My prior research and clinical training ideally situate me to investigate the anatomic and molecular mechanisms underlying the effects of Roux-en-Y gastric bypass (RYGB) on obesity and diabetes. Work detailed in my doctoral thesis from the laboratory of Morris White, PhD identified the first post-translational modification involved in the induction of insulin resistance associated with pre-diabetes, serine phosphorylation of insulin receptor substrate -1, as well as the responsible kinase and its cognate binding site. It further detailed the molecular mechanism of this modification; abrogation of the insulin-stimulated interaction between the insulin receptor and insulin receptor substrates, an interaction required for the insulin cellular response. This intensive basic research experience complements the surgical and technical expertise I obtained during my post-doctoral fellowship in the laboratory of Lee Kaplan, MD, PhD involving the development and evaluation of rodent models of bariatric surgery. Thus, I am uniquely trained to investigate the underlying physiologic and molecular mechanisms induced by anatomic manipulation of the gut during bariatric surgery.

Our novel surgical model of RYGB in mice is a valuable research tool through which these mechanisms can be directly investigated. At UT Southwestern (UTSW), I have already established a surgical facility in which we have performed RYGB and sham operations on over 100 animals, including various acquired and genetic mouse models of obesity and diabetes. These experiments have revealed novel findings, some of which are detailed in the Research Strategy of this proposal. These data demonstrate our technical capability and expertise to perform RYGB on genetically-engineered mice, to generate the number of animals required for the proposed studies, and to appropriately characterize their physiologic and metabolic phenotype.

In addition, I am currently in the ideal environment to execute the proposed investigations. First, I have access to the technically complicated phenotyping equipment required for the proposed experiments through the Mouse Metabolic Phenotyping Core Facility at UTSW. I am already quite familiar with this equipment and support staff as evidenced by the preliminary data outlined in the Research Strategy. Second, Joel Elmquist, DVM, PhD, my mentor, is a well-recognized leader in the neurobiology of obesity. He is also director of the Division of Hypothalamic Research, one of the research centers of the Taskforce for Obesity Research (TORS) at UTSW, the only obesity research center in the U.S. supported by the NIH Roadmap Initiative for Interdisciplinary Research. The author of over 125 manuscripts, Dr. Elmquist has overseen the development and characterization of numerous genetic models of obesity and diabetes. He is one of the leaders in the molecular dissection of neuroanatomic tracts involved in the regulation of energy balance and glucose metabolism through the use of tissue-specific, Cre-mediated modulation of gene expression. Germane to this proposal, the Elmquist laboratory has mouse lines with loxP-modified alleles of the melanocortin-4 receptor (MC4R) that can be reactivated (in an MC4R null background) or deleted (in a wild-type background) in a Cre-dependent, tissue-specific manner. The MC4R is a critical mediator of body weight regulation and glucose metabolism, and MC4R null mice fail to lose weight or improve diabetes after RYGB. Thus, the anatomic sites of MC4R expression and their functional consequence after RYGB can be directly investigated by RYGB-treatment of mice with tissue-specific reactivation of MC4R reactivation (in the MC4R null background). Similarly, the functional requirement of MC4R expression can be directly determined through tissue specific deletion of MC4R. These reagents, and others, are available to me from Dr. Elmquist. The use of these reagents is detailed in the experiments proposed in this application. Furthermore, Dr. Elmquist has overseen the career development of over 16 post-doctoral fellows, ensuring the quality of mentoring I will receive under his tutelage.

Third, the unique and supportive scientific environment at UTSW, exemplified by TORS, is crucial for my success and development as an investigator. I have full access to the entirety of the TORS faculty as well as their lab members. This faculty includes, among others, Michael Brown, Joseph Goldstein, David Mangelsdorf, Philipp Scherer, Jay Horton, Masashi Yanagisawa, Craig Malloy, Joyce Repa, Elizabeth Parks, and Helen Hobbs. This opportunity has already proven beneficial as evidenced by my ongoing collaborations involving the study of in vivo intermediary metabolism after RYGB with researchers in our Advanced NMR Imaging Center (Shawn Burgess), sexual dimorphism in the response to RYGB (Deborah Clegg), differential neuronal activation after RYGB (Carol Elias) and the effect of RYGB on ghrelin biology (Jeffrey Zigman). Access to this distinguished faculty through both formal and informal interactions provides an ideal learning environment for a junior investigator. My prior clinical and research training coupled with this unique and ideal environment ensure my continued success and career development. Obtaining the K08 Mentored Clinical Scientist Development Award will greatly facilitate this process by providing salary support and additional protected time to devote to my research efforts.
My primary career goal is to become an independent clinician-scientist and a leader in the fields of obesity, diabetes, and bariatric surgery. My professional training to date has already provided me with a strong foundation in basic research through which to attain these goals. Coupled with the development of our novel surgical model, I will be able to successfully investigate the anatomic, physiologic, and molecular mechanisms of RYGB. This research is critically important due to the magnitude of the obesity problem and the clinical efficacy of bariatric surgery. Knowledge of the mechanisms contributing to this efficacy will provide a better understanding of the pathophysiology of obesity and related disease. Importantly, such an understanding will also facilitate the development and evaluation of novel therapies that mimic the underlying physiologic mechanisms induced by bariatric surgery as well as its clinical efficacy. These therapies will be less-invasive and therefore safer, and more suitable for broad application to the large number of people at risk for obesity-related morbidity who do not meet the body weight requirements for bariatric surgery.

My interest in science began during college when I had the opportunity to perform basic research involving the organic synthesis of anti-neoplastic compounds. This experience introduced me to basic research as a career path and prompted my matriculation into the MD-PhD program at Harvard Medical School. I was first exposed to the plight of the obesity epidemic during my clinical training in medical school. My decision to work in the laboratory of Dr. Morris White at the Joslin Diabetes Center was prompted by an opportunity to combine my prior research experience in organic chemistry and my developing interest in the molecular mechanisms underlying the derangements of intermediary metabolism and cellular insulin responsiveness seen in diabetes. Due to the advancing acknowledgement and basic understanding of the role of gut-derived factors on the acute regulation of feeding behavior, energy balance, and glucose metabolism, I found myself interested in the profound, substantial, and sustained impact of bariatric surgery on body weight and metabolic disease towards the end of my doctoral thesis.

This newfound interest coincided with my return to clinical training and was the reason I chose to complete an internal medicine residency and gastroenterology fellowship. It further guided my research focus during post-doctoral fellowship, particularly towards the development of a mouse model of RYGB. My prior basic research training was instrumental in this decision, as I recognized the utility of an animal model of bariatric surgery. In combination with novel genetic models of obesity and diabetes, our model of RYGB is a robust tool for investigation of its underlying anatomic, physiologic, and molecular mechanisms. A detailed understanding of these mechanisms is integral to the development of targeted anti-obesity therapies of sufficient efficacy and safety.

This research opportunity arises at a crucial time in my career development. I have successfully transitioned to faculty member, both clinically and in a research capacity. At UT Southwestern, I have already established both a fully functional mouse microsurgery facility and the mouse RYGB model. To this end, we have already performed RYGB or sham operations on over 100 mice, including two genetic models of obesity and diabetes, demonstrating our technical proficiency with both wild-type and genetic mice. Furthermore, we have taken advantage of the substantial metabolic phenotyping capability of the UT Southwestern core facilities. Thus, we have both the technical capability and experimental proficiency to complete all of the goals and objectives outlined in this proposal.

The learning opportunities available to me at UT Southwestern are substantial. For instance, I do not have extensive experience in the care of genetic mouse models of obesity and diabetes or in their metabolic phenotyping. Therefore, the choice of my mentor, Dr. Elmquist, was calculated and instrumental to the success of my project and career development. Dr. Elmquist has successfully trained a number of doctoral and post-doctoral fellows in precisely the intellectual and technical capacity for which I strive. Furthermore, as director of the Division of Hypothalamic Research, he has also overseen the career advancement of division faculty since 2006. I am thus ideally situated to attain expertise in the metabolic phenotyping of genetic mouse models of obesity and diabetes – intellectual and technical expertise that will successfully complement my prior research experience in molecular mechanisms of diabetes and the development of rodent models of bariatric surgery. This unique combination of intellectual and technical expertise in molecular mechanisms of disease, models of bariatric surgery, care of genetic mice, and metabolic phenotyping will facilitate me in attaining my research goals during the K08 award period. The K08 CDA comes at a crucial time because it will aid my development in the following ways: 1) provide salary support that will guarantee the requisite protected time to achieve my career goals, 2) provide valuable research funds to continue novel experimentation, and 3) initiate my entry into the process of obtaining peer-reviewed research funding that is vital for my scientific independence. Obtaining the K08 award will greatly aid my current career development as well as future advancement.
My prior research training has involved both investigation of the molecular mechanisms of insulin resistance and the development of rodent models of bariatric surgery. This combination of research and technical expertise ideally situate me to investigate the anatomic, physiologic, and molecular mechanisms underlying the beneficial effects of Roux-en-Y gastric bypass. Furthermore, I am in the ideal position at UT Southwestern to perform the proposed experiments from both a technical and intellectual perspective.

The specific expertise that I propose to obtain during the award period involves (1) generation and study of genetic mouse models of obesity and diabetes, (2) metabolic profiling of mice, and (3) in vivo metabolism in post-surgical mice. This will involve husbandry and genotyping of mice, performing analyses of glucose metabolism, and complete metabolic phenotyping. I have full access to the requisite technical equipment and support staff. Importantly, I have access to faculty members of my division, additional TORS faculty members, and the large number of people who occupy their labs – all of whom have extensive experience in the specific expertise I propose to obtain during the award period. I have already begun to take advantage of this environment, and it has been instrumental in enabling me to obtain the preliminary data outlined in the Research Strategy component of this application. This enhanced skill and knowledge will complement my prior molecular and surgical expertise and will enable me to establish myself as a leader in the fields of obesity and diabetes, particularly as they relate to bariatric surgery. This over-arching focus will be coordinated within the Division of Hypothalamic Research under the supervision of my mentor, Dr. Elmquist.

Structured activities during the award period will include:

**Obesity and Metabolism:** UT Southwestern has a large and active community of researchers interested in weight regulation and metabolism as evidenced by the endowment of an NIH Roadmap grant to study obesity.

1. Mandatory participation in weekly 1.5 hour data presentation with members of the Division of Hypothalamic Research including members of the laboratories of Dr. Joel Elmquist and several colleagues including Drs. Jeffrey Zigman, Carol Elias, Roberto Coppari, and Joyce Repa.
2. Once a month this meeting is expanded to include presentations from members of the NIH Roadmap Grant on Obesity (TORS) including presentations from the laboratories of Drs. Michael Brown, Joseph Goldstein, David Mangelsdorf, Philipp Scherer, Jay Horton, Masashi Yanagisawa, Craig Malloy, Joyce Repa, Elizabeth Parks, and Helen Hobbs.
3. Weekly 1 hour meetings with Dr. Elmquist for mentorship of my research project. These meetings will include counseling about academic skills required for success as an individual researcher, including – but not limited to – the following: Instruction and supervision in grant and manuscript writing skills; advice and discussion of issues concerning responsible conduct of research; development of administrative and leadership skills as they pertain to research.
4. “in-house” peer review of all manuscripts and grant applications.
5. Individual meetings as needed with members of the Elmquist laboratory experienced in listed techniques including metabolic analysis, neuroanatomical tracing, dual label immunohistochemistry, and viral mediated gene transfer.
6. The career development plan includes access to my Division Chief, Dr. Rockey, for mentorship involving issues surrounding the conduct of research and development of administrative and leadership skills.

**Course Work:** Several courses have been included in my training plan to take advantage of the new Department of Clinical Sciences (http://www.utsouthwestern.edu/utsw/home/educ/CBSCS/). The first year includes courses in Biostatistics, Research Design, and Grant Writing. The second year will involve Research Management. These courses provide advanced instruction in techniques relevant to basic researchers as well as clinical researchers. They will also offer me an opportunity to interact with my clinical research peers to consult on the design of their research studies. This is another way in which this award will allow me to fulfill my career goal of bridging the gap between basic science and clinical treatments.

**National Meetings:** Dr. Elmquist actively encourages participation in national meetings. This is evidenced by his support of travel to annual scientific symposia such as the Keystone Conference on obesity and diabetes as well as annual scientific meetings including, but not limited to, those of the American Gastroenterologic Association, American Diabetes Association, the Endocrine Society, and the Obesity Society.

**Clinical Work:** My ultimate professional goal is to use my clinical experiences with patients to inform my basic science research. Indeed the initial inspiration for this project came from my work involving gastroenterologic issues of obese patients before and after bariatric surgery. I propose to continue this experience in the bariatric center at UT Southwestern. My current clinical work involves 4 weeks per year managing the in-patient gastroenterology consultative service. In addition, I am involved in the delivery of out-patient consultative and endoscopic services one day per week. This level of commitment will not change in the event of receipt of this award.
Several opportunities exist at UT Southwestern for training in the Responsible Conduct of Research. All researchers at UT Southwestern are given formal instruction in ethical issues related to research and in issues of scientific integrity upon their entrance to the institution. This material is organized and directed by Dr. Fred Grinnell, Professor of Cell Biology, who is also a prominent and thought-provoking scholar and author of the book “The Scientific Attitude” (Guilford Press, New York, 1992). All researchers are also given a copy of the publication “On Being a Scientist”, published by the National Academy Press, and a compilation of case studies of research-related ethical issues put together by Saul Weingrad. Material from these publications is the basis for discussion classes. Topics covered include ethical issues in human genetics research, the practice of science, policies protecting human research subjects, clinical ethics in medicine, and law, literature, and medicine. I will also attend semi-annual seminars on the Ethical Conduct of Research sponsored by the Department of Medicine. At these seminars, trainees discuss cases contained in “Teaching the Responsible Conduct of Research through a Case Study Approach” (AAMC). Recent topics include data selection and retention, reporting preliminary results, dealing with suspicions of misconduct, and conflicts of interest.

A responsibility emphasized at UT Southwestern is that faculty members and institutional officials not only bear particular responsibility for their own ethical behavior but they should also set a positive example by their actions. All faculty members are required to commit appropriate time for adequate supervision of trainees, and to ensure that the research carried out by the trainees is performed in a manner that reflects high standards for the responsible conduct of science. Among other things, the necessity of entering all raw data into bound notebooks in readily retrievable form is emphasized. Such raw data cannot be excluded from analysis unless there are explicit reasons (entered in the notebook) for the exclusion. Furthermore, it is emphasized that authorship of a scientific paper should be limited to those who have contributed in a meaningful way to its intellectual content. All co-authors should have been directly involved in planning some component of the work, writing a draft or revising the article, and final approval of the version to be published.

With any ethical problem that may arise, I may report my concerns to the Director of Graduate Studies, to the Director of the relevant training program, or to the Director of Postdoctoral Research. In the unlikely event that all individuals are seen to be involved in the misconduct, I know to approach my mentor, the Department Chairman, or the Associate Dean for Research. If allegations of academic fraud do arise, the procedures published by the University will be initiated – policies taught to trainees in the mandatory seminars.

I am current in my Training in the Responsible Conduct of Research. I met the annual requirements for classes in this subject throughout my post-doctoral fellowship, and have completed one round of instruction at UT Southwestern since my arrival last year. The most recent class was Fall of 2009.

