PI: **Kalaitzoglou, Evangelia**	Title: Muscle -bone interaction and its role in diabetic bone disease of Type I diabetes	
Received: 03/11/2020	FOA: PA19-117 Clinical Trial:Not Allowed	Council: 10/2020
Competition ID: FORMS-E	FOA Title: Mentored Clinical Scientist Research Career Development Award (Parent K08 Independent Clinical Trial Not Allowed)	
1 K08 DK124566-01A1	Dual:	Accession Number: 4418094
IPF: 2793601	Organization: UNIVERSITY OF KENTUCKY	
Former Number: 1K08DK124566-01	Department: Pediatrics	
IRG/SRG: DDK-B	AIDS: N	Expedited: N
Subtotal Direct Costs (excludes consortium F&A) Year 1: 153,551 Year 2: 153,636 Year 3: 153,722 Year 4: 153,808 Year 5: 153,895	Animals: Y Humans: Y Clinical Trial: N Current HS Code: 30 HESC: N HFT: N	New Investigator: Early Stage Investigator:
Senior/Key Personnel:	*Organization:*	*Role Category:*
Evangelia Kalaitzoglou	University of Kentucky Research Foundation	PD/PI
John Fowlkes	University of Kentucky Research Foundation	Other Professional-Mentor
Philip Kern	University of Kentucky Research Foundation	Other Professional-Co-Mentor
Charlotte Peterson	University of Kentucky Research Foundation	Other Professional-Co-Mentor
Jeffry Nyman	Vanderbilt University Medical Center	Other (Specify)-Collaborator
Thomas Hawke	McMaster University	Other (Specify)-Collaborator

Reference Letters

Gerald Supinski	University of Kentucky	03/11/2020
Mary Beth Humphrey	OUHSC	03/11/2020
Susan Smyth	University of Kentucky	03/11/2020

Additions for Review

Accepted Publication Recent Publication

OMB Number: 4040-0010
Expiration Date: 12/31/2022

APPLICATION FOR FEDERAL ASSISTANCE
SF 424 (R&R)

3. DATE RECEIVED BY STATE	**State Application Identifier**

1. TYPE OF SUBMISSION*

❍ Pre-application ● Application ❍ Changed/Corrected Application

4.a. Federal Identifier
DK124566

b. Agency Routing Number

2. DATE SUBMITTED
2020-03-11

Application Identifier

c. Previous Grants.gov Tracking Number

5. APPLICANT INFORMATION **Organizational DUNS*:** 939017877

Legal Name*: University of Kentucky Research Foundation
Department:
Division:
Street1*: 500 South Limestone
Street2: 109 Kinkead Hall
City*: Lexington
County: Fayette
State*: KY: Kentucky
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 40526-0001

Person to be contacted on matters involving this application
Prefix: First Name*: Louetta Middle Name: Kasandra Last Name*: Hurst Suffix:
Position/Title: Research Administrator
Street1*: 500 South Limestone
Street2: 109 Kinkead Hall
City*: Lexington
County:
State*: KY: Kentucky
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 40526-0001
Phone Number*: 859-257-9420 Fax Number: 859-323-1060 Email: ospa@uky.edu

6. EMPLOYER IDENTIFICATION NUMBER *(EIN) or (TIN)** 1-616033693-C4

7. TYPE OF APPLICANT* H: Public/State Controlled Institution of Higher Education

Other (Specify):
Small Business Organization Type ❍ Women Owned ❍ Socially and Economically Disadvantaged

8. TYPE OF APPLICATION*

❍ New ● Resubmission
❍ Renewal ❍ Continuation ❍ Revision

If Revision, mark appropriate box(es).
❍ A. Increase Award ❍ B. Decrease Award ❍ C. Increase Duration
❍ D. Decrease Duration ❍ E. Other *(specify)* :

Is this application being submitted to other agencies?* ❍Yes ●No What other Agencies?

9. NAME OF FEDERAL AGENCY*
National Institutes of Health

10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER
TITLE:

11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT*
Muscle bone interaction and its role in diabetic bone disease of Type I diabetes

12. PROPOSED PROJECT
Start Date* Ending Date*
09/01/2020 08/31/2025

13. CONGRESSIONAL DISTRICTS OF APPLICANT
KY-006

SF 424 (R&R) APPLICATION FOR FEDERAL ASSISTANCE

14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION

Prefix: First Name*: Evangelia Middle Name: Last Name*: Kalaitzoglou Suffix:
Position/Title: Assistant Professor
Organization Name*: University of Kentucky Research Foundation
Department: Pediatrics
Division: Medicine
Street1*: 2195 Harrodsburg Road
Street2: Suite 125
City*: Lexington
County: Fayette
State*: KY: Kentucky
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 40504-3504
Phone Number*: 859-323-2232 Fax Number: Email*: Evangelia.kalaitzoglou@uky.edu

15. ESTIMATED PROJECT FUNDING

a. Total Federal Funds Requested*	$830,102.00
b. Total Non-Federal Funds*	$0.00
c. Total Federal & Non-Federal Funds*	$830,102.00
d. Estimated Program Income*	$0.00

16.IS APPLICATION SUBJECT TO REVIEW BY STATE EXECUTIVE ORDER 12372 PROCESS?*

a. YES ○ THIS PREAPPLICATION/APPLICATION WAS MADE AVAILABLE TO THE STATE EXECUTIVE ORDER 12372 PROCESS FOR REVIEW ON:
DATE:

b. NO ● PROGRAM IS NOT COVERED BY E.O. 12372; OR
○ PROGRAM HAS NOT BEEN SELECTED BY STATE FOR REVIEW

17. By signing this application, I certify (1) to the statements contained in the list of certifications* and (2) that the statements herein are true, complete and accurate to the best of my knowledge. I also provide the required assurances * and agree to comply with any resulting terms if I accept an award. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. (U.S. Code, Title 18, Section 1001)

● I agree*

** The list of certifications and assurances, or an Internet site where you may obtain this list, is contained in the announcement or agency specific instructions.*

18. SFLLL or OTHER EXPLANATORY DOCUMENTATION File Name:

19. AUTHORIZED REPRESENTATIVE

Prefix: First Name*: Kim Middle Name: C Last Name*: Carter Suffix:
Position/Title*: Associate Director
Organization Name*: University of Kentucky Research Foundation
Department: Sponsored Projects Administrat
Division: Research
Street1*: 500 South Limestone
Street2: 109 Kinkead Hall
City*: Lexington
County: Fayette
State*: KY: Kentucky
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 40526-0001
Phone Number*: 8592579420 Fax Number: 8593231060 Email*: ospa@uky.edu

Signature of Authorized Representative*
Kim C Carter

Date Signed*
03/11/2020

20. PRE-APPLICATION File Name:

21. COVER LETTER ATTACHMENT File Name:Cover_letter_RK08_0305201009017454.pdf

424 R&R and PHS-398 Specific
Table Of Contents

OMB Number: 4040-0010
Expiration Date: 10/31/2019

Project/Performance Site Location(s)

Project/Performance Site Primary Location

❍ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name:	University of Kentucky Research Foundation
Duns Number:	939017877
Street1*:	2195 Harrodsburg Road
Street2:	Suite 125
City*:	Lexington
County:	Fayette
State*:	KY: Kentucky
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	40504-3504
Project/Performance Site Congressional District*:	KY-006

Additional Location(s) File Name:

Funding Opportunity Number: PA-19-117. Received Date: 2020-03-11T14:42:33.000-04:00

OMB Number: 4040-0010
Expiration Date: 12/31/2022

RESEARCH & RELATED Other Project Information

1. Are Human Subjects Involved?* ● **Yes** ❍ **No**

1.a. If YES to Human Subjects

Is the Project Exempt from Federal regulations? ❍ Yes ● No

If YES, check appropriate exemption number: _ 1 _ 2 _ 3 _ 4 _ 5 _ 6 _ 7 _ 8

If NO, is the IRB review Pending? ❍ **Yes** ● **No**

IRB Approval Date: 08-22-2019

Human Subject Assurance Number 00005295

2. Are Vertebrate Animals Used?* ● **Yes** ❍ **No**

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? ❍ Yes ● No

IACUC Approval Date: 03-02-2020

Animal Welfare Assurance Number A3336-01

3. Is proprietary/privileged information included in the application?* ❍ **Yes** ● **No**

4.a. Does this project have an actual or potential impact - positive or negative - on the environment?* ❍ **Yes** ● **No**

