth and a committed transition from trainee to independent investigator. My long-term scientific goal is to become a respected and dynamic member of the scientific community who contributes to understanding the etiology of, and novel treatments for, metabolic diseases. A crucial component of this plan will be to recruit energetic and dedicated trainees and to provide a dynamic and well-funded environment in which they can thrive.

The objectives described above are highly dependent upon a successful transition from trainee to independent investigator. To attain these objectives I will obtain world-class training in the neuroendocrine regulation of glucose metabolism and energy balance at the Metabolic Diseases Institute of the University of Cincinnati. This award is essential to my future success for two reasons, 1) it will provide the vehicle that combines both prior and new training opportunities along with the chance to apply this training to novel and relevant questions in the field, & 2) it will provide a smooth and sustained transition to independence.

My prior training has provided me with a solid foundation in the field of glucose metabolism and energy balance and will contribute to the successful completion of this application. Throughout my training I have acquired a diverse skill set in energy metabolism research, endocrinology, biochemistry, and integrative physiology. This background has provided me with unique training and expertise in key research areas inherent to the successful completion of this application. My graduate training was conducted at the Indiana University School of Medicine, in the Department of Biochemistry and Molecular Biology. This program provided rigorous coursework in cellular signaling, enzymology, biochemistry, and protein structure/function. This core coursework was supplemented by additional courses with a focus in diabetes and obesity. These additional courses exposed me to the specific pathophysiology of diabetes and obesity, as well as introducing me to systems biology. My thesis research was conducted in the group of Dr. Jeffery Elmendorf. Research in the Elmendorf lab is focused on lipid rafts of the plasma membrane and their subsequent regulation of insulin-dependent and –independent glucose transport. This research highlighted the intricate regulation between plasma membrane cholesterol and the underlying cortical-actin cytoskeleton in the trafficking of glucose-transporter 4 containing vesicles. Within this framework, my thesis research focused on the inappropriate accumulation of membrane cholesterol in insulin-resistant skeletal muscle[1], and a subsequent reversal during stimulus of the AMP-dependent Kinase[2]. These studies provided me with a strong foundation in cell biology, membrane dynamics, intracellular signaling, and glucose transport.

To build upon this foundation I sought out training in systems biology that would allow me to apply this training to the regulation of glucose and energy metabolism. I was fortunate to secure a postdoctoral fellowship in the group of Dr. Matthias Tschöp at the Metabolic Diseases Institute of the University of Cincinnati. Professor Tschöp is a leader in the field of obesity research, and his group investigates the neuroendocrine regulation of energy and glucose homeostasis. My fellowship in the Tschöp group resulted in a successful transition to the field of systems biology. This transition is evidenced by publication of three first-author reports[3, 4] "Ghrelin receptor deficiency does not affect diet-induced atherosclerosis in low-density lipoprotein receptor-null mice", "Restoration of leptin responsiveness in diet-induced obese mice using an optimized leptin analog in combination with exendin-4 or FGF21", and "The role of adipose and hepatic atypical Protein Kinase C-lamda (PKC-λ) in the development of obesity and glucose intolerance" (*Adipocyte, in press*); a corresponding authorship "Caloric restriction chronically impairs metabolic programming in mice" (*Diabetes, in press*), as well as several contributing authorships[5, 6] and review articles[7-11].

Recently, Dr. Tschop left the U.S. to take a new position in Munich. At that point I made a choice to move to the laboratory of Dr. Randy Seeley. This proposal is a direct result of my decision to focus on bariatric surgery as a model that can help us understand how signaling from the gastrointestinal (GI) tract contributes to metabolic disease and its potential treatments. This training will also allow me to acquire a number of new research skills highlighted in this proposal. My time with Dr. Seeley has already been productive. Data obtained from my work with Dr. Seeley is currently being compiled for publication and comprises much of the preliminary data for this application. During this fellowship I also attended the Isotope Tracers In Metabolic Research: Principles and Practice of Kinetic Analysis (April 2010), a course that will be invaluable to the work proposed in this award. The skills I have acquired in the course of my postdoctoral training, including: rodent stereotaxic and bariatric surgeries, basic rodent phenotyping (insulin & glucose tolerance tests, feeding studies, etc…), indirect calorimetry, quantitative RT-PCR, and ELISA, provide a strong foundation for the experiments included in this proposal, as well as my future career as an independent researcher. Together

with my graduate work, this training represents a broad understanding of metabolic regulatory systems, as well as the tools to investigate these complex systems **from single molecules to whole body physiology.**

My unique background will insure that any finding will be appropriately and efficiently addressed. The scientific environment afforded at the Metabolic Diseases Institute (MDI) at the University of Cincinnati enhances this background. Investigators at the MDI conduct research focused on the genetic, molecular, cellular and physiologic mechanisms of metabolic disorders, neuropsychiatric disorders, cancer and cardiovascular disease. This faculty includes Drs. Randy Seeley, Stephen Benoit, David D'Alessio, Darleen Sandoval, James Herman, Silvana Obici, Randall Sakai, and Patrick Tso.

Altogether, this proposal describes a vehicle that combines both prior and new training opportunities along with the chance to apply this training to novel and relevant questions in the field and is essential in that it will provide a smooth and sustained transition from trainee to independence. **The successful completion of this proposal will significantly contribute to becoming an NIH-funded investigator and mentor.**

**INTRODUCTION TO REVISED PROPOSAL**

We would like to thank the reviewers for a thorough critique that clearly argued their concerns with the previous version of this application. We were pleased to see that the proposal was viewed as addressing a significant area, which could provide important information relevant to patient care. We were also appreciative that the reviewers gave both this investigator, and the research committee, high marks and recognized the stimulating and supportive research environment at the University of Cincinnati. Beyond these strengths the reviewers identified a number of specific issues that could improve the proposal. We have given these critiques serious consideration and tried to address each point in a substantive way. We have obtained additional data illustrating the feasibility of this proposal and feel we are in a stronger position to test our central hypotheses. Below is a point by point response to the central criticisms raised with changes throughout the grant noted in *italic blue text*.

**Reviewer 1:**

Although Reviewer 1 thought the application addressed a significant area, a concern was expressed that the three specific aims are labor intensive and require large numbers of animals. Moreover, there was a question of the ability of the animals to tolerate multiple surgeries.

- Upon reflection, we agree that the initial proposal was a bit too ambitious in terms of labor proposed. Thus, we have identified several studies that, while of significant interest, would be more appropriate as future or alternative considerations. Our follow-up studies have yielded much higher survival rates (initially 60%, now >80%) and thus we were also able to reduce the number of animals required in each group, resulting in a 33% total reduction. Finally, with regard to the multiple surgeries our group has recently published several studies utilizing combined bariatric-clamp studies and feel this is now a minor concern.

The reviewer also expressed that although the studies are hypothesis driven, although there is no central hypothesis to guide the project.

- We acknowledge that in an attempt to create three, independent aims we had under-emphasized the overarching hypothesis that guides this proposal. We have now revised the specific aims page to highlight the central hypothesis.

**Reviewer 2:**

A concern was expressed concerning the training record of the co-mentors as well as details about the remaining three members of an advisory committee.

- We apologize for these omissions and have expanded these components in the resubmission.

The reviewer also felt that it was unclear what new disciplines would be acquired, and that the training program was lacking in details of any formalized professional programs that would prepare him for a career in research.

- We thank the reviewer for pointing out the vague nature of these sections and have now clarified the novel training points. We have also added several formal training programs to this proposal that will prepare this investigator for the multifaceted demands of a career in research.