Outline of Activities by year:

<table>
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<tr>
<th>Activity</th>
<th>Year 1</th>
<th>Year 2</th>
<th>Year 3</th>
<th>Year 4</th>
<th>Year 5</th>
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<td>Care of Genetic Mouse Models</td>
<td>Metabolic Phenotyping using TSE Metabolic Cages</td>
<td>In Vivo Metabolism using NMR</td>
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<td>Weekly Meeting with Mentor</td>
<td>Weekly Obesity Alliance Meeting</td>
<td>Monthly TORS Works-In-Progress Meeting</td>
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<td>Specific Aim #3</td>
<td>Specific Aim #3</td>
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<td>Keystone DDW*</td>
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<td></td>
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<td>Write Paper #1</td>
<td>Write Paper #2</td>
<td>Write Paper #3</td>
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<td>Development of Research Program</td>
<td>Join Graduate Program</td>
<td>Hire Postdoc or Graduate Student</td>
<td>Submit R01</td>
<td>Revise R01</td>
<td>Submit R01A1</td>
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</table>
May 17, 2010

Re: Sponsor Letter for [redacted] M.D., Ph.D.
Applicant for the K08 Mentored Clinical Scientist Career Development Award

Dear Committee Members,

I am writing with great enthusiasm in support of Dr. [redacted] application for the NIH Mentored Clinical Scientist Career Development Award (K08). The purpose of the current proposal is to provide Vincent with advanced experience and assistance in transitioning to an independent faculty position. I encouraged Vincent to apply for this award for several reasons. First, Vincent is a highly motivated and talented scientist with great potential to become an independent scientist. I am confident that his future research on the mechanisms by which Roux-en-Y gastric bypass (RYGB) induces weight loss and improves diabetes will greatly contribute to the advancement of the field. Second, Vincent is at a critical stage of his career development, and additional training in my division will provide excellent opportunities for him to acquire the necessary technical skills, knowledge, and experience to develop his independent career. The support from a K08 award will certainly facilitate this. Finally, Vincent’s proposal is a logical extension of his previous work. The proposed studies are hypothesis driven and will likely advance our understanding about the central mechanisms underlying the anti-obesity effects of RYGB. I want to state definitively that I am committed to the training plan outlined below and to Vincent’s success as an independent investigator. This letter describes Vincent’s qualifications and potential as a candidate for the award, our plan for his training and career development, the research environment at UT Southwestern, and my experience as a mentor.

Applicant’s Qualifications and Potential for a Research Career

Vincent was recruited to the Department of Internal Medicine and Division of Hypothalamic Research at UT Southwestern in January 2009 from the Gastrointestinal Unit at Massachusetts General Hospital. As documented in his biosketch, he is extremely well-trained in both basic research and clinical medicine. Work detailed in his doctoral thesis, performed in the laboratory of Dr. Morris White, Ph.D. at the Joslin Diabetes Center and Harvard Medical School, identified the first molecular mechanism of cytokine-induced insulin resistance. After completing formal clinical training in gastroenterology, Vincent returned to the laboratory under the mentorship of Dr. Lee Kaplan, M.D., Ph.D. where he became interested in the physiologic mechanisms underlying the effects of RYGB on body weight and glucose homeostasis. In the Kaplan lab, he was involved in the development and initial characterization of the first mouse model of RYGB. Vincent was quite productive during this time, as evidenced by the multiple manuscripts he has under review summarizing his initial findings. One publication from his post-doctoral thesis involving an indwelling, endoluminal device that induces weight loss and improves diabetes when implanted in the duodenum of obese rats was named the “Most Outstanding Research Manuscript of the Year” by the Obesity Society in 2008.
Since coming to UT Southwestern, Vincent has established an animal surgical facility to continue his investigation into the mechanisms underlying RYGB. He is currently doing so through the use of various genetic mouse models of obesity and diabetes available here at UT Southwestern. Vincent brings a unique set of technical and intellectual skills to the field of obesity research owing to the novelty of his surgical model, clinical training in gastroenterology, and extensive prior training in the basic science of diabetes and metabolism. This is important and timely due to the pressing obesity epidemic. The value of Vincent’s model is that it will facilitate the identification of key molecular intermediates potentially amenable to therapeutic intervention for patients with obesity and related diseases. Based upon my close observations and interactions with Vincent, I am convinced that he is an excellent young scientist and an outstanding candidate for this award. The work he is proposing is hypothesis-driven and I am confident that it will produce an important series of findings. Moreover, his research effort will provide an intensive training experience in molecular endocrinology, energy balance, glucose homeostasis, intermediary metabolism, and neuroanatomy. Importantly, Vincent has taken a rigorous path in both his scientific and clinical training. I am committed to his development and I am confident that he has the skills, both personal and professional, to make substantial contributions to the fields of obesity and metabolism and to emerge as a leading independent investigator.

Training Plan, Environment, and Career Development Plan:

I would like to take this opportunity to explain what I foresee as my role in Vincent’s training. Specifically, I will serve as a mentor for Vincent and am committed to his scientific and professional development. As outlined below and in his application, I strongly believe that Vincent already has very unique skills and is poised to become a leader in the fields of obesity, diabetes, and bariatric surgery. However, he has the opportunity to further his training during the period of this award. Thus, I view him as an excellent candidate for a K08 award, and support at this stage of his career would go a long way towards his progression to an independent clinical scientist. I have particular expertise in the areas of feeding behavior, obesity, metabolism, systems neuroscience, and neuroanatomy. Additionally, a major focus of my laboratory’s research is the development of mouse models using advanced genetic expression techniques which allow for the precise spatial and temporal manipulation of the expression of several genes involved in the regulation of feeding and metabolism. Importantly, all of the mouse models and techniques that have been proposed in Vincent’s application are already well-established in my lab. In addition, I want to stress that Vincent’s developing research interests as an independent investigator coincide greatly with my own as well as those of the Division of Hypothalamic Research, which I direct. Thus, I believe that I am extremely well-suited to advise him during the work proposed in his current Mentored Clinical Scientist Career Development Award application. I am dedicated to doing everything I can to help ensure the success of this most talented young clinical scientist.

Vincent’s research training and career development plan is multifaceted and includes his proposed research project, training in the Responsible Conduct of Research, training in various experimental techniques, mentored meetings, and participation and presentation at seminars, national scientific meetings, and finally our divisional group meetings where he will present original data. The most important component of Vincent’s training will continue to be the research project that forms the basis of this proposal. As illustrated by several of our recent publications, we are routinely using the Cre-recombinase/loxP system to make neuron-specific gene modifications. We have generated both a number of mice with specific loxP-modified alleles as well as several models expressing Cre-recombinase under the control of specific promoters. Our list of genetic tools also includes a number of mouse lines obtained from our collaborators. Importantly for Vincent’s proposed studies, a mouse line with a loxP-modified MC4R allele whose expression can be “reactivated” in a Cre-dependent manner (loxTB-MC4R mice) has been generated in our lab. In Aim 1 of the proposal, Vincent can cross these mice with our ChAT-Cre mice in order to generate MC4R deficient mice that express MC4R exclusively in autonomic cholinergic preganglionic neurons (i.e., parasympathetic and sympathetic motor). Vincent can use these mice to directly address the role of MC4Rs on these neurons in the effects of RYGB on energy expenditure, body weight, and glucose metabolism. In Aim 2 of the proposal, Vincent can cross loxTB-MC4R mice with our Phox2B-Cre mice to
generate MC4R deficient mice that express MC4R exclusively in motor and sensory components of the vagus nerve. These mice can be used to directly address the role of MC4Rs on the vagus nerve in the effects of RYGB on glucose metabolism. Importantly, these mice will enable distinction of the effects of RYGB that involve parasympathetic vs. sympathetic motor neurons by comparison to results from Aim 1. In Aim 3, Vincent will take advantage of a mouse line with a unique molecular sensory vagotomy to investigate the role of sensory vagus neurons in the generation of the initial signal from the gut to the brain after RYGB. Vincent’s technical ability to perform the complicated RYGB procedure in mice is an invaluable asset as it enables direct investigation of the underlying anatomic and molecular requirements for the effects of RYGB on obesity and other related metabolic diseases, particularly diabetes. Clearly, gastric bypass is the most widely used and efficacious treatment for obesity. However, remarkably little is known regarding the mechanisms underlying its beneficial effects. We are excited about these experiments because they will advance knowledge of the effects of both MC4R and components of the autonomic nervous system on obesity and glucose metabolism in the context of RYGB. Given the potential training Vincent will get during the mentored phase, I expect he will be able to independently accomplish this goal within the proposed timeline. In summary, I am extremely enthusiastic about the studies Vincent has proposed because the potential importance of these experiments is quite high, and they certainly will expand Vincent’s training as a mentored junior faculty member and facilitate his transition to independent faculty. Furthermore, I expect the proposed studies will generate several more high profile papers for Vincent in the next few years, which will definitely increase the likelihood for him to successfully compete for R01 funding in the future and make him a uniquely qualified and promising member of the obesity and diabetes scientific communities.

A key part of Vincent’s training will be his participation in various laboratory meetings, seminars, and scientific conferences. This is already underway. For example, Vincent attends my laboratory’s weekly group meeting and will continue to present either original data or a review of a topical paper from the literature on a regular basis. He also regularly attends and presents at the weekly “Works in Progress” meeting of the Division of Hypothalamic Research, a meeting at which the faculty members of the division and their lab members attend. In addition, once a month our laboratory hosts a group meeting for the investigators in our P50 Roadmap Grant from the NIH (see Taskforce on Obesity Research at UT Southwestern (TORS; http://www8.utsouthwestern.edu/utsw/home/research/TORS/index.html). The Roadmap awards were very competitive and our proposal was the only grant awarded in obesity and metabolism. In addition to myself, presenters at this meeting include members of the laboratories of Michael Brown and Joseph Goldstein, Helen Hobbs, Jay Horton, Masashi Yanagisawa, Andrew Zinn, Joyce Repa, David Mangelsdorf, Carol Elias, Joe Takahashi, Philipp Scherer, Jeffrey Zigman, and Roberto Coppari.

Through my experience at mentoring at all levels, including pre-doctoral, post-doctoral, and at the junior faculty level (I have been fortunate enough to mentor 20 different individuals in the previous 11 years), I plan to provide and/or stipulate the following specific elements for Vincent’s career development:

- Daily access to me for discussion of research
- Counseling about academic skills required for success as an individual researcher, including – but not limited to – the following:
  - Instruction and supervision in grant and manuscript writing
  - Advice and discussion of issues concerning responsible conduct of research
  - Development of administrative and leadership skills as they pertain to his research and his role as an academician in the Medical Center
- “In-house” peer review of all manuscripts and grant applications
- Mandatory participation in our highly structured research journal club (in which topical research is presented, results reviewed, and future research topics are discussed)

Additionally, relationships of the applicant to specific collaborators within the medical center will be nurtured. Key individuals for his research include: Michael Brown, Joseph Goldstein, Jay Horton, Philipp Scherer, David Mangelsdorf, Carol Elias, Jeffrey Zigman, Shawn Burgess, and Joyce Repa.
A committee to review and monitor the progress of the applicant has been set up and will oversee Vincent’s career development. This committee will consist of individuals with a broad range of experience, including that in molecular genetics, neuroscience, cell biology, and pharmacology. The committee will meet semi-annually over the next 5 years, and will provide formal feedback to Vincent.

Vincent’s training plan also includes opportunities to participate in several seminars, offered by UT Southwestern Medical School, that are specifically designed for career development and training in the Responsible Conduct of Research. Topics in career development include principal investigator responsibilities, grant writing, manuscript writing, funding opportunities, and academic advancement. Topics in the responsible conduct of research include training in ethics, the use of experimental animals, biochemical hazard safety and radiation safety. These seminars provide regular updates regarding local and national research policies and procedures. Further, my interactions with Vincent will include group lab meetings as well as both formal and informal individual meetings. During these meetings, we will specifically address Vincent’s research projects, the responsible conduct of research, issues related to his career development, and his overall progress. Specifically, we will discuss, in detail, data collection, analysis, presentation, and publication. We will establish focused goals on the formulation of testable hypotheses, for the completion of the proposed projects, and their eventual publication in peer-reviewed journals. The expectation will be at least one manuscript per year per aim of this proposal. While I will continue to spend a substantial amount of time with Vincent going over his data and talking about published studies, I will also encourage him to actively contribute in choosing and designing new experiments and thinking through the narratives that will underlie future papers. In addition, we will work on improving his manuscript and grant writing skills. To facilitate his work, I will freely share any and all laboratory reagents with Vincent, including novel genetic mouse models of obesity and diabetes, and Vincent is free to continue working with these models once he establishes his own independent research program. These types of interactions will all facilitate continued career development and advancement.

As a faculty member in the Division of Digestive and Liver Diseases, Vincent has the opportunity to dedicate some of his time to clinical ventures. He will have a half-day a week out-patient clinic in the Division of Digestive and Liver Diseases, with a concentration on general and obesity-related gastroenterology. I view this as a major strength since it gives Vincent an excellent opportunity to maintain patient contact, provide a much needed clinical service, and inform his basic research with clinically relevant observations. His teaching load during this time will be minimal, and limited to lectures and seminars focused on his area of clinical and/or research expertise. Importantly, Vincent will dedicate at least 75% effort to research.

My Mentoring Experience:

My laboratory currently consists of six postdoctoral fellows, three graduate students, seven instructors, and four research assistants. I have trained 2 pre-doctoral students and 18 post-doctoral fellows over the past 11 years. A representative seven of my previous trainees are listed below.

<table>
<thead>
<tr>
<th>Trainee</th>
<th>Training Period</th>
<th>Current Affiliation</th>
</tr>
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<tbody>
<tr>
<td>Yong Xu, PhD</td>
<td>2006-2010</td>
<td>Assistant Professor, Baylor College of Medicine</td>
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<tr>
<td>Jennifer Hill, PhD</td>
<td>2003-2009</td>
<td>Assistant Professor, Center for Diabetes and Endocrine Research, University of Toledo College of Medicine</td>
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<tr>
<td>Jacob N. Marcus, PhD</td>
<td>2002-2006</td>
<td>Research Scientist, Merck Research Laboratories, West Point, PA</td>
</tr>
<tr>
<td>Jeffrey Zigman, MD, PhD</td>
<td>2001-2006</td>
<td>Assistant Professor, Department of Internal Medicine, UT Southwestern Medical Center</td>
</tr>
</tbody>
</table>
I am familiar with the challenges that those seeking an academic career must overcome in order to become truly independent. To this end, mentor-trainee interactions include group lab meetings as well as both formal and informal individual meetings. I will hold regular meetings with Vincent in all of these settings. I am committed to helping Vincent successfully navigate the transition from mentored clinical scientist to independent investigator and I am confident he has the ability to do so.

Conclusion:

My work with Vincent up to the present has shown him to be a highly talented scientist of exceptional promise. His self-directed move into research that combines the fields of bariatric surgery and energy homeostasis underscores this fact. He has demonstrated that by the end of this mentored award, he will be well prepared to start an independent research program that will make valuable contributions to both fields. I am happy to provide annual evaluations of Vincent’s progress as required in the annual progress report. He has gained a broad and unique perspective from his extensive background in gastroenterology, bariatric surgery, and diabetes and from the training in neuroanatomy and molecular neuroendocrinology in our group. I sincerely believe he is poised to fill an important niche in the field of metabolism that will ultimately lead to the design of effective treatment strategies for obesity and diabetes.