4.b. If yes, please explain:

4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an environmental assessment (EA) or environmental impact statement (EIS) been performed? ❍ Yes ❍ No

4.d. If yes, please explain:

5. Is the research performance site designated, or eligible to be designated, as a historic place?* ❍ **Yes** ● **No**

5.a. If yes, please explain:

6. Does this project involve activities outside the United States or partnership with international collaborators?* ❍ **Yes** ● **No**

6.a. If yes, identify countries:

6.b. Optional Explanation:

	Filename
7. Project Summary/Abstract*	Abstract_Summary_RK08_0305201009017455.pdf
8. Project Narrative*	Project_narrative_022520_RK081009017456.pdf
9. Bibliography & References Cited	References_RK08_030520_SC1009017458.pdf
10.Facilities & Other Resources	facilites___other_resources_0304201009017457.pdf
11.Equipment	Equipment_RK08_0304201009017459.pdf

Funding Opportunity Number: PA-19-117. Received Date: 2020-03-11T14:42:33.000-04:00

Summary

Bone fracture incidence observed in individuals with Type 1 diabetes (T1D) is much higher compared to the general population. The burden of diabetic bone disease is partially due to lack of evidence to support targeted prevention and interventions to reduce fractures in this population. Furthermore, those with T1D exhibit skeletal muscle dysfunction associated with decreased muscle strength and muscle mass. Skeletal muscle and bone communication is a potential modifiable factor that may contribute to development of diabetic musculoskeletal disease. More specifically, the candidate proposes that a myokine called myostatin, is directly involved in development of diabetic bone disease and may associate with skeletal muscle dysfunction. The role of myostatin in T1D and how it affects the musculoskeletal system in this disease are gaps in knowledge that will be addressed with this proposal. Specifically, the three aims of this proposal are: 1. Quantify the relationship between myostatin levels in serum and skeletal muscle, and bone parameters of humans with and without T1D; 2. Quantify the relationship between myostatin levels in serum and muscle and the bone phenotype of mice with insulin-deficient diabetes; 3. Evaluate whether inhibition of myostatin is beneficial for prevention of DBD in insulin-deficient diabetes; and lastly 4: Determine the mechanism of action of myostatin on osteoblastic bone cells under normoglycemic and hyperglycemic conditions. Myostatin, which is thought to negatively affect both bone and skeletal muscle, may serve both as a marker of musculoskeletal function and surrogate for risk for fracture in those with T1D. Additionally, it may offer an opportunity for targeted intervention to prevent or improve musculoskeletal dysfunction associated with T1D. The knowledge gained from these studies will set the ground for future studies in musculoskeletal health in diabetes and will offer the candidate an opportunity to transition towards an independent career in the abovementioned field.

This proposal presents a five-year research career development program focused on the study of muscle and bone interactions in Type 1 diabetes; and specifically, how muscle derived molecules, called myokines contribute to diabetic bone disease, a serious and emerging complication of Type 1 diabetes. The candidate currently holds a position as an Assistant Professor of Pediatrics in the Division of Pediatric Endocrinology at the University of Kentucky. She has 75% protected time for research, independent office space, laboratory space and access to all equipment and resources offered by the Barnstable Brown Diabetes Center. The candidate is strongly committed to an academic career in the field of musculoskeletal research in diabetes and is supported by her mentors and her department. The proposed study and the complementary didactic work will provide the candidate with research skills in basic, translational and clinical research thereby enabling to transition to an independent clinician scientist.

Project Narrative

Individuals with Type 1 diabetes have higher fracture rates and smaller and weaker muscles compared to those without diabetes, even in young ages. With this project we will try to identify whether a molecule that comes from skeletal muscle, called myostatin, is involved in the increased risk of fractures and the poor quality of skeletal muscle in young individuals with Type 1 diabetes.

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Facilities and Resources

Overview: Established in 1865, the University of Kentucky (UK) is a public land grant university dedicated to improving people's lives through excellence in education, research and creative work, service, and health care. Research and academic activities at UK span all 16 colleges, approximately 80 multidisciplinary research centers, and 30 core research facilities. UK faculty and staff brought in over $333.8 million in new sponsored project awards in FY 2018. Of that total, UK was awarded $200 million in grants and contracts from federal agencies. Additionally, UK is home to one of only 22 medical centers in the United States that have earned the National Cancer Institute Cancer Center Designation, has a federally funded Alzheimer's Disease Center, and has been awarded a Clinical and Translational Science Award from the NIH. UK HealthCare encompasses the University of Kentucky's clinical enterprise and affiliated teaching hospitals: the 724-bed UK Albert B. Chandler Hospital and Kentucky Children's Hospital and the 221-bed UK Good Samaritan Hospital. UK Hospitals provide the only Level 1 Trauma Center for both adult and pediatric patients in central and eastern Kentucky and the only Level IV neonatal intensive care unit in the region. These hospitals are supported by a physician group practice that numbers more than 800 members from over 80 specialties, subspecialties, and clinical services to meet the advanced care needs of the Commonwealth of Kentucky. Capping a decade of growth, UK HealthCare had total system discharges for FY 2017 exceeding 38,000. Outpatient visits totaled more than 1.5 million.

Laboratories:

1) The Barnstable Brown Pediatric Diabetes Research (BBPDR) laboratory, directed by Dr. Fowlkes, is located on the 4th floor in the Charles T. Wethington (CTW) building on the UK campus and occupies 1224 sq ft of wet lab space and includes benches, work stations, and desks for up to 6 individuals. Within the laboratory, there are 2 fume hoods and a fully equipped tissue culture facility with a laminar flow hood and 2 incubators. The BBPDR laboratory also has its own adjacent microscope suite, as well as a large equipment room. The laboratory also supports a fully equipped work- station for cell and tissue processing for cyto/histochemistry and immunofluorescence experiments. **Dr. Kalaitzoglou has dedicated laboratory space in the BBPDR in addition to adjacent office space (100 sq. ft.) and access to all equipment and resources within the BBPDR laboratory.**

2) The Center for Muscle Biology (CMB) core laboratories, including the Immunohistochemistry and Image Facility, are directed by Dr. Peterson. They occupy approximately 1000 sq ft, and are also located on the 4th floor of the CTW building, down the hall from the BBPDR. This space includes benches and desks for training muscle researchers to work with muscle tissues and to perform molecular, immunohistochemical analyses and enzyme assays. Autoclave facilities and shared equipment rooms are provided. **For this project, Dr. Kalaitzoglou will have access to all laboratory space and resources for the proposed skeletal muscle analyses, in addition to interaction and instruction by the staff in Dr. Peterson's laboratory.**

Clinical Sites and Clinical Research Facilities:

1) The Barnstable Brown Diabetes Center (BBDC) is a multidisciplinary unit at the University of Kentucky devoted to clinical and basic research, training and service, directed by Dr. John Fowlkes, who is Dr. Kalaitzoglou's primary mentor. The BBDC is located in a new state-of-the-art facility, about a 10 minute drive from the UK main campus. In the Center Clinics diabetes care delivery is provided across the lifespan under a comprehensive care model engaging pediatric and adult endocrinologists alongside advanced practice nurses, social workers, mental health providers, and certified diabetes educators, to more than 7,500 adults and 2,500 pediatric patients annually. To accomplish this seamless care model, the Center provides unique waiting areas for adult and pediatric patients and families, an independent registration desk, intake and check out areas that are age-friendly, core lab services, and an integrated information technology platform. The Center is also physically contiguous with other key health care

components, such as pharmacy, radiology, family medicine, physical therapy, eye care, and dentistry, making it a truly unique “one stop shop” for diabetics requiring expanded health care beyond their diabetes care. For the last year, over 5,000 individuals have been seen in the outpatient clinics for diabetes. The Center is a leader in diabetes education and hosts a Diabetes Education Accreditation Program by the American Association of Diabetes Educators. In addition, faculty in the Center conduct research and provide services as part of a patient education program built around the Center’s Disease Control Diabetes Prevention Recognition Program (DPRP), which is focused on topics such as nutritional management, blood glucose monitoring, medication administration, and exercise planning**. The BBDC is where Dr. Kalaitzoglou carries out her outpatient clinical duties and the primary recruitment area for the proposed study. Dr. Kalaitzoglou and the research coordinator for her study also have office space at BBDC.**