The reviewer also highlighted that a method for direct measurement of NMDAR knockout and knockdown was not provided.

- This very valid concern has now been addressed.

The reviewer noted that the candidate remains a postdoctoral fellow who can only hope for advancement if the proposal is funded, and this indicates a lack of commitment on the part of the institution.

- The investigator has recently been promoted to assistant professor on the research-track, thus this should no longer be a concern.

Finally, concerns regarding feasibility of labor and a clear central hypothesis have been addressed in response to Reviewer 1.

**Reviewer 3:**

Although Reviewer 3 felt this was an exciting proposal there was a primary concern regarding institutional support.

- We would like to thank the reviewer for their concern in this important aspect of the K01 and its role as a developmental mechanism. As noted in comments to Reviewer 2, this investigator has recently been promoted to assistant professor on the research-track. Furthermore, a new letter of support from the institute now more clearly outlines their commitment to the development of this research.

**Title: Duodenal nutrient exclusion enhances glucose metabolism via CNS regulation**

Currently considered the gold standard in bariatric surgical procedures, Roux-en-Y Gastric Bypass (RYGB) stimulates a considerable and sustained weight-loss in obese individuals. This reduced adiposity is often accompanied by profound improvement in glucose control of type 2 diabetic subjects. While some of the effects of RYGB on glucose control are secondary to reduced body weight, dramatic changes in gut nutrient sensing/presentation and hormone profile may contribute to the resolution of T2D in many individuals. Accumulating evidence indicates that duodenal nutrient exclusion (DNE) may be an important contributor to the metabolic benefits of RYGB. We can directly test such DNE without gastric restriction or altering the path of ingested nutrients using duodenal, barrier-endolumenal sleeves (DES) that prevent ingested nutrients from interacting with the upper intestine.

Early pre-clinical experiments in rodents and clinical experiments in humans point to beneficial effects of DES in individuals with impaired glucose homeostasis. We have successfully established a rodent model of a DES that will allow careful mechanistic testing of the effects of a DES not possible in humans. In our hands, we observe that DES results in a small decrease in fat mass and a clear improvement in glucose tolerance, which is above and beyond the effect of the weight loss alone. This proposal will test several specific hypotheses as to the mechanism by which DNE exerts these beneficial effects.

Emerging data supports an intimate communication between the small intestine and the central nervous system (CNS) that is an important contributor to normal regulation of glucose. This communication appears to originate in vagal afferents of the small intestine. Furthermore, it is dependent upon glutamate (NMDA) receptors of intestinal-vagal afferents terminating in the hindbrain. Melanocortin-4 receptors (MC4R) in the nucleus of the solitary tract (NTS) modulate glutamatergic transmission, which may enhance these vagal afferent signals. Importantly, the contribution of this circuit to normal glucose regulation is lost in rats fed a high-fat diet. ***The central hypothesis of this application is that: duodenal nutrient exclusion, via MC4R mediated modulation of glutamatergic transmission from vagal afferents, enhances hindbrain regulation of hepatic glucose metabolism.*** To test our hypothesis we will pursue three specific aims:

**1: To identify the mechanisms by which DES improves glucose regulation.** Increased hepatic glucose production is a signature element of patients with type-2 diabetes and in rats placed on a high-fat diet. We will directly measure the ability of DES to reduce hepatic glucose production and alter insulin stimulated glucose disposal using hyperinsulinemic-euglycemic clamps. Additionally, we will determine DES's effect on insulin secretion using hyperglycemic clamps.

**2: To test the hypothesis that the enhanced glucose tolerance observed during duodenal nutrient exclusion is mediated by increased nutrient sensing via glutamatergic neurons of the NTS.** We will test this aim by infusing lipid, protein, or carbohydrate into the duodenum of rats with either DES or sham procedure. Hyperinsulinemic-euglycemic clamps will be used to assess glucose metabolism in response to the varying macronutrients. The role of glutamatergic vagal-afferents will be assessed by local blockade of NMDA receptors in the NTS as well as selective removal of NMDA receptors from vagal afferents of the NTS via viral delivery of shRNA in the nodose ganglion.

**3: To test the hypothesis that brainstem melanocortin receptor signaling is necessary for the benefits of duodenal nutrient exclusion.** We will test this aim by conducting DES implant or the sham procedure in populations of wild-type and MC4R deficient rats. We will also utilize melanocortin pharmacology in DES and sham rats to agonize and antagonize MC4R of the NTS. We will further dissect this system by selectively removing NMDA and/or MC4 receptors from vagal afferent terminals via viral delivery of shRNA in the nodose ganglion. Endpoints for these chronic studies include body and fat mass, humoral measurements and hyperinsulinemic-euglycemic clamps.

While DES is already in clinical development for the treatment of impaired glucose metabolism, we remain hindered by a poor understanding of the mechanistic basis for its beneficial effects. These studies will not only inform our understanding of DES, but will also provide insight into other bariatric procedures such as RYGB that include DNE as a component. Importantly, this proposal will provide an excellent training vehicle for Dr. Habegger. His prior training provides a solid foundation in glucose and energy metabolism as well as systems biology. This proposal will expand upon that foundation and provide rigorous training in neuroanatomy and the central processes that regulate glucose and energy metabolism. This additional training will also serve to facilitate his transition to becoming an independent investigator. The current proposal will expand his technical skill set and move his work into exploring key aspects of the neuroendocrine systems that regulate multiple aspects of energy and glucose regulation.

# Research Strategy

## SIGNIFICANCE

Although diverse in structure and execution, bariatric interventions such as RYGB and DES promote decreased food intake, body and fat mass. Furthermore, these interventions increase glucose tolerance independent of reduced adiposity. Anatomically, RYGB and DES converge in the context of duodenal nutrient exclusion, shedding light on a possible shared mechanism. Our strategy will probe the underlying mechanisms of duodenal nutrient exclusion to highlight components of enhanced glucose tolerance and elucidate CNS targets of nutrient sensing. Therefore, these studies will provide insight into novel therapies that are less invasive and more effective in the treatment of metabolic diseases.

**Roux en-Y gastric bypass (RYGB):** Current medical and lifestyle interventions offer only modest efficacy in the treatment of obesity[12, 13]. However, bariatric interventions have proven to be highly efficacious and often carry the beneficial side effect of T2D resolution[14]. Among the current options for bariatric intervention, RYGB surgery is one of the most commonly used procedures[15]. It is of interest to note that among the current surgical options, not all procedures are equally efficacious. RYGB involves creating a small stomach pouch (~5% of the original volume) and rerouting nutrients through the intestine; such that nutrients are bypassed from most of the stomach, the entire duodenum, and the most proximal region (15-20 cm) of the jejunum[16]. RYGB results in weight loss of 45 kg (60% excess body weight) on average and a resolution of T2D in approximately 80% of patients[14]. RYBG has also been demonstrated to reduce a wide-range of obesity-related co-morbidities including: T2D, cardiac dysfunction, hypertension, arthritis, infertility, dyslipidemia, nonalcoholic steatohepatitis, sleep apnea, and venous stasis ulcers[17-19].