Finally, I would like to note that Vincent has been a welcome addition to our research program. He is very collaborative and his friendly disposition permits him to interact and work well with others. He is bright and extremely dedicated to his work, and I have no doubt that he will be successful in his endeavors. Although Vincent himself is a “trainee”, his unique background has provided fascinating new avenues of research in my laboratory. Vincent’s accomplishments have already been impressive and, in combination with his work habits and personality, bode well for a career in biomedical research in obesity, diabetes, metabolism, and bariatric surgery.

In summary, Vincent is at a critical stage in his career and I strongly believe that he is an ideal and outstanding candidate for the K08 Award from the NIH. He is highly motivated and committed to clinically-informed basic science research, and I strongly believe Vincent is a clinical scientist of remarkable abilities and potential. I would argue that the novelty of his proposed experiments will greatly accelerate his scientific development and will propel him to the forefront of research in bariatric surgery, obesity, and diabetes. A K08 Award would provide critical support for Vincent to develop new and necessary technical and analytical skills needed to appropriately perform studies related to the molecular, cellular, neuroanatomical, and physiologic aspects of the regulation of obesity and diabetes after RYGB. UT Southwestern provides Vincent access to a truly unique academic environment that includes leaders in the fields of neuroanatomy, obesity, and metabolism. We also have in place the resources to facilitate his success. Furthermore, I am strongly committed to Vincent’s career development. I expect that over the next few years Vincent will publish several important papers in high impact journals in addition to the manuscripts currently under review. Together, these elements provide a unique opportunity for Vincent and all of the necessary ingredients for Vincent’s successful transition from mentored faculty to an independent investigator and clinical scientist.
Please feel free to contact me if you have any further questions.

Sincerely,

Joel K. Elmquist, D.V.M., Ph.D.
Professor and Director
Division of Hypothalamic Research
Departments of Internal Medicine and Pharmacology
Description of Institutional Environment

The Southwestern Medical College was established in 1943 as the 68th medical school in the United States. In 1949 it became the second medical school in The University of Texas system and was renamed Southwestern Medical School of The University of Texas, and later to The University of Texas Southwestern Medical School in 1954. In 1972, it became a full-fledged health science center, and was renamed The University of Texas Southwestern Medical Center at Dallas in 1987. It includes three degree-granting institutions (Southwestern Medical School, Southwestern Graduate School of Biomedical Sciences, and Southwestern Allied Health Sciences School), which train over 3,400 medical, graduate, and allied health students, residents, and postdoctoral fellows annually.

UT Southwestern ranks among the top academic medical centers in the nation. It supports nearly 2,000 research projects annually, totaling more than $155 million, and it hosts a Howard Hughes Medical Institute. UT Southwestern was ranked in the top eight American institutions (1994) in research impact of federally funded American universities. A 1997 study published in *Applied Clinical Trials* ranked UT Southwestern fourth among over 7,000 U.S. institutions in the number of investigators conducting studies sponsored by pharmaceutical companies. It is one of 33 sites with a National Institutes of Health-approved Medical Scientist Training Program leading to M.D., Ph.D. degrees.

Further evidence of the excellence of this institution is based on the fact that UT Southwestern has 4 active Nobel Laureates (more than any other medical school in the world), 20 members of the National Academy of Sciences, 14 members of the American Academy of Arts and Sciences, and 19 members of the Institute of Medicine. UT Southwestern has approximately 5,000 employees and an annual operating budget of almost $500 million. Finally, there are approximately 45 endowed Research Centers and Chairs at UT Southwestern.

The 90-acre campus is located 3 miles north of downtown Dallas, with more than 2 million square feet of teaching and laboratory space. Faculty members and residents provide care for more than 75,000 hospitalized patients and oversee more than 1.4 million outpatient visits a year. The medical complex includes University Hospitals (Zale Lipshy and St. Paul), Parkland Memorial Hospital, Children’s Medical Center, UT Dallas Callier Center for Communications Disorders, Texas Woman’s University Institute of Health Science, Southwestern Institute of Forensic Sciences, and the City of Dallas Health Department. The South Campus is composed of approximately 15 buildings which are home to administrative offices, the UTSW Student Center, numerous Medical School Research Buildings, and two animals care facilities. These include the Danciger Building (H), the Green Research Building (Y), the Greed Science Building (L), the Laboratory Research and Support Building (JA), and the Jonsson Building (K). The North Campus is composed of 5 new biomedical research towers constructed since 1995. These include the Hamon Biomedical Research Building (NA), the Simmons Biomedical Research Building (NB), the Seay Biomedical Research Building (NC), the Biomedical Research Building (ND), and the Clements Advanced Imaging Research Center (NE).

Clinical activities at UT Southwestern occur at 3 primary sites, including University Hospital, Parkland Memorial Hospital (PMH), and the Department of Veterans Administration Medical Center (VAMC) (part of the integrated residency program). The Division of Digestive and Liver Diseases has a major presence at each of these teaching hospitals, including active clinical and translational research at each.

Basic Obesity and Metabolism Resources

As one of the world’s foremost research institutions, UT Southwestern focuses on attracting and retaining preeminent researchers, while maintaining an environment that promotes research in diverse areas. For basic or clinical research, UT Southwestern, an institution that fosters multidisciplinary approaches and rigorous scientific training, provides an ideal environment.

UT Southwestern has had a dedicated focus on obesity and the associated constellation of metabolic disorders referred to as the metabolic syndrome (atherogenic dyslipidemia, hyperglycemia, hypertension, prothrombotic and proinflammatory states). The Taskforce for Obesity Research at UT Southwestern (TORS) brings together investigators from diverse disciplines to examine the behavioral, metabolic, and molecular
mechanisms that cause obesity and the metabolic syndrome. TORS integrates the traditionally disparate disciplines of neuroendocrinology, genetics, lipid metabolism, intermediary metabolism, and clinical epidemiology into a cohesive center to study obesity and the metabolic syndrome. The major focus of the Division of Hypothalamic Research is on the brain and liver, organs that play central roles in the development of obesity and its adverse metabolic consequences. The primary goal of this taskforce is to elucidate the molecular mechanisms by which the brain regulates food intake and energy expenditure, and to determine how dysregulation of glucose and lipid metabolism in the liver causes the metabolic syndrome. Its long-term goal is to develop more directed approaches to prevent obesity and treat the metabolic complications of this disorder. In recognition of the superb collaborative research environment, UT Southwestern has been awarded a NIH Roadmap Grant to support the research activities of our rare and unique interdisciplinary research team that focuses on the study of obesity and its metabolic complications. The success of this application was due to the participation of more than 30 investigators from 12 different scientific disciplines. Of the numerous applications received, ours was the only one chosen for funding that focused on obesity. Importantly, this award strengthens the interactions of Dr. Elmquist’s lab with that of Dr. Scherer of the Department of Internal Medicine, ensuring the investigation of the neural basis of obesity remains a priority for the UT Southwestern Taskforce for Obesity Research.

Another resource supporting this proposal is the Division of Hypothalamic Research, which was established in 2006 to bring together scientists interested in understanding the mechanisms underlying hypothalamic function. The hypothalamus is one of the most evolutionarily conserved regions of the mammalian brain. Dysfunction of its central regulation results in obesity and type 2 diabetes, both of whose prevalence continue to rise at alarming rates. To understand the causes and to develop treatments for these and related diseases, it is first necessary to unravel the central pathways regulating energy homeostasis. Thus, one of the major goals of the research programs in the Division of Hypothalamic Research is to understand the molecular and neuroanatomic basis for coordinated control of body weight and glucose homeostasis. The Division hopes to make discoveries that will help combat the growing problems of obesity and diabetes. The Division is in the Department of Internal Medicine, but also includes investigators from several other clinical and basic science departments throughout the university, including Joel Elmquist, Joyce Repa, Jay Horton, David Mangelsdorf, Masashi Yanagisawa and Andrew Zinn.

Finally, the applicant will also benefit from interactions with researchers in the Touchstone Diabetes Center at UTSW. Members of the Center include Deborah Clegg, Philipp Scherer, and Roger Unger, whose research focuses on both basic and clinical aspects of type I and type II diabetes and questions related to the impact of diabetes and obesity on cardiovascular disease outcome and cancer incidence. Particularly, the Mouse Metabolic Phenotyping Core operated by the Touchstone Diabetes Center and the Division of Hypothalamic Research provides a full spectrum of services for the metabolic phenotyping of preclinical models and assay capabilities for clinical samples. The applicant has had full access to the Core for his previous studies and he will continue to benefit from the comprehensive metabolic analyses for the current proposal.

**Additional Laboratories:** Dr. Elmquist has 2000 sq ft in the newly formed Division of Hypothalamic Research, of which he is the Director. The overall center occupies approximately 14,000 sq ft and is fully equipped for histology, physiology (including rat and mouse telemetry transmitters and antennae), and biochemistry. All essential basic wet laboratory equipment is readily available and access to shared equipment in core molecular biology facilities is contiguous. A new Arcturus laser capture microscopy system is available for isolation of tissues for gene chip and quantitative PCR.

**Animals:** A core animal housing facility is maintained by UT Southwestern Medical Center. Most obesity and metabolism investigators on our south campus (where the Division of Hypothalamic Research is located) use the conventional animal facility in the K building or the barrier animal facility in the JA building. This is in addition to the dedicated animal survival surgical facility that Dr. [name] maintains in the Department of Surgery Care Animal Surgery Facility. Maintenance and veterinary care is provided by the Animal Resource Center of UT Southwestern. There are also animal procedure rooms and surgery rooms within each of these animal housing facilities.
Core Facilities: The Mouse Metabolic Phenotyping Core is jointly run by Drs. Joel Elmquist, D.V.M., Ph.D., Jay Horton, M.D. and Philipp Scherer, Ph.D. and offers a number of services for complete metabolic phenotyping of rodents, particularly mice. These include:

1. Measurements of up to 36 standard serum chemistries and 5 hormones, with additional chemistries, metabolites, and hormones available on request.
2. 36 metabolic chambers that measure calorimetry, activity, food, and water consumption.
3. Body composition analysis (DEXA, MRI and a rodent CT Scanner)
4. A Seahorse instrument to measure mitochondrial function in tissue culture cells.
5. A STARR MouseOx instrument to measure Oxygen Saturation (SpO2) (The MouseOx™ provides real-time percent oxygen saturation of functional arterial hemoglobin, heart rate (the MouseOx™ provides real-time cardiac pulse rate and respiratory rate).
7. Rodent insulin clamping core organized by Drs. Jay Horton and Philipp Scherer.

See more specific information regarding the metabolic phenotyping core facility at: http://www.utsouthwestern.edu/utsw/home/research/metstudcore/index.html

In addition, UT Southwestern has all standard state-of-the-art Core facilities that include facilities for Analytical Ultracentrifugation, DNA Microarray, DNA Sequencing, Electron Microscopy, Flow Cytometry, Live Cell Imaging, Mouse MRI, Protein Chemistry, Rapid Biochemical Kinetics, Synthetic Chemistry, Structural Biology and the Transgenic Model Generation. These facilities are open to all investigators for a nominal fee.

Computers: Dr. maintains four computers between his office and laboratory (two desktops and two laptops), all of which are linked to the UT Southwestern network and, thus, a host of printers and scanners are also available for his use. These include, but are not limited to, four laser printers (HP 6MP, HP 5M, HP 3500N, HP 2300N) and two scanners (Umax Powerlook 1100 and an HP Scanjet 4C). In addition, the laboratory area has 15 networked Macintosh (6iMacs, 2 Macintosh G3’s, 2 Macintosh G4’s, and 2Macintosh G5’s) and 3 PC platform computers present. A Novell server containing 500 GB of hard disk space has been added as part of the unit. In the administrative space within the laboratory, there is also a Xerox 5614 copier. An additional computer resource room (300 sq. ft.) contains multiple personal computers available to the PIs and their colleagues, with connections to the Internet.

Major equipment:

Aguirre Laboratory: Dr. has been allotted approximately 500 sq feet of laboratory space on the 6th floor of the Y building, which he has equipped with two microfuges, a thermocycler, a -80C freezer, a -20 freezer, a standard refrigerator, and typical laboratory disposables. A further 1,500 square feet of bench space including a fully outfitted cell culture room, animal surgery room, equipment room, conference room, and a work area for microscopy is also present in shared areas for his daily use. These areas are within the space allotted for the Division of Hypothalamic Research and are occupied by the laboratories of all the investigators in the Division as well as substantial common equipment and experimental space.

Common: Major equipment within the common laboratory space of the Division of Hypothalamic Research includes the following: a Beckman Avanti J-25 equipped with dedicated Beckman JE-6B elutriator rotor, tissue culture hoods, CO2 incubators, dedicated surgical area equipped with three Gilson 4 channel peristaltic pumps and a Zeiss CS1 O/R Microscope for microdissection, Sorvall RT 7 table top centrifuge, New Brunswick rotating water bath, four dual chamber copper lined Revco Incubators, a Forma dry incubator, Eppendorf 5417 table top refrigerated centrifuge, 2 non-refrigerated Eppendorf table top centrifuges, Ericomp Deltacycler II dual block thermocycler, Perkin Elmer Prism 7000 real-time PCR machine, Berthold Technologies Orion L microplate luminometer, Biometra thermocycler, Spectronic Genesys 5 spectrophotometer, Molecular Devices microplate reader, Mettler microbalance, Mettler analytical balance6 gel electrophoresis glass plate systems, 5 power supplies, gel dryer linked to a dedicated vacuum pump, Taylor-Wharton 3K cryopreservation system, 2 Revco -80 °C freezers, Revco -20 °C freezer, and a standard refrigerator. All equipment needed for histology
(including freezing microtomes, cryostats, shakers, a laser capture microscope, a Zeiss Apotome microscope, etc.) is also available. Additionally, all equipment needed to run western blotting and immunohistochemistry, as well as perform in situ hybridization histochemistry is available for my use. A Nikon Photomat TE300 inverted microscope with available epifluorescence (equipped with a Nikon 1200 DXM digital camera linked to a dedicated Gateway E-4400 computer and ACT-1 imaging software as well as MetaVue image analysis software) is present within the laboratory space. Adjacent to the laboratory are a walk-in cold room linked to a walk in -20 °C freezer, autoclave, and dishwasher. Available to the investigator on the same floor of the K Research Building are the following: a Beckman L8 Ultracentrifuge, a Reichert cryotome, a darkroom facility with Kodak X-Omat film developers, and a Phosphorimager.

**Surgical Facility:** Dr. [REDACTED] has dedicated space within the Animal Surgery Core of the Department of Surgery in the H Building at UTSW. In this facility, he has established a survival microsurgical suite which includes an anesthesia machine with adjoining induction chamber that is able to induce and maintain two animals simultaneously. The apparatus is configured to supply scavenged inhaled anesthesia to two adjacent microsurgical stages. Both of these stages are equipped with identical Zeiss automated microsurgical OPMI MDU Microscopes with foot-pedal controls for zoom and focus. This facility enables the performance of up to 8 surgical procedures per day.
June 6, 2010

Institutional Commitment to Research Career Development of [redacted], M.D., Ph.D.

