

AWARD NUMBER: W81XWH-17-1-0133

TITLE: Metabolic Characterization of a Putative Novel Antidiabetic Drug, GTI-421

PRINCIPAL INVESTIGATOR: Brian DeBosch

CONTRACTING ORGANIZATION: Washington University, St. Louis, MO 63110

REPORT DATE: May 2018

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

REPORT DOCUMENTATION PAGE				Form Approved OMB No. 0704-0188	
Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.					
1. REPORT DATE May 2018		2. REPORT TYPE Annual		3. DATES COVERED 15 April 2017 – 14 April 2018	
4. TITLE AND SUBTITLE Metabolic Characterization of a Putative Novel Antidiabetic Drug, GTI-421				5a. CONTRACT NUMBE	
6. AUTHOR(S) Dr. Brian DeBosch, MD, PhD E-Mail: bdebosch@wustl.edu				5b. GRANT NUMBER W81XWH-17-1-0133	
				5c. PROGRAM ELEMENT NUMBER	
				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Washington University One Brookings Drive Campus Box 1054 St. Louis, Mo. 63130-4862				8. PERFORMING ORGANIZATION REPORT NUMBER	
				10. SPONSOR/MONITOR'S ACRONYM(S)	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Material Command Fort Detrick, Maryland 21702-5012				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT The purpose of this project is to define and characterize a novel putative metabolic therapy. Specifically we seek to characterize a compound, GTI-421, which inhibits hepatic glucose transport by establishing the therapeutic efficacy of GTI-421, and define its mechanism of action. The premise of this project is that hepatic glucose transporter (GLUT) blockade mimics the beneficial effects of generalized fasting. Finding a GLUT inhibitor is therefore expected to enhance hepatic and extrahepatic metabolism through canonical fasting response pathways. We have made significant strides toward our stated goals during this reporting period. Our major objectives and advances as they relate to these advances are described herein.					
15. SUBJECT TERMS <i>Diabetes, metabolic syndrome, non-alcoholic fatty liver disease, obesity, metabolism, glucose transport, hepatic steatosis, fibroblast growth factor 21.</i>					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT U	b. ABSTRACT U	c. THIS PAGE U	UU	63	USAMRMC
					19b. TELEPHONE NUMBER (include area code)
					Standard Form 298 (Rev. 8-98) Prescribed by ANSI Std. Z39.18

Table of Contents

	<u>Page</u>
1. Introduction.....	1
2. Keywords.....	2
3. Accomplishments.....	3
4. Impact.....	6
5. Changes/Problems.....	7
6. Products.....	8
7. Participants & Other Collaborating Organizations.....	9
8. Special Reporting Requirements.....	11
9. Appendices.....	12

1. INTRODUCTION

The purpose of this project is to define and characterize a novel putative metabolic therapy. Specifically we seek to characterize a compound, GTI-421, which inhibits hepatic glucose transport by establishing the therapeutic efficacy of GTI-421, and define its mechanism of action. The premise of this project is that hepatic glucose transporter (GLUT) blockade mimics the beneficial effects of generalized fasting. Finding a GLUT inhibitor is therefore expected to enhance hepatic and extrahepatic metabolism through canonical fasting response pathways. We have made significant strides toward our stated goals during this reporting period. These are outlined below.

2. KEYWORDS

Diabetes, metabolic syndrome, non-alcoholic fatty liver disease, obesity, metabolism, glucose transport, hepatic steatosis, fibroblast growth factor 21.

3. ACCOMPLISHMENTS

- **What were the major goals of the project?**

1. Quantify the therapeutic actions of GTI-421 in preclinical diabetic models.
2. Define the mechanisms by which GTI-421 induces hepatic FGF21 and PGC1a

- **What was accomplished under these goals?**

1. **Quantify the therapeutic actions of GTI-421 in preclinical diabetic models.**

Major activity 1

Define the role of GTI-421 on energy homeostasis in mice

Specific objective 1

Define the role of GTI-421 on thermogenesis in wild-type (WT) mice. To accomplish this, we treated mice with GTI-421 or vehicle (5 days) in drinking water, administered ad libitum. This addressed the question: can GTI-421 enhance energy balance in a WT mammalian model? We quantified thermogenesis, oxygen consumption and carbon dioxide exchange by indirect calorimetry in a metabolic cage.

Significant Results 1

We demonstrated that GTI-421 enhances thermogenesis and O₂-CO₂ exchange in WT mice. This is important because type 2 diabetes mellitus (T2DM) is a disease of caloric excess. GTI-421 treatment enhances caloric expenditure, which is one means to improve energy balance in states of caloric excess like T2DM. The data are depicted in Fig. 1

Major activity 2:

Define the role of GTI-421 on thermogenesis in an ob/ob diabetic mouse models.

Specific Objective 2

Define the role of GTI-421 on thermogenesis in an ob/ob diabetic mouse models. This addresses the question: can GTI-421 enhance energy balance in a genetic diabetic mouse model? This addresses a secondary question as well: Is the enhanced energy balance conferred by GTI-421 dependent on leptin signaling? To accomplish this, we treated ob/ob diabetic mice with GTI-421 or vehicle (5 days) in drinking water, administered ad libitum. We quantified thermogenesis, oxygen consumption and carbon dioxide exchange by indirect calorimetry in a metabolic cage.

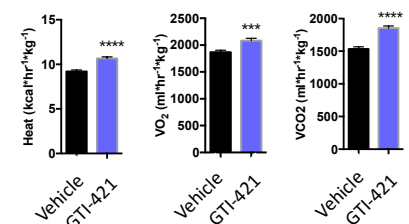
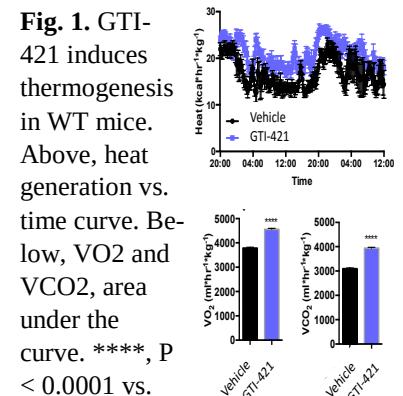
Significant Results 2

We demonstrated that GTI-421 enhances thermogenesis and O₂-CO₂ exchange in ob/ob diabetic mice. This is important because we validate that caloric excess. GTI-421 treatment enhances caloric expenditure, which is one means to improve energy balance in states of caloric excess like T2DM. The data are depicted in Fig. 2

2. **Define the mechanisms by which GTI-421 induces hepatic FGF21 and PGC1a**

Major activity 1

Define the mechanism of GTI-421 induction of hepatic FGF21



Specific objective 1

Define the mechanism of GTI-421 induction of hepatocyte FGF21 in isolated hepatocytes. We tested the hypothesis that GTI-421, a GLUT inhibitor, reduces hepatocyte energy production as a result of reduced substrate influx. We tested this by performing Seahorse respirometry in culture hepatocytes. We then directly tested whether FGF21 induction by GTI-421 is a result of reduced substrate influx by measuring FGF21 gene induction in the presence or absence of 5mM pyruvate. The pyruvate served as a substitute energy source downstream of the glucose transporter.

Significant Results 1

We demonstrated GTI-421, reduces maximal respiration and glycolytic rate, as quantified by extracellular acidification rate (ECAR) (Fig. 3). We then showed that FGF21 induction by GTI-421 depends on an energy deficit. This is based on the fact that pyruvate-mediated energy reconstitution in GTI-421-treated hepatocytes reversed the effect of GTI-421 on FGF21 induction. (Fig. 3). This is important because it verifies the mechanism of therapeutic efficacy of GTI-421, and thus opens the opportunity to generate more efficacious drug candidates that utilize the same, proven mechanism of action.

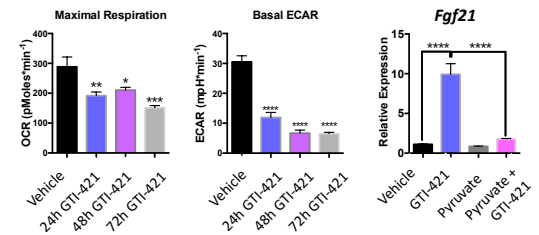


Fig. 3. GTI-421 induces energy deficits to induce FGF21 in hepatocytes. Left, maximal cellular oxygen consumption in hepatocytes treated with or without GTI-421. Middle, basal extracellular acidification rate (ECAR) in hepatocytes treated with or without GTI-421. Right, FGF21 expression in hepatocytes treated with GTI-421 with or without pyruvate energy rescue downstream of the glucose transporter. *, ***, ****, $P < 0.05$, < 0.001 , and < 0.0001 vs. vehicle.

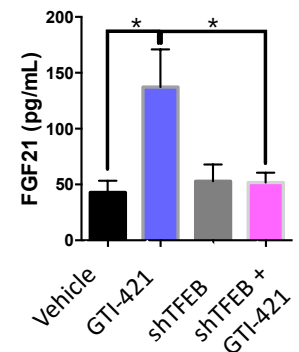
Specific objective 2

Define the mechanism of GTI-421 induction of hepatic FGF21 in mice. Data in objective #1 suggested that GTI-421 induces a hepatocyte starvation response to induce FGF21, and hepatic transcription factor EB (TFEB) is a component of the canonical hepatocyte fasting response that is partly responsible for FGF21 production in liver. We therefore tested the hypothesis that hepatic TFEB is required for the in vivo production of FGF21. We tested this by treating mice with GTI-421 in the presence or absence of AAV8-mediated hepatic TFEB-directed shRNA knockdown. We measured circulating FGF21 peptide in all mice.

Significant Results 2

We showed that GTI-421 induces circulating FGF21 peptide versus vehicle-treated controls. We also demonstrated that hepatic TFEB knockdown reverses this induction. The results suggest that hepatic TFEB is required for GTI-421-induced FGF21 in vivo (Fig. 4). These results are important because they suggest in vivo that the canonical hepatocyte fasting response, and its downstream signaling pathways, mediate the FGF21-producing effects of GTI-421. Given the known anti-diabetic effects of FGF21, our data suggest one possible downstream pathway by which GTI-421 confers enhanced peripheral metabolic effects.

Fig. 4. GTI-421 activates hepatic TFEB to induce FGF21 in vivo. Shown is FGF21 circulating peptide in mice treated with GTI-421 with or without shRNA directed against TFEB, delivered by AAV8. *, $P < 0.05$ vs. vehicle.



In summary, we have made significant strides on the two primary goals of this project. We demonstrated efficacy of our drug, GTI-421 to improve energy balance in normal and diabetic mouse models. We then showed the mechanism by which GTI-421 works: via introducing an energy deficit and activating canonical fasting pathways. Next steps in the coming reporting period will focus on PGC1a induction, and in vivo effects of GTI-421-induced

FGF21. These results advance the field by demonstrating that pharmacological glucose transporter inhibition is a viable, small-molecule-amenable therapeutic pathway against diabetes.

- **What opportunities for training and professional development has the project provided?**

From a professional development standpoint, I expanded my collaborative network by presenting this work at the 2017 Federation of American Societies for Experimental Biology Summer Research Conferences in Snowmass, CO. There I gained important collaborative contacts within my subfield. In addition, I was able to bring my graduate student trainee, Allyson Mayer, to present related work. She was honored at this conference with an abstract travel award for her presentation.

- **How were the results disseminated to communities of interest?**

I presented this work to the Washington University Medical Scientist Training Program trainees, who are training physician scientists aspiring to careers running independent laboratories.

- **What do you plan to do during the next reporting period to accomplish the goals?**

I plan to present this work during the summer of 2018 to Washington University Summer Biomedical Research Program. These are summer undergraduate research trainees, who aspire to careers in the medical sciences.

4. IMPACT

- **What was the impact on the development of the principal discipline(s) of the project?**

It was long held that a key starvation-induced process, autophagy (e.g. cellular self-eating) was responsible for the effects of blocking sugar transport in metabolic tissues like the liver. Our results refine this paradigm by demonstrating that the canonical fasting response strictly to energy deficit mediates the thermic effects of fasting. The resulting publication (in press) exhorts that autophagy-independent effects of fasting-mimetic therapies be explored and exploited as a target pathway moving forward.

- **What was the impact on other disciplines?**

Nothing to report.

- **What was the impact on technology transfer?**

Nothing to report.

- **What was the impact on society beyond science and technology?**

We anticipate that these enlightenments will improve human health by introducing a new therapeutic compound into the community. Ameliorating obesity is expected to greatly enhance the human experience, and the extent to which our citizens can regularly and fully participate in activities of daily living.

5. CHANGES/PROBLEMS

- **Changes in approach and reasons for change**

Nothing to report.

- **Actual or anticipated problems or delays and actions or plans to resolve them**

Nothing to report.

- **Changes that had a significant impact on expenditures**

We had some delay in obtaining ACURO approval of our animal protocol. Therefore, our expenditures were pushed back approximately 6 months. During that delay on animal work, we were able to complete much of the proposed in vitro mechanistic work, then validate these mechanisms in animals once the protocol was approved.

- **Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**

During the grant period we were required to update our protocol in regard to techniques used for other funded projects. No changes were made to our ACURO-approved protocol. All changes were approved by Washington University IACUC. Our new protocol is 20180031, approved February, 2018.

- **Significant changes in use or care of human subjects:**

Nothing to report.

- **Significant changes in use or care of vertebrate animals:**

Nothing to report.

- **Significant changes in use of biohazards and/or select agents**

Nothing to report.

6. PRODUCTS

- **Publications, conference papers, and presentations**

- 1. **Journal publications.**

- a. A. L. Mayer, Y. Zhang, E. H. Feng, C. B. Higgins, O. Adenekan, T. A. Pietka, W. L. Beatty, B. J. DeBosch, Enhanced Hepatic PPAR α Activity Links GLUT8 Deficiency to Augmented Peripheral Fasting Responses in Male Mice, **159**, 2110–2126 (2018). Federal support: Yes
 - b. Y. Zhang, C. B. Higgins, A. L. Mayer, I. Mysorekar, B. Razani, M. Graham, P. W. Hruz, B. J. DeBosch, Transcription Factor EB (TFEB)-dependent Induction of Thermogenesis by the Hepatocyte Solute Carrier 2A (SLC2A) Inhibitor, Trehalose, *Autophagy* (2018) *in press*. Federal support: Yes

- 2. **Books or other non-periodical, one-time publications.**

- Mayer AL and DeBosch BJ. “Glucose Transporters in Hepatic Fasting: Implications for Cardiometabolic Disease” (2018). Doctoral Dissertation, Ms. Allyson L. Mayer, Program in Developmental, Regenerative and Stem Cell Biology. Department of Biology & Biomedical Sciences, Washington University School of Medicine. Federal support: Yes.

- 3. **Other publications, conference papers, and presentations.**

- a. DeBosch BJ. “TFEB-dependent induction of thermogenesis by the SLC2A inhibitor, trehalose. Presented at the FASEB Summer Research Conference: Glucose Transport, Gateway to Systems Biology (2017). Publication status: *in press*
 - b. Mayer AL and DeBosch BJ. “Trehalose inhibits pressure overload-induced heart failure”. Presented at the FASEB Summer Research Conference: Glucose Transport, Gateway to Systems Biology (2017). Publication status: Under review for publication.

- **Website(s) or other Internet site(s)**

- Nothing to report

- **Technologies or techniques**

- Nothing to report.

- **Inventions, patent applications, and/or licenses**

- Nothing to report.

- **Other Products**

- 1. New animal model: For this project, we generated mice with liver-specific knockdown of the fasting-induced transcription factor, TFEB using AAV8-mediated TFEB-directed short-hairpin RNA (shRNA).

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

- What individuals have worked on the project?