2) The Center for Clinical and Translational Science (CCTS) is located in the UK hospital that is connected by a bridge to the CTW building, and only a five-minute walk from the research labs. It is funded by an NIH CTSA. The CCTS is dedicated to growing clinical and translational science research teams of the future, providing infrastructure needed to foster collaborations between basic and clinical scientists, and facilitating research translation. The CCTS routinely supports a wide variety of both inpatient and outpatient studies. CCTS staff perform blood draws and provide support for muscle biopsies. Examination rooms include a procedure supply cabinet, 10 Bergstrom-type 5 mm biopsy needles, and a bedside workstation with dissecting microscope and analytical balance for bedside tissue processing. The CCTS also houses the Body Composition Core, including a Lunar Prodigy DXA. **Muscle biopsies and DXA of human subjects in this project will be performed at the CCTS.**

Animal Research Facilities: The Division of Laboratory Animal Resources (DLAR) is the centralized core facility responsible for housing biomedical research animals at the UK. DLAR is fully accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC) and operates under a statement of assurance of compliance with the PHS Policy on the Humane Care and Use of Laboratory Animals on file with the Office of Laboratory Animal Welfare (A3336-01). UK’s Biological-Pharmaceutical vivarium, completed for occupancy in February 2010, is a 16,319 NASF animal facility located in the Lee T. Todd, Jr. Building. The new facility offers 13 animal holding rooms, 2 multi-station rodent surgical suites with instrument preparation and storage, 4 shared procedure rooms, 6 behavioral rooms, 8 additional procedure rooms contiguous with animal holding rooms, an imaging room, a necropsy suite, and a cage wash with bulk sterilizer. The facility was designed to provide a daily inventory of up to 2,000 sterile mouse cages and 11,000 IVC cages. All protocols involving vertebrate animals are performed under the oversight of the UK Institutional Animal Care and Use Committee (IACUC). **Animals included in this research proposal will be housed in UK’s Biological-Pharmaceutical vivarium, where most procedures will also be conducted. It is a ten minute enclosed walk from the research labs.**

Office Space/Computer: Full computer support are available; currently, Dr. Kalaitzoglou has dedicated access to a Laptop with docking abilities and dual monitors with additional data storage availability in the BBPDR and CMB Imaging Labs. She also has the necessary software for data analysis including Microsoft Office, GraphPad Prism, Endnote and Adobe Acrobat Pro. **Dr. Kalaitzoglou’s primary office space is adjacent to the BBPDR lab in the CTW building, with a separate phone and shared printer. Additional office space is available at the BBDC clinic area to both Dr. Kalaitzoglou and the BBDC research coordinator.** There is free access to shared printer and administrative support in the BBDC clinic area.

Other Resources:

Biostatistics, Epidemiology, and Research Design Core: Faculty from key academic disciplines in biostatistics, statistics, and epidemiology, led by core director, Richard Kryscio, PhD, form the CCTS Biostatistics, Epidemiology, and Research Design (BERD) Core. They serve the biostatistical, study design,

and data analysis needs of CCTS investigators. Four specialized BERD services include: 1) data analysis, 2) randomized clinical trial design, 3) population-based study design, and 4) observational study design. **This service will be available as consultation for clinical study design and data analysis.**

Office of Research Integrity (ORI). The office provides administrative assistance to UK's Institutional Review Boards (three medical, one non-medical) for the Protection of Human Research Subjects, the Radioactive Drug Research Committee (RDRC) and the Institutional Animal Care and Use Committee (IACUC). ORI also advises faculty, staff, and students regarding university and federal regulations; disseminates IRB, RDRC and IACUC application forms; prepares and maintains federally mandated reports; and assists in handling IRB, IACUC, and RDRC reports of noncompliance. ORI offers training in the ethical conduct of research involving human subjects, animals, and in handling allegations of research misconduct. **These services will be available for training and consultation for the duration of this project.**

Proposal Development Office (PDO). PDO assists faculty in securing extramural funding to support their scholarly activities. Specific functions include disseminating announcements of, and applications for funding opportunities; identifying potential funding sources; serving as a liaison with appropriate funding agencies; identifying potential collaborators on the UK campus; assisting individuals and groups of faculty in developing programs and proposals; reviewing proposal drafts; conducting seminars on funding strategies and grant proposals; conducting new faculty orientations; and coordinating multidisciplinary research proposal submissions. **Assistance from the PDO will be used during this award as consultation services will be utilized towards grant writing workshops and when preparing applications for future R-type funding.**

The Metabolic Core Facility is a part of the Center of Research on Obesity and Cardiovascular Disease (COCVD). The COCVD is a multidisciplinary collaborative project created to identify mechanisms linking the epidemic of obesity to the high incidence of cardiovascular diseases in the obese population. The Metabolic Core provides specialized equipment and expertise that allow investigators to gather data on the effects on metabolism of genetic and environmental interventions, and to examine how metabolic changes affect the development of obesity and cardiovascular disease. Test equipment of the Core Facility is located in the BioPharmacy DLAR facility and DLAR assigned space in the BBSRB Facility. All animals tested using COCVD equipment will have approved use of individual test procedures (TSE® LabMaster Calorimetry, ECHO-MRI Relaxometry) on their home research IACUC protocol. **This facility is where EchoMRI for assessment of body composition in animal studies will be performed.**

Vanderbilt University Medical Center - collaborating site for ex vivo skeletal analyses: Dr. Nyman (collaborator) has full access to *multiple* facilities on the joint campuses of VA Tennessee Valley Health Care System (TVHS), Vanderbilt University (VU), and Vanderbilt University Medical Center (VUMC) including the Vanderbilt University Institute of Imaging Science (VUIIS), the Vanderbilt Center for Bone Biology (VCBB), Vanderbilt Biophotonics Center (VBC), and the Orthopaedic Biomechanics Lab (OBL). The OBL (950 ft^2) is a bioengineering lab in the Department of Orthopaedic Surgery and Rehabilitation at VUMC. Managed by Dr. Nyman, the lab is entirely available to serve the needs of the proposed project. It houses the material testing equipment necessary to perform the mechanical tests on all bone samples. There are -20 °C and -80 °C freezers available for storing mouse femurs. Also, the lab has the tools and a dedicated dissection room for notching bone specimens. Lastly, the lab is adjacent to the VUIIS building that houses the imaging equipment (2 min. walk), and all bone specimens will be transported in an enclosed container designed to transport biohazardous tissues. **These facilities are where imaging and biomechanical testing of bone samples in the animal studies will be performed (Aim 2 and 3).**

Equipment

Center for Clinical and Translational Science

The following equipment will be used towards completion of **Specific Aim 1:**

Functional Assessment and Body Composition Core (FABCC)

- Lunar Prodigy DXA scanner

Clinical Research Unit (CRU)

Examination rooms include

- Procedure supply cabinet
- 10 Bergstrom-type 5 mm biopsy needles and necessary instruments for muscle biopsies
- Bedside workstation with dissecting microscope and analytical balance for bedside tissue processing

Laboratory processing and shipping area with freezer storage and dry ice is available as well as a nursing station on site.

Barnstable Brown Pediatric Diabetes Research Laboratory

The following equipment will be used towards completion and data analysis for **Specific Aims 2, 3, and 4:**

Fully equipped to complete cell culture experiments, protein and nucleic acid electrophoresis, RT-qPCR, immunohistochemistry amongst other techniques.