Candidate for Mentored Clinical Scientist Career Development Award (K08)
National Institutes of Health

Dear Committee Members:

I am delighted to write this statement of Institutional Commitment on behalf of [redacted], M.D., Ph.D., who is being considered for a Mentored Clinical Scientist Career Development Award (K08). Dr. [redacted] was recruited last year to join our faculty by Don Rockey, M.D., Chief of the Division of Digestive and Liver Diseases, and Dr. Joel Elmquist, D.V.M., Ph.D., Director of the Division of Hypothalamic Research. With this letter, I would like to reiterate the Department’s commitment to protect Vincent’s research time; we have agreed that he will have 75% protected time for research. Dr. Rockey will ensure that his clinical activities are commensurate with this commitment and in addition, will provide essential mentorship for his career development.

Dr. [redacted] as you are aware, has an outstanding record of accomplishment. He performed his doctoral studies with Morris White, Ph.D. on insulin resistance pathways; and more recently has focused his efforts on the epidemic of obesity. We are extremely enthusiastic about him being on our faculty at UT Southwestern.

Two points merit particular emphasis. First, UT Southwestern has a long history of accomplishment in nutrition and metabolism. These contributions started with the original contributions of a number of faculty members including Roger Unger, Scott Grundy, Michael Brown and Joseph Goldstein, and other outstanding investigators in the Department. The investigators are still active in this area, and moreover have been expanded more recently with the recruitment and development of an outstanding group of talented investigators including Jay Horton, Philipp Scherer, and Joel Elmquist. Due to the scope and quality of their interests, this powerful group of investigators has been awarded one of the coveted NIH Roadmap grants focusing on obesity and the metabolic syndrome. Accordingly, Dr. [redacted] background will be both appreciated and supported. Joel Elmquist, a recipient of the Ernst Oppenheimer Award for outstanding scientific achievement and director of the Division of Hypothalamic Research, will serve as his scientific preceptor and mentor. It is also notable that the Division and Department have made a substantial commitment to Dr. [redacted] future; he has and will be provided with substantial resources in addition to his time that will contribute to his success. As detailed in this application, Dr. [redacted] will be provided with the equipment, facilities, and resources necessary for a structured research career development experience. This includes appropriate office and laboratory space, equipment, and other resources and facilities (i.e., in particular, full access to metabolic core research facilities).

Second, Dr. [redacted] will benefit greatly from being engaged in an institution that is highly supportive of his research endeavors. As a highly active research institution, UT Southwestern, with its four active Nobel Laureates and 20 members of the prestigious National Academy of Sciences, is poised to lead the way in a new era of scientific discovery in the 21st century. We believe that
research is the cornerstone upon which world-class medical education and patient care are built. UT Southwestern ranked first among the world’s medical school in the production of highly cited research papers in molecular biology and genetics between 1992 and 2002. The elite rank was compiled by Science Watch, an independent publication that reports trends and performances in basic research. Investigations into the basic areas of metabolism, as in the application of Dr. [Redacted], are essential as we seek to keep UT Southwestern at the forefront of medical progress.

On behalf of the Division and Department, I am pleased to provide my strongest and unwavering support for Dr. [Redacted]. I believe that he is an absolutely ideal candidate for the Mentored Clinical Scientist Career Development Award and I am confident that he will be an outstanding representative of the goals and ideals that it represents.

Sincerely yours,

Don C Rockey, MD
Professor of Internal Medicine
Chief, Division of Digestive and Liver Diseases
Specific Aims

Bariatric surgery is emerging as the most effective therapy for obesity and diabetes resolution [1]. Recent studies suggest that Roux-en-Y gastric bypass (RYGB), the most commonly performed bariatric procedure in the US, differentially modulates neurohormonal pathways involved in energy balance and glucose homeostasis to induce weight loss and improve diabetes [2-11]. We have established a mouse model of RYGB to directly test this hypothesis. RYGB in obese mice induces substantial weight loss and improves diabetes. The mechanism of this weight loss is primarily an increase in energy expenditure (EE).

The melanocortin-4 receptor (MC4R) is a key regulator of energy balance that is expressed in the central nervous system. MC4R null mice and humans are hyperphagic, obese, and diabetic [12-16]. MC4R null mice also have reduced EE. Interestingly, RYGB fails to increase EE, induce weight loss, or improve diabetes in MC4R null mice. Thus, modulation of MC4R-dependent pathways is required to mediate the beneficial components of RYGB. Elucidating the key anatomic structures and neuronal populations involved in this process is of great interest to understand the physiologic mechanisms and clinical efficacy of RYGB.

The autonomic nervous system is another key player in the regulation of energy balance. The parasympathetic vagus nerve is a mixed nerve whose sensory component conveys afferent signals from the gut to the brain regarding the status of nutrient intake. The motor component of the vagus nerve provides innervation to target tissues, including the gut and liver, via preganglionic neurons originating in the brainstem. Sympathetic preganglionic neurons provide motor innervation to thermogenic brown adipose tissue. MC4Rs are expressed in afferent vagal neurons as well as vagal and sympathetic efferent preganglionic neurons. Thus, it is likely that these components are involved in the effects of MC4R on body weight and diabetes. Inasmuch as MC4Rs are expressed in these and other areas of the nervous system regulating energy balance, it is likely that gut-derived signals induced by RYGB communicate with MC4R-expressing neurons. In turn, these neurons initiate compensatory responses in glucose metabolism and EE via preganglionic parasympathetic and sympathetic neurons, respectively. It is also likely that the initial signal from the RYGB-manipulated gut to the brain occurs through afferent vagal neurons. We will test these hypotheses through the following aims:

Specific Aim 1: Determine if MC4Rs in autonomic preganglionic neurons mediate the effects of RYGB on EE, body weight, and glucose metabolism.

We hypothesize that RYGB induces endogenous agonism at MC4R on autonomic preganglionic neurons to increase EE, reduce body weight, and improve glucose homeostasis. We will directly test this hypothesis by performing RYGB on obese MC4R null mice that express MC4R exclusively on autonomic preganglionic neurons. If our hypothesis is correct, RYGB will increase EE, induce weight loss, and improve glucose metabolism in these mice.

Specific Aim 2: Determine if MC4Rs in the vagus nerve mediate the effect of RYGB on glucose metabolism.

We hypothesize that RYGB induces endogenous agonism at MC4R on autonomic preganglionic neurons to increase EE, reduce body weight, and improve glucose homeostasis. We will directly test this hypothesis by performing RYGB on obese MC4R null mice that express MC4R exclusively on autonomic preganglionic neurons. If our hypothesis is correct, RYGB will increase EE, induce weight loss, and improve glucose metabolism in these mice.

Specific Aim 3: Determine if afferent vagal neurons are required for the effects of RYGB on EE, body weight, and glucose metabolism.

We hypothesize that afferent vagal neurons contribute to the beneficial effects of RYGB on EE, body weight, and diabetes. We will directly test this hypothesis by performing RYGB on mice that lack vagal afferents due to Cre-mediated, tissue-specific expression of diphtheria toxin A. If our hypothesis is correct, the effects of RYGB on EE, body weight, and glucose metabolism will not occur, or be severely attenuated, in these mice.
Research Strategy: Significance

Obesity and Bariatric Surgery. Obese patients have substantially increased morbidity and mortality due to related comorbid conditions such as diabetes, cancer, and cardiovascular disease [17]. The care of patients with obesity has become a significant health and financial burden with no end in sight given the rising prevalence of childhood obesity [18]. Even modest weight loss significantly improves most measures of obesity-related risk. Unfortunately, current behavioral and pharmacologic therapies have limited efficacy and are seldom durable [19]. In contrast, bariatric surgery results in substantial and sustained weight loss, resolution of obesity-related comorbidity, and increased survival [1, 20]. However, this therapy is only available to patients with a BMI > 40 or BMI > 35 with significant comorbidity due to its associated risk. As the risk of developing obesity-related comorbidity increases with BMI from a value as low as 21 [21], there is a large percentage of the population at increased risk who are not candidates for bariatric surgery. For example, the 54% of the U.S. population that has a BMI between 25 and 35 [22]. Thus, less invasive therapeutic options of similar efficacy are needed, and their development will be facilitated through a detailed understanding of the mechanisms underlying bariatric surgery. Unfortunately, little is known regarding these mechanisms, and this lack of understanding remains a critical barrier in the fields of both bariatric surgery and obesity. The experiments detailed in this proposal will directly investigate anatomic and molecular mechanisms underlying the effects of Roux-en-Y gastric bypass (RYGB), the most commonly performed bariatric procedure in the U.S.

Potential mechanisms of bariatric surgery. Bariatric procedures can be divided into those that are purely restrictive, such as the adjustable gastric band, and those that involve gastrointestinal (GI) bypass, such as RYGB. After the purely restrictive bariatric procedures, glycemic improvements and disease resolution occur months after the procedure and in parallel with weight loss. In contrast, GI bypass procedures, particularly RYGB, lead to improvement in diabetes within days of surgery, well before any significant weight loss. These early metabolic improvements appear to be attributable to neuronal and/or hormonal changes induced by the anatomic rearrangement of the small bowel [2, 3]. Preliminary data from animals and humans support this hypothesis and further suggest that some of the long-term effects of RYGB on body weight and diabetes involve the direct neurohumoral modulation of central mechanisms governing energy balance and glucose homeostasis rather than simple changes in calorie intake due to gastric restriction or malabsorption resulting from intestinal bypass [4-11]. Reconstruction of the GI tract during RYGB likely alters the pattern of mucosal exposure to ingested luminal contents so as to differentially modulate gut-derived signals to central areas involved in the regulation of feeding, energy expenditure (EE), and glucose homeostasis. The clinical result is long-term weight loss and improved diabetes. Identification of these gut-derived mediators and their central targets will advance our understanding of the pathophysiology of obesity as well as facilitate the development of novel, less-invasive therapies. Likely mediators involved in the effects of RYGB include the melanocortin-4 receptor (MC4R) and the autonomic nervous system (ANS).

MC4R and body weight regulation. Body weight is determined by a balance of energy intake and expenditure. The brain detects alterations in this balance and coordinates biological responses that alter food intake, EE, and glucose metabolism to compensate for changes in fuel availability and body weight [23]. A key molecular mediator involved in this process is the MC4R, whose functional relevance has been demonstrated in numerous studies [12, 24]. For instance, central administration of MC4R agonists decreases food intake, increases EE, and improves glucose metabolism [12]. In support of their role in the regulation of body weight, MC4R null mice and humans are hyperphagic, obese, and diabetic [12-16]. MC4R null mice also have reduced EE. Interestingly, we have determined that RYGB fails to increase EE, induce weight loss, or improve diabetes in MC4R null mice (see Approach). Thus, the modulation of MC4R-dependent pathways is required for the beneficial effects of RYGB. Elucidating the key anatomic structures and neuronal populations involved in this process is of great interest to understand the physiologic mechanisms and clinical efficacy of RYGB.

The ANS is a critical mediator in the regulation of energy balance. For instance, the parasympathetic vagus nerve is a mixed nerve whose sensory component conveys afferent signals from the gut to the brain regarding the status of nutrient intake. These signals are initiated directly at afferent axon terminals within the gut wall or via gut-derived mediators such as cholecystokinin (CCK), peptide Y-Y, glucagon-like peptide 1 (GLP1), dietary lipids, and portal glucose that directly modulate the activity of afferent vagal neurons. Experimental studies demonstrate that intact vagal afferents are required for the effects of these mediators on feeding behavior and glucose homeostasis [25-28]. Information conveyed by vagal afferents is integrated by neurons of the central nervous system with analogous input from peripheral energy stores. These neurons, in turn, relay efferent signals to target tissues such as the gut, liver, and brown adipose tissue via autonomic preganglionic neurons which initiate compensatory changes in feeding behavior and EE to offset perturbations of energy balance and body weight. Studies demonstrate that vagal efferents are required for the regulation of...
feeding behavior and glucose homeostasis in response to these gut-derived mediators as well as signals of energy excess within the hypothalamus [24-32]. Pathologic dysregulation of these homeostatic mechanisms in obesity results in maintenance of elevated body mass and disruption of normal glucose homeostasis [23-28]. It is therefore likely that RYGB improves aspects of this regulation to mediate its beneficial effects.

The ElMquist lab has determined that MC4Rs are expressed in the ANS. For example, they are highly expressed in components of the vagus nerve, including secondary sensory neurons of the nucleus tractus solitarius (NTS), sensory afferents, and cholinergic preganglionic (i.e., motor) neurons emanating from the dorsal motor vagus (DMV) [33]. MC4R is also expressed in the sympathetic Raphe Pallidus (RP) and cholinergic preganglionic neurons of the intermediolateral nucleus (IML) [12, 24, 29, 30, 33, 34]. Administration of MC4R agonists to the dorsal vagus complex (i.e., the NTS and DMV) potently inhibits food intake [30] and administration to the RP increases sympathetic activity to thermogenic brown adipose tissue [24] demonstrating the functional relevance of MC4Rs in these areas. Inasmuch as MC4Rs are expressed in various areas of the nervous system regulating energy balance, it is likely that gut-derived signals induced by RYGB communicate with MC4R-expressing neurons via vagal afferents. In support of this hypothesis, weight loss of vagotomized obese rats is attenuated after RYGB [10]. It is further likely that sensory and motor autonomic neurons are the site of MC4R-dependent effects of RYGB on body weight and glucose metabolism.

**Summary.** This proposal outlines experiments that will directly investigate the roles of both MC4Rs and components of the ANS in the effects of RYGB on EE, body weight, and glucose metabolism. To address our Specific Aims, we will perform RYGB on novel genetic mouse models that enable us to directly investigate the physiologic relevance of MC4Rs and components of the ANS to the effects of RYGB. Results from the proposed experiments will advance our understanding of the pathophysiology of obesity, the anatomic and molecular mechanisms of weight loss and improved diabetes after RYGB, and the role of the ANS in the long-term regulation of body weight and glucose metabolism. MC4R agonists have well-characterized effects on energy balance and body weight, and our results may demonstrate their therapeutic potential for the sustained therapy of obesity and related metabolic disease. Furthermore, a detailed understanding of the mechanisms induced by RYGB will identify additional therapeutic targets whose pharmacologic modulation may mimic the profound clinical effects of bariatric surgery.

**Research Strategy: Innovation**

**A novel model of RYGB in mice.** To investigate mechanisms of weight loss and improved diabetes after bariatric surgery, we developed a novel model of RYGB in both rats and mice in my post-doctoral laboratory at Massachusetts General Hospital [5]. I have since successfully established the model in my laboratory at UT Southwestern (UTSW). Experiments detailed in this proposal involve the mouse model exclusively. In this model the gut is reconstructed in a Roux-en-Y fashion wherein the biliopancreatic and Roux limbs comprise 25% of total intestinal length (Fig 1), comparable to the human procedure. Gastric restriction is accomplished using a titanium ligating clip placed between the forestomach and glandular stomach. To control for intra-operative stress and peri-operative calorie restriction, sham operations (SO) are performed on obese mice in parallel. During the SO procedure, enterotomy and gastrotomy are introduced and repaired at locations corresponding to these manipulations in the RYGB procedure. Peri-operative care, dissection of abdominal viscera, and anesthesia exposure are standardized in both surgical groups. At UTSW, we have already performed RYGB on over 70 obese mice and SO on over 100 with an acceptable mortality of approximately 10%. We have sacrificed post-surgical animals for experimental purposes as long as 25 weeks after surgery. At the time of sacrifice, animals are healthy without evidence of intra-abdominal infection, abscess, or intestinal obstruction. The preliminary data described herein was generated exclusively in my laboratory at UTSW. Thus, we have extensive experience with this novel model as well as the phenotypic characterization of post-surgical animals. Importantly, our model enables the direct investigation of the anatomic, physiologic, and molecular requirements for the effects of RYGB.