These characteristics have established RYGB as the gold standard in bariatric interventions. However, the precise mechanisms that underlie the robust effects of RYGB on both body weight and glucose homeostasis are poorly understood. Recent research has focused on identifying the factor/s responsible for these benefits, in the hope that novel, less invasive therapies might be uncovered. Although at least some of the beneficial effects of RYGB on glucose control are secondary to reduced body weight, dramatic changes in nutrient presentation (or lack thereof) and gut hormone secretion may also contribute to these effects[20]. Studies in man have identified substantial changes in insulin-like growth factor 1, leptin, adrenocorticotropic hormone, ghrelin, and glucagon-like peptide-1(GLP-1)[16, 21] after RYGB. These humoral changes are absent in weight loss attributed to either restrictive bariatric procedures or hypocaloric diet[22, 23]. While it is likely to be a component of the overall effect, humoral reprograming alone is unlikely to account for the robust effects of RYGB on such a wide range of obesity related comorbidities. Consistent with this hypothesis, is the observation that RYGB profoundly alters circulating bile acid levels[26, 27] and microbiota of the gut[24], both of which may contribute to the overall phenotype. Taken together, these observations highlight the complex nature of RYGB, and provide multiple mechanisms for its effect on glucose metabolism.

**Duodenal nutrient exclusion (DNE):** As highlighted by the robust effects observed after RYGB, duodenal bypass offers a tempting option for treatment of metabolic defects. Consistent with this hypothesis, Rubino et al. provided a series of studies to support the “upper intestinal hypothesis”. This work centered on the duodenal-jejunal bypass (DJB) procedure, where the stomach is preserved and nutrients are routed through an anastomosis directly into the jejunum[25]. This procedure mimics the intestinal manipulation induced by RYGB while preserving normal gastric physiology. DJB ameliorated glucose intolerance in Goto-Kakizaki (GK) rats, a non-obese model of polygenic T2DM[25]. Subsequent studies found that DJB exerts its anti-diabetic effects via increased production of GLP-1 and glucose-dependent insulinotropic polypeptide (GIP) in jejunal enteroendocrine cells. Furthermore, this increased incretin production was associated with reduced beta-cell loss[26]. DJB has since been confirmed by independent investigation in GK[27] and Zucker Diabetic Fatty (ZDF) rats[28], as well as human subjects[29, 30]. Intriguingly, these effects can be reversed by the preservation of duodenal nutrient flow, in the presence of the jejunal anastomosis[31]. This finding lends further credence to the anti-diabetic effects of duodenal nutrient exclusion as opposed to acceleration of less-processed nutrients to the lower gut (“lower intestinal hypothesis”).

As an extension of DJB, a barrier device may stimulate similar enhancements in glucose metabolism. The duodenal endolumenal sleeve, as first described in a rodent model[32], is a flexible barrier device that prevents nutrient-mucosal contact. Implantation of this device in the duodenum of glucose intolerant rats resulted in small decreases in body weight, but profound improvements in glucose tolerance[32]. Ensuing studies described similar improvements in glucose homeostasis in pigs[33] and human subjects[34]. It is of interest to note that the placement and retrieval of this device is accomplished via endoscopy, and as such, this procedure represents a truly reversible therapy for the treatment of glucose intolerance. Though the site of action for these surgical

interventions is becoming clearer, the mechanism/s of action are still unknown. To date, the most promising hypothesis centers on an upper intestinal signal/s that is integrated by the CNS.

**Intestinal Nutrient Sensing:** Data outlined in the previous sections point to a robust association between duodenal nutrient exclusion and improved glucose metabolism. Furthermore this effect is most pronounced in individuals and animal models with impaired glucose homeostasis. As such, it is tempting to hypothesize that defects in duodenal nutrient sensing contribute to the development and/or progression of impaired glucose homeostasis and that duodenal nutrient exclusion contributes to the reversal of this defect/s. This hypothesis is bolstered by observations linking intestinal nutrient sensing to the central regulation of glucose and energy metabolism[35, 36]. In addition to their role as fuel, ingested nutrients also signal to the CNS, inhibiting further intake and allowing for appropriate energy balance[37].

Upper intestinal infusions of protein (peptone), carbohydrate (maltose) and lipid (intralipid) induce satiety in sham feeding rats[38]. Among these ingested nutrients, lipids have long been known to inhibit further food intake[39] and appear to be the most efficacious[38]. Intriguingly, this satiety has been linked to pre-absorptive signaling both by location and time[40]. Studies utilizing intra-duodenal infusion of lipid have repeatedly identified a satiety signal in rats[40] and man[41]. This signal was found to be dose dependent[40], and remain effective in rodent models that display chronic hyperphagia[42]. This signal is ablated by the addition of tetracaine, a local anesthetic, to the infused lipid[40]. This finding is consistent with the observation that satiety induced by intraluminal lipid infusion is not matched by intravenous administration of the same dose via the portal vein or vena cava[43]. *Taken together, these data suggest that upper intestinal-satiety signals are conveyed to the CNS by neuronal afferents, rather than a purely endocrine manner*.

New evidence demonstrates that intestinal (duodenal and jejunal) nutrient sensing is also a potent regulator of glucose homeostasis[36, 44]. Intriguingly, and in striking similarity to DJB[45], this regulation of hepatic glucose output was lost in rats fed a high fat diet[44]. Subsequent studies have elucidated a role for cholecystokinin (CCK) 1 receptor signaling in the duodenal-regulation of hepatic glucose production[46-48]. Vagal afferent terminals in the nucleus of the solitary tract (NTS) are populated by the *N*-Methyl-D-aspartic acid (NMDA) receptor and glutamatergic vagal input activates the primary response of NTS neurons[49, 50]. Intriguingly, NMDA receptors (NMDA-R) in the NTS are essential for CCK-mediated reduction in food intake[51] and have been implicated in central regulation of hepatic glucose output[52].

**Melanocortin-4 Receptor regulation of hepatic glucose production:** Melanocortin-4 receptor (MC4R), and signaling derived from this pathway, is a crucial regulator of energy and glucose homeostasis. The *in vivo* relevance of this regulation is evidenced by the severe obesity, hyperphagia, hyperglycemia, and hyperinsulinemia in MC4R knockout mice[53], which mirrors the physiology observed in human subjects with MCR4 frameshift mutations[54, 55]. Primarily identified as a hypothalamic regulator of food intake and adiposity, inhibition of MC4R by its endogenous antagonist, the Agouti-related protein (AgRP), stimulates food intake while reducing energy expenditure[56]. In addition to its role in the hypothalamus, populations of MC4R positive neurons can be identified throughout the CNS[57-59], including those of the NTS[60]. Re-expression of MC4R in cholinergic neurons of the dorsal motor nucleus of the vagus (DMV) rescues the defects in glucose homeostasis that are observed in global MC4R knockouts and supports a role for MC4R signaling in the regulation of hepatic glucose production[61]. It is of interest to note that MC4R expressing vagal efferents innervate peripheral tissues such as the stomach, liver and duodenum[62]; and that MC4R agonists potently modulate meal size, but not frequency, when administered to the brainstem[63]. This data demonstrates that hindbrain MC4R signaling enhances visceral signals such as satiety and nutrient load. Furthermore, MC4R agonism in the NTS increases presynaptic glutamatergic transmission from vagal afferents[63]. This modulation likely acts to enhance vagal afferent signals derived from the upper intestinal and provides a plausible link between melanocortin signaling and suppression of hepatic glucose production via the gut-brain-liver axis.