- Nuaire biosafety cabinet
- Air jacketed humidified CO2 incubators, liquid nitrogen cell storage unit
- Nikon Eclipse TS100 inverted microscope
- Sorvall refrigerated centrifuge
- Millipore Milli-Q A10 water purification system.
- Storage freezers and refrigerators (Thermo -80^{o}C, -20^{o}C and +4^{o}C)
- Bio-Rad protein and nucleic acid gel electrophoresis equipment
- MJ Research PTC-200 PCR thermal cycler
- Biotek EL800 absorbance plate reader
- Bio-Rad PW41 automatic plate washer
- Bio-Rad SmartSpec Plus UV/vis spectrophotometer
- ABI QuantStudio 5 (up to five color qPCR)
- Plexiglass mouse metabolism cages
- 2 chemical fume hoods
- Fisher Accumet pH meter.
- Zeiss Axio Observer inverted microscope, with an extensive array of objectives, 3 observation screens, and associated software for multi-color imaging and analyses

Center for Muscle Biology Laboratory

The following equipment will be used towards data analysis of skeletal muscle for **Specific Aims 1, 2 and 3:**

Immunohistochemistry (IHC) Lab: Fully equipped for carrying out IHC protocols and muscle tissue sectioning. Two cryostats are available in the Core:

- HM505E for core personnel
- Microm HM525NX for training and use by researchers. The HM525NX is user-friendly with a digital, touch-screen display and can be set for temperatures ranging between +5 and -35 ºC.

Other equipment includes

- shaking water baths,
- dry oven,

- hybridization oven,
- refrigerator and -20ºC freezer
- chemical fume hood outfitted with an H&E staining station and certified for satellite collection of hazardous waste.
- Microm HM315 microtome for sectioning of paraffin blocks is available upon request.

The IHC lab contains three microscopes:

- Zeiss Axioscop 40 teaching microscope with two binocular phototubes, pointer arrow and an LED light source.
- Olympus BX51 with brightfield capabilities and four fluorescent filters: DAPI, FITC, Texas Red and a Triple D/F/TXRD cube for simultaneously viewing all DAPI, FITC and Texas Red).
- Olympus SZ61 dissecting scope, equipped with an Olympus DP12 3.24 Mpixel digital camera.

Imaging Facility: The CMB imaging lab includes four fluorescent microscopes: 1) a Zeiss AxioImager MI upright scope; 2) an AxioObserver 7 inverted scope equipped for live-cell imaging; 3) an Olympus BX61VS; and 4) a Nikon upright scope.

- The Zeiss AxioImager M1 has a fully automated stage and filter wheel, and can acquire whole section, stitched images. This scope is equipped with four fluorescent filters (DAPI, FITC, TRITC and Cy5) and 4 air objectives (5x, 10x, 20x and 40x) plus a 100x oil objective.
- The Zeiss AxioObserver 7 inverted microscope is outfitted with a fully automated stage and motorized objective and reflector turret, allowing for whole section and multi-well scanning of samples. This scope is equipped with four fluorescent filters (DAPI, FITC, TRITC and Cy5) and 4 air objectives (2x, 10x, 20x and 40x).
- The Olympus BX61VS has a fully automated stage, which can hold up to five slides at once.
- The Nikon upright microscope is equipped with the CRi Nuance™ FX multispectral imaging system (Caliper Life Sciences, Hopkinton, MA).

A high-powered computing server (four CPUs) comprised of four Intel Xeon E5-4650L 2.6 GHz processors, 248 GB of RAM and 48 TB of total disk space will be available for processing large images, performing batch analysis of samples and handling large data.

Center of Research on Obesity and Cardiovascular Disease (COCVD)

The following equipment will be used for body composition analysis for Specific **Aim 3:**

- Echo-MRI-100™ Imager (Echo Medical System, Houston, TX)

Vanderbilt University Medical Center (VUMC) including the Tennessee Valley Healthcare System (TVHS) Lab, Vanderbilt University Institute of Imaging Science (VUIIS), the Orthopaedic Biomechanics Lab (OBL), and Vanderbilt Center for Bone Biology (VCBB)- Collaborating Site:

The following equipment will be used towards analysis of bone samples from **Specific Aims 2 and 3:**

VA TVHS

- A Speed-vac concentrator (Thermo-Fisher) with cold-trap is available to safely evaporate HCl acid after hydrolyzing bone for HPLC.
- Assorted equipment for characterizing bone samples include: Sorvall RC3B refrigerated centrifuge, various balances including a Cahn 24 automatic and Denver Instrument SI-215D electrobalance (0.0001g).
- The lab also has a laminar flow hood for mixing chemicals, a refrigerator for chemical storage, vacuum oven for hydrolyzing bone, storage cabinets for flammable liquids.
- A SPEX Freezer Mill is also available for turning bone into a fine powder from which proteins can be extracted.

VUIIS/CSAI

- The high throughput Scanco µCT50 is capable of acquiring high resolution (2-6 µm voxel size) images sufficient to determine mineralization density as well as architectural and structural characteristics of cortical bone and small mouse bones. There is also an earlier generation Scanco µCT40 available if needed. The VUIIS also houses vivaCT80 for *in vivo* low dose, high-resolution µCT imaging of rodents. The systems include a cluster of 8 HP Integrity computers with a total of 50 processor cores, >1 TB of RAM and 54 TB of storage on 4 duplicative RAID arrays. The Scanco software enables rapid and highly reproducible 3D analysis of trabecular and cortical bone as well as variety of synthetic materials using validated and widely accepted computations.
- A Faxitron LX-60 digital X-ray imaging system is available.

OBL

- For processing mineralized tissue and generating the specimens for mechanical testing, there is a diamond-embedded band saw (Exakt Systems), 2 low-speed, diamond-embedded circular saws (South Bay and Buehler Isomet), drill press with end-mill, and a lathe. Also, included is a histological grinding wheel along with mounting and embedding materials (part of the Exakt Systems) that produce reflective surfaces of bone for microscopic analysis of the micro-structure as well as near parallel ~100 micron sections. A metallurgic microscope (reflective light) and a transmission microscope with CCD camera (Zeiss Axio Imager) provide views of micro-features of bones.
- The two universal material testing machines - the MTS 858 Bionix and the Instron 8841 Dynamight - are servo-hydraulic, thereby capable of dynamic or cyclic testing. They acquire data at >100 Hz and test bone in flexure, tension, compression, or torsion. The latter machine has the necessary load cells and fixtures to test whole mouse bones.
- SRL Cannon camera are available for tracking crack growth in single edge notched beam specimens.
- The lab also has the reference point indentation instrument BioDent^{TM} (Active Life Scientific, Inc., Santa Barbara, CA) with stainless steel test probes (375 µm diameter, 90° cono-spherical, 2.5 µm radius tip).

VCBB

- Also available to the project is the Leica RM 2165 motorized microtome that cuts sections from plastic-embedded, undecalcified specimens and decalcified, paraffin specimens.
- Shandon Hypercenter XP tissue processor is also available to embed decalcified bone tissue.
- Autoclave and purified water are also available to the project.

OMB Number: 4040-0010
Expiration Date: 12/31/2022

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

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Project Role*: PD/PI Other Project Role Category:

Degree Type: MD Degree Year: 2008

Attach Biographical Sketch*: File Name: EK_Biosketch_0303201009017460.pdf

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

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Project Role*: Other Professional Other Project Role Category: Mentor

Degree Type: MD Degree Year: 1985

Attach Biographical Sketch*: File Name: 2020_RK08_FOWLKES_BIOSKETCH1009017461.pdf

Attach Current & Pending Support: File Name: Fowlkes_Other_Support_03_06_20201009017518.pdf

PROFILE - Senior/Key Person

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Project Role*: Other Professional Other Project Role Category: Co-Mentor

Degree Type: Degree Year:

Attach Biographical Sketch*: File Name: Kern_Biosketch_Eva_K_RK081009017462.pdf

Attach Current & Pending Support: File Name: Kern_Other_Support_3_10_201009017741.pdf

PROFILE - Senior/Key Person

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Project Role*: Other Professional Other Project Role Category: Co-Mentor

Degree Type: PHD Degree Year: 1984

Attach Biographical Sketch*: File Name: Peterson_Biosketch_2020_RK081009017463.pdf

Attach Current & Pending Support: File Name: Peterson_Support_030220201009017464.pdf

PROFILE - Senior/Key Person

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Project Role*: Other (Specify) Other Project Role Category: Collaborator

Degree Type: Degree Year:

Attach Biographical Sketch*: File Name: Biosketch_Nyman_2020_RK08_SC1009017465.pdf

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

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Project Role*: Other (Specify) Other Project Role Category: Collaborator

Degree Type: Degree Year:

Attach Biographical Sketch*: File Name: Tom_Hawke_Biosketch__RK08_0302201009017466.pdf

Attach Current & Pending Support: File Name:

OMB No. 0925-0001 and 0925-0002 (Rev. 09/17 Approved Through 03/31/2020)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. DO NOT EXCEED FIVE PAGES.