**Novel genetic tools to investigate the contributions of MC4Rs to the effects of RYGB.** MC4R is expressed in multiple areas of the brain and brainstem, any number of which could mediate the anti-obesity and anti-diabetic effects of MC4R agonists. The functional relevance of MC4R expression in these areas can now be tested using neuron-specific modulation of gene expression within the context of whole animals using Cre-mediated DNA recombination. The ElMquist lab has generated mice with an endogenous MC4R allele that is silenced by a loxP-modified transcriptional blocking sequence (loxTB-MC4R). The phenotype of these mice is identical to that of standard MC4R null mice - obese and diabetic, with hyperphagia and reduced EE. MC4R expression from this allele is “reactivated” by Cre-mediated DNA recombination. Thus, tissue-specific
Cre expression in loxTB-MC4R mice enables functional complementation of the MC4R null phenotype. In this model, restoration of MC4R expression in critical neurons in mice otherwise lacking MC4Rs should result in the prevention of obesity and/or diabetes.

This approach has two significant advantages. First, reactivation avoids the problem of functionally redundant pathways. As MC4R is expressed in multiple sites that could be involved in energy balance, functional redundancy of such vital biological pathways is likely. As a result, site-specific inactivation of MC4R expression would be expected to have little effect on body weight and glucose homeostasis. In support of this hypothesis, unilateral stereotactic lesions of the hypothalamus are often without effect. Secondly, reactivation restores MC4R expression only in sites with latent, genetic capacity to express MC4Rs. This is because Cre-mediated deletion of the transcriptional blocking sequence restores MC4R expression as driven by the endogenous allele. This technology has successfully been used in the Elmquist lab to demonstrate the role of MC4R expression in the paraventricular hypothalamic nucleus in the regulation of food intake and body weight. [34]. They have also used this technology to investigate the functional consequences of MC4Rs in ANS components. Germane to this proposal, reactivation of MC4R in parasympathetic and sympathetic cholinergic preganglionic neurons using Cre expression from the choline acetyl transferase (ChAT) locus in loxTB-MC4R mice (ChAT/MC4R mice) improves the reduced EE, hyperinsulinemia, and hyperglycemia characteristic of MC4R deficiency (unpublished data). These data demonstrate that MC4R in autonomic preganglionic neurons mediates effects of MC4R on EE and glucose metabolism. Whether the effect on glucose metabolism is secondary to the observed weight loss or a primary effect of MC4R reactivation is unknown.

To dissociate the effects of MC4R on parasympathetic vs. sympathetic cholinergic preganglionic neurons observed in ChAT/MC4R mice, MC4R expression can be selectively reactivated in parasympathetic brainstem neurons including sensory and motor components of the vagus nerve using Phox2B–Cre mice. Phox2B is expressed in parasympathetic preganglionic neurons, but not in sympathetic preganglionic neurons. MC4R reactivation in the vagus nerve of these mice attenuates the hyperinsulinemia of MC4R deficiency, but has no effect on the reduced EE, hyperphagia, or obesity (unpublished data). These data suggest that MC4R in vagal preganglionic efferents mediates effects of MC4R on glucose metabolism while MC4R in sympathetic preganglionic efferents mediates effects of MC4R on EE, body weight, and/or glucose metabolism. By using these parallel approaches, we can determine and dissociate the contributions of MC4R action on parasympathetic vs. sympathetic neurons in mediating metabolic improvements of RYGB found to be absent in MC4R null mice. We propose experiments using these novel models to directly investigate the physiologic relevance of MC4R in autonomic neurons for the effects of RYGB on EE, body weight, and diabetes.

**Novel genetic tool to investigate the role of sensory vagal afferents in the effects of RYGB.**

Progress in the understanding of the function of sensory vagal afferents has been limited by surgical and pharmacologic techniques of vagal manipulation that are technically challenging and lack specificity. To circumvent these issues, the Elmquist laboratory has established a well-characterized model of molecular sensory vagotomy using two mouse lines. LoxTB-DTA mice express Diptheria toxin subunit A under control of a ubiquitous promoter whose expression is dependent on Cre-mediated removal of a loxP-modified transcriptional blocker. This allows for cell-type specific neuronal ablation by expression of Diptheria toxin in a Cre-dependent manner. The well-characterized Nav1.8-Cre mouse expresses Cre recombinase under control of the Nav1.8 promoter. Nav1.8 is a sodium channel expressed only in peripheral sensory neurons, including the vast majority of vagal sensory neurons [35, 36]. LoxTB-DTA/Nav1.8-Cre (DTA/Nav) mice are viable, and the majority of vagal afferent neurons are deleted in these mice. These mice therefore enable direct investigation of the role that vagal afferents play in the effects of RYGB. The functional consequence of sensory vagotomy on the effects of RYGB will be determined using DTA/Nav mice in Specific Aim 3.

**Research Strategy: Approach**

We propose to directly investigate the role of MC4R and components of the ANS in the effects of RYGB on body weight and diabetes through the use of a novel model of RYGB in mice. To do so, we will take advantage of novel genetic tools of MC4R reactivation and molecular sensory vagotomy that allow us to directly investigate the functional consequences of MC4R expression in key autonomic components and sensory vagal afferents in the effects of RYGB, respectively. Most importantly, our results will demonstrate the potential clinical efficacy of pharmacologic modulation of vagal and MC4R action in the manner induced by RYGB. We have determined that RYGB fails to induce weight loss or improve diabetes in MC4R deficient mice. This observation enables us to directly investigate the functional relevance of MC4R expression in the effects of RYGB using novel genetic models that “reactivate” MC4R expression in a tissue-specific manner. In Specific Aim 1, we will perform RYGB on obese mice with reactivation of MC4R exclusively in autonomic
preganglionic neurons to test the hypothesis that MC4R in these neurons mediates the effects of RYGB on EE and body weight. In Specific Aim 2, we will perform RYGB on obese mice with reactivation of MC4R exclusively in sensory and motor fibers of the vagus nerve to test the hypothesis that MC4R in the vagus nerve mediates the effects of RYGB on glucose homeostasis. Comparison of the results from these two aims will allow us to discriminate the contribution of MC4R in sympathetic vs. parasympathetic neurons to the effects of RYGB. In Specific Aim 3, we will perform RYGB on a novel model of molecular sensory vagotomy to determine the contribution of afferent vagal neurons to the effects of RYGB on EE, body weight, and diabetes.

Preliminary Data

**RYGB in obese mice.** We have determined that RYGB in C57Bl/6 mice with diet-induced obesity induces substantial and sustained weight loss in comparison to SO animals (Fig 2A). The observed weight loss is due entirely to a decrease in fat mass (Fig 2B), with comparable decreases in both visceral and subcutaneous fat (Fig 2C). The mechanism of this weight loss is a significant increase in EE (Fig 2D) in the absence of changes in food intake (Fig 2E). RYGB also improves fasting blood glucose and glucose tolerance (Fig 3A-C). Fasting insulin and insulin levels after oral glucose administration are decreased, suggesting significant insulin sensitization (Fig 3B, D). Consistent with this hypothesis, insulin tolerance is improved after RYGB (Fig 3E). These parameters are similarly improved in SO animals subjected to comparable weight loss by calorie-restriction (WM-SO) suggesting that the anti-diabetic effect of this magnitude of weight loss is sufficient to similarly improve glucose metabolism (Fig 3A-E).

The typical response to substantial weight loss is a compensatory reduction of EE that serves to conserve energy and promote weight re-gain. The increased EE observed after RYGB therefore suggests that the effect on EE is direct and may be the primary cause of the observed weight loss. Consistent with this hypothesis, RYGB fails to induce a compensatory hepatic lipogenic gene response that would serve to promote weight re-gain, as observed in SO animals subjected to comparable weight loss (data not shown). These observations strongly support our overarching hypothesis that RYGB induces weight loss through direct modulation of central homeostatic mechanisms regulating energy balance and body weight. In support of this hypothesis, RYGB fails to induce weight loss or improve glucose metabolism in obese MC4R null mice (Fig 4). These data are incompatible with a simple anatomic model of RYGB-induced weight loss. Instead, they demonstrate that RYGB induces weight loss through the direct modulation of MC4R activity. RYGB must increase EE through induction of endogenous agonism at MC4R in key neurons governing energy balance.

**Specific Aim 1:** Determine if MC4Rs in autonomic preganglionic neurons mediate the effects of RYGB on EE, body weight, and glucose metabolism.

**Rationale:** Work in the Elmquist laboratory has found that both sympathetic and parasympathetic preganglionic neurons express MC4Rs. We can reactivate MC4R expression exclusively in autonomic preganglionic neurons using ChAT-Cre mice. Our preliminary results suggest that re-expression of MC4Rs in these neurons improves the decreased EE, hyperinsulinemia, and hyperglycemia characteristic of MC4R deficiency. These effects occur due to MC4R reactivation alone, in the absence of pharmacologic agonists or...
induction of endogenous agonism. Since RYGB fails to increase EE or induce weight loss in MC4R deficient mice, we hypothesize that RYGB induces endogenous agonism at MC4R on autonomic preganglionic neurons to increase EE and induce weight loss. In this model, anti-diabetic effects would occur via a direct effect of MC4R reactivation or as a secondary effect of weight loss. We will directly test this hypothesis by performing RYGB on obese MC4R null mice that express MC4R exclusively in autonomic preganglionic neurons (ChAT/MC4R mice).

**Specific Experiments:** ChAT/MC4R mice will be provided regular chow ad libitum from the time of weaning. When they reach a preoperative weight of 50g (approximately 12 weeks of age), age-matched animals will be randomly allocated to individual groups and subjected to RYGB or SO (n=15 per group). RYGB and SO will be performed on obese C57Bl/6 and MC4R null mice in parallel to control for the effects of RYGB on wild-type mice and the requirement of MC4R for the effects of RYGB on EE, body weight, and glucose metabolism, respectively. Animals will undergo phenotypic characterization as follows:

1. Weekly determination of body weight;
2. Body composition analysis including total lean and fat mass, as well as quantification of visceral and subcutaneous fat;
3. Measurement of food intake;
4. Measurement of EE, heat production, respiratory quotient, locomotor activity (x, y, and z planes), water intake, and simultaneous food intake;
5. Fed and fasted plasma glucose and insulin levels;

Post-operative body weight will be followed serially on a weekly basis. Characterization will begin after suitable post-operative recovery, which is defined as the post-operative week (POW) during which SO animals recover their pre-operative weight (typically occurs during POW 4). If RYGB induces weight loss in ChAT/MC4R mice, a second group of SO animals will be generated from age and weight-matched obese mice. The post-operative body weights of this group will be matched to those of RYGB-treated mice by daily calorie restriction. Based on prior experience, daily calorie restriction of approximately 15% is sufficient to induce target weight loss in SO mice. This experimental group will be used to control for the anti-diabetic effects of RYGB-induced weight loss vs. those due to reactivation of MC4R.

Phenotyping will proceed as follows. Total fat and lean mass will be measured by Bruker NMR, and visceral and subcutaneous fat quantified by computed tomography at the beginning of POW 5. During the remainder of POW 5, food intake will be measured over 5 consecutive days. 24-hour, light-cycle, and dark-cycle intake will be measured, and data will be analyzed as total intake and normalized per body weight in the event that RYGB induces weight loss. During POW 6, animals will undergo metabolic phenotyping (respiratory quotient, oxygen consumption, CO2 production, heat production, activity [x, y and z planes], simultaneous food intake, fluid intake) in the TSE Comprehensive Laboratory Animal Monitoring System over 5 consecutive days after 5 days of acclimation. Data will be analyzed per 24-hour period, light-cycle, and dark-cycle. Oxygen consumption and CO2 production will be normalized to measured lean body mass to account for the observed differences in body weight.

During POW 7, glucose metabolism will be evaluated. Tail blood will be collected during ad libitum feeding and after overnight fasting (from the start of the dark cycle) to measure fed and fasted glucose and insulin. All mice will undergo glucose tolerance testing (GTT) and insulin tolerance testing (ITT). GTT will be performed after an overnight fast by assessing blood glucose values from tail blood at time 0, 15, 30, 45, 60, and 120 minutes after glucose administration by oral gavage (1mg/kg body weight). Tail blood will also be collected at these time points to measure glucose-stimulated insulin levels. ITT will be performed in ad libitum-feeding mice three hours after the start of the light cycle. Blood glucose will be measured from tail vein blood 0, 15, 30, 45, and 60 minutes after administration of insulin by intraperitoneal injection (0.75 U/kg body weight). For all measures, the significance of the difference in means between groups will be evaluated by Student's t-test. Differences in curves (ITT and GTT) will be assessed by ANOVA with Bonferroni multiple comparison post-test, and as mean AUC using Student's t-test. All studies are designed to yield at least n=10 for each experimental condition and assay, which allows for a detection of a 20% change in blood glucose levels upon ITT/GTT with >80% power at the p<0.05 significance level.

**Expected results, potential problems, alternative strategies, and benchmarks for success:** We predict that RYGB will induce weight loss in obese ChAT/MC4R mice. Furthermore, EE will be increased in RYGB-treated ChAT/MC4R mice in comparison to SO ChAT/MC4R and RYGB-treated MC4R null mice. We expect that the increase in EE will be comparable to that seen in RYGB-treated wild-type mice, and that the magnitude of weight loss will also be comparable. This should be the case since the predominant mechanism
of weight loss in RYGB-treated wild-type mice is increased EE. If RYGB induces weight loss in ChAT/MC4R mice, we expect that it will also improve glucose metabolism and that glucose metabolism will be similarly improved in weight-matched SO ChAT/MC4R mice. We have found this to be the case in RYGB-treated rats and mice with diet-induced obesity (see Preliminary Data section of Research Strategy: Approach) suggesting that the insulin-sensitizing effects of this magnitude of weight loss confounds evaluation of concurrent improvements in glucose metabolism that may be independent of changes in body weight. If particular indices of glucose metabolism are improved in RYGB-treated but not weight-matched SO ChAT/MC4R animals, they will have occurred as a direct result of MC4R reactivation. Whether we find this to be the case or not, our experimental design will allow us to directly assess this. We will use an alternative experimental design to circumvent this issue in Specific Aim 2 where we will test the hypothesis that RYGB improves glucose metabolism through MC4R activation independent of changes in body weight.

The most significant potential problem will be if RYGB fails to induce weight loss or improve glucose metabolism in ChAT/MC4R mice, suggesting that expression of MC4R in autonomic preganglionic neurons is not sufficient for these effects of RYGB and that alternative sites of MC4R expression are required. This would prompt further investigation using alternative genetic mouse models of MC4R reactivation. One potential site is the RP, which expresses MC4R, receives projections from hypothalamic neurons that express the endogenous MC4R ligand, and innervates sympathetic preganglionic neurons of the IML. The RP is activated by thermogenic stimuli and induces brown fat thermogenesis [22, 31], and injection of pharmacologic MC4R agonists into the RP increases sympathetic activity to BAT [24]. Thus, the RP is a key site of MC4R expression that may be involved in the effects of RYGB on EE. This hypothesis could be directly tested through the reactivation of MC4R in the RP of loxTB-MC4R mice using either adenovirus or tissue-specific expression of Cre recombinase. A likely alternative site involved in the effects of MC4R and RYGB on glucose metabolism is the vagus nerve, and the functional relevance of vagal MC4R expression on the effects of RYGB will be directly addressed in Specific Aim 2.