Name:	Cassie Higgins
Project Role:	Research Associate
Researcher Identifier (e.g. ORCID ID):	n/a
Nearest person month worked:	12
Contribution to Project:	Mrs. Higgins aided with data collection and analysis/interpretation on the role and mechanism of GTI-421 in thermogenesis (Aim 1), and on mechanisms of GTI-induced FGF21 (Aim 2).
Funding Support:	Children's Discovery Institute MI-FR-2014-426

Name:	Yiming Zhang
Project Role:	Technician II
Researcher Identifier (e.g. ORCID ID):	n/a
Nearest person month worked:	12
Contribution to Project:	Mr. Zhang aided with data collection and analysis/interpretation on the role and mechanism of GTI-421 in thermogenesis (Aim 1), and on mechanisms of GTI-induced FGF21 (Aim 2).
Funding Support:	Children's Discovery Institute MI-FR-2014-426

Name:	Allyson Mayer
Project Role:	Graduate Student
Researcher Identifier (e.g. ORCID ID):	n/a
Nearest person month worked:	4
Contribution to Project:	Ms. Mayer aided with data collection and analysis/interpretation on the role and mechanism of GTI-421 in thermogenesis (Aim 1).
Funding Support:	National Science Foundation DGE-1143954 Washington University NIGMS Institutional Training Grant in Cell and Molecular Biosciences T32GM007067

- **Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**

Nothing to report.

- **What other organizations were involved as partners?**

Nothing to report.

8. SPECIAL REPORTING REQUIREMENTS

- **Collaborative Awards:** N/A
- **Quad Charts:** N/A

9. APPENDICES

- CV – See attached
- IACUC Approval Institutional Protocol– See attached
- IACUC Approved Amendments/Modification- See attached
- ACURO Updated Form– See attached
- Washington University Facility Inspection from the USDA- See attached

Curriculum Vitae

Brian DeBosch, M.D., Ph.D.

Address and Telephone Numbers

University: Washington University in St. Louis
School of Medicine
Department of Pediatrics
Gastroenterology, Hepatology and Nutrition
660 S. Euclid Ave.
St. Louis, MO 63110
Campus Box 8086

Phone: 314-454-6173
Fax: 314-454-2412
email: deboschb@wustl.edu

Present Positions

Assistant Professor of Pediatrics, Gastroenterology, Hepatology and Nutrition
Diabetes Research Center

Education and Training

1997 - 2001 BS, *Summa Cum Laude Distinction*, Cellular & Molecular Biology, University of Michigan, Ann Arbor, MI

2001 - 2008 MD, Medical Scientist Training Program, Washington University School of Medicine, St. Louis, MO

2001 - 2008 PhD, *Olin Fellowship*, Molecular Cell Biology, Washington University School of Medicine, St. Louis, MO/USA

2008 - 2010 Residency, St. Louis Children's Hospital, St. Louis MO

2010 - 2014 Fellowship - Accelerated Research Pathway, Washington University School of Medicine, St. Louis MO

Academic Positions and Employment

- 2014 - 2016 Instructor in Pediatrics, Gastroenterology, Hepatology and Nutrition, Washington University in St. Louis, St. Louis, MO
- 2015 - Pres Diabetes Research Center, Washington University in St. Louis, St. Louis, MO
- 2016 - Pres Assistant Professor of Pediatrics, Gastroenterology, Hepatology and Nutrition, Washington University in St. Louis, St. Louis, MO

Appointments and Committees

NIH Study Sections

- 2016 - Pres National Science Foundation Graduate Research Fellowship Selection Panel
- 2017 - Pres Ad Hoc Grant Reviewer Longer Life Foundation
- 2017 - Pres BJH-ICTS T1/T2 Study Section
- 2018 - Pres Internal Selection Study Section: NIH grant T32 DK077653
- 2018 - Pres WUSTL BioSURF Grant Review Committee

University Affiliations

- 2010 - Pres Ad hoc interviewer - Division of Pediatric Gastroenterology, Hepatology & Nutrition Fellowship Recruitment
- 2011 - Pres Washington University Dept. of Pediatrics Academic Careers Retreat Planning Committee
- 2015 - Pres Washington University Dept. of Pediatrics MSTP/DBBS Faculty Showcase Planning Committee
- 2016 - Pres WUPS Liaison, Division of Gastroenterology, Hepatology & Nutrition
- 2016 - Pres Assistant Director - Pediatric Gastroenterology, Hepatology & Nutrition Fellowship Admissions Committee
- 2017 - Pres Ad hoc interviewer, Medical Scientist Training Program Admissions
- 2018 - 2018 Translational Genomics Focus Group

Hospital Affiliations

- 2014 - Pres Ad Hoc Interviewer - SLCH Residency Recruitment

Thesis Committees (*Chair) Advisor

- 2015 - Pres Allyson Mayer Kelle Moley

Scholarship Oversight Committees

- 2014 - 2016 Rachel Kassel MD, PhD* (Advisor: Edward Pearce / Rodney Newberry)

2015 - Pres Kim Liss, MD* (Advisor: Brian Finck, PhD)
2016 - Pres Sakil Kulkarni, MD (Advisor: David Rudnick, MD, PhD)
2017 - Pres Brendan Harris (Advisor: Mark Manary)

Licensure and Certifications

2008 - Pres Basic Life Support
2008 - 2010 Neonatal Resuscitation Program
2008 - 2010 Pediatric Advanced Life Support
2013 - Pres American Board of Pediatrics
2015 - 2016 MO Physician & Surgeon License #2011025489
2015 - Pres Pediatric Gastroenterology, Hepatology & Nutrition

Honors and Awards

1997 National Merit Scholar
1997 Regents-Alumni Scholar, University of Michigan, Ann Arbor
1997 UPAAM Outstanding Youth of Michigan Scholar
1997 - 2001 Academic Merit Scholar, College of Literature, Science & Arts, University of Michigan, Ann Arbor
1997 - 2001 Ford Endowed Merit Scholar
1997 - 2001 James B. Angell Scholar, University of Michigan, Ann Arbor
1998 - 2001 Class Honors Award, University of Michigan, Ann Arbor
1999 Sophomore Honors Award, University of Michigan, Ann Arbor
1999 - 2001 Telluride Association Scholar
2000 - 2001 Barry M. Goldwater Scholar
2001 - Pres Delta Phi Alpha National German Honor Society
2001 National Council on Undergraduate Research Award
2001 - 2001 Highest Honors Thesis Distinction, University of Michigan, Ann Arbor
2001 - 2001 Summa Cum Laude, University of Michigan, Ann Arbor
2011 Triple Crown Award - St. Louis Children's Hospital
2013 - 2015 Digestive Diseases Research Center P&F Award

2014 - Pres	Faculty Scholar - Children's Discovery Institute
2014 - Pres	Nutrition & Obesity Research Center P&F Award
2014 - Pres	Scholar - Child Health Research Center of Washington University School of Medicine
2015 - Pres	Early Investigator Award - the Endocrine Society
2015 - 2018	American Gastroenterological Association / Gilead Sciences Research Scholars Award in Liver Disease
2015	St. Louis Children's Hospital Recognition Award
2016 - Pres	Robert Wood Johnson Foundation Scholar - Amos Medical Faculty Development Program
2017 - Pres	United States Department of Defense Discovery Award
2017 - Pres	Inductee, Society for Pediatric Research
2018 - 2018	American Gastroenterological Association (AGA) / American Association for the study of Liver Diseases (AASLD) Conference Travel Award
2018 - 2018	St. Louis Children's Hospital Triple Crown Award for Safe, Effective and Exceptional Patient Care
2018 - Pres	Co-Inventor (Paul Hruz, MD, PhD), LEAP Inventor Challenge - Washington University School of Medicine Skandalaris Center: "Novel Treatment of Fatty Liver Disease"

Editorial Responsibilities

Editorial Ad Hoc Reviews

2005 - Pres	Journal of Molecular and Cellular Cardiology
2014 - Pres	Biochem Biophys Acta
2014 - Pres	Diabetes
2015 - Pres	ISME Journal
2016 - Pres	American Journal of Physiology Gastrointestinal & Liver Physiology
2016 - Pres	Scientific Reports
2016 - Pres	Hepatology
2016 - Pres	American Journal of Physiology - Cell Physiology
2016 - Pres	Autophagy
2016 - Pres	American Journal of Physiology - Endocrinology & Metabolism

2016 - Pres Journal of Nutritional Biochemistry

2018 - Pres Nature Cell Death & Disease

Professional Societies and Organizations

2010 - Pres American Gastroenterological Association

2010 - Pres NASPGHAN

2015 - Pres The Endocrine Society

2017 - Pres American Heart Association, Member

Major Invited Professorships and Lectures

2014 Spontaneous, Early-Onset Metabolic Syndrome in Mice Lacking the Enterocyte Urate Transporter, SLC2A9 / Washington University Digestive Disease Research Core Center Speaker Series

2015 Treatment for Pediatric NAFLD: Sweet new therapies are coming through the portal! / Pediatrics Grand Rounds, Washington University School of Medicine

2016 - 2016 International Trehalose Symposium: "Trehalose, Cellular Action and Therapeutic Possibilities"

Research Support

Governmental Support

Discovery Award (DeBosch - NA)

2/1/2017- 7/31/2018

Department of Defense CDMRP

Metabolic Characterization of a Putative Novel Antidiabetic Drug, GFI-421

The goal of this project is to characterize the novel GLUT inhibitor, GTI-421 in mitigating NAFLD and type 2 diabetes mellitus in cell culture and in vivo diabetic models.

Non-Governmental Support

Interdisciplinary Research Initiative MI-II-
2017-593 (DeBosch - NA)

1/1/2017- 12/31/2019

Children's Discovery Institute

Prevention and Treatment of Hepatic Steatosis through Selective Targeting of GLUT8

The goal of this project is to identify novel therapies for NAFLD based on targeted inhibition of the sugar transporter, GLUT8.

Harold Amos (DeBosch - Principal
Investigator)

1/1/2016- 12/31/2019

RWJF

Role of GLUT8 in Hepatic Metabolic Regulation

The goal of this project is to determine the mechanism by which GLUT8 regulates hepatocellular autophagy and the underlying signaling and structural mechanisms that drive this regulation using in vitro and in vivo models.

FR MD-FR-2014-426 (DeBosch - Principal
Investigator)

7/1/2014- 6/30/2019

CDI

Role of Enterocyte GLUT9 in Intestinal Urate Handling and Energy Homeostasis

AGA-Gilead RSA (DeBosch - Principal
Investigator)

10/1/2015- 9/30/2018

American Gastroenterological Association

Role of GLUT8 in hepatic autophagic and metabolic regulation

Completed Support

P30 P30DK056341

11/1/2015- 10/31/2016

NIDDK / Washington University Nutrition
Obesity Research Center

Metabolic Regulation by Hepatic GLUT8

P30 DK052574

5/1/2013- 4/30/2015

NIDDK

Role of enterocyte GLUT9 in uric acid transport and metabolic homostasis

K12 5 K12 HD000850-29

7/1/2013- 6/30/2014

NICHD

Role of enterocyte GLUT9 in mammalian metabolic homeostasis

K12 5K12HD000850-27

7/1/2011- 6/30/2013

NICHD Pediatric Scientist Development
Program

Role of Enterocyte GLUT7 in Mammalian Metabolic Homeostasis

In this project, we are characterizing the role of the enterocyte glucose and fructose transporter, GLUT7, in enterocyte fructose absorption and fructose-induced metabolic syndrome. We are testing the hypothesis that alterations in enterocyte absorption can modulate the development of obesity and metabolic syndrome.

Current Trainees

2016	Xia Qian, MD, PhD, Postdoctoral Fellow, Signaling mechanisms in trehalose action
2014	Allyson L. Mayer B.S., Graduate Student, Mechanisms of Hepatocyte Hexose Sensing
2016	Daisy Zhou, MD, House Staff, Pediatrics
2014	Emily Feng, Other, Mechanisms of Non-Alcoholic Fatty Liver Disease

Clinical Responsibilities

2014 - Pres	Assistant Professor of Pediatrics and Cell Biology and Physiology, Pediatrics, Gastroenterology, Hepatology & Nutrition, Washington University School of Medicine / St. Louis Children's Hospital
-------------	---

Teaching Responsibilities

2010 - Pres	Ad Hoc Lecturer, General Pediatrics Rotation - Pediatric Residency
-------------	--

- 2014 - Pres Clinical Case Discussion in Pediatric Gastroenterology, Pediatrics - 3rd Year Clinical Rotation
- 2015 - 2016 Course Director, Studies that changed the art and practice of Pediatric GI
- 2016 - Pres Small Group Discussion Leader, Bio 5011 Ethics
- 2016 - Pres Discussion Group Leader, Molecular Cell Biology - Bio 5068
- 2018 Course Director, Research Methods in Pediatric Gastroenterology Course #Pending

Publications

1. DeBosch BJ, Baur E, Deo BK, Hiraoka M, Kumagai AK. Effects of insulin-like growth factor-1 on retinal endothelial cell glucose transport and proliferation. *J Neurochem*. 2001;77(4):1157-67. PMID:[11359881](#)
2. DeBosch BJ, Deo BK, Kumagai AK. Insulin-like growth factor-1 effects on bovine retinal endothelial cell glucose transport: role of MAP kinase. *J Neurochem*. 2002;81(4):728-34. PMID:[12065632](#)
3. Muslin AJ, DeBosch B. Role of Akt in cardiac growth and metabolism. *Novartis Found Symp*. 2006;274:118-26; discussion 126-31, 152-5, 272-6. PMID:[17019809](#)
4. DeBosch B, Treskov I, Lupu TS, Weinheimer C, Kovacs A, Courtois M, Muslin AJ. Akt1 is required for physiological cardiac growth. *Circulation*. 2006;113(17):2097-104. doi:[10.1161/CIRCULATIONAHA.105.595231](#) PMID:[16636172](#)
5. DeBosch B, Sambandam N, Weinheimer C, Courtois M, Muslin AJ. Akt2 regulates cardiac metabolism and cardiomyocyte survival. *J Biol Chem*. 2006;281(43):32841-51. doi:[10.1074/jbc.M513087200](#) PMCID:[PMC2724003](#) PMID:[16950770](#)
6. Lau JM, Jin X, Ren J, Avery J, DeBosch BJ, Treskov I, Lupu TS, Kovacs A, Weinheimer C, Muslin AJ. The 14-3-3tau phosphoserine-binding protein is required for cardiomyocyte survival. *Mol Cell Biol*. 2007;27(4):1455-66. doi:[10.1128/MCB.01369-06](#) PMCID:[PMC1800730](#) PMID:[17145769](#)
7. DeBosch BJ, Muslin AJ. Insulin signaling pathways and cardiac growth. *J Mol Cell Cardiol*. 2008;44(5):855-64. doi:[10.1016/j.yjmcc.2008.03.008](#) PMCID:[PMC2442827](#) PMID:[18423486](#)
8. DeBosch BJ, Brown D, Tse G, Hua M, Kodner IJ. Fetus-saving Caesarian rejection by pregnant woman: a case study. *Surgery*. 2009;145(1):6-8. PMID:[19093328](#)
9. Avery J, Etzion S, DeBosch BJ, Jin X, Lupu TS, Beitinjaneh B, Grand J, Kovacs A, Sambandam N, Muslin AJ. TRB3 function in cardiac endoplasmic reticulum stress. *Circ Res*. 2010;106(9):1516-23. doi:[10.1161/CIRCRESAHA.109.211920](#) PMCID:[PMC2913227](#) PMID:[20360254](#)
10. Etzion S, Etzion Y, DeBosch B, Crawford PA, Muslin AJ. Akt2 deficiency promotes cardiac induction of Rab4a and myocardial β -adrenergic hypersensitivity. *J Mol Cell Cardiol*. 2010;49(6):931-40. doi:[10.1016/j.yjmcc.2010.08.011](#) PMCID:[PMC2975863](#) PMID:[20728450](#)