NAME: Kalaitzoglou, Evangelia

eRA COMMONS USER NAME (credential, e.g., agency login): evangelia.kalaitzoglou

POSITION TITLE: Assistant Professor of Pediatrics

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE (if applicable)	END DATE MM/YYYY	FIELD OF STUDY
National and Kapodistrian University of Athens, Medical School, Athens	MD	08/2008	N/A
Penn State University, Hershey Medical Center, Hershey, PA	Resident	06/2012	Pediatrics
Oklahoma University Health Sciences Center (OUHSC), Oklahoma City, OK	Fellow	06/2015	Pediatric Endcocrinology

A. Personal Statement

I am a Pediatric Endocrinologist and a physician scientist, with specific interest in Type 1 diabetes and related complications, especially those affecting the musculoskeletal system. In addition to extensive training in treating patients with Type 1 diabetes, I have recently started focusing on the effects of insulin-dependent diabetes on muscle and bone interactions. I would like to further advance my knowledge in this area, as there are no current guidelines dictating patient care regarding bone and muscle health in individuals with Type 1 diabetes. The focus of my research is to delineate the effects of diabetes on bone and muscle and the relationship between muscle and bone disease in different diabetic animal models, and in humans. The role of cytokines released by muscle cells, also known as myokines, as signaling molecules that could affect bone morphology and repair in a diabetic environment is not well known. Our animal models, including the model of streptozotocin-induced diabetes, are associated with deficits in muscle and bone. My studies are aimed towards describing the effects of myostatin on bone and muscle in a diabetic environment and the underlying mechanisms. To achieve this goal, I have assembled a mentoring team with expertise in bone health, diabetes mellitus, and muscle physiology. My collaborators will assist with interpretation of the data, offering their relative expertise and/or resources towards this goal. The rich mentoring team and resources at the University of Kentucky will allow me to complete this research project and move my career onto an independent scientist trajectory

1. Fowlkes JL, Bunn RC, Ray PD, Kalaitzoglou E, Uppuganti S, Unal M, Nyman JS, Thrailkill KM. Constitutive activation of MEK1 in osteoprogenitors increases strength of bone despite impairing mineralization. Bone. 2020 Jan;130:115106. PMCID: PMC6914252.
2. Kalaitzoglou E, Fowlkes JL, Popescu I, Thrailkill KM. Diabetes pharmacotherapy and effects on the musculoskeletal system. Diabetes Metab Res Rev. 2019 Feb;35(2):e3100. PMCID: PMC6358500.
3. Nyman JS, Kalaitzoglou E, Clay Bunn R, Uppuganti S, Thrailkill KM, Fowlkes JL. Preserving and restoring bone with continuous insulin infusion therapy in a mouse model of type 1 diabetes. Bone Rep. 2017 Dec;7:1-8. PMCID: PMC5508511.
4. Kalaitzoglou E, Popescu I, Bunn RC, Fowlkes JL, Thrailkill KM. Effects of Type 1 Diabetes on Osteoblasts, Osteocytes, and Osteoclasts. Curr Osteoporos Rep. 2016 Dec;14(6):310-319. PMCID: PMC5106298.

B. Positions and Honors

Positions and Employment

2015 - Assistant Professor of Pediatrics, University of Kentucky, Lexington, KY

Other Experience and Professional Memberships

2013 -	Member, Pediatric Endocrine Society (PES)
2013 -	Member, The Endocrine Society
2016 -	Member, PES International Council
2016 -	Member, PES Training Council
2017 -	Member, American Diabetes Association
2017 - 2017	Reviewer, American Diabetes Association
2017 - 2020	Reviewer, Pediatric Endocrine Society (PES)
2019 - 2020	Member, European Society for Pediatric Endocrinology

Honors

2014	Oral Presentation Award, OUHSC Pediatric Research Day
2014	Young Investigator Travel Grant, ASBMR
2014 - 2015	Travel Award, Pediatric Endocrine Society (PES)
2016	Travel Grant in Orthopaedic Research Translation, ORS/OREF
2017	Helmsley Charitable Trust Award in Type 1 Diabetes, The Endocrine Society
2017	Award of Outstanding Research Presentation, Barnstable Brown Diabetes Research Day
2019	KL2 Exchange Program, selection as KL2 Visiting Scholar, Indiana University/University of Kentucky

C. Contribution to Science

1. The impact of insulin or other diabetes related treatments on diabetic bone disease: Type 1 diabetes, a chronic disease characterized by insulin deficiency, is associated with decrease in bone mass accrual and increased risk of fracture, a condition termed diabetic bone disease. Several modifiers of diabetes control, such as insulin and oral hypoglycemic agents can have variable effects on the developing skeleton of individuals with type 1 diabetes. Recently, I have had the opportunity to work with Drs. Fowlkes and Thrailkill and have participated in studies involving treatment of streptozotocin-induced diabetes in mice with insulin and/or SGLT2 inhibitors. The results of these studies highlight the beneficial effects of insulin treatment on the bone phenotype of diabetic animals and the possible effects of SGLT2 inhibitors on the diabetic skeleton. Further studies are needed to elucidate the mechanisms responsible for these effects and their relative contributions to diabetic bone disease.

    a. Kalaitzoglou E, Fowlkes JL, Popescu I, Thrailkill KM. Diabetes pharmacotherapy and effects on the musculoskeletal system. Diabetes Metab Res Rev. 2019 Feb;35(2):e3100. PMCID: PMC6358500.
    b. Nyman JS, Kalaitzoglou E, Clay Bunn R, Uppuganti S, Thrailkill KM, Fowlkes JL. Preserving and restoring bone with continuous insulin infusion therapy in a mouse model of type 1 diabetes. Bone Rep. 2017 Dec;7:1-8. PMCID: PMC5508511.
    c. Thrailkill KM, Nyman JS, Bunn RC, Uppuganti S, Thompson KL, Lumpkin CK Jr, Kalaitzoglou E, Fowlkes JL. The impact of SGLT2 inhibitors, compared with insulin, on diabetic bone disease in a mouse model of type 1 diabetes. Bone. 2017 Jan;94:141-151. PMCID: PMC5826569.
    d. Kalaitzoglou E, Popescu I, Bunn RC, Fowlkes JL, Thrailkill KM. Effects of Type 1 Diabetes on Osteoblasts, Osteocytes, and Osteoclasts. Curr Osteoporos Rep. 2016 Dec;14(6):310-319. PMCID: PMC5106298.

2. The role of the innate immune system in osteoarthritis: Osteoarthritis is a very common disease that affects both weight bearing and non-weight bearing joints. Dysregulation of the innate immune system associated with aging and adiposity can influence the development and progression of osteoarthritis. While working with Dr. Humphrey and Dr. Griffin we attempted to delineate the role of Toll-Like Receptor 4 (TLR4) and its negative regulator DNAX-activating protein of molecular mass 12 kilodaltons (DAP12) in the development of osteoarthritis. We believe that TLR4 ligands and inflammatory cytokines result in pro-inflammatory signals, driving macrophage-mediated, chronic inflammation and osteoarthritis.

a. Kalaitzoglou E, Lopes EBP, Fu Y, Herron JC, Flaming JM, Donovan EL, Hu Y, Filiberti A, Griffin TM, Humphrey MB. TLR4 Promotes and DAP12 Limits Obesity-Induced Osteoarthritis in Aged Female Mice. JBMR Plus. 2019 Apr;3(4):e10079. PMCID: PMC6478583.
b. Kalaitzoglou E, Griffin TM, Humphrey MB. Innate Immune Responses and Osteoarthritis. Curr Rheumatol Rep. 2017 Aug;19(8):45. PubMed PMID: 28718060.