Another consideration is that MC4R will also be reactivated in the pedunculopontine and lateral dorsal tegmental areas, areas which co-expression of ChAT and MC4R [32, 37]. We do not expect this to confound our results as these areas are not thought to be major contributors to energy balance or glucose metabolism, but rather are involved in the regulation of state control [38, 39]. MC4R and ChAT are no so-expressed in other cholinergic neurons. A role for these neurons in the effects of MC4R and RYGB on EE and glucose metabolism could also be eliminated using mice with reactivation of MC4R in the RP.

Benchmarks for success include the timely 1) generation of the requisite number of study animals, 2) completion of all surgeries, and 3) completion of the phenotypic characterization of post-surgical animals. To ensure similarity of genetic backgrounds, MC4R null and ChAT/MC4R mice will be generated as littermates. These mice have already been back-crossed to genetic purity and have been successfully bred for several lines of experimentation. Therefore, we do not foresee an inability to sufficiently expand these lines. In addition, our surgical facility is appropriately equipped to generate the requisite number of animals as we have already demonstrated for several other experimental groups, including genetically-manipulated mice. Furthermore, we are experienced and proficient at performing the proposed phenotypic characterization of post-surgical animals as we have done so for more than 100 animals. Our final benchmark for success will be completion of the study, including sacrificing animals and harvesting tissues for future studies.

**Specific Aim 2: Determine if MC4Rs in the vagus nerve mediate the effect of RYGB on glucose metabolism.**

**Rationale:** Metabolic improvements in mice with MC4R re-expression in autonomic preganglionic neurons could be due to MC4Rs expressed in parasympathetic or sympathetic preganglionic neurons (or both). To dissociate these possibilities, we can selectively reactivate MC4R expression exclusively in sensory and motor components of the vagus nerve using Phox2B-Cre mice (Phox/MC4R mice). MC4R reactivation in these components of the vagus nerve improves the hyperinsulinemia of MC4R null mice, without effect on food intake, EE, or body weight. Importantly, Phox2B is NOT expressed by sympathetic preganglionic neurons. By using these parallel approaches we will disassociate the contributions of MC4R action on parasympathetic vs. sympathetic preganglionic neurons in mediating the metabolic improvements of RYGB that we have found is absent in mice lacking MC4Rs. As in ChAT/MC4R mice, these effects occur due to reactivation alone, in the absence of administration of pharmacologic agonists or the induction of endogenous agonism. We hypothesize that RYGB induces endogenous agonism at MC4R on the vagus nerve to mediate its effects on glucose metabolism. We further predict that this effect will be independent of changes in EE and body weight.
We will directly test this hypothesis by performing RYGB on obese MC4R null mice that express MC4Rs in Phox2B neurons, including sensory and motor neurons of the vagus.

Specific Experiments: Phox/MC4R mice will be provided regular chow ad libitum from the time of weaning. When they reach a preoperative weight of 50g, age-matched animals will be randomly allocated to individual groups and subjected to RYGB or SO (n=15 per group). RYGB and SO will be performed in parallel on obese C57Bl/6 and MC4R null mice to control for the effects of RYGB on wild-type mice and the requirement of MC4R for the effects of RYGB on glucose metabolism, respectively. If RYGB induces weight loss in Phox/MC4R mice, a second group of SO animals will be weight-matched to RYGB-treated mice by calorie restriction to control for anti-diabetic effects of weight loss vs. those due to reactivation of MC4R.

After suitable post-operative recovery, all animals will undergo phenotypic characterization as tabulated and described in detail in Specific Aim 1. Data analysis, including statistical methods, will also be performed as described in Specific Aim 1.

Expected results, potential problems, alternative strategies, and benchmarks for success: We predict that RYGB will improve glucose metabolism but not induce weight loss in obese Phox/MC4R mice. Specifically, we expect that RYGB will improve fasting glucose, fasting insulin, glucose tolerance, and glucose-stimulated insulin levels. Of course, it is possible that MC4R reactivation will improve some, but not all, of these parameters. We will experimentally determine the MC4R-dependency of these effects by comparison to RYGB-treated MC4R null mice. If RYGB induces weight loss in Phox/MC4R mice, weight-matched SO Phox/MC4R mice will be used to distinguish anti-diabetic effects secondary to weight loss vs. those resulting from MC4R reactivation. If this is the case, our experimental design will also enable us to distinguish anti-diabetic effects of RYGB-induced weight loss that are MC4R-independent through the use of SO MC4R null mice in which comparable weight loss to that observed in RYGB-treated wild-type mice has been induced by caloric restriction. Most importantly, due to the pattern of MC4R reactivation in Phox/MC4R and ChAT/MC4R mice, we will be able to differentiate the functional effects of MC4R expression in parasympathetic vs. sympathetic cholinergic preganglionic neurons after RYGB.

The most significant potential problem is if RYGB does not improve glucose metabolism in Phox/MC4R mice. If so, our alternative strategy will be to investigate other areas of MC4R expression potentially involved in the regulation of glucose metabolism including the enteric nervous system, sympathetic efferent neurons, and alternative hypothalamic and extra-hypothalamic nuclei. The Elmquist lab currently has mouse lines with loxP-modified MC4R alleles that can be reactivated or deleted by Cre-mediated recombination as well as many of the appropriate transgenic Cre-expressing mice that would enable such an investigation, all of which are available to me as needed.

An additional consideration is that the expression of Phox2B overlaps with autonomic control sites that also express MC4R [40, 41]. This includes sympathetic postganglionic neurons in ganglia where MC4Rs are expressed during development [42]. We do not believe this to be confounding since MC4R action at these sites would require a peripheral source of an MC4R agonist. Furthermore, preliminary data in ChAT/MC4R mice suggest that effects of cholinergic MC4Rs occur through sympathetic preganglionic neurons rather than post-ganglionic. In addition, the Elmquist lab is addressing the functional effects of MC4R in adrenergic, noradrenergic, and dopaminergic neurons by crossing loxTB-MC4R mice with tyrosine hydroxylase-Cre mice that will reactivate MC4R in dopaminergic and catecholaminergic neurons as well as the sympathetic post-ganglionic neurons in question. Preliminary data suggest this restoration has little effect on body weight or glucose homeostasis.

Our benchmarks for success again include timely and successful generation of study animals, completion of the requisite number of surgeries, and completion of the phenotypic characterization of postsurgical animals. We have extensive experience in all of these areas, and do not anticipate problems in achieving these benchmarks.

Specific Aim 3: Determine if afferent vagal neurons are required for the effects of RYGB on EE, body weight, and glucose metabolism.

Rationale: Vagal afferents relay signals regarding the status of nutrient intake from the gut to the brain and brainstem that have well-characterized effects on feeding behavior, energy balance, and glucose metabolism [25-28]. Many of these effects are blunted in obesity and experimentally blocked by surgical or pharmacologic sensory vagotomy. We therefore hypothesize that afferent vagal neurons relay the initial signal from the RYGB-manipulated gut to the brain and brainstem, and that they are required for the effects of RYGB on EE, body weight, and glucose metabolism. We will directly test this hypothesis by performing RYGB on mice that lack vagal afferents due to Cre-mediated, tissue-specific expression of diphtheria toxin A (DTA/Nav
mice). We predict that some (if not all) of the effects of RYGB on EE, body weight, and glucose metabolism will be attenuated in obese DTA/Nav mice.

**Specific Experiments:** DTA/Nav mice will be provided high fat diet (HFD; 60% kcal from fat) ad libitum from weaning. When they reach a preoperative weight of 50g, age-matched animals will be randomly allocated to individual groups and subjected to RYGB or SO (n=15 per group). RYGB and SO will be performed on obese C57Bl/6 mice with diet-induced obesity in parallel to control for the effects of RYGB on EE, body weight, and glucose metabolism in wild-type mice. In the event that RYGB induces weight loss in DTA/Nav mice, an additional group of SO animals will be generated and weight-matched to RYGB-treated animals to control for anti-diabetic effects of post-operative weight loss. In the event that RYGB reduces food intake in DTA/Nav mice, a third group of SO animals will be pair-fed to RYGB-treated animals to determine the contribution of caloric restriction to the observed weight loss and to control for potential anti-diabetic effects resulting from calorie restriction itself.

After suitable post-operative recovery, animals will undergo phenotypic characterization as tabulated and described in detail in Specific Aim 1. Data analysis, including statistical methods, will also be performed as described in Specific Aim 1.

**Expected results, potential problems, alternative strategies, and benchmarks for success:** We predict that DTA/Nav mice will not lose weight nor improve glucose metabolism after RYGB, or that the magnitude of these effects will be substantially mitigated. Failure of RYGB to induce these effects in DTA/Nav mice will prove our hypothesis and demonstrate that the initial signal from the RYGB-manipulated gut to the brain and brainstem is relayed by sensory vagal afferents and that these neurons are required for the effects of RYGB on body weight and diabetes. A partial effect will suggest that vagal afferent signaling is involved, but additional RYGB-induced mechanisms exist. If RYGB does induce weight loss in DTA/Nav mice, our experimental design will allow us to determine the relative contribution of vagal afferents to the effects of RYGB by direct comparison with RYGB-treated wild-type mice.

The most significant potential problem is if RYGB induces weight loss and improves diabetes in DTA/Nav mice, and the magnitude of the response is comparable to that observed in RYGB-treated obese wild-type mice. If this is the case, we will confirm deletion of vagal afferents in DTA/Nav mice before discarding our hypothesis. We will do so by 1) performing immunohistochemistry on nodose ganglia (i.e., the ganglion in which soma of vagal afferent neurons reside) looking for Nav1.8 and Cre expression and 2) evaluating the ability of CCK to inhibit food intake, an effect requiring vagal afferents. If vagal afferents are sufficiently deleted, we will have disproven our hypothesis and will investigate alternative mechanisms of gut-brain communication required for the effects of RYGB. Candidates include various gut-derived peptide hormones, the enteric nervous system, bile salts, and dietary macromolecules themselves. The role of these mediators in the effects of RYGB can be investigated using genetically-modified mice that are deficient in the expression, function, and/or release of these mediators. A number of these genetic models are available to me at UTSW or via collaboration. If vagal afferents are not sufficiently deleted, we will test our primary hypothesis using an alternate approach of sensory vagotomy such as pharmacologic sensory deafferentation or surgical deafferentation. Despite their limitations, these are well-characterized methods of vagal deafferentation.

An additional consideration is potential confounding metabolic effects of the peripheral sensory impairment of DTA/Nav mice. Defects in cold and inflammatory pain as reported in DTA/Nav mice [36] can be expected to play a minor role in the regulation of energy balance. However, we do not believe this would be an issue as pharmacologic and surgical ablation of vagal sensory neurons also results in peripheral sensory impairment and has been utilized in numerous metabolic studies [27, 28, 37, 43, 44], including two involving gastric bypass [10, 11], without obvious confounding. Furthermore, SO DTA/Nav animals will serve as a control for the effects of peripheral sensory impairment on the measured parameters of energy balance and glucose metabolism. If, after our data analysis, we feel that non-vagal, peripheral sensory impairment is confounding we will avoid ablation of peripheral sensory neurons via injection of adenoviral vectors expressing the Cre-recombinase directly into the nodose ganglion of loxTB-DTA mice prior to surgery. The Elmquist lab has extensive experience involving the stereotactic injection of CNS and peripheral nervous system structures, including the nodose ganglion. Thus, we feel that this issue can be successfully addressed, if necessary.

Benchmarks for success again include the timely and successful generation of a sufficient number of study animals, completion of the required number of surgeries, and completion of the phenotypic characterization of post-surgical animals. The DTA/Nav mice have already been created, validated, and are currently undergoing phenotypic characterization in the Elmquist laboratory. Thus, I do not foresee the ability to generate study animals as a problem. We have extensive experience in the remainder of these areas, and do not anticipate problems achieving these benchmarks.
F. Vertebrate Animals

**Detailed description of the proposed use of animals** – We have proposed the use of both wild-type and genetically-altered mice in these studies. For wild-type study groups, obesity will be induced using a 60% high fat diet in a C57black background. Animals will be started on special diet regimen at 6 weeks of age and will be allowed to come to a pre-operative weight of 50g (approximately 18 weeks of age). To allow for appropriately powered experimental groups, 15 animals will be used per study group (SO and RYGB), resulting in a total of 30 male DIO mice to be used in the studies proposed in Specific Aims 1, 2, and 3. Genetically-altered animals, to be used in studies proposed in Specific Aims 1, 2, and 3, will be bred in house, requiring 10 mice per line as designated breeders to maintain these lines, resulting in 20 male and 20 female animals total to be used for breeding purposes. Weanlings of the appropriate genotypes will be maintained on a standard rodent diet (ChAT/MC4R, Phox/MC4R, and MC4Rnull) or placed on 60% high fat diet (DTA/Nav) and allowed to come to pre-operative weight of 50g (approximately 12 or 23 weeks of age, respectively). To allow for appropriately powered experimental groups, 15 male mice from each line will be used per experimental group, resulting in the following totals - 45 total from the ChAT/MC4R line (SO, RYGB, and WM-SO), 45 total from the Phox/MC4R line (SO, RYGB, and WM-SO), 30 total from MC4Rnull line (SO and RYGB), and 60 total from the DTA/Nav line (SO, RYGB, WM-SO, and PF-SO) – resulting in a total of 180 male, genetically-altered animals. Additional operational groups will be generated as needed or determined during data collection and analysis. Additional groups will be added as multiples of 15, consistent with our initial power calculations.

**Justification for the use of animals** –

The rodent is the closest species to humans on which the surgical interventions we propose can be performed. Similarities with humans regarding their physiology and anatomy make this species the ideal one to be used for biomedical research due to their size, relative ease of care, short life-span, and relatively short reproductive cycle. Most importantly, the ability to genetically modify this species allows for the investigation of candidate molecules in particular effects observed after RYGB. There are no in vitro, ex vivo, nor computer-based alternative to RYGB. Furthermore, the nature of this work requires the use of intact organs, and therefore, it is not feasible to do these types of studies with cultures of isolated cells, established cell lines, or computer simulation systems. Rodents make good models for the study of regulation of energy homeostasis because, like humans, they are mammals and thus have similar nervous systems, including central brain nuclei and neurotransmitters. Likewise, they have similar digestive systems, similar energy storage mechanisms, and a similar complement of nutritionally sensitive hormones to those of humans. Relevant to our studies, the rodent centers implicated in behavioral, neuroendocrine and autonomic regulation appear to have both anatomical and functional similarities to those of human beings. Many previous studies on the development of obesity in mouse models have been performed, and this makes it possible for the current work to be integrated into a growing knowledge base, making research progress faster and more efficient. Additionally, the mouse has been used reliably and repeatedly in the past as a species in which to make these types of genetic/transgenic modifications. The number of animals proposed represents the minimal number of animals required to reliably test each hypothesis based on both the inherent variability of the proposed
studies and expected results (powered to detect a 20% effect size of total body weight, blood glucose, and area under the curve).