11. DeBosch BJ, Chi M, Moley KH. Glucose transporter 8 (GLUT8) regulates enterocyte fructose transport and global mammalian fructose utilization. *Endocrinology*. 2012;153(9):4181-91. doi:[10.1210/en.2012-1541](https://doi.org/10.1210/en.2012-1541) PMCID:[PMC3423610](https://pubmed.ncbi.nlm.nih.gov/PMC3423610/) PMID:[22822162](https://pubmed.ncbi.nlm.nih.gov/22822162/)
12. DeBosch BJ, Chen Z, Finck BN, Chi M, Moley KH. Glucose transporter-8 (GLUT8) mediates glucose intolerance and dyslipidemia in high-fructose diet-fed male mice. *Mol Endocrinol*. 2013;27(11):1887-96. doi:[10.1210/me.2013-1137](https://doi.org/10.1210/me.2013-1137) PMCID:[PMC3805847](https://pubmed.ncbi.nlm.nih.gov/PMC3805847/) PMID:[24030250](https://pubmed.ncbi.nlm.nih.gov/24030250/)
13. DeBosch BJ, Chen Z, Saben JL, Finck BN, Moley KH. Glucose transporter 8 (GLUT8) mediates fructose-induced de novo lipogenesis and macrosteatosis. *J Biol Chem*. 2014;289(16):10989-98. doi:[10.1074/jbc.M113.527002](https://doi.org/10.1074/jbc.M113.527002) PMCID:[PMC4036240](https://pubmed.ncbi.nlm.nih.gov/PMC4036240/) PMID:[24519932](https://pubmed.ncbi.nlm.nih.gov/24519932/)
14. DeBosch BJ, Kluth O, Fujiwara H, Schürmann A, Moley K. Early-onset metabolic syndrome in mice lacking the intestinal uric acid transporter SLC2A9. *Nat Commun*. 2014;5:4642. doi:[10.1038/ncomms5642](https://doi.org/10.1038/ncomms5642) PMCID:[PMC4348061](https://pubmed.ncbi.nlm.nih.gov/PMC4348061/) PMID:[25100214](https://pubmed.ncbi.nlm.nih.gov/25100214/)
15. Omurtag K, Esakky P, DeBosch BJ, Schoeller EL, Chi MM, Moley KH. Modeling the effect of cigarette smoke on hexose utilization in spermatocytes. *Reprod Sci*. 2015;22(1):94-101. doi:[10.1177/1933719114533727](https://doi.org/10.1177/1933719114533727) PMID:[24803506](https://pubmed.ncbi.nlm.nih.gov/24803506/)
16. DeBosch BJ, Heitmeier MR, Mayer AL, Higgins CB, Crowley JR, Kraft TE, Chi M, Newberry EP, Chen Z, Finck BN, Davidson NO, Yarasheski KE, Hruz PW, Moley KH. Trehalose inhibits solute carrier 2A (SLC2A) proteins to induce autophagy and prevent hepatic steatosis. *Sci Signal*. 2016;9(416):ra21. PMCID:[PMC4816640](https://pubmed.ncbi.nlm.nih.gov/PMC4816640/) PMID:[26905426](https://pubmed.ncbi.nlm.nih.gov/26905426/)
17. Mayer AL, Higgins CB, Heitmeier MR, Kraft TE, Qian X, Crowley JR, Hyrc KL, Beatty WL, Yarasheski KE, Hruz PW, DeBosch BJ. SLC2A8 (GLUT8) is a mammalian trehalose transporter required for trehalose-induced autophagy. *Sci Rep*. 2016;6:38586. PMID:[27922102](https://pubmed.ncbi.nlm.nih.gov/27922102/)
18. Yiming Zhang, Cassandra B. Higgins, Allyson L. Mayer, Indira U. Mysorekar, Babak B. Razani, Mark J. Graham, Paul W. Hruz, Brian J. DeBosch . Transcription Factor EB (TFEB)-dependent Induction of Thermogenesis by the Hepatocyte Solute Carrier 2A (SLC2A) inhibitor, Trehalose *Autophagy*. 2018.
19. Mayer AL, Zhang Y, Feng EH, Higgins CB, Adenekan O, Pietka TA, Beatty WL, DeBosch BJ. Enhanced Hepatic PPAR α Activity Links GLUT8 Deficiency to Augmented Peripheral Fasting Responses in Male Mice *Endocrinology*. 2018.

 Washington University in St. Louis
Institutional Animal Care and Use Committee

Animal Welfare Assurance # A-3381-01
April 30, 2018

To: Brian J Debosch, MD, Ph.D.
From: Brian Finck, Ph.D., Chair
Subject: Approval of Protocol for Experiments Utilizing Animals

Agency/Title: DEPARTMENT OF DEFENSE
Metabolic Characterization of a Putative Novel Antidiabetic Drug, GTI-421

The Institutional Animal Care and Use Committee of Washington University has reviewed this protocol for the use of animals in conjunction with the research project named above. Federal laws and guidelines require that institutional animal care and use committees review ongoing projects annually. For the first two years after initial approval of this protocol, you will be asked to submit an annual progress report describing any changes in this protocol.

Approval Date: 02/20/2018

Approval No. 20180031

Expiration Date: 02/20/2021

Approved with Addendum

SPECIES:

Mouse

It is your responsibility to see that all persons who use animals under your direction understand and follow the approved protocol. Should it become necessary to make substantial changes in this protocol, you must submit a new protocol. **Failure to comply with these provisions can result in suspension of the research.**

Campus Box 1054, One Brookings Drive St. Louis, Missouri 63130,
(314) 362-3229, Fax: (314) 747-6695, <http://asc.wustl.edu>

Table of Contents

[PI Contact Record](#)

[I. Protocol Title/Info](#)

[II. Brief Summary](#)

[III. Outline of Animal Procedures](#)

[IV. Protocol Funding](#)

[Department of Defense--IV.\)a.\) Protocol Agency Funding List](#)

[Department of Defense--IV.\)a.\)1.\) Protocol Agency Funding Source](#)

[Pediatrics - GI--IV.\)b.\) Protocol Department Funding List](#)

[Pediatrics--IV.\)b.\)1.\) Protocol Department Funding Source](#)

[Pediatrics - GI--IV.\)b.\)1.\) Protocol Department Funding Source](#)

[IV.\)c.\) Protocol Private/Commercial Funding](#)

[V. Collaboration](#)

[V.\)b.\) Inside Collaboration](#)

[VI. Antibodies Use](#)

[VII Databases Searched](#)

[VIII Use of Hazardous Agents](#)

[VIII.\)a.\) Hazardous Use Info](#)

[IX. Species Use Question](#)

[IX.\)a.\) Housing Outside Central Animal Holding Facility](#)

[IX.\)b.\) Type of Animal Use](#)

[IX.\)c.\) Use of Human Clinical Areas](#)

[IX.\)d.\) Transportation of Animals](#)

[IX.\)e.\) Animal Species List](#)

[Mouse--IX.\)e.\)1.\) Species Information](#)

[Mouse--IX.\)e.\)2.\) Justification For Number Requested](#)

[Mouse--IX.\)e.\)3.\) Justification For Choice of Species](#)

[Mouse--IX.\)e.\)6.\) Housing Location](#)

[Mouse; B J C Insitute Of Health @ W U S M--IX.\)e.\)6.\)A.\) Protocol Housing Location Information](#)

[Mouse; CSRB Basement--IX.\)e.\)6.\)A.\) Protocol Housing Location Information](#)

[Mouse--IX.\)e.\)7.\) Use Location](#)

[Mouse; McDonnell Pediatric Research Bldg--IX.\)e.\)7.\)A.\) Protocol Use Location Info](#)

[Mouse; B J C Insitute Of Health @ W U S M--IX.\)e.\)7.\)A.\) Protocol Use Location Info](#)

[Mouse; CSRB Surgery--IX.\)e.\)7.\)A.\) Protocol Use Location Info](#)

[Mouse; CSRB Surgery--IX.\)e.\)7.\)A.\) Protocol Use Location Info](#)

[Mouse--IX.\)e.\)9.\) Drugs](#)

[Mouse; Isoflurane--Drug Information](#)

[Mouse; GTI-421--Drug Information](#)

[Mouse; Antisense Oligonucleotide--Drug Information](#)

[Mouse--IX.\)e.\)10.\) Hazardous Agents](#)

[Mouse; DNA \(recombinant\)--Biohazard Agents Information](#)

[Mouse; Adeno-associated virus--Biohazard Agents Information](#)

[Mouse--IX.\)e.\)11.\) Non-Surgical Procedures](#)

[Mouse--IX.\)e.\)11.\)A.\) Non-Surgical Procedure Information](#)

[Mouse--IX.\)e.\)12.\) Surgical/Experimental Procedures](#)

[Mouse; transverse aortic constriction--IX.\)e.\)12.\)A.\) Surgical/Experimental Procedures](#)

[Mouse--IX.\)e.\)13.\) Breeding Information](#)

[Mouse--IX.\)e.\)14.\) Humane Use Animal Categories](#)

[Mouse--IX.\)e.\)15.\) Euthanasia](#)

[Mouse; Carbon dioxide overdose followed by cervical dislocation--IX.\)e.\)15.\)A.\) Euthanasia Method Information](#)

[Information](#)

[Mouse--IX.\)e.\)16.\) SOP Exemptions](#)

[X. Schedule B Questions](#)

[XI.\) Personnel List](#)

[Debosch, Brian J--XI.\)b.\) Personnel Information](#)

[Mayer, Allyson--XI.\)b.\) Personnel Information](#)

[Higgins, Cassandra--XI.\)b.\) Personnel Information](#)

[Zhang, Yiming--XI.\)b.\) Personnel Information](#)

[Hard copy signature page - CLICK ON "PRINT TO HTML"](#)

[Hard copy signature](#)

PI Contact Record

Certain contact information may be added, edited and saved only by the ASC Staff please contact them for assistance with these fields.

Principal Investigator	Debosch, Brian J
Protocol Application Number	20180031
Document Type	New

Employee ID	
PI	Debosch, Brian J
Primary Role	Principal Investigator
Department	Washington University / Pediatrics - GI
Campus	Medical School
Preferred Contact Method (Phone, Email, Cell, etc.)	Email
Phone	314-747-2692
Emergency Phone	314-503-4308
Home Phone	636-778-1536
Cell Phone	314-265-0090
Pager	314-424-3012
Fax	314-454-2412
Email	DeBosch_B@kids.wustl.edu
Degree	MD PhD
Experience and Qualification	19 years of mouse handling experience. Took Gail Moore's techniques course.

[Table of Contents](#)

I. Protocol Title/Info

YOU MUST CLICK ON "SAVE AND CONTINUE" BETWEEN ALL SECTIONS OR YOUR INFORMATION WILL BE LOST, BECAUSE THE SOFTWARE DOES NOT AUTOMATICALLY SAVE. CLICKING ON "FINISH LATER" WILL ALLOW YOU TO RETURN TO THE CURRENT SECTION WHEN YOU LOGIN AGAIN BUT WILL NOT AUTOMATICALLY SAVE THE INFORMATION.

This title is a reference for this specific protocol and need not match the grant name. You will be prompted to enter the granting agency(s) and their title(s) at a later time.

Protocol Title

Enter a title for this protocol
Glucose transporters in murine metabolic control
Notes - For ASC/DCM Use Only
Are you renewing a protocol?
Yes
If you are renewing a protocol please enter the number of the Protocol you are renewing.
20150011

[Table of Contents](#)

II. Brief Summary

Brief Summary: Briefly explain the significance of the study in layman language. Summarize proposed use of animals, including the general goals and experimental design. Provide rationale and scientific objectives of the study. If this is a **protocol renewal** for an expiring protocol, please include a progress report on your research for the past three years even if no 'new' experiments are planned. Please state if new procedures will be added to this protocol renewal and describe in the appropriate section(s).

Brief Summary

Diabetes and obesity result from disorders in whole-body metabolism, and are major causes of morbidity and mortality in the Western and Developing worlds. I am trying to understand how sugar transporters in the liver and intestine control whole-body metabolism. The specific goal of my research is to understand what specific substances these sugar transporters transport, how these sugar transporters are regulated by circulating hormones and in various disease states, and how altered regulation of these transporters can cause, propagate or mitigate metabolic disease.

I study sugar transporters in the Glucose Transporter (GLUT) family of sugar transporter homologs. I showed that two particular members of the GLUT family - GLUT8 and GLUT9 - are quite important in controlling basal mammalian metabolism as well as the metabolic response to a high-sugar diet.

My experimental objectives are:

- 1) Understand how liver and intestinal GLUT8 controls sugar metabolism at baseline and under high-sugar dietary conditions.
- 2) Understand how liver and intestinal GLUT9 controls basal and high-calorie dietary conditions.
- 3) Understand the role of fasting liver metabolism in heart failure

I propose two main experiments:

1) Breed and maintain wild-type and whole-body GLUT8-deficient mice and use these mice to define the mechanisms by which GLUT8 in the liver controls metabolism. To work these pathways out, we will administer GTI-421 drug (outlined further in (III.)) to block hepatic glucose transport, or AAV8-based shRNA or antisense oligonucleotide to alter downstream signaling partners of the GLUT cascade. I will also sacrifice these mice and study isolated liver cells and isolated intestinal epithelial cells - the cells that line the intestine, which are responsible for nutrient absorption in culture. Lastly, to understand the role of liver GLUTs in heart dysfunction after pressure overload, we will subject wild-type and GLUT inhibitor-treated mice to transverse aortic constriction in the presence or absence of AAV8-delivered shRNA or antisense oligonucleotide (directed against downstream intermediaries) to work out these signaling pathways. This is important because using liver signaling pathways to block metabolic diseases like fatty liver disease and diabetes; and to block heart dysfunction may offer new treatments for human disease.

2) Breed and maintain whole-body GLUT9 mutant mice, which will have engineered cut-sites ("floxed" sites) that will delete the GLUT9 gene specifically when crossed with mice that express the "Cre" gene (which excises the gene only in tissues of interest). Mice will be maintained on regular chow or high-fructose diet and sacrificed for tissue sampling and analysis. This is important because enhancing uric acid excretion may be a novel way to treat metabolic disease in human patients.

PROGRESS REPORT 2018:

- 1) DeBosch BJ, Heitmeier MR, Mayer AL, Higgins CB, Crowley JR, Kraft TE, et al. Trehalose inhibits solute carrier 2A (SLC2A) proteins to induce autophagy and prevent hepatic steatosis. Sci Signal [Internet]. 2016 Feb 23;9(416):ra21-ra21.
- 2) Mayer AL, Higgins CB, Heitmeier MR, Kraft TE, Qian X, Crowley JR, et al. SLC2A8 (GLUT8) is a mammalian trehalose transporter required for trehalose-induced autophagy. Sci Rep [Internet]. 2016;6(December):38586.

In this interval we showed that GLUT8 blockade in the liver by trehalose induces hepatic fasting responses, including autophagic flux, AMPK signaling and mTOR blockade. We then showed that GLUT8 is required for AMPK activation and autophagic flux in hepatocytes, but not in neuron. We conclude that GLUT8 may act as a signaling transceptor to activate hepatic fasting responses in vivo.

[Table of Contents](#)

III. Outline of Animal Procedures

Include the major thrust of the experimental procedures, e.g., antibody production, ablation behavior study, surgical removal, etc., but do not give details such as names of anesthetics, analgesics, tranquilizers, etc. Experimental design and science will be reviewed only as relevant to Animal Studies Committee concerns. If this is a **protocol renewal**, please indicate if any of these experiments were completed in the last three years.

Brief outline of animal procedures

The major thrust of our experiments is to produce either wild-type, GLUT8- or GLUT9-mutant liver and intestinal tissue for analysis after sacrifice. To that end, we will breed GLUT8- GLUT9- and WT litters and subject them to either chow or high-sugar diet for up to 24 weeks in order to induce a metabolic syndrome-like state (see DeBosch, et al 2012). Post-mortem enteric and hepatic tissues will be excised and analyzed.

The goal of our next set of experiments on this project is to understand the mechanistic bases for metabolic changes in context of enterohepatic glucose transport inhibition. To that end, administration of glucose transporter inhibitor GTI-421 will be required in the context of genetic knockout models, or genetic knockdown using antisense oligonucleotides or AAV-mediated shRNA delivery. Tail snip-based DNA isolation and genotyping of each animal will be required to ensure appropriate genetic manipulations were achieved.