3. Evaluation of body composition throughout the lifespan: This product was a result of collaboration with Dr. David Fields during my fellowship at OUHSC and resulted in a review identifying the best methods for measurement of body composition through different stages in life. The conclusions of our review are that air displacement plethysmography is the best available method to track body composition throughout the lifespan.

a. Fields DA, Gunatilake R, Kalaitzoglou E. Air displacement plethysmography: cradle to grave. Nutr Clin Pract. 2015 Apr;30(2):219-26. PubMed PMID: 25761768.

Complete List of Published Work in MyBibliography:

https://www.ncbi.nlm.nih.gov/myncbi/evangelia.kalaitzoglou.1/bibliography/public/

D. Additional Information: Research Support

Ongoing Research Support

Clinician Scientist Development Award (SChoLAR) Kalaitzoglou (PI) 07/01/18-08/30/20
University of Kentucky-College of Medicine,
The role of myostatin in muscle-bone interactions and diabetic bone disease in Type 1 diabetes
Role: PI

Children's Miracle Network Research Fund Kalaitzoglou (PI) 11/17/17-11/17/20
The role of myostatin in muscle-bone interactions in Type 1 diabetes
Role: PI

OMB No. 0925-0001 and 0002 (Rev. 09/17 Approved Through 03/31/2020)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. DO NOT EXCEED FIVE PAGES.

NAME: JOHN FOWLKES

eRA COMMONS USER NAME (credential, e.g., agency login): meggers1

POSITION TITLE: Director, Barnstable Brown Diabetes Center and Professor of Pediatrics

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
West Texas State University, Canyon, TX	BS	1981	Biology & Chemistry
University of Texas Health Science Center at San Antonio, TX	MD	1985	Medicine
University of Texas Health Science Center at San Antonio, TX	None	1988	Pediatric Residency
Duke University Medical Center, Durham, NC	None	1992	Fellowship: Pediatric Endocrinology

A. Personal Statement

I am a pediatric endocrinologist and clinician scientist, with significant experience in project management and research leadership, having carried out research in basic, translational and clinical arenas for over 25 years. In particular, my research group has made significant findings concerning nephropathy and bone disease in conjunction with diabetes. I have been the principal investigator of NIH-funded projects related to these areas since 1999. My administrative experience includes a position as Chief, Department of Pediatrics at the University of Arkansas from 2001 to 2014 and leadership of a focus group on diabetes and endocrinology for the Arkansas Biosciences Institute from 2007 to 2014. As of January 1, 2015, I relocated to the University of Kentucky and assumed the position as Director of the Barnstable Brown Diabetes Center. In my current role, I am responsible for cultivating and supporting basic, translational, and clinical research related to diabetes and its complications. Over the last few years, I have begun to work with a collaborative group (Drs. Bunn and Thrailkill, now at University of Kentucky) and Dr. Nyman (Vanderbilt) to better understand the skeletal fragility observed in diabetes. Over the course of these collaborative efforts, we have been able to better define the roles that deficiencies of insulin and IGF-I play in the pathogenesis of this disorder, and how replacement of these same hormones may prove beneficial in preventing or reversing diabetic bone disease. Recently, our group along with other primary investigators in the field, along with my co-Editor, Dr. Lecka-Czernik, produced an up-to-date and comprehensive text (a first of its kind) on diabetic bone disease (Diabetic Bone Disease: Basic and Translational Research and Clinical Applications, Springer, 2016). As a clinician scientist, I believe I bring a unique and informed perspective to this problem. Thus, as a pediatric endocrinologist and physician scientist working in diabetes-related complications, I believe my background makes me a very appropriate and timely mentor to Dr. Kalaitzoglou. Since her arrival at UK, Dr. Kalaitzoglou has become an integral force within the research efforts of the Barnstable Brown Diabetes and Obesity Research Center. I look forward to working with her as she now develops her own independent and unique research efforts to understand the impact of muscle/bone interactions in Type 1 diabetes. I am fully supportive of her research career and I look forward to mentoring her throughout the tenure of this K08 award and to promoting her research path to independence.

a) Thrailkill, K.M., Lumpkin, C.K., Jr., Bunn, R.C., Kemp, S.F., and Fowlkes, J.L.: Is insulin an anabolic agent in bone: Dissecting the diabetic bone for clues. American Journal of Physiology - Endocrinology & Metabolism 289:E735-745, 2005. PMCID: PMC2387001.

b) DIABETIC BONE DISEASE: BASIC AND TRANSLATIONAL RESEARCH AND CLINICAL APPLICATIONS. Lecka-Czernik, B. and Fowlkes, J.L. (Editors). Publisher: Springer US. 2016.
c) Kalaitzoglou, E., Popescu, I., Bunn, R.C., Fowlkes, J.L., Thrailkill, K.M.: Effects of Type 1 Diabetes on Osteoblasts, Osteocytes, and Osteoclasts. Curr Osteoporos Rep. 2016 Oct 4. PMCID: PMC27704393.
d) Kalaitzoglou, E., Fowlkes, J.L., Popescu, I., Thrailkill, K.M.: Diabetes pharmacotherapy and effects on the musculoskeletal system. Diabetes Metab Res Rev. 2018 Nov 22:e3100. PMCID: PMC30467957.

B. Positions and Honors

Positions and Employment

1992-1994	Associate in Pediatrics, Duke University Medical Center, Department of Pediatrics, Division of Pediatric Endocrinology, Durham, NC
1994-1996	Assistant Professor of Pediatrics, Duke University Medical Center, Department of Pediatrics, Division of Pediatric Endocrinology, Durham, NC
1996-2000	Assistant Professor of Pediatrics, University of Kentucky Medical Center, Department of Pediatrics, Division of Pediatric Endocrinology, Diabetes and Metabolism, Lexington, KY
1997-2000	Assistant Professor of Physiology, University of Kentucky Medical Center, Department of Physiology, Lexington, KY
2000-2001	Associate Professor of Pediatrics, University of Kentucky Medical Center, Department of Pediatrics, Division of Pediatric Endocrinology, Diabetes and Metabolism, Lexington, KY
2000-2001	Associate Professor of Physiology, University of Kentucky Medical Center, Department of Physiology, Lexington, KY
2001-2014	Professor of Pediatrics, Chief, Division of Pediatric Endocrinology and Diabetes (tenure awarded in 2007), University of Arkansas for Medical Sciences, Department of Pediatrics
2007-2015	Vice Chair for Research, Department of Pediatrics, University of Arkansas for Medical Sciences, Little Rock, AR
2015-Present	Professor, Department of Pediatrics, University of Kentucky, Lexington, KY
2015-Present	Professor, Pharmacology and Nutritional Sciences, University of Kentucky, Lexington, KY
2016-Present	Professor, Department of Medicine, University of Kentucky, Lexington, KY
2015-Present	Director, Barnstable Brown Kentucky Diabetes and Obesity Center, University of Kentucky, Lexington, KY

Other Experience and Professional Memberships

- Memberships in professional or honor societies: AϕA; Beta Beta Beta; Alpha Chi; Phi Beta Sigma; American Society for Bone and Mineral Research (ASBMR); American Diabetes Association (ADA); The Endocrine Society; Pediatric Endocrine Society (PES); Society for Pediatric Research (SPR).
- Editorial Boards: ENDOCRINOLOGY (2000-2004), ENDOCRINE (2000-2010)
- NIH Study Sections: 2005, 06, 09, 10, 11, 12, 13, 14 – ad hoc reviewer for SBSR, NAME, SBDD, MOSS, and/or EMNR-T; 2013-2017 - permanent member SBSR.

Honors

1993	March of Dimes Birth Defects Foundation, Basil O'Connor Starter Scholarship Research Award
2001	Endowed Professorship – Barnstable-Brown
2007-2014	Endowed Chair - James Hamlen Professor of Pediatric Endocrinology
2015-present	Endowed Chair - Barnstable Brown Kentucky Diabetes and Obesity Center.