**Information on the veterinary care of the animals involved** – All animals will be housed in the UT Southwestern Medical Center Animal Resource Center (ARC). The ARC is fully accredited by the American Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) and conforms to federal guidelines outlined in “Guide for the Care and Use of Laboratory Animals.” Professionally trained, full-time staff and a veterinarian will care for the animals. Animals are routinely screened for infectious diseases.

**Procedures for minimizing discomfort, pain, and injury** –
Animals will be closely monitored throughout the peri-operative and post-operative periods for signs of discomfort, pain, and/or distress - indicated by labored breathing, increased respiratory rate, and increased heart rate peri-operatively; and decreased activity, abnormal responsiveness to external stimuli, extreme weight loss, increased respiratory rate/labored breathing, increased hear rate, failure to eat, and failure to groom post-operatively. Animals exhibiting signs of complications such as aspiration, bleeding, and serious infection will be euthanized via inhalant anesthesia followed by exsanguination. Dehydration will be addressed by intraperitoneal injection of isotonic solution. If the surgical procedure results in significant debilitation (i.e. neurologic deficits after recovery from anesthesia), the animal will be euthanized as described below. For RYGB or Sham surgery, animals will be induced and maintained using 1-4% isoflurane on a closed, scavenged circuit. Analgesia will be provided via IM injection of buprenorphine (0.05-0.1mg/kg) peri-operatively and post-operative analgesia will be provided by IP injection of buprenorphine (0.05-0.1mg/kg), as needed, in the 48 hours following surgery.

**Methods of euthanasia** -
Animals will be euthanized via inhalant anesthesia (isoflurane dosed to effect) followed by exsanguination, to minimize suffering and assure death. This method has been approved by the UT Southwestern Medical Center Institutional Animal Care and Use Committee (IACUC) and is consistent with the recommendations of the American Veterinary Medical Association (AVMA) Guidelines on Euthanasia.
Cover Letter

1. Title:

   Melanocortin-4 Receptor-dependent regulation of energy balance after gastric bypass in mice

2. Funding Opportunity:

   Mentored Clinical Scientist Research Career Development Award (K08), PA-10-059

3. Please assign this application to the following:

   Institutes/Centers

   National Institute of Diabetes and Digestive and Kidney Diseases - NIDDK

   Scientific Review Groups

   Integrative Physiology of Obesity and Diabetes - IPOD

   Cellular Aspects of Diabetes and Obesity Study Section – CADO

The reasons for this request are:

   The NIDDK is the most appropriate Institute/Center because the work detailed in this application are integrally related to the pathophysiology of diabetes and obesity, and how it is positively impacted by surgical manipulation of the Digestive tract as it occurs during bariatric surgery. The IPOD and CADO study sections are the most appropriate because the work described herein involves investigation into the physiologic and cellular mechanisms of energy balance and glucose metabolism as they relate to the pathophysiology of diabetes and obesity and are improved after bariatric surgery.

4. List of individuals who should not review this application:

   Lee M. Kaplan

   The reason for this request is that Dr. Kaplan was a prior mentor during my post-doctoral work. He was directly involved in the intellectual process behind the development and evaluation of our rodent models of Roux-en-Y gastric bypass and remains a scientific collaborator and mentor to me, to this day.

5. Agency approval is not required for this PA.

6. List of Referees:

   Jay Horton, M.D.
   Division of Digestive and Liver Diseases
   Molecular Genetics
   Department of Medicine
UT Southwestern Medical Center
Dallas TX

Philipp Scherer, Ph.D.
Division of Endocrinology
Cell Biology
Touchstone Diabetes Center
Department of Medicine
UT Southwestern Medical Center
Dallas TX

Shawn Burgess, Ph.D.
Advanced Imaging Resource Center
Department of Pharmacology
UT Southwestern Medical Center
Dallas TX

Morris White, Ph.D.
HHMI
Division of Endocrinology
Department of Medicine
Children’s Hospital
Harvard Medical School
Boston, MA
**Project Title**: Melanocortin-4 Receptor-dependent regulation of energy balance after gastric bypa

**SRG Action**: Impact/Priority Score: 10

**Human Subjects**: 10-No human subjects involved

**Animal Subjects**: 30-Vertebrate animals involved - no SRG concerns noted

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**ADMINISTRATIVE BUDGET NOTE**: The budget shown is the requested budget and has not been adjusted to reflect any recommendations made by reviewers. If an award is planned, the costs will be calculated by Institute grants management staff based on the recommendations outlined below in the COMMITTEE BUDGET RECOMMENDATIONS section.

**ADMINISTRATIVE NOTE**
RESUME AND SUMMARY OF DISCUSSION:

This application was submitted in response to program announcement PA-10-059 entitled "Mentored Clinical Scientist Research Career Development Award (Parent K08)". The goal of this application is to investigate the hypothesis that Roux-en-Y gastric bypass may mediate its effects on body weight, metabolic rate, and diabetes through MC4R in certain autonomic components. Strengths of the application include: the candidate; the letters of support; the career development plan; the environment and institutional support; and the research plan which is supported by preliminary data. Negligible weaknesses were identified which did not decrease the merit of the application. Weaknesses of the application include: applicability of the animal model to the human response to RYGB; and the research plan is ambitious. Overall this application is rated exceptional.

DESCRIPTION (provided by applicant): The combination of my prior research and clinical training uniquely situate me to investigate how Roux-en-Y gastric bypass (RYGB) causes weight loss and improves diabetes. Specifically, the surgical expertise that I obtained during my post-doctoral fellowship involving bariatric surgery in rodents complements the intensive basic science training in molecular mechanisms of diabetes that I received during my doctoral training. This surgical and intellectual expertise poises me to perform disease-oriented research investigating the physiologic and molecular effects of bariatric surgery on obesity and related metabolic disease, as proposed in this application. My immediate career goals include the further development of my research laboratory in terms of data, reagents, publications, and additional funding. My recent faculty appointment and substantial commitment of the Department of Medicine to my research efforts have greatly facilitated this process. My long-term career goals include continued publication of my data in peer-reviewed scientific journals, to further establish myself as an independent scientist, and to become recognized as a leader in the fields of obesity, diabetes, and gastrointestinal regulation of metabolism. Realizing these achievements will ensure my continued career advancement, particularly the transition from assistant to associate professor, and beyond. The research environment at UT Southwestern, particularly as coordinated within the Taskforce for Obesity Research at UT Southwestern (TORS), provides me with the ideal opportunity to attain these goals. Specifically, my daily exposure to the TORS faculty, and the UT Southwestern faculty at large, will provide the experience and mentoring I need to ensure my continued development as a clinical scientist. My career development will involve both informal interactions with my mentor, TORS faculty, and members of their laboratories as well as more structured opportunities that include weekly meetings with my mentor, regular participation and presentation at divisional and departmental WIP ("works-in-progress") meetings, departmental seminars, and international/national scientific symposia. I will also have the opportunity to lecture trainees regarding my clinical and research expertise, and attend classes involving biostatistics, research design, scientific grant writing, research management, and the Responsible Conduct of Research. In addition, I will have access to the full complement of technical capabilities represented in the TORS laboratories as well as the TORS Mouse Metabolic Phenotyping Core Facility, the extensive use of which is detailed in this proposal. Thus, UT Southwestern is the ideal environment to help me in achieving my goals. My prior training ensures that I will maximize these opportunities, and any others that may arise. I have focused my research efforts on the problem of obesity because of the magnitude of its public health threat. While even modest weight loss improves patient outcomes, most current behavioral and medical therapies are ineffective. In contrast, bariatric surgery induces substantial weight loss and improvement of related comorbidities. Unfortunately, due to its inherent risk, it is only available to the severely obese. Safer options that may be more broadly applied are needed. We have developed a technique to perform RYGB, the most commonly performed bariatric procedure in the US, on obese mice to understand how it so effectively induces weight loss and improves diabetes. This weight loss occurs largely due to an increase in metabolic rate. Additionally, we have determined that RYGB fails to induce weight loss or improve diabetes in genetically-manipulated mice that lack the melanocortin-4 receptor (MC4R), an important regulator of body weight. This finding enables us to
directly investigate the role of MC4R and putative target tissues in the beneficial effects of RYGB. It will further facilitate the development of safer, less-invasive therapies that may mimic the clinical efficacy of bariatric surgery. The MC4R is expressed in structures of the autonomic nervous system (ANS) that are involved in the regulation of body weight and glucose metabolism (which is dysregulated in diabetes), including the vagus nerve and motor neurons of the sympathetic nervous system (SNS). The vagus nerve relays information regarding nutrient intake to the brain and is involved in the responsive modulation of feeding behavior and glucose metabolism. Recent genetic experiments in mice have demonstrated that MC4R in the vagus nerve and motor neurons of the SNS are involved in the regulation of glucose metabolism and metabolic rate, respectively. These observations suggest that RYGB may mediate its effects on body weight, metabolic rate, and diabetes through MC4R in these autonomic components. In this application, we propose experiments to directly investigate this hypothesis. To address our aims, we will perform RYGB on mice that have been genetically-engineered to express MC4R exclusively in these components of the ANS. Complete phenotypic characterization of post-surgical animals will identify target tissues and MC4R-dependent physiologic mechanisms through which RYGB induces weight loss, increases metabolic rate, and improves diabetes. Medications that modulate MC4R function are already in clinical trials. These findings are critical in that they will identify target tissues to which pharmacologic therapy that modulates MC4R function may be directed, thereby facilitating the development of pharmacologic agents with increased specificity of action and clinical efficacy while limiting the risk of side effects.

PUBLIC HEALTH RELEVANCE: Obesity has become a significant health and economic burden worldwide. Unfortunately, the most efficacious and durable therapy for weight loss, bariatric surgery, is only available to the severely obese due to its inherent risk. Given the large number of people at significant obesity-related risk who do not meet the weight requirements for surgery, safer therapeutic options are critically needed. We have developed a technique to perform gastric bypass in obese mice to investigate the mechanisms of weight loss after bariatric surgery. An understanding of such mechanisms will facilitate the development of less-invasive, safer therapies for obesity and related disease that may be more broadly applied.

CRITIQUE 1:

Candidate: 1
Career Development Plan/Career Goals /Plan to Provide Mentoring: 2
Research Plan: 3
Mentor(s), Co-Mentor(s), Consultant(s), Collaborator(s): 1
Environment  Commitment to the Candidate: 1

Overall Impact:
This application contains many strengths and seems likely to help Dr. [redacted] develop as an independent investigator. The candidate is well trained in both basic research and clinical medicine to undertake research on the effects of bariatric surgery on weight loss and reversal of diabetes. He was trained to understand molecular mechanisms of diabetes as he investigated serine phosphorylation of IRS-1 in the laboratory of Dr. Morris White. He learned the surgical techniques to do RYGB in animal models in the laboratory of Dr. Lee Kaplan. In his laboratory at UTSW Dr. [redacted] has established all of the techniques to do the surgery and investigate weight loss metabolism in a mouse model. The advantage of using a mouse model is that he can use the very powerful array of genetically manipulated mice that are available at UTSW to study mechanisms. The only reservation is applicability of the animal model to the human response to RYGB. Humans have a rapid and durable reversal of diabetes that does not seem to be related to weight loss. The reversal of diabetes in mice seems to be related to weight loss. Dr. [redacted] mentor, Dr. Joel Elmquist is a world renowned scientist in CNS regulation of food intake, energy expenditure and obesity. Dr. Elmquist is very experienced as a mentor and is fully committed to mentoring and supporting Dr. [redacted]. The specific expertise that Dr. [redacted] plans to obtain during the award period involves (1) generation and study of genetic mouse models of
obesity and diabetes (2) metabolic profiling of mice, and (3) in vivo metabolism in post-surgical mice. His training plan also involves course work and participation in national meetings. Dr. has a tenure track position, a laboratory and a strong commitment from the institution.

1. Candidate:

Strengths

- Dr. received excellent training for his Ph.D. degree in the laboratory of Dr. Morris White. They published a very important paper on the role of serine phosphorylation of IRS-1 in insulin resistance. His training in basic science is a strong component in preparing him for a career in research. He understands and is able to study the molecular mechanisms of diabetes.

- Ph.D. and M.D. training for Dr. were performed at Harvard Medical School. He interned and was resident at Brigham and Woman’s Hospital with a fellowship in gastroenterology at Massachusetts General.

- Dr. did postdoctoral training with Dr. Lee Kaplan where he developed an animal model of gastric bypass. They used this model to study weight loss and the reversal of diabetes in response to the surgery. Dr. also investigated an endoluminal sleeve to see if the diabetes reversal could be accomplished by keeping food from contact with the foregut.

- Dr. has a good publication record. Up to 2002 (representing his Ph.D. training) he had 7 papers published in good journals and 3 were first authored. Since 2008 (after his fellowship) he has 3 papers, with 1 first authorship. He was awarded “outstanding Research Manuscript of the Year” by the Obesity Society and “Poster of Distinction” from the American Gastroenterologic Association for his work on the endoluminal sleeve.

- In 2009 Dr. took a tenure track position as assistant professor in the Department on Internal Medicine, University of Texas Southwestern Medical Center. He has established his laboratory and has set up a surgery suite where he has performed approximately 100 RYGB.

- The letters of recommendation from Dr. Philipp Scherer, Dr. Jay Horton, Dr. Morris White and Dr. Shawn Burgess were uniformly outstanding.

- With the experience of the candidate and the training planned for this award it seems likely that Dr. will become an independent investigator.

Weaknesses

- None

2. Career Development Plan/Career Goals & Objectives/Plan to Provide Mentoring:

Strengths

- The specific expertise that Dr. plans to obtain during the award period involves (1) generation and study of genetic mouse models of obesity and diabetes (2) metabolic profiling of mice, and (3) in vivo metabolism in post-surgical mice.

- He will participate in weekly 1-2 hour data presentation with members of the Division of Hypothalamic Research, once a month meeting of the TORS group, weekly meeting with Dr. Elmquist and “in-house” peer review of all manuscripts and grant applications. He will also have access to the Division Chief, Dr. Rockey, for mentorship involving development of administrative and leadership skill.

- In the first year of the award Dr. will take courses in Biostatistics, Research Design and Grant Writing. The second year will involve Research Management.

- Dr. will participate in national meetings, such as Keystone Conferences on obesity and diabetes as well as annual scientific meetings including, but not limited to, those of the
American Gastroenterologic Association, American Diabetes Association, the Endocrine Society, and the Obesity Society.

- Dr. [name redacted] will continue to do clinical work to integrate patients into his research. His current clinical work involves 4 weeks per year managing the inpatient consultative service and in the delivery of out-patient consultative and endoscopic services one day per week.
- The candidate presents a very reasonable time line of training and activities toward becoming an independent investigator.
- A course on training in Responsible Conduct of Research (which is presented biannually) is part of the training plan.
- A committee will be established to review and monitor the progress of the applicant and will oversee his career development. The committee will consist of individuals with a broad range of experience including molecular genetics, neuroscience, cell biology, and pharmacology. The committee will meet semi-annually over the term of the grant.