Briefly, GTI-421 will be delivered orally ad libitum at a dose of 10mg/kg/day. This dosage is based on empirically determined food consumption rates for each cohort, on which we base drug wt/wt concentration in preparing the chow with GTI-421.

Antisense oligonucleotides (ASOs) are obtained from IONIS pharmaceuticals and will be delivered in sterile saline (0.9%) by intraperitoneal injection at a dose of 25mg/kg/day three times weekly throughout the duration of the drug intervention protocol. shRNA will be delivered by adenovirus-associated virus, obtained and purified by Vector Biolabs (Philadelphia, PA).

Genotyping is performed by guidelines published by the WUSTL IACUC protocols. Briefly, 0.5mm tail tip is excised at age < 21 days for DNA extraction and genotyping PCR. Bleeding is controlled, if necessary upon close monitoring, by continued manual pressure to the wound site until hemostasis is obtained.

PROGRESS REPORT:
In the last three years we have made progress using each of the above techniques. However, none are completely "completed" or obsolete in our laboratory. All the above procedures are active in our laboratory at present.

[Table of Contents](#)

IV. Protocol Funding

Based on the boxes checked below, additional screens will appear to further identify the fund sources for this proposal. Complete subsequent pages as appropriate.

How will this protocol be funded? Check all applicable boxes

Agency Funding	Yes
If this protocol is part of a Training Grant or Program Project Grant, list title of the overall project and name of Program Director.	
Departmental Funding	Yes
Private/Commercial Funding	Yes
Fellowship	
Gift	
Other	

[Table of Contents](#)

IV.)a.) Protocol Agency Funding List

Please verify that all granting agency information listed below is correct

Select	Fund Souce/Agency	Grant Number	Fund Title
21563	Department of Defense		DEPARTMENT OF DEFENSE Metabolic Characterization of a Putative Novel Antidiabetic Drug, GTI-421

[Attachments List](#)

File Spec	Description	Created
-----------	-------------	---------

[Table of Contents](#)

IV.)a.)1.) Protocol Agency Funding Source

If this protocol is part of a Training Grant or Program Project Grant, list the title of the overall project, the name of the Program Director and your project title.

Funding Source/Agency	Department of Defense			
Fund Title	DEPARTMENT OF DEFENSE Metabolic Characterization of a Putative Novel Antidiabetic Drug, GTI-421			
Funding Application Due Date	/ /			
Funding Grant Number, if known		Sponsored Projects #		
Currently Funded				
PI on Grant (If different than PI on Protocol) First Name		Middle Initial	Last Name	

[Table of Contents](#)

IV.)b.) Protocol Department Funding List

Select	Organization Name	Department Name	Department Fund Title	Sponsored Project #
PEDS	Washington University / Pediatrics	Pediatrics	Departmental Startup Funds	

PEGI	Washington University / Pediatrics - GI	Pediatrics - GI	WASHINGTON UNIVERSITY DRC Mechanisms of trehalose action in modulationg hepatic and extrachepatice metabolism	
------	--	-----------------	---	--

Attachments List

File Spec	Description	Created
-----------	-------------	---------

[Table of Contents](#)

IV.)b.)1.) Protocol Department Funding Source

Organization	Washington University / Pediatrics
Dept Name	Pediatrics
Department Funding Title	Departmental Startup Funds
Sponsored Projects #	

[Table of Contents](#)

IV.)b.)1.) Protocol Department Funding Source

Organization	Washington University / Pediatrics - GI
Dept Name	Pediatrics - GI
Department Funding Title	WASHINGTON UNIVERSITY DRC Mechanisms of trehalose action in modulationg hepatic and extrachepatice metabolism
Sponsored Projects #	

[Table of Contents](#)

IV.)c.) Protocol Private/Commercial Funding

Protocol Private/Commercial Funding

Company Name	Children's Discovery Institute
Funding Application Due Date	/ /
Private/Commercial Funding Title	Role of Enterocyte GLUT9 in Intestinal Urate Handling and Energy Homeostasis AMERICAN GASTROENTEROLOGICAL SOCIETY AND GILEAD SCIENCES Role of GLUT8 in hepatic autophagic and metabolic regulation ROBERT WOOD JOHNSON FOUNDATION Role of GLUT8 in Hepatic Metabolic Regulation CHILDRENS DISCOVERY INSTITUTE Prevention and Treatment of Hepatic Steatosis through Selective Targeting of Glut8 ICTS - WASHINGTON UNIVERSITY Hepatic Starvation Responses Induced By Trehalose In Migrating Hepatic Steatosis
Sponsored Project Fund Number	

Attachments List

File Spec	Description	Created
-----------	-------------	---------

[Table of Contents](#)

V. Collaboration

Please give details of any collaboration in this section. If you have more than one collaboration, add the second, third, etc., in the text box since there is room for only one. Do not delete previously approved collaboration information

Details on Inside Collaboration would only be required if transferring animals from another protocol.

Collaboration with another institution should be reported to the ASC, and proof of the institution's standing with regards to OLAW, USDA and AAALAC, INT. may be required.

Will you be collaborating with:	
An outside institution	NO
Another colleague within the institution	YES

V.)b.) Inside Collaboration

If animals used under this protocol are obtained from other protocols, state protocol number and briefly describe association (e.g. obtaining transgenic mice from protocol #).

Collaborating PI Name First Name	Kelle	Initial	H	Last name	Moley
Protocol #	20150070				
Please describe the association between this protocol and the one listed above	We both study GLUT8- and GLUT9-deficient mice. We will therefore transfer mice between protocols as experiments and mouse needs dictate.				
	Second collaboration: MCPC – Weinheimer PI, protocol number 20150143. The MCPC will be performing surgeries to examine cardiac function of our mice.				

Attachments List

File Spec	Description	Created
-----------	-------------	---------

[Table of Contents](#)

VI. Antibodies Use

Please refer to ASC guideline if antibodies will be used.

[Adjuvants for Immunostimulation \(Freund's\) HTML](#)

[Adjuvants for Immunostimulation \(Freund's\) PDF](#)

Protocol Antibodies Use

Will Antibodies be obtained from an outside source?	NO
---	----

[Table of Contents](#)

VII Databases Searched

Protocol DB Searched - Database

Alternatives:

Consideration of alternatives to each procedure that may cause pain or distress (**Pain Class D and/or E**) must state sources consulted. Reduction, replacement and refinement must be addressed.

Here is a link to a list of databases to assist you in your search:

[Databases Page](#)

Databases Searched (Minimum of two required): Please list the Titles of the Databases Searched
Pubmed Medline

Key Words and/or Search Strategy Used:(Include the species for this protocol and the keywords animal welfare and animal research alternatives.)
Mouse animal welfare animal research alternatives transverse aortic constriction Mouse animal welfare animal research alternatives genotyping

The searches were performed on (use this format for the date (Month/Day/Year))	02/02/2018
--	------------

Years of experience in this field
21

Please select yes to indicate your acceptance of the statement of necessity for your protocol research.

Based on the years of experience in this field indicated above, in conjunction with periodic consultation of bibliographic sources and a number of references, I believe there is no alternative to performing the potentially painful/distressful procedure(s) contained in this protocol in order to achieve the scientific objectives of my research.
Yes

Please indicate the time range covered in your search (include both the start year and the end year, i.e. 1985-2013)
1990-2018

[Table of Contents](#)

VIII Use of Hazardous Agents

Use of hazardous agents must be stated and the agents identified. For projects employing radioactive materials, infectious agents, hazardous chemical agents, recombinant DNA, carcinogens, human tissue, human embryonic stem cells, and/or human pluripotent stem cells (including induced pluripotent stem cells) a copy of this protocol will be forwarded to the Microbiological Safety Committee, the Radiation Safety Committee and/or the Human Embryonic Stem Cell Review Committee. Final approval will not be granted until the protocol has been approved by the appropriate Safety/Review Committee(s).

Will you be using agents potentially hazardous to humans or animals?	YES
--	-----

Occupational Health Information

Risk Category	
---------------	--

[Table of Contents](#)

VIII.)a.) Hazardous Use Info

Please indicate if you will be using any of the following hazardous agents in this protocol. Specific questions pertaining to each of them will be asked in another section.

Infectious Agents will be used?	YES
Radioactive Materials will be used?	NO
Hazardous Chemical will be used?	YES
Human Tissue, human embryonic stem cells, and/or human pluripotent stem cells (including induced pluripotent stem cells)?	NO

[Table of Contents](#)

IX. Species Use Question

ANSWER YES TO THE QUESTION "WILL YOU BE USING LIVE ANIMALS IN THIS PROTOCOL"?
Please answer the following questions concerning anticipated activities for this protocol.

Will you be using Live Animals in this protocol?	YES
Please provide the rationale for the use of animals in your research.(The rationale should include reasons why computer models, tissue culture or other	Metabolism is a complex interplay between tissues within an organism. We study metabolism from two vantage points: 1) We study metabolism in cell culture (where variables can be controlled and 2) We study metabolism in context within the live mammal to determine whether our reductionist cell culture model can be validated in the animal model. In order for computer models to exist, a priori information must inform the model. That is the purpose of

In vitro models cannot be used.)	these experiments, which elucidate the role of sugar transporters in mammalian metabolism - that others (in vivo and in silico biologists alike) may understand better how these transporters function.
Will any drugs, including controlled substances, be administered to animals?	YES
Will videos, photographs or electronic media of experimental animals, be recorded?	No
If yes, please describe use and the method of dissemination. Recorded materials must be stored in a secure location (e.g. locked desk or cabinet in a locked room).	
Requested Support: If there are any special services or support that you would like to discuss with the Division of Comparative Medicine, such as suggestions for anesthesia, euthanasia, or other concerns; please describe.	

[Table of Contents](#)

IX.)a.) Housing Outside Central Animal Holding Facility

If you request housing outside of the central animal holding facility greater than 12 hours for nonrodent mammals or 24 hours for rodents, provide scientific justification. The ASC strongly discourages lab housing of animals but will evaluate requests on an individual case basis.

[Housing of Warm-Blooded Vertebrates HTML](#)

Will rodents be housed outside the central facility for more than 24 hours?	NO
Location	
Scientific Justification for housed outside more than 24 hours	

Will non-rodent mammals or non-rodent vertebrate species be housed outside the central facility for more than 12 hours?	NO
Location	
Scientific Justification for housed outside more than 12 hours	

[Table of Contents](#)

IX.)b.) Type of Animal Use

Identify all types of animal use for this protocol.

Which of the following describes the type of animal use proposed in this application?
(check all that apply)

Research Type (Core,Basic,Field)	Basic
Instructional or Training	
Testing (toxicology, etc.)	Yes
Services - Sentinels	
Services - Other	
Other (Specify)	

IX.)c.) Use of Human Clinical Areas

Will animals be imaged or treated in Human Clinical areas?	NO
--	----

[Table of Contents](#)

IX.)d.) Transportation of Animals

Transportation of animals outside the central facility must follow guidelines set by the ASC. Please refer to the ASC guideline:

[Animal Transport and Use in Public Access Areas, Section A - HTML](#)

[Animal Transport and Use in Public Access Areas, Section A - PDF](#)

Will you be moving animals through public access areas?	YES
Describe methods and containment to be utilized. Include the route and elevator(s) to be utilized.	Ice-cream containers with air-holes punched in the lids will be used to transport any live animals to the laboratory. All containers will be carried in a black garbage bag provided by the animal facility to conceal contents. I will use the BJCIH elevators from the basement to the 2nd floor bridge/skywalks to travel to the MPRB where my laboratory is located. To reach the Cardiovascular Phenotyping Core, we will take our mice either from our BJCIH facility or from the CSRB basement using the underground walkways (key access) to the CSRB elevators. Those will be taken to the first floor CSRB back entrance to the Core. Dr. Kovacs or Mrs. Weinheimer are notified in advance of arrival and will permit entry into the Core thereafter.
Will live animals be transported to your laboratory?	Yes
Location of laboratory (Building and Room Number)	MRPB 5th floor lab entry #3

[Table of Contents](#)

IX.)e.) Animal Species List

Indicate which species you propose to use for this study and follow the directions below to choose the correct button.

1) IF A FIELD STUDY PROTOCOL: Please choose "FIELD STUDY" as the species

2) "ADD SPECIES" Button: Click and "add species" and it will open a new page where you can choose a species. Once you have chosen the species, it will disappear from the drop-down list.

3) "EDIT SPECIES" Button: If you are renewing a protocol, click the "select" button and then click on "edit species", so the original information can be downloaded and available to edit. You will then proceed through each section of your expiring protocol information to perform edits and updates (i.e., numbers, procedures, etc).

4) "COPY SPECIES" Button: Click the "select" button of the species you want to copy (i.e., you want to add rat vs. mouse and edit doses, etc.) and you will open a new page asking you to choose the new species (remember that the original species will have disappeared from the drop-down list). You will then go through each section for the new species to edit the information, as necessary.

5) "REMOVE SPECIES" Button: Click the "select" button to remove a specific species from the protocol.

REMEMBER TO "SAVE AND CONTINUE" BETWEEN EACH SECTION OR YOUR DATA WILL BE LOST.

Protocol Animal Species List

Species
Mouse

[Table of Contents](#)

IX.)e.)1.) Species Information

Choose proposed species from the pick list. If species is not available, contact the ASC office at 362-3229.

Species Activities

Will drugs be administered on this Species?	Yes
Will animals be euthanized?	Yes
If not, what will be done to animals after the experiment or project is completed?	
Will non-surgical procedures (i.e. blood collection, tissue collection, etc.) be performed?	Yes
Will surgeries be performed?	Yes
Will animals be restrained?	No
Will Hazardous Agents be used?	Yes
Breeding	YES

Please indicate the total number of animals you will need for all three years of the protocol.

Species	Mouse
Total number of requested animals	7200

[Table of Contents](#)

IX.)e.)2.) Justification For Number Requested

Justify the number of animals to be used. Include the definition of experimental groups and animals needed per group based on statistical criteria.

Include estimated number of animals for breeding.

Species Name	Mouse
--------------	-------

What is your justification for number of animals requested?
For metabolic experiments, our typical experimental design is to run a minimum of four groups: an untreated control, a positive control for stimulus, negative inhibitor control and a stimulus + inhibitor experimental arm. We on average run 3 cohorts for diet, drug and antisense optimization at n = 15 per experimental group per cohort, then run a minimum of 3 optimized experimental replicate cohorts (therefore, 6 total cohorts per experiment, including optimization runs). We will run GTI-421 studies with and without antisense oligo and shRNA-based knockdown for mechanistic studies. To assure that our results are not due to non-specific gene knockdown by our constructs, we run 2-3 different target-based antisense oligos and 2-3 different shRNA. In sum, for our metabolic studies, 6 cohorts per experiment X (GTI alone; GTI with and without 3 Antisense constructs; GTI with and without 3 shRNA constructs for a total of 7 major experiments) = 42 cohorts of mice. 42 major experimental cohorts X minimum 60 mice per cohort (at four groups, n = 15 per group) = 2520 mice.
TAC studies are designed similarly. Our typical design is to run a minimum of four groups: an untreated control, a TAC control for

stimulus, negative inhibitor control and a TAC + inhibitor experimental arm. We again, on average, will run 3 cohorts for diet, drug and antisense optimization at n = 15 per experimental group per cohort, then run a minimum of 3 optimized experimental replicate cohorts (therefore, 6 total cohorts per experiment, including optimization runs). We will run GTI-421 studies with and without antisense oligo and shRNA-based knockdown for mechanistic studies. Again, to assure that our results are not due to non-specific gene knockdown by our constructs, we run 2-3 different target-based antisense oligos and 2-3 different shRNA. In sum, for our metabolic studies, 6 cohorts per experiment X (GTI alone; GTI with and without 3 Antisense constructs; GTI with and without 3 shRNA constructs for a total of 7 major experiments) = 42 cohorts of mice. 42 major experimental cohorts X minimum 60 mice per cohort (e.g. four groups, n = 15 per group) = 2520 mice.