C. Contribution to Science

Our Diabetes Research Working Group has explored several major areas of science related to diabetes and its complications. Highlights are presented below:

Diabetic bone disease. Our group is one of only a handful that has developed extended and clinically relevant mouse models to study diabetic bone disease, which is now a widely recognized complication of diabetes in humans. Our group demonstrated that diabetes impairs regeneration of bone and makes bone

more fragile and fracture-prone. In addition we have superimposed diabetes onto relevant genetically-modified mouse models in order to study the mechanisms by which insulin and IGF-1 may prevent and/or improve diabetic bone disease.

a) Thrailkill, K.M., Liu, L., Wahl, E.C., Bunn, R.C., Perrien, D.S., Cockrell, G.E., Skinner, R.A., Hogue, W.R., Carver, A., Fowlkes, J.L., Aronson, J., and Lumpkin, C.K., Jr.: New bone formation is impaired in a model of Type I Diabetes Mellitus. Diabetes 54:2875-2881, 2005. PMCID: PMC16186388.
b) Nyman, J.S., Bunn, R.C., Even, J.L., Jo, C-H, Herbert, E.G., Murry, M.R., Cockrell, G.C., Wahl, E.C., Lumpkin, C.K., Jr., Fowlkes, J.L., Thrailkill, K.M.: Increasing duration of Type 1 diabetes perturbs the strength-structure relationship of mouse bone and increases brittleness. Bone. 48:733-740, 2011. PMCID: PMC3062641.
c) Thrailkill, K., Bunn, R.C., Lumpkin, Jr., C.K., Wahl, E., Cockrell, G., Morris, L., Kahn, C.R., Fowlkes, J., Nyman, J.S.: Loss of insulin receptor in osteoprogenitor cells impairs structural strength of bone, J Diabetes Res, Epub May 18, 2014. PMCID: PMC4052184.
d) Iyer, S., Han, L., Ambrogini, E., Yavropoulou, M., Fowlkes, J., Manolagas, S.C., Almeida, M.: Deletion of FoxO1, 3 and 4 in osteoblast progenitors attenuates the loss of cancellous bone mass in a mouse model of Type 1 Diabetes. J Bone Miner Res. Aug 4. doi: 10.1002/jbmr.2934, 2016. PMCID: PMC5492385.

Effects of insulin and IGF-1 on the skeleton and diabetic bone disease. We have shown that insulin and IGF-1 signal transduction is functional in osteoblast precursors and throughout osteoblast differentiation *in vitro*. Our work over the past few years has demonstrated that *in vivo* the diabetic bone phenotype can be markedly improved by insulin and IGF-1. These improvements can be seen at the level of osteoblastogenesis (i.e., RUNX2- and RUNX2-regulated genes), microarchitecture (uCT and histomorphometry), strength and toughness (bone quality indicators), and de novo bone regeneration (distraction osteogenesis).

a) Fowlkes, J.L., Liu, L, Wahl, E.C., Coleman, H.N., Cockrell, G.E., Perrien, D. S., Lumpkin, C.K. Jr.: Runt-related transcription factor 2 (RUNX2) and RUNX2-related osteogenic genes are down regulated in regenerate bone in a model of type 1 diabetes mellitus while local adipogenesis is enhanced. Endocrinology. 149:1697-704, 2008. PMCID: PMC2276714.
b) Fowlkes, J.L., Nyman, J.S., Bunn, R.C., Jo, C-H., Wahl, E.C., Liu, L., Cockrell, G.E., Morris, L.M., Lumpkin, Jr. C.K., Thrailkill, K.M.: Osteo-promoting effects of insulin-like growth factor I (IGF-I) in a mouse model of type 1 diabetes. Bone. 57: 36-40, 2013. PMCID: PMC3789626.
c) Nyman, J.S., Kalaitzoglou, E., Bunn, C.R., Uppuganti, S., Thrailkill, K.M., Fowlkes, J.L. Preserving and restoring bone with continuous insulin infusion therapy in a mouse model of type 1 diabetes. Bone Rep. 2017 Jul 4;7:1-8. PMCID: PMC5508511.
d) Thrailkill, K.M., Nyman, J.S., Bunn, R.C., Uppuganti, S., Thompson, K.L., Lumpkin, C.K. Jr, Kalaitzoglou, E., Fowlkes, J.L. The impact of SGLT2 inhibitors, compared with insulin, on diabetic bone disease in a mouse model of type 1 diabetes. Bone. 2017 Jan;94:141-151. PMCID: PMC5826569.

Regulation of insulin-like growth factor action and bioavailability. Our group was the first to identify the matrix metalloproteinases (MMPs) as insulin-like growth factor binding protein (IGFBP) proteinases. Furthermore, we have shown that degradation of IGFBPs results in the release of bioactive IGFs. This phenomenon may be important in a variety of physiologic (e.g., cellular growth and differentiation) as well as pathophysiologic conditions (e.g., cancer and fibrosis).

a) Fowlkes J.L., Enghild J.J., Suzuki K., Nagase H. Matrix metalloproteinases degrade insulin-like growth factor-binding protein-3 in dermal fibroblast cultures. Journal of Biological Chemistry 269:25742-25746, 1994.
b) Fowlkes J.L., Serra D.M., Rosenberg C.K., Thrailkill K.M. Insulin-like growth factor (IGF)-binding protein-3 (IGFBP-3) functions as an IGF-I reversible inhibitor of IGFBP-4 proteolysis. Journal of Biological Chemistry 270: 2748127488, 1995.
c) Fowlkes J.L., Serra D.M., Enghild J.J., Suzuki K., Nagase H. Regulation of insulin-like growth factor (IGF)-I action by matrix metalloproteinase-3 involves selective disruption of IGF-I/IGF-binding protein-3 complexes. Endocrinology,145:620-626, 2004.

d) Bunn R.C., Green L.D., Overgaard M.T., Oxvig C., Fowlkes J.L. IGFBP-4 degradation by pregnancy-associated plasma protein-A in MC3T3 osteoblasts. Biochemical and Biophysical Research Communications, 325:698-706, 2004.

Complete List of Published Work in MyBibliography:

https://www.ncbi.nlm.nih.gov/sites/myncbi/john.fowlkes.1/bibliography/49510304/public/?sort=date&direction=ascending

D. Research Support

Ongoing Research Support

2R56 DK084045-05A1 (NIH/NIDDK) Fowlkes (PI) 04/01/18-03/31/20
The Insulin/IGF Axis in Diabetic Osteopathy
The goal of this project is to understand the role of insulin and IGF-I signaling in osteogenesis by identifying signaling pathways utilized by insulin and IGF-I to regulate osteoblastic gene expression, characterizing skeletal strength and quality parameters in mice deficient for insulin and/or IGF-I receptors, and investigating the utility of insulin and IGF-I in preventing diabetic osteopathy.
Role: PI

1UL TR001998-01 (NIH/NCATS) Kern (PI) 09/15/16 – 05/31/20
Kentucky Center for Clinical and Translational Science
The University of Kentucky (UK) Center for Clinical and Translational Science (CCTS) is an integrated home for clinical and translational research to promote scientific progress and discoveries at every phase of the translational continuum.
Role: Co-I and Director of Translational Research Development Team (TRiDENT).

1P30ES026529-01A1 (NIH/NIEHS) Shi (PI) 05/01/17-03/31/22
Center for Appalachian Research in Environmental Sciences.
The University of Kentucky Center for Appalachian Research in Environmental Sciences (UK-CARES) is an Environmental Health Sciences Core Center to enhance research capacity focused on major environmental health impacts to air and water quality that have been implicated in environmentally induced disease.
Role: Co-I and Co-leader of Epigenetics and Metabolic Disorders Research Interest Group.

1P20GM121299-01A1 (NIH/NIGMS) Sundaram (PI) 02/15/18 – 01/31/23
Appalachian Center for Cellular Transport in Obesity Related Disorders (ACCORD)
This COBRE will identify novel targets that can potentially be modeled as new therapeutics to ameliorate and/or prevent obesity related disorders in West Virginia/Central Appalachia, and across the country
Role: Co-I, mentor.