*Taken together these observations suggest that duodenal nutrient exclusion, induced by diverse bariatric procedures, results in a similar enhancement in glucose metabolism. Furthermore, emerging data demonstrates that nutrient, and specifically lipid, sensing by the upper intestine is a crucial regulator of glucose homeostasis and energy balance that is lost during high-fat feeding. However, the contribution of the gut-brain-liver axis to the enhancement in glucose metabolism after duodenal-nutrient exclusion is still poorly understood. The studies described in this application will address this contribution, as well as test the role of the melanocortin signaling system in the regulation of hepatic glucose production by the gut-brain-liver axis.*

**INNOVATION**

***Conceptual:*** The association between gut nutrient signaling and overall glucose homeostasis is a clinically relevant and active area of research. While the benefits of duodenal-nutrient exclusion on glucose metabolism are well documented, the underlying mechanism/s for these enhancements is poorly understood. Likewise, nutrient sensing from the upper intestine, and its regulation of hepatic glucose production, has been mapped through the CNS, but has yet to be directly linked to the benefits of many bariatric interventions. This proposal aims to investigate the important mechanistic relationship between duodenal nutrient exclusion and regulation of hepatic glucose production via the gut-brain-liver axis. These experiments will identify novel signaling pathways stimulated/inhibited by duodenal nutrient exclusion in the central control of glucose metabolism, thereby identifying new therapeutic targets in the treatment of glucose intolerance.

***Technical:*** During his postdoctoral training in the lab of Dr. Tschöp, the candidate developed the surgical skills necessary to conduct several bariatric interventions, including the implant of duodenal-barrier sleeves in adult rats; as well as the expertise to conduct well-executed studies of glucose, lipid, and energy metabolism. He is now well positioned to use these innovative techniques in the proposed experiments to investigate the role of duodenal-nutrient sensing and central integration of visceral afferent signaling in the regulation of glucose metabolism. The studies described herein will provide extensive training opportunity for the candidate in the field of neuroscience and its application to the study of metabolism. The intersection of cutting edge rodent surgical techniques and world-class neuroscience research under Drs. Seeley and Herman insure not only the novelty of this application, but also its success.

## APPROACH

***Specific Aim 1*: To identify the mechanisms by which DES improves glucose regulation.**

***Overall rationale:*** Consistent with previous data[32], we have strong preliminary evidence that a duodenal barrier endosleeve (DES) stimulates reduced body (Figure 1a & b) and fat mass (Figure 2 a & b) in DIO and Zucker Diabetic (ZDF) rats. This exclusion resulted in enhanced glucose tolerance (Figure 3a & b) that was, at least in part, independent of adiposity as demonstrated by the significant enhancement over fat mass-matched controls (Sham-pf). Specific Aim 1 is designed to determine the component/s of glucose metabolism responsible for the enhanced glucose metabolism. We will conduct hyperinsulinemic-euglycemic and hyperglycemic clamps in rats after DES implant. This paradigm will allow us to delineate the contribution of hepatic insulin sensitivity, as well as other insulin-sensitive tissues, in the enhanced glucose tolerance observed after DES implant.

***Experiment 1.1:* The influence of DES on hepatic glucose output in obese, high-fat diet (HFD) fed rats. *Design:*** This experiment will test the hypothesis that DES stimulates an enhancement of hepatic insulin sensitivity that is resistant to the effects of HFD. *This experiment will require 48, 10-week old Long-Evans rats,* divided into 4 groups. All rats will be provided *ad lib* high-fat butter diet ([HFD] 4.54 kcal/g; 41% fat; Research Diets, New Brunswick, NJ) for 8 weeks, previously shown to produce diet induced obesity (DIO) and metabolic impairments. Upon reaching

**Figure 1:**

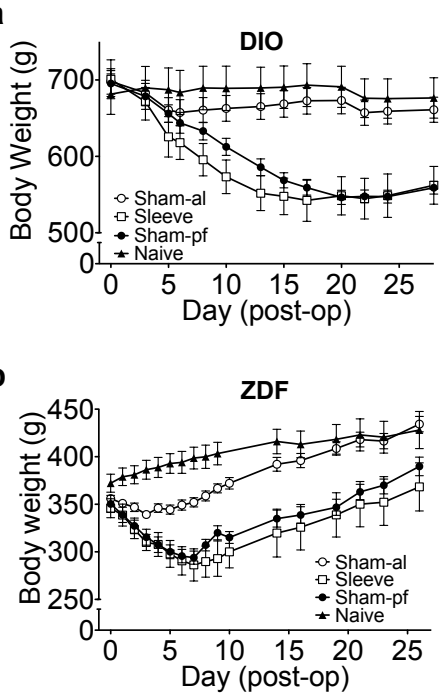


Figure 1: DES reduces body weight via reduced food intake. Body weight in grams of DIO and ZDF rats for 27 days post-op. Sham-al (ad lib), Sham-pf (pair fed to DES rats).

**Figure 2:**

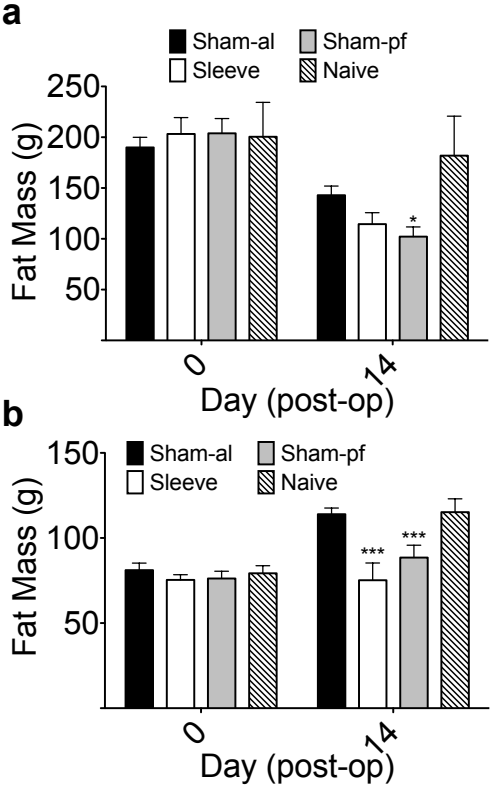


Figure 2: DES reduces fat mass. Fat mass in DIO (a) and ZDF rats (b) before and 14 d after implant. Sham-al (ad lib).* $p<0.05$, *** $p<0.001$ vs Sham-al

DIO, Twelve rats will be implanted with DES, 24 will receive a sham procedure to match the DES implant, and 12 will be left with no intestinal intervention (naïve). Twelve of the sham animals will be fed *ad lib,* while the remaining 12 will be fed the amount of food eaten by the DES group (pair-fed). All sham and DES animals will receive rigorous post-operative care including liquid diet (osmolite), administration of a non-steroidal anti-inflammatory, an opioid pain medication, and subcutaneous warm saline. Following postoperative recovery (~3 days), all rats will be returned to HFD for the duration of the experiments. Ten days after the bariatric procedure, catheters will be implanted in the carotid artery and jugular vein of all rats. Body weight and food intake will be measured daily. 15-19 days postoperatively, when improvements in intraperitoneal (ip) glucose and meal tolerance are observed with DES, the rats will be fasted for 4 h and then undergo a hyperinsulinemic-euglycemic clamp with a primed, continuous infusion of [3-3H]glucose and 2-[1-14C]Deoxy-D-glucose, as previously described[64]. *Plasma samples (25 µl whole blood/time point) for insulin will be taken at 0, 2, 5, 10, 15, 30, and 45min.* Surgeries will be staggered to allow 4 rat clamps/day.