We request additional mice to accommodate our breeding protocol. We require 20 breeders to generate genetic models used in subsequent studies. This is based on 4 genotypes entering our colonies, with 5 breeding cages each. These will produce on average (6 mice per litter) 120 mice every 2 months for experimentation (720 / year). Over the remaining life of this protocol (3yr) this will be an additional 2160 mice. Total mice on the protocol is now 2520 + 2520 + 2160 = 7200 mice

Attachments List

File Spec	Description	Created
-----------	-------------	---------

[Table of Contents](#)

IX.)e.)3.) Justification For Choice of Species

Justify the appropriateness of the species selected.

Species Name	Mouse
--------------	-------

Provide rationale for the use of the specific species selected:

Mice are well-established and well-accepted models of mammalian metabolism. Moreover the array of reagents available to study murine models allows us to perform the highest-quality mechanistic studies possible with an array of genetic tools, the mouse genome sequence available, and commercial antibodies readily made for widespread use. There are no lower vertebrate model systems currently in use to model mammalian metabolism that are accepted in the field.

[Table of Contents](#)

IX.)e.)6.) Housing Location

Species Name	Mouse
--------------	-------

Select	Facility Name
	B J C Insitute Of Health @ W U S M
	CSRB Basement

[Table of Contents](#)

IX.)e.)6.)A.) Protocol Housing Location Information

Species Name	Mouse
--------------	-------

Facility Name	B J C Insitute Of Health @ W U S M
Location Type	HOUSING

[Table of Contents](#)

IX.)e.)6.)A.) Protocol Housing Location Information

Species Name	Mouse
--------------	-------

Facility Name	CSRB Basement
Location Type	HOUSING

[Table of Contents](#)

IX.)e.)7.) Use Location

Species Name	Mouse
--------------	-------

Select	Facility Name	Room No
	B J C Insitute Of Health @ W U S M	0B146
	CSRB Surgery	1108
	McDonnell Pediatric Research Bldg	05303
	CSRB Surgery	1124, 1125, 1110

[Table of Contents](#)

IX.)e.)7.)A.) Protocol Use Location Info

When entering Room Numbers please follow the University Space System's naming conventions.

Species Name	Mouse
--------------	-------

Facility Name	McDonnell Pediatric Research Bldg
Facility Room Number	05303
Location Type	USE
Location where experimental procedures will be performed	Yes
Location where surgeries will be performed	No
Location where postoperative recovery will be monitored after survival surgeries	No

[Table of Contents](#)

IX.)e.)7.)A.) Protocol Use Location Info

When entering Room Numbers please follow the University Space System's naming conventions.

Species Name	Mouse
--------------	-------

Facility Name	B J C Insitute Of Health @ W U S M
Facility Room Number	0B146
Location Type	USE
Location where experimental procedures will be performed	Yes
Location where surgeries will be performed	No
Location where postoperative recovery will be monitored after survival surgeries	No

[Table of Contents](#)

IX.)e.)7.)A.) Protocol Use Location Info

When entering Room Numbers please follow the University Space System's naming conventions.

Species Name	Mouse
--------------	-------

Facility Name	CSRB Surgery
Facility Room Number	1108
Location Type	USE
Location where experimental procedures will be performed	YES
Location where surgeries will be performed	YES
Location where postoperative recovery will be monitored after survival surgeries	YES

[Table of Contents](#)

IX.)e.)7.)A.) Protocol Use Location Info

When entering Room Numbers please follow the University Space System's naming conventions.

Species Name	Mouse
Facility Name	CSRB Surgery
Facility Room Number	1124, 1125, 1110
Location Type	USE
Location where experimental procedures will be performed	Yes
Location where surgeries will be performed	Yes
Location where postoperative recovery will be monitored after survival surgeries	Yes

[Table of Contents](#)

IX.)e.)9.) Drugs

Please list the drugs that will be administered to animals in this study.

Species Name	Mouse
--------------	-------

Agent	Type	Drug Dosage	Route of Administration	Frequency of Administration	Admin Reason
Antisense Oligonucleotide	Other	25mg/kg/day; ~200ul/mouse, At 10ul/gram Mouse Weight	IP	Twice Weekly	Genetic knockdown of hepatic metabolic pathway intermediates considered to play a role in the GTI-421 and other generalized physiological fasting response.
GTI-421	Other	2-20mg/kg/day In 1% Weight/weight Chow	PO	Ad Libitum (we Based The 10mg/kg/d Dose On Avg Daily Feed Mass	Anti-diabetic and anti-steatotic properties with unknown mechanism of action. We are administering this Rx into mouse genetic models to understand its mode of action.
Isoflurane	Anesthetic	5% Inhaled Isoflurane X 5minutes	IH (inhalation)	Once - Lethal	Isoflurane administered for analgesia preceding cervical dislocation and primary murine hepatocyte isolation protocol. Dose based on IACUC guidelines in - http://animal.research.uiowa.edu/iacuc-guidelines-anesthesia

[Table of Contents](#)

Drug Information

For assistance in determining species-appropriate drugs and dosages, contact ARC veterinarian...

Drug Name	Isoflurane
Drug Type	Anesthetic
If controlled substance, please provide an estimate of total volume required for use in this species during the course of this protocol. If not a controlled substance please enter 0. Click here for the Controlled Substance List.	0
Route of administration (check all applicable)	IH (inhalation)
Dose (volume and concentration)	5% Inhaled Isoflurane X 5minutes
Frequency of Administration	Once - Lethal
Reason for Administration	Isoflurane administered for analgesia preceding cervical dislocation and primary murine hepatocyte isolation protocol. Dose based on IACUC guidelines in - http://animal.research.uiowa.edu/iacuc-guidelines-anesthesia

Species Name	Mouse
--------------	-------

Drug Information

For assistance in determining species-appropriate drugs and dosages, contact ARC veterinarian...

Drug Name	GTI-421
Drug Type	Other
If controlled substance, please provide an estimate of total volume required for use in this species during the course of this protocol. If not a controlled substance please enter 0. Click here for the Controlled Substance List.	N/A
Route of administration (check all applicable)	PO
Dose (volume and concentration)	2-20mg/kg/day In 1% Weight/weight Chow
Frequency of Administration	Ad Libitum (we Based The 10mg/kg/d Dose On Avg Daily Feed Mass
Reason for Administration	Anti-diabetic and anti-steatotic properties with unknown mechanism of action. We are administering this Rx into mouse genetic models to understand its mode of action.

Species Name	Mouse
---------------------	-------

Drug Information

For assistance in determining species-appropriate drugs and dosages, contact ARC veterinarian...

Drug Name	Antisense Oligonucleotide
Drug Type	Other
If controlled substance, please provide an estimate of total volume required for use in this species during the course of this protocol. If not a controlled substance please enter 0. Click here for the Controlled Substance List.	0
Route of administration (check all applicable)	IP
Dose (volume and concentration)	25mg/kg/day; ~200ul/mouse, At 10ul/gram Mouse Weight
Frequency of Administration	Twice Weekly
Reason for Administration	Genetic knockdown of hepatic metabolic pathway intermediates considered to play a role in the GTI-421 and other generalized physiological fasting response.

Species Name	Mouse
---------------------	-------

IX.)e.)10.) Hazardous Agents

For projects employing radioactive materials, infectious agents, hazardous chemical agents, recombinant DNA, carcinogens, human tissue and/or human embryonic stem cells, a copy of this protocol will be forwarded to the Microbiological Safety Committee, the Radiation Safety Committee and/or the Human Embryonic Stem Cell Review Committee. Final approval will not be granted until the protocol has been approved by the appropriate Safety/Review Committee(s).

Species Name	Mouse
--------------	-------

[Table of Contents](#)

Biohazard Agents Information

Biohazardous agents administered to animals must be defined, including safety plan for personnel

Hazardous Agent	DNA (recombinant)
Category	
Class/Biosafety Level	
Agent Biosafety Committee #	
Expires On	/ /

Species Name	Mouse
--------------	-------

[Table of Contents](#)

Biohazard Agents Information

Biohazardous agents administered to animals must be defined, including safety plan for personnel

Hazardous Agent	Adeno-associated virus
Category	All
Class/Biosafety Level	
Agent Biosafety Committee #	
Expires On	/ /

Species Name	Mouse
--------------	-------

[Table of Contents](#)

IX.)e.)11.) Non-Surgical Procedures

Please check the "SELECT" box to add and describe a non-surgical procedure that you will conduct.
(DO NOT UNCHECK THE CHECK BOX UNLESS YOU WANT TO DELETE THE PROCEDURE)

Select	Procedure Name	Procedure Description	Additional ?s
YES	**NON-Surgical Procedure 01		YES
YES	**NON-Surgical Procedure 02		YES
YES	**NON-Surgical Procedure 03		YES
YES	**NON-Surgical Procedure 04		YES
YES	**NON-Surgical Procedure 05		YES

Species Name	Mouse
--------------	-------

[Table of Contents](#)

IX.)e.)11.)A.) Non-Surgical Procedure Information

Please answer the following questions regarding the non-surgical procedure(s) you will be conducting.

Species Name	Mouse
--------------	-------

**NON-Surgical Procedure 01	
A. Describe all procedures and	Mouse blood will be collected from the facial vein prior to sacrifice. No more than 200 microliters will be collected at any given time. Rapid collection through the facial vein is required because we are measuring

experimental manipulations.	transient, unstable metabolites that can change quickly over time in a euthanized animal (e.g. serum lactate, pyruvate).
B. For each species, list anesthetics, analgesics and paralyzing agents used. For each, list agent, dose and route of administration.	No analgesia will be utilized.
C. List probable clinical response to and potential complications of the experiments and indicate how these complications will be managed.	The most likely complication of this procedure is collecting insufficient blood from the facial vein on one side of the animal. This is a rare complication in experienced hands (e.g. one in every 50 mice will require a second puncture for an experienced handler). In these cases the contralateral facial vein will be probed for blood for a total collection of no more than 200uL blood per mouse. No mouse will be punctured more than twice on a given day. Mice will be sacrificed immediately following the procedure.
D. Ascites production used to produce antibodies requires justification as to why in vitro systems cannot be used.	
E. State duration and endpoints of experiment (e.g., animals will have an echocardiogram at 6 weeks and be euthanized to collect tissue).	Mice will be euthanized to collect tissue at approximately 12 weeks of age (older if maintained on a specialized diet, such as a high-sugar diet).
**NON-Surgical Procedure 02	
A. Describe all procedures and experimental manipulations.	Mice will be maintained on a chow or high-sugar diet (60% fructose diet, obtained from Harlan Teklad) for up to 24 weeks.
B. For each species, list anesthetics, analgesics and paralyzing agents used. For each, list agent, dose and route of administration.	No analgesics are to be utilized for this procedure.
C. List probable clinical response to and potential complications of the experiments and indicate how these complications will be managed.	The most likely response is that the high-sugar diet induces a metabolic syndrome-like state (e.g. dyslipidemia, high blood pressure, possibly high fasting blood sugar). The animals do not appear obese or overtly "ill" on this diet (e.g. picture up to 30% of American adults is walking around with metabolic syndrome without knowing they have this condition)
D. Ascites production used to produce antibodies requires justification as to why in vitro systems cannot be used.	
E. State duration and endpoints of experiment (e.g., animals will have an echocardiogram at 6 weeks and be euthanized to collect tissue).	Animals 6wk in age will be placed on diet for up to 24 weeks and euthanized for tissue collection.
**NON-Surgical Procedure 03	
A. Describe all procedures and experimental manipulations.	Mice will be administered several agents to determine the mechanism of action of fasting responses in modulating diabetic progression. GTI-421 is a novel anti-diabetic compound that blocks hepatic glucose transporters and mimics fasting responses. Therefore, we will not have to fast or food restrict mice to obtain the fasting-like effect. Instead, we will administer this drug to mimic fasting responses. We will administer this drug pulverized into powder form through the diet for up to 24 weeks, PO, Ad libitum. ASOs will be administered at up to 10ug/kg/day in 50-150uL normal saline IP once daily three times weekly for up to four weeks. Adeno-associated virus will be administered at 10 ¹¹ particles per animal, 50-100uL in saline, one dose, per tail vein injection.
B. For each species, list anesthetics, analgesics and paralyzing agents used. For each, list agent, dose and route of administration.	No analgesics are to be utilized for these procedures
C. List probable clinical response to and potential complications of the experiments and indicate how these complications will be managed.	In preliminary in vitro studies, GTI-421 activates canonical hepatic starvation pathways, including FGF21. Therefore we anticipate fat loss and increased heat production in mice treated with this compound. Antisense oligonucleotides and adeno-associated virus will also be administered. Based upon published studies demonstrating minimal effects in control mice regarding histological inflammation, cytokine release, etc. we do not anticipate significant inflammatory or malaise responses to AAV administration. ASO administration similarly has been used in histological studies in the liver and does not show any demonstrable effects on hepatic histology, inflammation or cytokine release. Animals will be monitored daily (including weekends and holidays) by DCM Vet staff. DeBosch Lab personnel will monitor at 3-5x/weekly during experimental protocols involving administering these agents. Mice dosed with experimental agents will be directly observed behaviorally for normal movement, activity, breathing, sociability within the cage during and after agents are administered. In addition, mice will be weighed 1-3x/week as part of the monitoring procedure. Evidence of weight loss below 90% of initial weight will prompt closer observation. In consultation with DCM veterinary staff and after interpretation of weight loss with

	other clinical symptoms of the mouse (behavior, sociability, appropriate feeding mass of ~1 pellet or 5g/mouse/day), mice having lost significant weight will be killed to avoid undue suffering as necessary. In addition, DeBosch Lab personnel will be on call 24hr/day, 7d/week to respond to any concerns raised by DCM staff. If any of the agents are found to unexpectedly make the animals "sick" (loosely defined by presence of hunching, fluffing, lost grooming, unanticipated weight loss, any concerns by DCM or DeBosch Lab staff), the situation will be remedied by euthanizing the animal(s) in question.
D. Ascites production used to produce antibodies requires justification as to why in vitro systems cannot be used.	
E. State duration and endpoints of experiment (e.g., animals will have an echocardiogram at 6 weeks and be euthanized to collect tissue).	Animals will be euthanized to collect tissue at a maximum timepoint of 24wk of age, depending on specific experimental protocol upon assay optimization.
**NON-Surgical Procedure 04	
A. Describe all procedures and experimental manipulations.	Tail clipping for genotyping. We will clip tails of mice at age 20 days or less. From: Jones, Carissa P., Scott Carver, and Lon V. Kendall. "Evaluation of Common Anesthetic and Analgesic Techniques for Tail Biopsy in Mice." Journal of the America Association for Laboratory Animal Science 51.6 (2012): 808-14. Procedure: All mice to be genotyped are age 20 days or younger. Gently, manually restrain animal. Snip tail with sanitized sharp scissors or disposable blade, length < 5mm will be snipped. Tail tip placed into a tissue collection tube. Check animal for bleeding, if bleeding occurs: Apply pressure to the cut portion of the tail with gauze until bleeding has stopped. Return animal to its cage Clean off biologic material (e.g. blood or fur) from scissors and sanitize after each snipping.
B. For each species, list anesthetics, analgesics and paralyzing agents used. For each, list agent, dose and route of administration.	No Analgesia required
C. List probable clinical response to and potential complications of the experiments and indicate how these complications will be managed.	Typical response to tail clipping is for 1-10uL bleeding, then coagulation at the wound site. If bleeding above 10uL without immediate coagulation occurs: we will apply pressure to the cut portion of the tail with gauze until bleeding has stopped.
D. Ascites production used to produce antibodies requires justification as to why in vitro systems cannot be used.	
E. State duration and endpoints of experiment (e.g., animals will have an echocardiogram at 6 weeks and be euthanized to collect tissue).	The duration of this experiment on the animal lasts about 60 seconds to handle the mouse, then obtain tail clippings. Post-monitoring will last approximately 3-5' to assure bleeding is adequately coagulated at the wound. Tail tissue endpoint will be PCR genotyping.
**NON-Surgical Procedure 05	
A. Describe all procedures and experimental manipulations.	Anesthetized mice will be imaged in the left lateral decubitus position on a Sequoia cardiac echocardiography machine (Acuson Co, Malvern, Pa) equipped with a 15-MHz linear transducer (15-L8). Two-dimensional parasternal long- and short-axis views were recorded, as was 2D targeted M-mode tracings throughout the anterior and posterior LV walls.
B. For each species, list anesthetics, analgesics and paralyzing agents used. For each, list agent, dose and route of administration.	Per MCPC protocol.
C. List probable clinical response to and potential complications of the experiments and indicate how these complications will be managed.	An adverse response to anesthesia is theoretically possible. This would be manifest by an anaphylactoid reaction and sudden death. (I have never observed an anesthesia reaction in mice post-echocardiography, however.) If this complication does occur, the mice will appear acutely ill clinically, hunched and fluffed, non-social, low locomotion. In this case the mouse will be sacrificed immediately to avoid pain and suffering. Mice will be monitored the same day following echo to assure clinical stability post-procedurally.
D. Ascites production used to produce antibodies requires justification as to why in vitro systems cannot be used.	
E. State duration and endpoints of experiment (e.g., animals will have an echocardiogram at 6 weeks and be euthanized to	Echo takes 15-45'. The mice are sacrificed the next day for tissue harvest and post-mortem analysis.