Weaknesses
- None

3. Research Plan:

Strengths
- This research project is a logical extension of Dr. [name redacted] previous work.
- The proposal is hypothesis driven and will likely advance our understanding about the central mechanisms underlying the anti-obesity effects of RYGB.
- One of the strengths of the research plan is the availability of mouse lines with loxP-modified alleles of the melanocortin-4 receptor (MC4R) that be reactivated (in a MC4R null background) or deleted (in a wild-type background) in a Cre-dependent, tissue-specific manner. The MC4R is a critical mediator of body weight regulation and glucose metabolism, and MC4R null mice fail to lose weight or improve diabetes after RYGB. Thus, the anatomic sites of MC4R expression and their functional consequence after RYGB can be directly investigated by RYGB-treatment of mice with tissue-specific reactivation of MC4R reactivation (in the MC4R null background). Similarly, the functional requirement of MC4R expression can be directly determined through tissue specific deletion of MC4R.

Weaknesses
- The only reservation is applicability of the animal model to the human response to RYGB. Humans have a rapid and durable reversal of diabetes that does not seem to be related to weight loss. The reversal of diabetes in mice seems to be related to weight loss.

4. Mentor(s), Co-Mentor(s), Consultant(s), Collaborator(s):

Strengths
- Dr. Joel K. Elmquist, is a Professor, Internal Medicine, Pharmacology, & Psychiatry University of Texas, Southwestern Medical Center, as well as director of the Division of Hypothalamic Research.
- Dr. Elmquist is a world renowned scientist in the area of central regulation of food intake, energy expenditure and obesity. He has published more than 125 manuscripts.
- Dr. Elmquist is a very experienced mentor and has mentored 20 individuals in the past 11 years.
- Funding for Dr. Elmquist’s research is outstanding. The titles of grants are: (1) Leptin action and central melanocortin systems, (2) Interactions of leptin and central serotonin systems, (3) CNS pathways regulating ghrelin effects on body weight, (4) Central mechanisms regulating energy homeostasis and (5) High fat diet induces alterations in gene expression in nonhuman primates.
Dr. Elmquist research group has developed and characterized numerous genetic models of obesity and diabetes and these model animals will be available to the candidate. In particular the Elmquist laboratory has mouse lines with loxP-modified alleles of the melanocortin-4 receptor (MC4R) that can be reactivated (in a MC4R null background) or deleted (in a wild-type background) in a Cre-dependent, tissue-specific manner.

Weaknesses

- None.

5. Environment and Institutional Commitment to the Candidate:

Strengths

- Dr. [redacted] has been allotted approximately 500 sq feet of laboratory space that has been equipped with the equipment that he will need through start up funds from the University. In addition he has dedicated space within the Animal Surgery Core of the Department of Surgery for his RYGB procedures.
- Dr. Don C. Rockey, Chief, Division of Digestive and Liver Diseases, wrote a strong letter of commitment for the Department of Internal Medicine. Dr. [redacted] will be allowed 75% of his time for research.
- The University of Texas, Southwestern Medical Center has many strengths that will enhance Dr. [redacted] chances to be a successful scientist. The research environment is coordinated within the Taskforce for Obesity Research (TORS). TORS is the only obesity research center supported by the NIH Roadmap Initiative for Interdisciplinary Research. Faculty associated with TORS, and available to Dr. [redacted], includes, among others, Michael Brown, Joseph Goldstein, David Mangelsdorf, Philipp Scherer, Jay Horton, Masashi Yanagisawa, Craig Malloy, Joyce Repa, Elizabeth Parks, and Helen Hobbs.
- Another resource to support the candidate is the Division of Hypothalamic Research, which is headed by Dr. Elmquist, his mentor.
- Dr. [redacted] also has the benefit from interactions with researches in the Touchstone Diabetes Center, whose members include Deborah Clegg, Philipp Scherer and Roger Unger.
- The Mouse Metabolic Phenotyping Core Facility will be very beneficial for Dr. [redacted] research.

Weaknesses

- None.

Protections for Human Subjects:

Not Applicable (No Human Subjects)

Data and Safety Monitoring Plan (Applicable for Clinical Trials Only):

Not Applicable (No Clinical Trials)

Vertebrate Animals:

Acceptable

- The animal facilities and care are outstanding. Description, justification, veterinary care, procedures for minimizing discomfort, pain, and injury and euthanasia were points that were adequately covered by the applicant.

Biohazards:

Not Applicable (No Biohazards)

Training in the Responsible Conduct of Research:

Acceptable
Comments on Frequency (Required):

- The applicant is currently taking the course Training in the Responsible Conduct of Research and he met the annual requirements for classes in this subject throughout his post-doctoral fellowship. His most recent completed class was 2009.

Budget and Period of Support:
Recommend as Requested

CRITIQUE 2:

Candidate: 1
Career Development Plan/Career Goals /Plan to Provide Mentoring: 1
Research Plan: 2
Mentor(s), Co-Mentor(s), Consultant(s), Collaborator(s): 1
Environment Commitment to the Candidate: 1

Overall Impact:
This is an outstanding proposal aimed at discerning the role of the MC4R expressed in the autonomic nervous system in mediating the beneficial effects of bariatric surgery on weight loss and glucose homeostasis. The Candidate has a tenure-track position in the Dept of gastroenterology at UTSWD and has chosen as mentor Dr. Elmqquist who has an outstanding record in the study of obesity and metabolism using genetic mouse models. This plan very clearly will launch the independent career of the Candidate. The research plan is very ambitious but extremely well organized and mindful of possible pitfalls.

1. Candidate:

Strengths

- Dr [Redacted] has an excellent research record, having completed a very successful MD-PhD program. His graduate mentor was Dr Morris White with whom he has published many articles on peer-reviewed journals on the role of IRS phosphorylation as mechanism of insulin resistance. As postdoctoral fellow, he has worked with Dr Lee Kaplan at Mass General and has developed a very original gut device that induces weight loss in obese animals.

- He has been recruited by the Gastroenterology department of UT Southwestern in Dallas since 2009. Since then he has developed an original line of research to study the mechanisms by which gastric bypass induces weight loss.

- His previous research achievements demonstrate his high potential to quickly become an independent and productive physician scientist.

- Letter of recommendation from former and current mentors are extremely laudatory.

Weaknesses

- None noted.

2. Career Development Plan/Career Goals & Objectives (K24 Plan to Provide Mentoring):

Strengths

- Dr [Redacted] has laid out a very thoughtful and structured plan that includes course work, interaction with mentors that will substantially contribute to his scientific growth.

- Considering his prior training, the current career development plan focuses on giving the Candidate fresh new tools in the field of obesity and mouse genetics that would greatly enhance his independent career.

Weaknesses
None noted.

3. Research Plan:

Strengths

- Based on Dr. preliminary observation that obese MC4R null mice do not lose weight after gastric bypass, specific aims are designed to ask the following questions: (SA1) whether the reactivation of MC4R in preganglionic neurons restores the RYGB efficacy on weight loss and/or glucose tolerance; (SA1) whether the reactivation of MC4R in vagal neurons restores the RYGB efficacy on weight loss and/or glucose tolerance; (SA3) whether afferent vagal fibers are required for the beneficial effects of RYGB on weight loss or glucose homeostasis. These aims are well constructed and feasible.

- Although this proposal requires the use of many mouse line with genetic manipulations, the laboratory of Dr. Elmquist is well equipped to rapidly generate these models. Also The Candidate has shown that he has successfully set up in his new lab the bariatric surgery techniques.

- Possible pitfalls are adequately addressed

Weaknesses

- The research plan is rather ambitious since it involves the use of many mouse lines which should then undergo a delicate surgical procedure to be evaluated for many outcomes (energy balance and glucose metabolism)

4. Mentor(s), Co-Mentor(s), Consultant(s), Collaborator(s):

Strengths

- Dr. Elmquist is a world expert in the genetic manipulation of mice for the study of neurendocrine control of energy balance. He is clearly an outstanding mentor for this Candidate

- Drs. Hortons and Scherer are also outstanding additional mentors and collaborators for training Dr. in the metabolic phenotyping of these mice.

Weaknesses

- None noted.

5. Environment and Institutional Commitment to the Candidate:

Strengths

- Institutional commitment is outstanding. Dr. was recruited as a tenure-track member of the gastroenterology division and with his own start-up fund.

Weaknesses

- None noted.

Protections for Human Subjects:
Not Applicable (No Human Subjects)

Data and Safety Monitoring Plan (Applicable for Clinical Trials Only):

Vertebrate Animals:
Acceptable

Biohazards:
Not Applicable (No Biohazards)

Training in the Responsible Conduct of Research:
Comments on Format (Required):
  • Acceptable
Comments on Subject Matter (Required):
  • Acceptable
Comments on Faculty Participation (Required; not applicable for mid- and senior-career awards):
  • Acceptable
Comments on Duration (Required):
  • Acceptable
Comments on Frequency (Required):
  • Acceptable

Select Agents:
Not Applicable (No Select Agents)

Resource Sharing Plans:
Acceptable

Budget and Period of Support:
  • adequate

CRITIQUE 3:

Candidate: 1
Career Development Plan/Career Goals /Plan to Provide Mentoring: 2
Research Plan: 2
Mentor(s), Co-Mentor(s), Consultant(s), Collaborator(s): 2
Environment Commitment to the Candidate: 1

Overall Impact:
This is an extremely well conceived grant application from an outstanding young investigator. Dr. [redacted] is exceptionally well trained clinically and in both molecular biology and translational research. He was very productive as a graduate student and as a post-doctoral fellow. He has chosen an excellent mentor who can help round out his expertise by working on projects involving genetic mouse models and neurophysiology and neuroendocrinology. The research plan is carefully thought out, and he has anticipated problems and has considered alternate approaches, as well as benchmarks to assess his progress. The research environment for studying the molecular aspects of obesity is unmatched, and the mentoring program is carefully considered.

1. Candidate:

Strengths
  • [redacted] received MD, PhD at Harvard, working Dr Morris White’s lab on molecular studies of IRS-1 and publishing several high quality papers, including two first authored papers in JBC
  • He did his residency in internal medicine at Brigham and Women’s, then did his GI fellowship at MGH, learning gastric bypass surgery in Dr Lee Kaplan’s lab.
  • As postdoc, helped develop intraluminal device that induced weight loss, and his MS won an award from the Obesity Society
• He is an Assistant Professor at UT Southwestern and he is clearly committed to an academic research career
• Four very enthusiastic letters of recommendation attest to his great potential. They also mention great scientific leadership skills.

Weaknesses
• None noted.

2. Career Development Plan/Career Goals & Objectives (K24 Plan to Provide Mentoring):

Strengths
• He has received outstanding training in molecular biology and in animal models of clinical investigation and animal surgery. The K08 will allow him to obtain training in genetic mouse models, metabolic phenotyping and neurophysiology. This will help round out his expertise.
• Career development plan includes all necessary training in responsible conduct of research
• Numerous structured meetings, including frequent meetings with his mentor and other scientists in TORS
• Committee to review and monitor his progress that will meet semi-annually with PI to assess progress. Committee not yet established.

Weaknesses
• Little in the way of formal coursework is described

3. Research Plan:

Strengths
• Important clinically relevant model of obesity. Could answer questions regarding how bariatric surgery works.
• Appropriate set of studies to expand PI’s expertise into neurophysiology and genetics.
• PI developed model of Roux-en-Y gastric bypass in rodents with rigorous sham controls. Method is working at UT-SW
• Mentor has MC4R knock-out mice which allow for tissue-specific reactivation, allowing PI to tease out which neurons (sympathetic vs. parasympathetic) are mediating which effects. PI also has knock-out mice which can ablate vagal afferents using Diphtheria toxin.
• Excellent preliminary results showing that the model of RYGB in diet-induced obese mice causes them to lose weight and increase energy exposure
• Most importantly, in MCR4 knock-outs, RYGB does not lead to weight loss
• Multiple controls, including pair-feeding controls, in different genetic backgrounds will be used.
• Extensive cutting-edge phenotyping of treated mice will be performed, including body composition, oxygen consumption and 3-dimensional motion monitoring, GTT, and ITT.
• Very sophisticated discussion of interpretation of experiments, alternate plans and benchmarks for success.

Weaknesses
• None noted.

4. Mentor(s), Co-Mentor(s), Consultant(s), Collaborator(s):

Strengths
• Joel Elmquist DVM, PhD will be the primary mentor. He has trained more than 30 post-doctoral fellows. He is an eminent and productive neurophysiologist with substantial NIH funding. He directs the Center for Hypothalamic Research.
• Very strong supporting letter with plans for close mentorship

Weaknesses
• None noted.

5. Environment and Institutional Commitment to the Candidate:

Strengths
• Absolutely superb environment to study obesity and metabolism. UT Southwestern has an extraordinary cadre of scientists interested in obesity, diabetes and metabolism
• They have a Roadmap Grant to support a “Taskforce for Obesity Research at UT Southwestern” that includes a Nutrition Center, a diabetes center and the Center for Hypothalamic Research which houses Dr. [REDACTED].
• Members of this TORS include Drs. Brown, Goldstein, Mangelsdorf and many others
• Clear commitment to career development and 75% protected time
• [REDACTED] also provides unique skills to his unit (surgical and metabolic)

Weaknesses
• None noted.

Protections for Human Subjects:
Not Applicable (No Human Subjects)

Data and Safety Monitoring Plan (Applicable for Clinical Trials Only):

Vertebrate Animals:
Acceptable

Biohazards:
Not Applicable (No Biohazards)

Training in the Responsible Conduct of Research:
Acceptable

Comments on Format (Required):
• Discussion classes and semiannual seminars are held. Reading material is provided.

Comments on Subject Matter (Required):
• Comprehensive set of issues are discussed

Comments on Faculty Participation (Required; not applicable for mid- and senior-career awards):
• Faculty lead seminars and teach by example

Comments on Duration (Required):
• This is not discussed

Comments on Frequency (Required):
• Seminars semi-annually. Not clear how often discussion groups are held

Select Agents:
Not Applicable (No Select Agents)
Resource Sharing Plans:
Acceptable

- He will not be generating new animal models, but will be using existing ones provided by mentor

Budget and Period of Support:
Recommend as Requested

THE FOLLOWING RESUME SECTIONS WERE PREPARED BY THE SCIENTIFIC REVIEW OFFICER TO SUMMARIZE THE OUTCOME OF DISCUSSIONS OF THE REVIEW COMMITTEE ON THE FOLLOWING ISSUES:

VERTEBRATE ANIMAL (Resume): ACCEPTABLE

SCIENTIFIC REVIEW OFFICER'S NOTES:

The plans outlined in the application to obtain training in the responsible conduct of research are adequate to satisfy this requirement.

COMMITTEE BUDGET RECOMMENDATIONS: The budget was recommended as requested.

Recommended direct cost levels are estimated and are subject to further adjustment based on the Institute’s standard budget calculation practices.


The impact/priority score is calculated after discussion of an application by averaging the overall scores (1-9) given by all voting reviewers on the committee and multiplying by 10. The criterion scores are submitted prior to the meeting by the individual reviewers assigned to an application, and are not discussed specifically at the review meeting or calculated into the overall impact score. For details on the review process, see http://grants.nih.gov/grants/peer_review_process.htm#scoring.
MEETING ROSTER

Diabetes, Endocrinology and Metabolic Diseases B Subcommittee
National Institute of Diabetes and Digestive and Kidney Diseases Initial Review Group
NATIONAL INSTITUTE OF DIABETES AND DIGESTIVE AND KIDNEY DISEASES DDK-B

October 20, 2010 - October 21, 2010

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