***Experiment 1.2:*** **The influence of DES on hepatic glucose output in a rat model of T2D.**

***Design:*** This experiment will test the hypothesis that DES stimulates an enhancement of hepatic insulin sensitivity in rats with *diet-independent* impairments of glucose metabolism. This experiment will utilize the Zucker Diabetic fatty (ZDF) rat model. ZDF rats are characterized by impaired glucose tolerance at an early age that is associated with hyperglycemia, insulin resistance and hyperinsulinemia. The ZDF phenotype is primarily driven by a genetic defect affecting the extracellular domain of the leptin receptor. This results in hyperphagia[65], *obesity, and frank diabetes* while on a normal chow diet[66].

Thus we can use this model to investigate *diet-independent* effects of the DES. *This experiment will require 48, 10-week old ZDF rats, divided into 4 groups. Twelve rats will be implanted with DES*, 24 will receive a sham procedure to match the DES implant, and 12 will be left with no intestinal intervention (naïve). Twelve of the sham animals will be fed *ad lib,* while the remaining 12 will be fed the amount of food eaten by the DES group (pair-fed). Post-operative care will be administered as in experiment 1.1. Following postoperative recovery (~3 days), all rats will be fed standard chow diet throughout the experiment. Ten days after the bariatric procedure, catheters will be implanted in the carotid artery and jugular vein of all rats. Body weight and food intake will be measured daily. 15-19 days postoperatively, when improvements in intraperitoneal (ip) glucose and meal tolerance are seen with DES, the rats will be fasted for 4 h and then undergo a hyperinsulinemic-euglycemic clamp as in experiment 1.1. *Plasma samples (25 µl whole blood/time point) for insulin will be taken at 0, 2, 5, 10, 15, 30, and 45min.* Surgeries will be staggered to standardize post-operative time and to allow 4 rat clamps per day.

**Figure 3:**

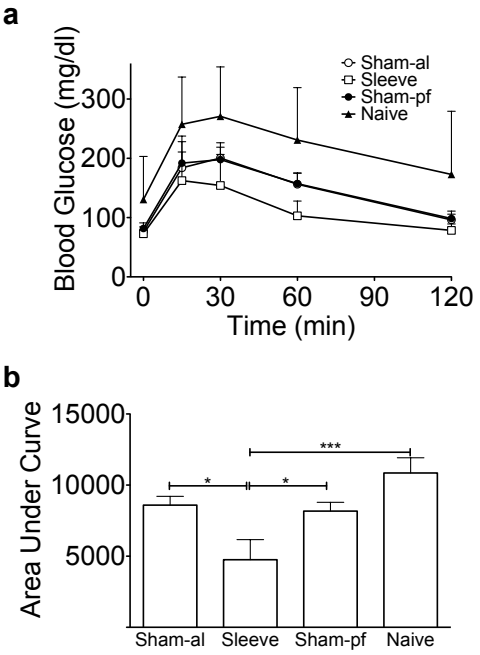


Figure 3: DES enhances glucose tolerance. Glucose excursion (a) and area under the curve analysis (b) of ZDF rats 14 days post-op. Sham-al (ad lib), Sham-pf (pair fed to DES rats).* p<0.05, *** p<0.001 vs Sham-al

***Experiment 1.3:*** **Insulin-stimulated glucose uptake after DES.**

***Design:*** This experiment will build upon observations obtained in experiments 1.1 & 1.2 and will the test the hypothesis that DES stimulates an enhancement of insulin action in adipose tissue and skeletal muscle. Furthermore, this experiment will assess if these effects will occur in the presence of leptin-signaling deficiency and programed, β-cell failure. Following the conclusion of the hyperinsulinemic-euglycemic clamp in experiments 1.1 & 1.2, rats will be sacrificed. Trunk-blood, liver, skeletal muscle (quadriceps) and adipose tissue (inguinal and epididymal) will be collected. A portion of the liver, skeletal muscle and adipose tissue will be analyzed for radiolabeled glucose uptake, while the rest will be frozen for later analysis.

***Interpretation and potential pitfalls: Experiments 1.1-3***

The Seeley Lab has invested considerable time and effort perfecting rodent models of bariatric surgery. We have established rat models of Ileal transposition[67], VSG[64, 68], and RYGB[64]. In the last year we have developed a rat models of adjustable gastric banding and, more importantly, DES. As such, we do not anticipate any technical difficulties with these surgeries. Our laboratory has a long history of assessing food intake and body weight, and we have successfully implemented hyperinsulinemic-euglycemic clamp analyses into several of our recent reports[64, 69]. Consequently, this experiment represents a significant training experience for Dr. Habegger, who will learn the vascular surgeries and clamp techniques. *Dr. Sandoval will oversee the training of Dr. Habegger on these new techniques*.

**Figure 4:**

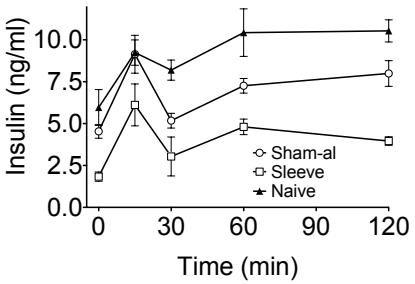


Figure 4: DES enhances independent of increased plasma insulin. mixed-meal stimulated insulin excursion of ZDF rats 19 days post-op. Sham-al (ad lib)

The expected outcome of this experiment is that the implant of DES will result in reduced hepatic glucose output; resulting in overall improvements in glucose metabolism. It is further expected that this enhancement will be contributed to by both fat-mass dependent and independent mechanisms, as evidenced by the intermediate effect of pair feeding on glucose tolerance (Figure 3a & b). This result would support the hypothesis that upper intestinal nutrient sensing, through its CNS-mediated regulation of hepatic glucose output, is a critical modulator of overall glucose metabolism.

*Although our preliminary data would suggest otherwise (Figure 4), it is possible that the enhanced glucose tolerance in DES rats is mediated by a hypersecretion of insulin in response to glucose load. This is especially possible if the peak insulin secretion occurs before 15min in the DES rats. If we find that insulin sensitivity is not regulated by DNE, we will test the hypothesis that DES stimulates an enhancement of insulin secretion via a hyperglycemic clamp. This strategy will allow us to determine if DES-stimulated DNE acts on the pancreas to alter hormone secretion and subsequent benefits in glucose homeostasis.*

Although a potential difficulty with these experiments is the animal's ability to handle two surgeries (DES implant plus carotid and jugular catheterization). *We have now published several reports combining bariatric and vascular surgeries and are confident that this is a strategy, which will be successful. Specifically, we have proposed to stagger the surgeries by 10 days, as this is the time point where the animal's body weight stabilizes. We plan to have 8 animals per group successfully clamped. In order to accomplish this, we will perform the DES in 12 animals. Typical survival rates for this surgery are ~80%. These strategies (increased n per group, increased time between surgeries, etc…) will also be utilized in subsequent experiments (Experiments 2.1a-c, 2.2, 3.1 & 3.2) to insure that the studies will attain the necessary statistical power*.

A potential confounding factor to these studies is the contribution of insulin secretion to the overall enhancement of glucose tolerance. This concern will be specifically addressed by the analysis of insulin secretion and C-peptide accumulation during the first 45 min of the hyperinsulinemic-euglycemic clamp as described in experiment 1.1 and glucose-stimulated insulin-secretion and C-peptide accumulation during hyperglycemic clamp in experiment 1.4. Additionally, HFD has been reported to impair duodenal lipid sensing in rats fed HFD[44]. However, our utilization of diet-independent, glucose-intolerant rodent models in experiments 1.2-.4 will address both of these concerns.