IX.)e.)12.) Surgical/Experimental Procedures

Species Name	Mouse
--------------	-------

Surgery Type
transverse aortic constriction

Protocol Surgery Type - Multiple Major Surgeries

Multiple Surgeries

No animal should undergo more than one operative procedure without strong scientific justification. Multiple surgeries may be justified when separate surgical sessions are needed to repair/replace implanted device, to create and later repair a lesion, to obtain serial tissue samples, or to perform other surgical procedures that require a time intermission. Performing surgery on more than one body site during a single anesthetic session does not constitute multiple surgeries.

Will any animal undergo more than one surgical procedure?	NO
---	----

Provide clinical/scientific justification for multiple surgeries.	
---	--

Attachments List

File Spec	Description	Created
-----------	-------------	---------

IX.)e.)12.)A.) Surgical/Experimental Procedures

Surgical Locations

Select location(s) in which surgeries are performed

Surgical Location	Room Number
B J C Insitute Of Health @ W U S M..0B146	0B146

Species Name	Mouse
--------------	-------

Type of surgery/experiment to be performed	transverse aortic constriction
A. Describe surgery in detail , including presurgical preparation, anesthetic regimen, and surgical technique. For Survival Surgeries address the following: 1.Include the aseptic methods to be utilized. 2.Describe methods to alleviate postoperative pain.	Surgery will be performed by the mouse cardiovascular phenotyping core using their existing protocols. All mice will be returned to our care after 24 hours monitoring in the MCPC. We will assume care of post-op animals for any needed suture removal and post-op monitoring from that time forward. Animals will be housed in the CSRB basement until an end echo is performed. All mice will receive post-op analgesics administered by the MCPC after this procedure. Analgesics will be Buprenex or Buprenorphine-SR according to the MCPC protocol.
B. For each agent used, list dose and route of administration.	As per mouse cardiovascular phenotyping core protocol
C. State criteria to assess level of anesthesia (loss of pain reflex, respiratory/circulatory function, etc.).	As per mouse cardiovascular phenotyping core protocol
D. State probable clinical responses and potential complications and indicate how these will be controlled.	The most likely clinical response for mice undergoing this proedure is to undergo adaptive cardiac hypertrophy to compensate for the increased afterload induced by TAC (see DeBosch,et al, Circulation 2006). However, in some cases, acute heart failure can occur infrequently (~5%)in which the mice fail to adequately compensate immediately after the procedure. In these cases, the decompensation is clinically obvious: the mouse will become less active, respiratory rate elevates, and the mouse will hunch and fluff within the first 24hr post-operatively. We will therefore monitor the mice daily after the procedure on post-op days 1-3, then every other day throughtout the remainder of the protocol. In those cases, mice will be sacrificed to avoid pain and suffering.

E. State duration and endpoints of experiment.	As per mouse cardiovascular phenotyping core protocol. Duration will be 2 weeks, and mice will be sacrificed 2 weeks post-operatively and tissues harvested for post-mortem analysis.
Is this a Survival or Non-survival Type of Surgery/Experiment	Survival

[Table of Contents](#)

IX.)e.)13.) Breeding Information

If breeding mice, strain choice may require written authorization from vendor

Species Name	Mouse
--------------	-------

Will breeding colony be maintained?	YES
Will <u>transgenic</u> breeding be used?	YES

Strain/stock	Generation	Breeding Scheme	Genetic Monitoring Program	Sub line/Sub strain Designation
C57/b6 / Vil-cre		Either	YES	
C57/b6 / Glut9(flox)		Either	YES	
C57/b6 / Lc3-gfp / Glut8(ko)		Either	YES	
Sv129 / Glut8(ko)		Either	YES	
C57/b6 / Fgf21(flox)		Either	YES	

[Table of Contents](#)

IX.)e.)14.) Humane Use Animal Categories

The USDA categories marked as required (with a red *) below were determined to be appropriate based on the procedures you reported. Please enter the number of animals requested for each of the required categories. The total of the required fields may not exceed your total requested number.

Class C Description Examples

Animals upon which teaching, research, experiments, or tests were conducted involving no pain, distress, or use of pain relieving drugs.

- *euthanasia for tissue collection
- *blood/body fluid collection
- *adjuvant administration
- *antibody production
- *sight restriction
- *behavioral training
- *hypothermia (conscious)
- *hyperthermia (conscious)
- *food/water deprivation <18 hours
- *physical restraint <12 consecutive hours
- *escapable pain induction.

Class D Description Example

Animals upon which experiments, teaching, research, surgery, or tests were conducted involving accompanying pain or distress to the animals and for which appropriate anesthetic, analgesic or tranquilizing drugs were used.

- *surgery (survival and/or nonsurvival)
- *death as endpoint, with pain or distress relief
- *full thickness burn studies
- moribund state as endpoint
- *food/water deprivation 18-48 hours.

Class E Description Examples

Animals upon which teaching, experiments, research, surgery, or tests were conducted involving accompanying pain or distress to the animals and for which the use of appropriate anesthetic, analgesic or tranquilizing drugs would have adversely affected the procedures, results, or interpretation of the teaching, research, experiments, surgery or tests.

- *death as an endpoint
- *inescapable or chronic pain induction
- *food/water deprivation >48 hours
- *physical restraint >12 consecutive hours

Species Name	Mouse
---------------------	-------

Pain Level	# of Animals	Description
C	7000	
D	200	

Total # Requested for Species	7200
--------------------------------------	------

[Table of Contents](#)

IX.)e.)15.) Euthanasia

State agent, dose and route of administration. Contact DCM for assistance if required. Animals that appear to be in severe pain or distress or which are moribund will be euthanized unless scientific justification is provided by the investigator and approved by the ASC. Any experiment that involves death as a dependent variable must be scientifically justified.To view the American Veterinary Medical Association report on euthanasia [AVMA Website - Euthanasia Report](#)

Species Name	Mouse
---------------------	-------

Select	Euthanasia Method
104	Carbon dioxide overdose followed by cervical dislocation
135	Inhalant anesthetic overdose followed by cervical dyslocation

[Table of Contents](#)

IX.)e.)15.)A.) Euthanasia Method Information

To view the American Veterinary Medical Association report on euthanasia [AVMA Website - Euthanasia Report](#)

Species Name	Mouse
---------------------	-------

Euthanasia Method	Carbon dioxide overdose followed by cervical dislocation
Euthanasia Drugs: List drugs and the dose to be administered.	CO2 - lethal dosage as administered in SMART boxes in the BJCIH animal housing facilities. Approximately 5 minutes CO2 exposure.
Any experiment that involves death as a dependent variable must be scientifically justified.	

[Table of Contents](#)

IX.)e.)15.)A.) Euthanasia Method Information

To view the American Veterinary Medical Association report on euthanasia [AVMA Website - Euthanasia Report](#)

Species Name	Mouse
---------------------	-------

Euthanasia Method	Inhalant anesthetic overdose followed by cervical dyslocation
Euthanasia Drugs: List drugs and the dose to be administered.	Isoflurane / isothesia 5% inhaled.
Any experiment that involves death as a dependent variable must be scientifically justified.	

[Table of Contents](#)

IX.e.)16.) SOP Exemptions

Please specify any Non-Standard Operating Procedures that may be performed with this species on this protocol.

- Examples Include:
- Non DCM-approved vendor/source for animals
 - Circumvention or alteration of Quarantine
 - Alteration or restriction of diet (fasting)
 - Alteration or restriction of water
 - Irregular Cage Changing
 - Bedding materials
 - Weaning Dates (>21 days for mice or rats)
 - Cage Population Density - e.g., single housing
 - Animal Identification Method
 - Environmental Parameters, such as reversed light cycles, humidity levels, etc...

Species Name	Mouse
--------------	-------

Will there be any SOP Exemptions for this species?	YES
Will any animals be housed one per cage?	
If yes, please describe these exemptions and provide a justification for them.	My mice will be placed on a special (high-sugar) diet (Obtained from Harlan Teklad, # TD89247)in order to induce metabolic disease. This is required so that we can study the role of our sugar transporters, GLUT8 and GLUT9, in initiating or protecting from metabolic disease. Our mice do not spontaneously develop metabolic disease in the absence of this diet. In addition, we place mice on regular rodent chow mixed with GTI-421. This mode of delivery is chosen for two reasons: 1) PO route of delivery for this drug we have determined because it delivers drug most efficiently to the liver via the portal circulation, where the efficacy is greatest and 2) PO route was thought to be the most humane route that induces the lowest achievable pain for administration to complete our studies in a rigorous manner.

[Table of Contents](#)

X. Schedule B Questions

For projects employing radioactive materials, infectious agents, hazardous chemical agents, recombinant DNA, carcinogens, human tissue, human embryonic stem cells, and/or human pluripotent stem cells (including induced pluripotent stem cells), the ASC will forward a copy of this protocol to the Microbiological Safety Committee, the Radiation Safety Committee and/or the Human Embryonic Stem Cell Review Committee. Final approval will not be granted until the protocol has been approved by the appropriate Safety/Review Committee(s).

SCHEDULE B

Check off and complete if any of the following will be used: immunizations, biological fluid collections, tumors and/or cell lines, DNA, human tissue, human embryonic stem cells, and/or human pluripotent stem cells (including induced pluripotent stem cells), radioactive materials or biohazardous agents.

Immunizations will be used
No
Provide the following Information Species: Immunogen: Primary adjuvant: Booster adjuvant: Route of administration: Injection site: Volume per site: # of sites injected: # of boosters:

Frequency of boosters:
Biological fluids (blood, lymph, bile, CSF, etc.) will be collected
Yes
Provide the following details Species: Types of fluids collected: Volume & frequency: Site of collection:
Species: mouse Fluid: Blood Volume: < 200microliters, frequency: once Site of collection: left or right facial vein
Use of Tumors, Cell Lines and/or Ascites (including hybridoma inoculation for monoclonal antibody production).
No
Provide the following information Species inoculated: Species of tumor origin or cell line: Inoculation site: Endpoint for (tumor) harvest/passage:
For hybridomas, indicate priming agent, dosage and route of administration:
If tumors or cell lines (including embryonic stem cells and/or human pluripotent stem cells) are used in rodents, please indicate explicitly whether or not these have been tested and found to be free of rodent viruses and/or mycoplasmas. Mouse/Rat Antibody Production Test (MAP, RAP) is used to test cells or tumors for the presence of rodent viruses before using the cells in mice or rats. Cells may carry viruses (e.g. mouse hepatitis virus) that could become active in inoculated mice or rats. The MAP test is performed by DCM (362-3125), and consists of inoculating mice/rats with the experimental cells, collecting serum four weeks after inoculation, and testing for viral antibody.
With respect to tumor induction procedures (for acquisition of cells or study of tumor response) please specify: Species: Carcinogen: Route of administration: Tumor type: Site of development:
Radioactive materials / Irradiation will be used with live animals. If answered YES, your protocol will be sent to the Radiation Safety Committee for approval.
No
List species exposed, Isotope, Quantity (mCi) and/or Irradiator used (by location).
Infectious agents will be used in live animals. If answered YES, your protocol will be sent to the Microbioloical Safety Committee for approval.
No
List biological agents and species exposed.
Recombinant DNA will be used in this animal study. If answered YES, your protocol will be sent to the Microbiological Safety Committee for approval.
Yes
Carcinogens or hazardous chemical agents will be used in animals. If answered YES, your protocol will be sent to the Microbiological Safety Committee for approval.
No
List agent(s) and species.
Adeno-associated virus will be used to obtain differential CNS, cardiac, liver and CNS transgenic expression. This will be used in species: mouse.
Human tissue will be used in live animals.
No
Tissue has been /will be tested for human pathogens
No
Human embryonic stem cells and/or human pluripotent stem cells (including induced pluripotent stem cells) will be used in this animal study?
No
For projects employing radioactive materials, infectious agents,

hazardous chemical agents, recombinant DNA, carcinogens, human tissue, human embryonic stem cells, and/or human pluripotent stem cells (including induced pluripotent stem cells) please:

1. Describe expected clinical effects and expected mortality.

Expected mortality from AAV infection: none
Clinical effects: Minimal, which has facilitated approval of AAV vector use in multiple human clinical trials (Daya and Berns, Clin Microbiol Rev 2008). Some reports of transient transaminase elevations were detected in some small human trials (Daya and Berns, 2008), and slight elevations of IL-10 were noted in other groups, however, no description of clinically significant symptoms have been reported.

2. State experimental endpoint criteria (e.g., tumor size, percentage body weight gain or loss, inability to eat or drink, behavioral abnormalities, clinical symptomatology, or signs of toxicity) when the administration of tumor cells, biologics, infectious agents, radiation or toxic chemicals are expected to cause significant symptomatology or are potentially lethal.

N/A - the agents administered are not expected to cause symptomatology or lethality.

3. List the criteria to be used to determine when euthanasia is to be performed. Death as an endpoint must always be scientifically justified.

Euthanasia is not expected in our protocol, nor do we expect to observe in any of our experimental conditions clinical symptoms that would require euthanasia. However, if any of the following are observed, we will consider euthanizing experimental animals in conjunction with our veterinary technician and/or attending DVM:
1) exhibiting malaise, systemic illness or depression (e.g. hunched, fluffed demeanor)
2) weight loss > 20% from initial weight
3) Irreparable injury
4) At the recommendation for any indication, by the DVM or Vet Tech.

[Table of Contents](#)

XI.) Personnel List

BEFORE A PROTOCOL CAN BE REVIEWED BY THE VETERINARIAN, SCHEDULE A FORMS MUST BE COMPLETED, ATTACHED AND SPECIFIC TO THE PROTOCOL. PLEASE MAKE SURE ALL PROCEDURES INCLUDE SPECIES, AND IF SURGICAL PROCEDURES ARE TO BE PERFORMED, THE NAME OF THE PROCEDURE, TRAINING TYPE AND YEARS OF EXPERIENCE.

Complete an UPDATED [Schedule A \(Training Documentation Log\)](#) form and attach it to this protocol
Name all personnel working on this project. The individual(s) listed on this protocol must be aware of responsibilities to the institution and the animals in his/her care. The principal investigator certifies that personnel involved in the project demonstrate competence with techniques described in the protocol including those to minimize animal pain and distress. Please complete one form for each individual listed on the project.

Protocol Personnel List

Name	Role	Phone	Email Id	Primary Contact	Requestor	Copy Requestor on All Email
Debosch, Brian J	Principal Investigator	314-747-2692	DeBosch_B@kids.wustl.edu			
Higgins, Cassandra	Administrative Assistant	314 7477363	orf_c@kids.wustl.edu			
Mayer, Allyson	Responsible Person	314 362-1997	a.mayer@go.wustl.edu			
Zhang, Yiming	Administrative Assistant	314-747-2692	yiming.zhang@wustl.edu			

Attachments List

File Spec	Description	Created
20180031_1_0001_20150011_3_0001_Yiming_Zhang.pdf		01/30/2018
20180031_1_0001_20150011_3_0001_schedule_A_DeBosch_update.docx		01/30/2018
20180031_1_0001_20150011_3_0001_schedule_A_Higgins_update.docx		01/30/2018
20180031_1_0001_20150011_3_0001_schedule_A_Mayer_update.docx		01/30/2018

[Table of Contents](#)

XI.)b.) Personnel Information

Person Information

Name	Debosch, Brian J
Role	Principal Investigator
Organization/Department	Washington University / Pediatrics - GI
Campus	Medical School
Phone	314-747-2692
Email	DeBosch_B@kids.wustl.edu
Fax	314-454-2412
Emergency Phone	314-503-4308
Home Phone	636-778-1536
Cell Phone	314-265-0090
Pager	314-424-3012
Card Access ID	
Is there a schedule A attached for this person?	Yes

Protocol Related Activities

Will the person be handling animals?	YES
Will person have access to controlled substances?	NO

Species - Protocol Data

Species Name
Mouse

Please review and update person's degrees

Degrees
MD PhD

Please review and update person's experience and qualifications

Experience and Qualification
19 years of mouse handling experience. Took Gail Moore's techniques course.

Please review and update person's address information

Risk Category

Attachments List

File Spec	Description	Created
-----------	-------------	---------

[Table of Contents](#)

XI.)b.) Personnel Information

Person Information

Name	Mayer, Allyson
Role	Responsible Person
Organization/Department	Washington University / Obstetrics/Gynecology
Campus	Medical School
Phone	314 362-1997
Email	a.mayer@go.wustl.edu
Fax	
Emergency Phone	
Home Phone	
Cell Phone	
Pager	
Card Access ID	
Is there a schedule A attached for this person?	No

Protocol Related Activities

Will the person be handling animals?	YES
Will person have access to controlled substances?	NO

Species - Protocol Data

Species Name
Mouse

Please review and update person's degrees

Degrees
DBBS

Please review and update person's experience and qualifications

Experience and Qualification
1.5 years in lab. Learned mouse handling from Dr. DeBosch (protocol PI), Gail Moore's animal techniques course and from Michaela Reid, Moley lab manager

Please review and update person's address information

Risk Category

Attachments List

File Spec	Description	Created
-----------	-------------	---------

[Table of Contents](#)

XI.)b.) Personnel Information

Person Information

Name	Higgins, Cassandra
Role	Administrative Assistant
Organization/Department	Washington University / Pediatrics
Campus	Medical School
Phone	314 7477363
Email	orf_c@kids.wustl.edu
Fax	
Emergency Phone	3147477363
Home Phone	
Cell Phone	
Pager	
Card Access ID	
Is there a schedule A attached for this person?	No

Protocol Related Activities

Will the person be handling animals?	YES
Will person have access to controlled substances?	NO

Species - Protocol Data

Species Name
Mouse

Please review and update person's degrees

Degrees
B.S.

Please review and update person's experience and qualifications

Experience and Qualification
Research tech I X 13months. Completed Gail Moore's animal handling / techniques course. Received training from Dr. DeBosch (protocol PI), and from Michaela Reid, Lab manager in Kelle Moley's laboratory

Please review and update person's address information

Risk Category

Attachments List

File Spec	Description	Created
-----------	-------------	---------

[Table of Contents](#)

XI.)b.) Personnel Information

Person Information

Name	Zhang, Yiming
Role	Administrative Assistant
Organization/Department	Washington University / Pediatrics
Campus	Medical School
Phone	314-747-2692
Email	yiming.zhang@wustl.edu
Fax	
Emergency Phone	
Home Phone	
Cell Phone	
Pager	
Card Access ID	
Is there a schedule A attached for this person?	Yes

Protocol Related Activities

Will the person be handling animals?	YES
Will person have access to controlled substances?	NO

Species - Protocol Data

Species Name
Mouse

Please review and update person's degrees

Degrees
B.S. - Biochemistry

Please review and update person's experience and qualifications

Experience and Qualification
Undergraduate degree in biochemistry 2015 Undergraduate research in plant biology, laboratory of Jay Thelen, PhD, Biochemistry, U of Missouri, Columbia 2 years experience in industrial research laboratory setting (EMG, Columbia, MO).

Please review and update person's address information

Risk Category	
---------------	--

Attachments List

File Spec	Description	Created
-----------	-------------	---------

[Table of Contents](#)

Hard copy signature page - CLICK ON "PRINT TO HTML"

Please print and sign and date this form and fax it to the Animal Studies office, FAX #(314) 747-6695.

[Table of Contents](#)

Hard copy signature

Principal Investigator	Debosch, Brian J
Protocol Application Number	20180031
Document Type	New

PLEASE EMAIL IACUC@WUSTL.EDU OR FAX THE SIGNATURE PAGE TO THE IACUC OFFICE AT 314-747-6695 (USE ALL SEVEN NUMBERS)
Assurances: Please read the following assurances carefully. By submitting this protocol you agree to the following statements.

Procedures involving animals will be designed and performed with due consideration of their relevance to human or animal health, the advancement of knowledge, or the good of society.

1. I accept responsibility for assuring that this study will be conducted in accord with the requirements set forth in the Animal Welfare Act, the Guide for the Care and Use of Laboratory Animals, and guidelines of Washington University.

2. The research proposed is not unnecessarily duplicative of previously reported research.

3. The procedures described within this protocol match with those described in the grant application(s) related to this protocol.

4. Changes in procedures will not be implemented unless approved by the IACUC. Amendment forms are available from the IACUC office or from the [IACUC Website](#).

5. All staff involved with this project have received information and training on the care and use of laboratory animals and have been given specific material and instruction on the use of the animals employed in this project. A copy of Schedule A must be completed by each person handling animals in this project.

6. Anesthesia, analgesia, sedation, tranquilization, and euthanasia will be used to relieve pain or distress, so long as these agents do not compromise the validity of the experimental data and withholding them has been justified scientifically.

7. Appropriate steps have been/will be taken to avoid or minimize exposure of persons to any hazardous agents used in this project. All staff have been informed of the Washington University Occupational Health Program. Information and health programs are available on the [DCM Website](#)

Principal Investigator		DATE	
ASC CHAIRMAN'S SIGNATURE			

[Table of Contents](#)

ACURO Animal Use Appendix for Research Involving Animals
FULL VERSION

*****NOTE: ACURO will ONLY review protocols that have been approved by your IACUC. *****

****Animal work MAY NOT be initiated until the awardee receives ACURO approval. ****

Animal work initiated without ACURO approval is noncompliant and may not be funded.

Institutions using DOD funds to support the use of animals in research, development, testing and evaluation (RDT&E) projects must provide electronic copies of the following documents to the US Army Medical Research and Materiel Command (USAMRMC), Office of Research Protections (ORP), Animal Care and Use Review Office (ACURO) for review and approval prior to initiation:

A. a copy of the **IACUC-approved institutional protocol(s)** and documentation of initial IACUC approval- Note: If the protocol expires within the next 60 days, please wait to submit until your protocol is renewed.

B. a copy of all existing IACUC-approved protocol amendments or modifications and documentation of IACUC approval (**future modifications or amendments** must be reviewed and approved by ACURO **PRIOR** to implementation)

C. completed corresponding ACURO Animal Use Appendix for each IACUC-approved protocol.

D. a copy of the research site's most recent facility inspection report from the USDA (This requirement also applies to all subcontractors using animals in support of DoD-funded projects or programs)

Specific information requested in the following animal use Appendix is derived from requirements in the Animal Welfare Regulations (AWRs), the *Guide for the Care and Use of Laboratory Animals*, and other applicable Federal and DOD regulations. The DOD policies and requirements for the use of animals in DoD-supported RDT&E are described in DoD Instruction 3216.01, *Use of Animals in DOD Programs*, dated September 13, 2010 and Army Regulation 40-33, *The Care and Use of Laboratory Animals in DOD Programs*, dated February 16, 2005. These requirements differ from those of other funding agencies. Use of the Appendix is intended to meet the requirements of these documents.

Questions concerning animal use and review should be directed to the USAMRMC ACURO:

Phone: 301-619-6694

Email: Usarmy.detrick.medcom-usamrmc.other.acuro@mail.mil

~~~~~  
~~

**Each section of this Appendix must be completed.** To assist you in completing this appendix, instructions and explanations are provided as *hidden, red and italicized text*. To view the instructions and/or examples for each section, select the “**Show/Hide ¶**” button on your tool bar (the button itself appears as the ¶ symbol). To print the hidden text, select “Print Hidden Text” in the print options section. It is important that you carefully **read the instructions** for each paragraph to ensure you provide a comprehensive response. Begin typing responses after the colon (“:”) for each section to ensure your typing is not within the hidden text.

Submit electronic copies of the appendix only; please **do not** submit printed copies to ACURO.

**Refer to the cover page for directions on viewing the hidden text in this document.  
WHEN COMPLETING THIS APPENDIX, INCLUDE ONLY ANIMALS, EXPERIMENTS AND  
PROCEDURES THAT ARE FUNDED BY THIS GRANT/CONTRACT**

**1. DOD Funding of Protocol**

☒ Yes ☐ No The attached IACUC-approved protocol contains experiments that are not funded by this DOD award. If all experiments within the protocol are funded, ! You must use the **abbreviated version** of the Appendix.



**2. Administrative Data:**

|                                                    |                                                                                |
|----------------------------------------------------|--------------------------------------------------------------------------------|
| DOD Award PI Name:                                 | Brian DeBosch MD, PhD                                                          |
| DOD Award PI Email:                                | deboschb@wustl.edu                                                             |
| DOD Award PI Phone:                                | 314-747-2692                                                                   |
| DOD Funding Source:                                | Dept. of the Army -- USAMRAAPeer Reviewed Medical<br>Discovery Award /Proposal |
| DOD Grant/Award/Proposal Number:                   | #PR162041                                                                      |
| Institution Where Animal Studies are<br>Conducted: | Washington University                                                          |
| IACUC Protocol PI Name                             | Brian DeBosch MD, PhD                                                          |
| IACUC Protocol PI Email                            | deboschb@wustl.edu                                                             |
| IACUC Protocol PI Phone                            | 314-747-2692                                                                   |
| IACUC Protocol Title                               | Metabolic Characterization of a Putative Novel<br>Antidiabetic Drug, GTI-421   |
| IACUC Protocol Number                              | 20180031                                                                       |
| Attending Veterinarian Name                        | Ken Boschert, DVM                                                              |
| Attending Veterinarian Email                       | Boscherk@wustl.edu                                                             |
| Attending Veterinarian Phone                       | 314-362-3773                                                                   |
| IACUC Administrator or Chair Name                  | Janice L. Witte                                                                |
| IACUC Admin or Chair Email                         | witte@acuc.wustl.edu                                                           |
| IACUC Admin or Chair Phone                         | 314-362-3229                                                                   |
| Institutional Grants Manager Name                  | Teri Medley                                                                    |
| Grants Manager Email                               | researchgrants@wusm.wustl.edu                                                  |
| <b>Grants Manager Phone</b>                        | 314-747-4134                                                                   |

**3. Brief Objective Summary:**

Our objective is to define the role of the hepatic glucose fasting in mitigating diabetes and cardiovascular disease. We use cell culture and animal models of disease to define the pathways that contribute to cardiometabolic disease, and to define efficacy of novel therapeutics generated within our lab.

**4. Total Number of Animals Used (by Species) and USDA Pain/Distress Category- Note: All animals proposed for use must be accounted for in this section, including species not currently regulated by the USDA.**

**USDA Pain/Distress Category Definitions:**

- **Column B:** Animals being bred and animals being held for use but not yet used for such purposes.
- **Column C:** Animals that will experience no more than slight or momentary pain or distress
- **Column D:** Animals that will potentially experience accompanying pain or distress and for which appropriate anesthetic, analgesic, or tranquilizing drugs **WILL** be used.
- **Column E:** Animals that will potentially experience accompanying pain or distress and for which appropriate anesthetic, analgesic, or tranquilizing drugs **WILL NOT** be used.

| SPECIES | HIGHEST USDA PAIN/DISTRESS CATEGORY (B, C, D, E*) See table below for definitions | TOTAL NUMBER                                                                                                                                                                                   |
|---------|-----------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Mouse   | B                                                                                 | 30 (5X3 breeding pairs for each of the three genetic lines used: WT, FGF21LKO, PGC1aLKO). Db/db line will be purchased for each experiment from the Jackson Laboratory)                        |
| Mouse   | C                                                                                 | 1a: 50 (n = 10, five experimental groups)<br>1b: 60 (n = 10, six groups)<br>2a: 65 (n = 10, six in vivo groups + 5 mice for primary hepatocyte derivation)<br>2b: 60 mice (n = 10, six groups) |
| Mouse   | D/E                                                                               | 0                                                                                                                                                                                              |

**5. Statistical/Other Basis for Number of Animals Used:**

Prior pilot experiments in our lab or in the literature justified the above numbers requested.

**6. Column E only- If any animals are listed in USDA Column E (Unalleviated Pain or Distress), the PI must provide a scientifically valid justification for withholding pain relieving medication:**  
N/A

**7. Consideration of Alternatives to Painful Procedures (Columns D or E only).** If there are no animals listed in USDA Column D or E, mark this section "N/A." If any animals are listed in USDA Column D or E, the PI must perform this literature search. **You MUST provide a narrative summary of the results of the literature search for alternatives to painful procedures.** The Animal Welfare Act regulations specifically state that the P.I. must provide a narrative description of the methods and sources that he/she used to determine that alternatives to the painful or distressful procedure(s), including those in which pain or distress is alleviated, were not available: **DOD regulations require this for all animals undergoing painful procedures including species not covered by the Animal Welfare Act.**

N/A

## 8. Rationale for Using Animals:

Determination of insulin sensitivity and differences in genetic susceptibility for developing diabetes are well-established within the rodent model. We employ a cell culture system for reductionist studies as part of the current proposal, however, it is not possible to fully mimic all characteristics of mammalian physiology *in vitro*. There are many advantages in using this animal model system to study *in vivo* insulin resistance and glucose uptake. This includes the ability to perform studies within a well-defined genetic background, the ability to control for possible confounding variables such as diet and exercise.

## 9. Animal Model Rationale:

Mice are a mammalian model system with powerful genetic tools available. Their metabolism and physiology model that of humans closely, rendering drug effects found in this model highly likely to be translatable to humans patients.

## 10. Animal Procurement:

☒ Yes ☐ No Does the protocol involve Animal Welfare Act-regulated species?

☒ Yes ☐ No If so, are the animals obtained legally from suppliers licensed by the USDA?  
If the supplier claims exemption from USDA licensing, provide confirmation from the research site's IACUC that the exemption criteria have been met.

☐ Yes ☒ No Are wildlife species are used? If so, provide IACUC assurance that animals have been obtained legally and provide copies of all applicable capture and use permits.

## 11. Experimental Design:

**1a. Wild-type (WT) mice exposed (4wk) to a high-fat diet and also db/db mice will be subjected to vehicle or GTI-421 IP injections twice daily for two wk prior to metabolic assays**

**1b. WT, and liver-specific FGF21- and PGC1alpha-deficient** mice are exposed 2wk to GTI-421 prior to metabolic assay. Db/db mice treated with scrambled or FGF21- or PGC1alpha-specific antisense oligonucleotide every other day for 2wk are treated 2wk with GTI-421 prior to metabolic assay

2a.i. WT mice with no prior interventions are sacrificed and primary murine hepatocytes are isolated post-mortem for *in vitro* experimentation. Briefly, primary hepatocytes isolated post-mortem are treated with or without GTI-421 in the presence or absence of transfection with adenovirus encoding GLUT2 or GLUT8 or in the presence or absence of pyruvate prior to marker gene expression assays probing PGC1a, FGF21 and PPARg target genes.

2.a.ii. WT mice are treated with or without AAV encoding GLUT2, GLUT8 or green fluorescent protein (GFP) prior to treatment with or without GTI-421. Post-mortem analysis of liver tissue by gene expression assay will be undertaken to measure PGC1a, FGF21 and PPARg target genes.

2b. db/db mice treated with Adeno-associated virus encoding GLUT8, GLUT2 or GFP (negative control) are subjected to 2wk GTI-421 prior to sacrifice and target gene assays in dissected liver tissue.

**12. Specific Procedures / Technical Methods:** In the subparagraphs listed below, provide a complete description of all procedures the animals will experience

**a. Animal Observations and Health Status Assessment Criteria:**

Animals will be assessed at least once daily following procedural initiation. Animals observed to be hypomotile, hunched, fluffed/undergroomed fur and malaise will be considered to have declining health status. An attending veterinarian performs rounds daily as well to assess health of all animals, including weekends and holidays.

Breeder mice and their progeny are examined daily by veterinary technicians for signs of illness. The veterinary technicians notify the PI of any animals exhibiting illness or distress. All mice will be euthanized prior to harvest of tissues for analysis.

**b. Anesthesia/Analgesia/Chemical Restraint and /or Non-pharmaceutical Methods of Relieving Pain or Distress:**

|                          |     |                                     |    |                                                                                |
|--------------------------|-----|-------------------------------------|----|--------------------------------------------------------------------------------|
| <input type="checkbox"/> | Yes | <input checked="" type="checkbox"/> | No | Anesthetics/Analgesics/Tranquilizers will be used to relieve pain or distress. |
| <input type="checkbox"/> | Yes | <input checked="" type="checkbox"/> | No | Non-pharmaceutical methods will be used to relieve pain or distress.           |
| <input type="checkbox"/> | Yes | <input checked="" type="checkbox"/> | No | Drugs will be used for chemical restraint.                                     |

If "Yes" to any of the above,

**i. Describe methods or strategies planned to effectively relieve pain and/or distress:**

No survival procedures are performed on our animals for purposes of this funded proposal. We will perform routine handling of animals to calm them before potentially mildly painful protocols are initiated, such as intraperitoneal drug injection. Therefore, no analgesia will be required during these manipulations.

**ii. Intraprocedural Observations: list the observational or monitoring criteria used to assess depth of anesthesia while the procedure is being performed and/or to determine if animals are experiencing pain or distress and require additional anesthetics, analgesics, tranquilizers or non-pharmaceutical pain relief:**

Not applicable.

**c. Paralytic Agents (Note: the use of paralytic agents without anesthesia is prohibited):**

|                          |     |                                     |    |                                                     |
|--------------------------|-----|-------------------------------------|----|-----------------------------------------------------|
| <input type="checkbox"/> | Yes | <input checked="" type="checkbox"/> | No | Paralytic agents will be used during this protocol. |
|--------------------------|-----|-------------------------------------|----|-----------------------------------------------------|

If "Yes," describe the following:

**i. Rationale for using paralytic agents:**

**ii. Paralytic Agent Protocol (e.g., drug, dose, frequency of injection, etc.):**

**iii. Monitoring methods to ensure adequate depth of anesthesia while animal is under influence of paralytic agents:**

**d. Surgery:**

|                          |     |                                     |    |                                                                         |
|--------------------------|-----|-------------------------------------|----|-------------------------------------------------------------------------|
| <input type="checkbox"/> | Yes | <input checked="" type="checkbox"/> | No | Surgical procedures are performed on live animals during this protocol. |
|--------------------------|-----|-------------------------------------|----|-------------------------------------------------------------------------|

If "Yes," describe the following:

**i. Pre-operative Considerations and Animal Preparation:**

**ii. Surgical Procedures:**

**iii. Immediate and Long-Term Post-operative Monitoring/Observations/Treatment:**

**e. Multiple Major Survival Surgeries (performed on the same animal):**

☐ Yes ☒ No Multiple major survival surgeries will be performed on the same animal.

If "Yes," provide a scientifically valid justification: Be sure to include time between surgeries.

**f. Biosamples:**

☒ Yes ☐ No Biosamples are collected from animals during this protocol.

If "Yes," state the frequency, volume, harvest site, and collection method for each sample type collected ante-mortem (before the animal is euthanized)

Insulin and glucose tolerance testing will be performed as we have done previously (DeBosch et al Endocrinology 2012; Molecular Endocrinol 2013). This requires that approximately 25-35uL blood be drawn during a two hour testing period from a small 1-2mm nick in the distal, lateral portion of the tail. Collection will be into a glucometer strip, as is done for patients with diabetes mellitus checking their serum glucose levels.

**g. Adjuvants:**

☐ Yes ☒ No Adjuvants are used in animals during this protocol.

If "Yes," list any adjuvants used and the plan for their use:

**h. Genotyping/DNA Analysis:**

☐ Yes ☒ No Genotyping/DNA analysis will be performed on animals during this protocol.

If "Yes," describe any methods used for genotyping or other DNA analysis:

**i. Agents Administered to Animals:** In this section, list all substances, compounds, or special diets administered to animals by any method.

| Agent         | Dose/Volume                        | Route of Administration |
|---------------|------------------------------------|-------------------------|
| GTI-421       | 10mg/kg BID X 2 wk                 | IP                      |
| AAV8          | 10 <sup>9</sup> PFU per mouse once | Tail Vein               |
| High fat diet | Ad libitum x 4 weeks               | PO                      |
|               |                                    |                         |

**j. Prolonged Restraint:**

☐ Yes ☒ No Animals will undergo prolonged restraint (as defined by institutional IACUC policies or the Animal Welfare Act regulations) during this protocol.

If "Yes," justify and describe restraint and duration in detail:

**k. Behavioral Studies or Behavioral Modification Techniques:**

☐ Yes ☒ No Animals will be involved in behavioral studies or undergo behavioral modification during this protocol.

:

**I. Other Procedures (e.g., electrocardiograms, radiology or other imaging procedures, tissue perfusions, stress induction, etc.):**

Indirect calorimetry (TSE Phenomaster Systems) will be performed on test mice. This involves no pain-induction, injections, agent administrations. It is carried out by transferring mice from their cage to a calorimeter cage. This cage is essentially identical to the standard housing cages in our DCM facility, however, the external portion of the cage has gas sampling apparatus attached. The calorimeter is in the adjacent facility to where non-experimental mice are housed, therefore, the change in environment is minimal.

**13. Endpoints for Animals:**

- a. Study Endpoint:** State the projected study endpoint for each animal, animal group, or experiment (e.g., survival until the specified time point and then euthanasia, recovery, death without early euthanasia)

All mice will be euthanized for tissue harvest. The mice will be 8- to 10-weeks-of-age at the time of tissue harvest.

**b. Humane Early Endpoints:** Define specific, and objective criteria that will be used to determine early removal (for example, percentage of weight loss, tumor size, number of abdominal taps, abdominal distension, loss of locomotion, significant lowering of body temperature, decreased food or water consumption, decreased activity, etc.).

We will subject mice to humane early endpoints, after close monitoring if we observe any of the following: hunching, fluffing, lost grooming, unanticipated weight loss, any concerns by DCM or DeBosch Lab

**c. Death as an Endpoint:** Specifically address and scientifically justify any proposal in which animals are allowed to die as a result of the experimental procedures without the benefits of treatment or early euthanasia.

N/A

**14. Euthanasia:**

Mice will be sacrificed by cervical dislocation prior to tissue harvest.

**15. Literature Search for Unnecessary Duplication:** This search is required for all animal use proposals.

- a. Source(s) Searched :**  
PUBMED, MEDLINE

- b. Date(s) of Search :**  
5/3/18

- c. Years Covered by Search :**  
01/01/2008 – 5/3/2018

- d. Keywords Used and/or Search Strategy Please note that this is not a search for alternatives and alternatives terms should not be used here. :**

GTI-421 AND  
Hepatic Metabolism OR  
Diabetes mellitus OR  
Non-alcoholic Fatty Liver Disease

**Results of Search :** No hits. This is consistent with the fact that we identified this novel compound through our proprietary screen.

## 16. Qualifications:

### STUDY PERSONNEL QUALIFICATIONS/TRAINING

| Protocol activity or procedure (e.g., tail vein injections, euthanasia) | Name of person performing activity | Qualifications or experience of person performing activity in the proposed species (e.g., research technician; 2 yrs experience with intracranial surgical procedure; performed IP injections on 100s of mice ) | <b>Specific</b> training in this activity or procedure (e.g., rodent handling class; trained to do surgical procedure by PI; aseptic surgical techniques training; rabbit intubation) |
|-------------------------------------------------------------------------|------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Tail vein injections for viral construct transduction                   | Brian DeBosch, MD, PhD             | P.I. 19 yrs experience in this procedure, performed injections on > 1000 mice. Completed refresher course through Washington University DCM 6/2012 (Gail Moore, course instructor)                              | Rodent handling class; trained to do procedure by undergraduate PI in 1998;                                                                                                           |
|                                                                         | Cassandra Higgins, BS              | Technician, 4yrs experience with this procedure; performed procedure on > 100 mice, Completed Washington Univ DCM course 11/2014.                                                                               | Rodent handling class; trained to do procedure by DeBosch (PI) in 2014.                                                                                                               |
| Indirect Calorimetry                                                    | Brian DeBosch, MD, PhD             | P.I. 6 yrs experience in this procedure, performed IP indirect calorimetry > 100 mice. Completed refresher course through Washington University DCM 6/2012 (Gail Moore, course instructor)                      | Rodent handling class; trained to do procedure by Core Facility Director in 2012 (Trey Coleman, PhD);                                                                                 |
|                                                                         | Cassandra Higgins, BS              | Technician, 4yrs experience with this procedure; performed procedure on > 50 mice, Completed Washington Univ DCM course 11/2014.                                                                                | Rodent handling class; trained to do procedure by DeBosch (PI) in 2013 and by Core Facility Director (Sangeeta Gupta, PhD) in 2014.                                                   |
| Intraperitoneal injection (ITT and GTT assays)                          | Brian DeBosch, MD, PhD             | P.I. 19 yrs experience in this procedure, performed IP injections on > 500 mice.                                                                                                                                | Rodent handling class; trained to do procedure by                                                                                                                                     |

|  |                          |                                                                                                                                                                                                                                              |                                                                                                          |
|--|--------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------|
|  | Cassandra Higgins,<br>BS | Completed refresher course through Washington University DCM 6/2012 (Gail Moore, course instructor)<br><br>Technician, 4yrs experience with this procedure; performed procedure on > 100 mice, Completed Washington Univ DCM course 11/2014. | undergraduate PI in 1998;<br><br>Rodent handling class; trained to do procedure by DeBosch (PI) in 2014. |
|  |                          |                                                                                                                                                                                                                                              |                                                                                                          |

**\*\*\*\*The following questions refer to the Research Site and Protocol PI.\*\*\*\***

Information and/or documents required in these questions should be obtained from the research site's IACUC or veterinary staff. Documents may be provided directly to ACURO by the Protocol PI or institution staffs.

**17. Institutional Accreditation / Assurances:**

**a. Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC) Accreditation** (do NOT provide AAALAC correspondence):

☒ Yes ☐ No Animal work is being performed at an AAALAC International-accredited facility.

**b. Public Health Service Animal Welfare Assurance Statement:**

☒ Yes ☐ No Animal work is being performed at a PHS-assured facility.

**c. Non-accredited, Unassured Facilities:** If neither above apply to the facility where animal work is being performed, submit a statement signed by the Institutional Official that states the care and use of animals will be conducted in accordance with the National Research Council's 2011 *Guide for the Care and Use of Laboratory Animals* and applicable Federal and DoD regulations.

**18. Continuing Review:**

☒ Yes ☐ No Does your IACUC conduct continuing review of all animal use protocols, no less than once per year, in accordance with the Animal Welfare Act Regulations and Department of Defense requirements? All animal use protocols funded by the DOD, even in part, must be reviewed by the IACUC no less than annually, including those that do not utilize USDA regulated species.

**19. Institution's Veterinary Care:** Provide a brief description of the veterinary care plan at the research site.

**a. Describe routine care of animals by vivarium staff**

Mice will be housed in a state of the art, pathogen-free animal housing barrier facility maintained by the Washington University Medical School. This facility provides animal breeding, housing, experimentation bays, and a complete full-time veterinary staff. Mice will be examined daily (including weekends and

holidays) by veterinary technicians to ensure that the mice appear well and have adequate food and water. A full time attending veterinarian is on staff full-time.

**b.** Describe weekend, holiday, and emergency care of animals by vivarium staff.

Mice will be examined daily (including weekends and holidays) by veterinary technicians to ensure that the mice appear well and have adequate food and water.

**c.** Identify whether the attending veterinarian is on staff full-time or by contract. If contracted, address frequency of visits to the facility.

A full time attending veterinarian is on staff full-time.

### **Protocol Principal Investigator Assurances:**

The law specifically requires several written assurances from the P.I. Please read and sign the assurances as indicated (this page may be photocopied and signed).

As the Principal Investigator on this protocol, I acknowledge my responsibilities and provide assurances for the following:

**A. Painful Procedures:** I assure that discomfort and injury to animals will be limited to that which is unavoidable in the conduct of scientifically valuable research and that analgesic, anesthetic, and/or tranquilizing drugs will be used where indicated and appropriate to minimize pain and/or distress to animals.

**B. Animal Use:** The animals authorized for use in this protocol will be used only in the activities and in the manner described herein, unless a modification is specifically approved by the IACUC and the U.S. Army Medical Research and Materiel Command **Animal Care and Use Review Office** (ACURO) prior to its implementation.

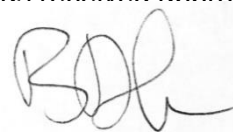
**C. Duplication of Effort:** I have made a reasonable, good faith effort to ensure that this protocol is not an unnecessary duplication of previous experiments.

**D. Statistical Assurance:** I assure that I have consulted with a qualified individual who evaluated the experimental design with respect to the statistical analysis, and that the minimum number of animals needed for scientific validity will be used.

**E. Training:** I verify that the personnel performing the animal procedures/manipulations/observations described in this protocol are technically competent and have been properly trained to ensure that no unnecessary pain or distress will be caused to the animals as a result of the procedures/manipulations.

**F. Responsibility:** I acknowledge the inherent moral, ethical and administrative obligations associated with the performance of this animal use protocol, and I assure that all individuals associated with this project will demonstrate a concern for the health, comfort, welfare, and well-being of the research animals. Additionally, I pledge to implement animal use alternatives where feasible, and conduct humane and lawful research.

**G. Scientific Review:** This proposed animal use protocol has received appropriate peer scientific review, and is consistent with good scientific research practice.



Brian DeBosch, MD, PhD 5/10/2018

(Protocol Principal Investigator Name) (Protocol Principal Investigator Signature and Date)



## Inspection Report

Washington University  
660 S Euclid  
Campus Box 8106  
Saint Louis, MO 63110

Customer ID: 1444

Certificate: 43-R-0008

Site: 001

WASHINGTON UNIVERSITY

Type: FOCUSED INSPECTION

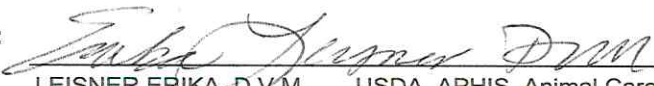
Date: 07-FEB-2018

No non-compliant items identified during this inspection. This was a focused inspection limited to paperwork.

This inspection and exit interview were conducted with facility representatives. An exit interview was completed on February 8, 2018.

### Additional Inspectors

Snow William, Veterinary Medical Officer

Prepared By:   
LEISNER ERIKA, D.V.M. USDA, APHIS, Animal Care

Date:  
08-FEB-2018

Title: VETERINARY MEDICAL OFFICER 6037

Received By:   
KENNETH BOSCHERT, DVM

Title: INTERIM DIRECTOR/ATTENDING VETERINARIAN

Date:  
08-FEB-2018