***Specific Aim* 2: To test the hypothesis that the enhanced glucose tolerance observed during duodenal nutrient exclusion is mediated by increased nutrient sensing via glutamatergic neurons of the NTS.**

***Overall rationale:*** Emerging data demonstrates that the upper intestine senses nutrients[35, 36, 44] and relays this information to the CNS[44]. *Vagal afferent terminals in the nucleus of the solitary tract (NTS) are populated by the N-Methyl-D-aspartic acid (NMDA) receptor and glutamatergic vagal input activates the primary response of NTS neurons[49, 50]. Intriguingly, NMDA receptors (NMDA-R) in the NTS have been implicated in central regulation of hepatic glucose output[52]*. Thus, it is hypothesized that this gut-brain axis, and particularly glutamatergic neurons of the NTS, are crucial in the maintenance of glucose homeostasis and energy balance. Specific Aim 2 is designed to investigate the role of glutamatergic NTS-neurons in enhanced glucose metabolism observed after duodenal nutrient exclusion. Aim 2 will also evaluate the role of these neurons when the duodenum is challenged with varying macronutrients. This paradigm will serve to test the hypothesis that DES augments upper intestinal signals integrated by glutamatergic neurons of the NTS as an important regulator of glucose and energy balance.

***Experiment 2.1a:* The role of DNE on hepatic glucose output**

**Figure 5:**

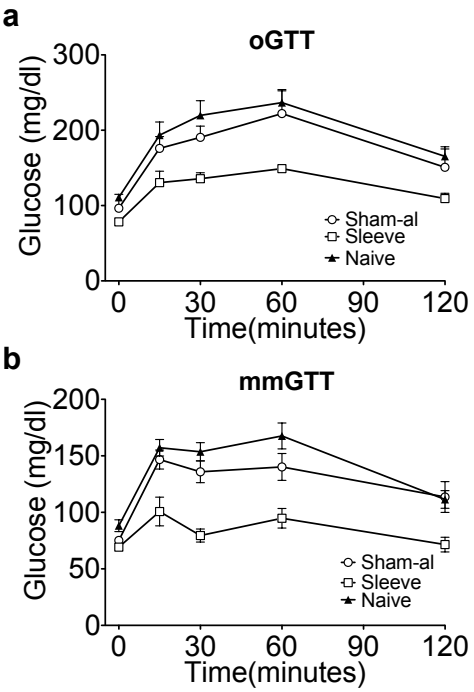


Figure 5: DES enhances glucose tolerance. Glucose excursions following 2g/kg oral glucose challenge (a) or mixed-meal challenge (b) in ZDF rats at 19 and 41 days post-op. Sham-al (ad lib)

**during duodenal lipid infusion.**

***Design:*** Previous reports suggest that lipid sensing by the intestine triggers activation of a gut-brain-liver signaling pathway, ultimately resulting in decreased hepatic glucose output[44]. Similarly, intestinal infusion of lipid, peptide, or carbohydrate stimulates satiety via a gut-brain axis[38]. Emerging data demonstrates that NMDA receptors (NMDA-R) of the NTS are necessary for transmission of nutrient sensing[46, 51, 52]. This experiment will test the hypothesis that duodenal **lipid** sensing stimulates an enhancement of hepatic insulin sensitivity associated with activation of NMDA receptors of the NTS. This experiment will utilize acute injection of the NMDA-R antagonist, MK-801, into the NTS to prevent NMDA signaling. *This experiment will require 144, 18-week old Long-Evans rats*, divided into 12 groups. All rats will be maintained on chow diet throughout the experiment. *48 rats will be implanted with DES* while 96 will be given the appropriate sham procedure. All rats will be implanted with duodenal infusion catheters in a simultaneous procedure. Catheters will be externalized in the scapular region of the back to allow access for infusion of macronutrient directly into the upper intestine. Post-operative care will be administered as in experiment 1.1. Following postoperative recovery (~3 days), all rats will be fed standard chow diet. All DES and 48 sham rats will be fed *ad lib* (sham-al), while 48 sham rats will be pair fed to the DES group (sham-pf). Ten days after the bariatric procedure, catheters will be implanted in the carotid artery and jugular vein of all rats. Bilateral catheters will be implanted into the NTS of all rats (as previously described[46]) concurrent to the vascular catheterization. Body weight and food intake will be measured daily. 15-19 days post-DES implant, when improvements in intraperitoneal (ip) glucose and meal tolerance are seen with DES, the rats will be fasted for 4 h. DES, sham-al and sham-pf rats will each be matched into either MK-801 or Vehicle groups. *Five minutes prior to insulin infusion, rats will receive an infusion of either 0.9% NaCl or 0.03 ng/min MK-801 in 0.9% NaCl (i.e. 48 MK/DES, 48 NaCl/DES, 24 MK/sham-al, 24 NaCl/sham-al, 24 MK/sham-pf, and 24 NaCl/sham-pf). This dose was found to be efficacious in similar rodent models of insulin sensitivity[36, 44].* Hyperinsulinemic-euglycemic clamp will be conducted as previously described[64]. During the final 30 minutes of clamp rats will be infused with the 0.9 g/h of intralipid or 0.9% NaCl via duodenal catheter to evaluate the role of duodenal lipid sensing on hepatic insulin sensitivity. Surgeries will be staggered to standardize post-operative time and to allow 4 rat clamps per day.

***Experiment 2.1b:*** **The role of DNE on hepatic glucose output during duodenal protein infusion.**

***Design:*** This experiment builds upon experiment 2.1a & b and will test the hypothesis that duodenal **protein** sensing stimulates an enhancement of hepatic insulin sensitivity via activation of NMDA receptors of the NTS. This experiment will require *144, 18-week old Long-Evans rats, divided into 12 groups. Rats will be maintained on chow throughout the experiment. 48 rats will be implanted with DES* while 96 will be given the appropriate sham procedure. Rats will be grouped and treated as in experiment 2.1a. However, for the final 30 minutes of the hyperinsulinemic-euglycemic clamp peptone or 0.9% NaCl will be infused into the duodenum at a rate of 2.1 g/h. Surgeries will be staggered to standardize post-operative time and to allow 4 rat clamps per day.

***Experiment 2.1c:*** **The role of DNE on hepatic glucose output during duodenal carbohydrate infusion.**

***Design:*** This experiment builds upon experiment 2.1a and will test the hypothesis that duodenal **carbohydrate** sensing stimulates an enhancement of hepatic insulin sensitivity via activation of NMDA receptors of the NTS. *This experiment will require 144, 18-week old Long-Evans rats, divided into 12 groups. Rats will be maintained on chow throughout the experiment. 48 rats will be implanted with DES* while 96 will be given the appropriate sham procedure. All rats will be grouped and treated as in experiment 2.1a. However, for the final 30 minutes of the hyperinsulinemic-euglycemic clamp maltose or 0.9% NaCl will be infused into the duodenum at a rate of 2.6 g/h. Surgeries will be staggered to standardize post-operative time and to allow 4 rat clamps per day.

***Interpretation and potential pitfalls: Experiments 2.1a-c***

The NTS, vascular, and bariatric surgical interventions described in *Experiments 2.1a-b* are common to the Seeley lab and as such, we do not anticipate any technical difficulties with these surgeries. *However, the duodenal and vascular implant surgeries are new to Dr Habegger, thus these experiments represent a critical training opportunity. Drs. Seeley and Sandoval of the Career Advisory Committee will conduct this training.*

Based on published reports[44], the expected outcome of this experiment will be an enhancement in hepatic insulin sensitivity, and specifically reduced hepatic glucose output, in lipid infused rats as compared to their vehicle-infused controls. Duodenal infusions of lipid, protein, and carbohydrates are known to induce similar signaling pathways in the stimulation of satiety, although proteins and carbohydrates are less effective than lipid[38]. Thus, we expect duodenal infusion of all macronutrients to increase hepatic insulin sensitivity, with lipids having the greatest effect. As NMDA receptors of the NTS are implicated in this gut-brain-liver axis, we expect that the effects of macronutrients on hepatic insulin sensitivity will be abrogated in rats administered the NMDA-R antagonist MK-801. A caveat of these experiments is that the enhanced glucose handling may be independent of hepatic glucose production. However, these experiments will be informed by those of Aim 1

and the primary endpoint (or tissue of enhanced insulin action) will be adjusted based on this knowledge.

Our preliminary data showing DES rats are more glucose tolerant, supports recent findings demonstrating inhibition of endogenous glucose production induced by jejunal nutrient sensing[36]. This data shows that gut-brain-liver regulation is active ***in spite of duodenal bypass*** *(DJB)*. Our expectation is that rats with DES will mimic that anatomy and physiology of DJB, and thus, will display enhanced of hepatic insulin sensitivity. We expect that the duodenal exclusion induced by DJB or DES act to enhance the nutrient signal derived from the jejunum. Future studies will utilize alternate placement (duodenal vs. jejunal vs. ileal) of nutrient infusion catheters in the presence or absence of DES to specifically test this hypothesis. In regards to the central integration of these signals, we expect that the effects of DES on hepatic insulin sensitivity will be abrogated in rats administered the NMDA-R antagonist MK-801.

***Experiment 2.2:*** **The role of vagal afferent NMDA receptors in DES enhancement of glucose and energy metabolism.**

***Design:*** This experiment will test the hypothesis that the beneficial, and persistent, effects of DES on glucose and energy balance are dependent on activation of NMDA-R of the NTS. In this experiment we will utilize shRNA technology to knockdown NMDA-R specifically in vagal afferents of the NTS. As opposed to the pharmacological blockade of NMDA-R, which will prevent glutamatergic signaling throughout the hindbrain, this paradigm will allow us to directly test the hypothesis that NMDA-R ***on vagal afferents*** are responsible for DES-stimulated enhancement of glucose and energy balance. *This experiment will require 72, 10-week old Long-Evans rats divided into 6 groups: 12, DES+scramble; 12, DES+shNMDAR; 12, Sham+scramble; 12, Sham+shNMDAR; 12, pair-fedSham+scramble; and 12, pair-fedSham+shNMDAR.* All rats will be provided *ad lib* HFD for 8 weeks. Upon reaching DIO, rats will be grouped as described above. The rats will be anesthetized, the nodose ganglion will be exposed, and shNMDAR or shScramble will be administered bilaterally, as previously described[70]. Rats will recover for 7 days and the appropriate bariatric surgical intervention performed. Post-operative care will be administered as in experiment 1.1. Following postoperative recovery (~3 days), all rats will be returned to HFD. On bariatric postoperative day-14 rats will be fasted for 4 h and then undergo i.p. glucose-tolerance test (ipGTT). Rats will recover for 7 days and then will be administered liquid diet (mixed meal challenge) via oral gavage (mmGTT). Blood glucose will be measured via hand-held glucometer at 0, 15, 30, 60, and 120min in both GTTs. *Plasma samples (50 µl whole blood/time point) for insulin and C-peptide will be taken at 0, 15, 30, and 60min in both GTTs.* Surgeries will be staggered to standardize post-operative time and allow for 12 rats per day. Body weight and food intake will be followed throughout the study. Fat and lean mass will be measured before ganglion injection and 28 days after bariatric intervention. On day 30, we will inject a lethal dose of FatalPlus and perfuse animals with paraformaldehyde. C-fos immunohistochemistry, as described in[71], will be used to evaluate the activation of NTS neurons.

**Figure 6:**

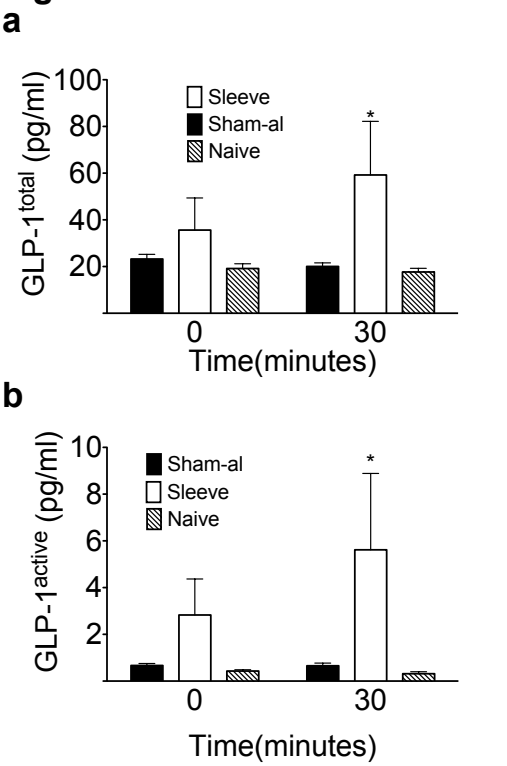


Figure 6: DES increases plasma GLP-1 levels. Plasma GLP-1 in 4 h fasting (a) or after a mixed-meal challenge (b) in ZDF rats at 19 days post-op. Sham-al (ad lib). * p<0.05 vs Sham-al

***Interpretation and potential pitfalls: Experiment 2.2***

The Seeley Lab routinely investigates the neuronal response to feeding and energy balance. Furthermore, Drs. Seeley and Herman have successfully utilized lentiviral-shRNA to deliver and knockdown target genes of the CNS[72]. While we have not delivered these viral reagents directly to the nodose ganglian, our collaborator Dr. Powley has generously agreed to consult regarding training and interpretation. Dr. Powley has published extensively on the relationships between vagal afferents and gastrointestinal function[70, 73-76], and routinely uses injection of this ganglion in his research. As such, we do not anticipate any technical challenges with this experiment. Dr. Habegger has limited experience and/or formal training in neuroanatomy and therefore, this represents an important new training opportunity. Specifically, these experiments provide Dr. Habegger with the opportunity to acquire new training in manipulation of vagal afferents via injection of the nodose ganglion. *He will also gain additional training in rodent stereotaxic surgeries, CNS tissue preparation &*

*immunohistochemistry, and creation/implementation of lentivirus shRNA constructs under Dr. Herman.*

NMDA-R signaling has been implicated in CNS regulation of glucose metabolism[52] and energy balance via feeding[49]. Thus, the expected outcome of these experiments is that we will be able to confirm the role of vagal afferent NMDA-R in the beneficial effects of DES. Specifically, we expect that NMDA-R knockdown in DES rats will ablate the beneficial effects of DES on glucose metabolism and energy balance, resulting in DES rats of similar body weight, fat mass, and glucose tolerance as compared to the sham controls.

A possible confounding factor in these studies is the reported impairment of duodenal lipid sensing in rats fed HFD[44]. Thus a lack of effect of NMDA knockdown on DES rats may be incorrectly interpreted as an NMDA-R independent effect. We will however, be informed of this caveat by studies proposed in Aim 1. If we find in experiment 1.1 that the effects of DES are ablated by HFD feeding, we will use our diet independent model of T2D, ZDF rats, in this experiment (2.2). *Similarly, lack of efficient NMDA-R knockdown will be difficult to differentiate from a negative result. To address this concern we will remove the nodose ganglion from each rat and assess both the expression and the receptor protein level at the end of the study.* Another possible component of the enhanced glucose and energy metabolism may be associated with altered secretion of gut-hormones such as GLP-1, PYY, and Ghrelin. Previous reports have associated each of these hormones with DNE[77]. Furthermore, we find that DES stimulates a significant increase in both total and active forms of plasma GLP-1 after a meal (Figure 6a & b). Future studies will address these observations through a similar strategy invoked in Aim 2. Specifically, pharmacological inhibition of central and peripheral GLP-1 receptors via extendin-9 will be used to test the role of this signaling pathway in the enhanced glucose handling after DES.

***Specific Aim* 3: Test the necessity of the melanocortin-signaling pathway for the benefits of duodenal nutrient exclusion.**

***Overall rationale:*** The melanocortin signaling system is a well-characterized regulator of both glucose metabolism and energy balance (reviewed in [78]). Genetic ablation of the melanocortin-4 receptor results in severe obesity, hyperphagia, hyperglycemia, and hyperinsulinemia[53], and importantly mirrors the physiology of human subjects with MCR4 mutations[54, 55]. *Emerging data demonstrates that MC4R signaling in the NTS increases presynaptic glutamatergic transmission from vagal afferents[63] and this signaling may enhance visceral signals such as satiety, and possibly the weight-independent effects of RYGB on glucose homeostasis[79].* Specific Aim 3 is designed to investigate the role of MC4R signaling in DES enhancements of glucose metabolism and energy balance.

***Experiment 3.1:* The role of MC4R signaling in DES enhancement of glucose and energy metabolism.**

***Design:*** Our group possesses a unique tool in the MC4R knockout ($MC4R^{-/-}$) rat, as well as the surgical expertise to utilize this model. These rats were developed and characterized by Mul et al. and display a similar phenotype to that of the MC4R knockout mouse[80]. Specifically, these rats display elevated body weight, food intake, and white adipose mass[80]; subsequent studies has also identified considerable glucose intolerance[81].

This experiment will test the hypothesis that the beneficial, and persistent, effects of DES on glucose and energy balance are dependent on MC4R signaling. *This experiment will require 36 $MC4R^{-/-}$ rats and 60 wild type (WT) littermates. $MC4R^{-/-}$ rats will be divided into 3 groups of 12 (DES, ad lib Sham, and pair-fed Sham). WT rats will be divided into 6 groups of 12. Three groups will receive only DES or Sham procedures (DES, ad lib Sham, and pair-fed Sham), while the other 3 will also receive chronic cannulation in the forth ventricle (DES+i4vt$^{SHU9119}$, ad lib Sham+i4vt$^{SHU9119}$, and pair-fed Sham+i4vt$^{SHU9119}$). Pharmacological MC3R/MC4R blockade via SHU9119 (0.50 nmol/day) will be administered via Alzet osmotic mini-pump[60]. Pair-fed Sham animals will be fed to match DES rats, respective to their appropriate genotype/treatment.* Post-operative care will be administered as in experiment 1.1. Following postoperative recovery (~3 days), all rats will be returned to chow for the duration of the experiment. On bariatric postoperative day-14 rats will be fasted for 4 h and then undergo ipGlucose tolerance test (ipGTT). Rats will recover for 7 days and then will be administered liquid diet (mixed meal challenge) via oral gavage (mmGTT). Blood glucose will be measured via hand-held glucometer at 0, 15, 30, 60, and 120min in both GTTs. *Plasma samples (50 µl/time point) for insulin and C-peptide will be taken at 0, 15, 30, and 60min in both GTTs.* Surgeries will be staggered to standardize post-operative time and allow for 12 rats per day. Body weight and food intake will be followed throughout the study. Fat and lean mass will be measured before ganglion injection and 28 days after bariatric intervention. On day 30, we will inject a lethal dose of FatalPlus and perfuse the animals with paraformaldehyde. C-fos immunohistochemistry, as described in[71], will be used to evaluate the activation of NTS neurons.

***Interpretation and potential pitfalls: Experiments 3.1***

As with experiment 2.2, we predict no technical difficulties in completing this experiment.

MC4R signaling is essential to many of the systems regulating glucose metabolism and energy balance.

Thus, our expectation is that WT DES rats will display a decreased body weight and enhanced glucose tolerance, while these effects will be blunted or even ablated in MC4R$^{-/-}$ and i4vt$^{SHU9119}$ DES rats. However, a recent publication in these rats has found that the effects of vertical sleeve gastrectomy (VSG) are independent of MC4R signaling[81]. *It is possible that like VSG, the effects of DES will be independent of MC4R signaling. This experimental paradigm is designed to assess the role of hindbrain MC4R signaling as compared to whole body knockout of MC4R. Therefore, we may be able to identify a subset of the DES-stimulated effects that segregate based on the level of blockade (i.e. whole body vs. hindbrain).* Future studies will utilize the technical skills acquired in Aim 2 (lentiviral delivery of shRNA to vagal afferents via the nodose ganglion, and ICV delivery of MC4R antagonists [SHU-9119]) to address specific neuronal populations.

***Experiment 3.2:*** **The role of MC4R signaling and DES on hepatic glucose output.**

***Design:*** This experiment will test the hypothesis that DES stimulates an enhancement of hepatic insulin sensitivity that is dependent on MC4R signaling. *This experiment will require 36 MC4R$^{-/-}$ rats and 60 wild type (WT) littermates. Rats will be grouped as described in experiment 3.1. Pair-fed* Sham animals will be fed to match DES rats, respective to their appropriate genotype. Post-operative care will be administered as in experiment 1.1a. Following postoperative recovery (~3 days), all rats will be returned to chow for the duration of the experiment. Ten days after the bariatric procedure, catheters will be implanted in the carotid artery and jugular vein of all rats. Body weight and food intake will be measured daily. 15-19 days postoperatively, when improvements in intraperitoneal (ip) glucose and meal tolerance are observed with DES, the rats will be fasted for 4 h and then undergo a hyperinsulinemic-euglycemic clamp as previously described[64]. *Plasma samples (25 µl whole blood/time point)* for insulin will be taken at 0, 2, 5, 10, 15, 30, and 45min. Surgeries will be staggered to standardize post-operative time and to allow 4 rat clamps per day.

***Interpretation and potential pitfalls: Experiments 3.2***

The vascular, and bariatric surgical interventions described in Experiment 3.2 are common to the Seeley lab and as such, we do not anticipate any technical difficulties with these surgeries. As with the experiments of Aims 1 & 2 this experiment represents a significant training experience for Dr. Habegger, who will learn the vascular surgeries and clamp techniques needed throughout his career.

MC4R signaling is essential to many of the systems regulating glucose metabolism and as such, our expectation is that WT DES rats will display an enhanced hepatic insulin sensitivity (as evidenced by decreased hepatic glucose output during the clamp), while these effects will be blunted or even ablated in MC4R$^{-/-}$ and i4vt$^{SHU9119}$ DES rats. *This experimental paradigm is designed to assess the role of hindbrain MC4R signaling as compared to whole body knockout of MC4R. Thus, it is possible that a subset of the DES-stimulated effects will segregate based on the level of blockade (i.e. whole body vs. hindbrain). Future studies will utilize the technical skills acquired in Aim 2 (lentiviral delivery of shRNA to vagal afferents via the nodose ganglion, and ICV delivery of MC4R antagonists [SHU-9119] to the NTS) to address these specific neuronal populations.*

***Proposed Timeline:***