Completed Research Support (within 3 years)

R01 DK084045-03 Fowlkes (PI) 05/23/12-03/31/18
The Insulin/IGF-I Axis in Diabetic Osteopathy
The goal of this project was to understand the role of insulin and IGF-I signaling in osteogenesis by identifying signaling pathways utilized by insulin and IGF-I to regulate osteoblastic gene expression, characterizing skeletal strength and quality parameters in mice deficient for insulin and/or IGF-I receptors, and investigating the utility of insulin and IGF-I in preventing diabetic osteopathy.
Role: PI

OMB No. 0925-0001 and 0925-0002 (Rev. 09/17 Approved Through 03/31/2020)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: **Philip A. Kern, MD**

eRA COMMONS USER NAME: PAKern

POSITION TITLE: Professor of Medicine

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Tufts University, Medford, MA	BS	06/1974	Chemistry & Biology
New York Medical College, Valhalla, NY	MD	06/1978	Medicine
Montefiore, Albert Einstein College of Medicine, Bronx, NY	Residency	06/1981	Internal Medicine
University of Colorado School of Medcine, Denver	Fellowship	06/1984	Endocrinology & Metabolism

A. Personal Statement

My current leadership positions include the Directorship of the Center for Clinical and Translational Science (CCTS) and Associate Provost for Clinical and Translational Science, where I report directly to the Provost and to the University Vice President for Research. The CCTS is a University of Kentucky (UK) campus-wide Center that houses the institutional Clinical and Translational Science Award (CTSA); it is supported by the CTSA grants (UL1/KL2/TL1) and institutional funds with a total yearly budget over $10 million. The mission of the CCTS is to stimulate innovative translational science on campus; develop the translational workforce; stimulate team science, in part through a robust pilot grant program; develop efficiencies and improved strategies for translational research; and generally serve as a nexus at UK and in the Central Appalachian region for research that improves health in the community. As I was the inaugural Director of UK's Barnstable Brown Diabetes and Obesity Center, founded in 2009, I relinquished this responsibility in 2015 to concentrate on leadership demands of the CCTS and my own research.

I have a long history of studying adipose and muscle biology and I am engaged in both basic and clinical research related to obesity, metabolic syndrome, inflammation, lipid metabolism, diabetes and insulin resistance, as outlined below. I have become recognized as an important collaborator and advisor on many other investigators' grant applications and I have played a crutial role in career development of numerous trainees, including Dr. Eva Kalaitzoglou. I have been meeting with her regularly and am happy to help guide her career development. In recognition of my research achievements, I am honored to have been selected by my peers and UK leadership as a 2019 University Research Professor, the premier research recognition at our institution. Below are recent collaborative publications with scientists at UK.

a. Sui Y, Liu Z, Park S-H, Thatcher SE, Zhu B, Fernandez JP, Molina H, **Kern PA**, Zhou C. 2018. IKKβ is β-catenin kinase that regulates mesenchymal stem cell differentiation. J. Clin. Invest. Insight 3:e96660, 1-22. PMCID: PMC5821193

b. Walton RG, Kosmac K, Mula J, Fry CS, Peck BD, Groshong JS, Finlin BS, Zhu B, **Kern PA**, Peterson CA. 2019. Human skeletal muscle macrophages increase following cycle training and are associated with adaptations that may facilitate growth. Sci Rep. 9:969. PMCID: 6353900

c. Nicholas DA, Proctor EA, Agrawal M, Belkina AC, Van Nostrand SC, Panneerseeian-Bharath L, Jones AR, IV, Raval F, Ip BC, Zhu M, Cacicedo J, Habib C, Sainz-Rueda N, Persky L, Sullivan PG, Corkey BE, Apovian CM, **Kern PA**, Lauffenburger DA Nikolajczyk BS. 2019. Fatty Acid Metabolites Combine with Reduced β Oxidation to Activate Th17 Inflammation in Human Type 2 Diabetes. Cell Metabolism 30:447-461. PMID:31378464

d. Verma N, Liu M, Ly H, Loria A, Campbell KS, Bush H, **Kern PA**, Jose PA, Taegtmeyer H, Bers DM, Despa S, Goldstein LB, Murray AJ, Despa F. 2020. Diabetic Microcirculatory Disturbances and Pathologic Erythropoiesis Provoked by Deposition of Amyloid-Forming Amylin in Red Blood Cells and Capillaries. Kidney Int. 97:143-155. PMC6943180

B. Positions and Honors

Positions and Employment

1984-1995 Assistant / Associate Professor of Medicine, UCLA/Cedars-Sinai Med Center, Los Angeles, CA
1995-2009 Professor of Medicine, University of Arkansas for Medical Sciences, Little Rock, AR
1995-2007 Associate Chief of Staff, Research, Central Arkansas Veterans Healthcare System, Little Rock
2007-2009 Assistant Dean Clinical Research, College of Medicine, University of Arkansas Medical Sci.
2009-2015 Director, Barnstable Brown Diabetes and Obesity Center, University of Kentucky, Lexington, KY
2009-present Professor, Department of Medicine, Division of Endocrinology, University of Kentucky
2009-present Director, Center for Clinical and Translational Science, University of Kentucky, Lexington, KY
2009-present Associate Provost for Clinical and Translational Science; University of Kentucky, Lexington, KY

Active State Medical Licensure: Kentucky and Arkansas

Board Certification: Internal Medicine, 1981, Endocrinology and metabolism, 1983

Honors

1974 Tufts University, Magna Cum Laude.
1978 New York Medical College, Alpha Omega Alpha
1983-1985 NIH National Research Service Award
1986-1989 JDF Career Development Award
1991-1996 American Heart Association Established Investigator Award
1992 American Society for Clinical Investigation
2004 Association of American Physicians
2007-2019 Best Doctors in America®
2015 Fred & Marcielle de Beer Award, Barnstable Brown Diabetes and Obesity Center
2019 University Research Professor Award, University of Kentucky

Other Experience and Professional Memberships

1996-2000 VA Merit Review Council and Appeals Committee
1996-2000 Research Grant Review Panel, American Diabetes Association
2000-2002 Associate Chair / Chair, American Diabetes Association Research Grant Review Panel
2001-2006 VA Merit Review Endocrinology and Metabolism Study Section
2005-2006 VA Merit Review Endocrinology and Metabolism Chairman
2007 NIH Review CTSA Study Section
2008 NIH Review CADO Study Section
2010-2015 NIH Review CIDO Study Section
2012-2015 NIH Review CIDO, Chairperson
2015-2019 NIH Review, Ad hoc study sections; NHLBI Mentored Patient-Oriented Research; Molecular transducers of physical activity. Sleep and diabetes; Sleep apnea and diabetes. Member conflict SEP: Obesity and diabetes

C. Contribution to Science

1. **Lipoprotein lipase (over 50 publications).** Much of my career has focused on adipocyte biology and the regulation of lipoprotein lipase (LPL). Early experiments used different forms of adipocyte preparations in cell culture, and in the late 1980's, I began performing fat and muscle biopsies in humans with a goal of more directly studying LPL and other genes involved in the regulation of the abnormal metabolic state that ultimately leads to type 2 diabetes. My overall goal was the translation of adipose tissue and muscle gene expression to physiological and clinical conditions. The studies on LPL examined the effects of obesity, diabetes, feeding, exercise, weight loss and other drugs and conditions on LPL. Much of LPL expression was controlled at the level of translation, and careful biochemical experiments determined that LPL translation repression in response to cAMP agonists and thyroid hormone was regulated by a trans-acting inhibitory RNA binding complex that bound to the LPL 3'UTR. We determined that this RNA binding complex was composed of the C and R subunits of PKA, as well as A kinase anchor protein, which tethers PKA to structures in the cell. Among the many clinical studies on LPL, one study used this information to

shed light on a known LPL variant. LPL S447X is a gain of function variant that confers increased LPL activity (and CHD protection) on subjects. We showed that this increase in LPL activity is likely due to improved LPL translation due to a lower binding affinity for the inhibitory RNA binding complex.

a. **Kern PA**, Ong JM, Saffari B, Carty J. 1990. The effects of weight loss on the expression of adipose tissue lipoprotein lipase in very obese humans. N. Engl. J. Med. 322:1053-1059.
b. Ranganathan G, Phan D, Pokrovskaya ID, McEwen JE, Li C, **Kern PA**. 2002. The translational regulation of lipoprotein lipase by epinephrine involves an RNA binding complex including the catalytic subunit of protein kinase A. J Biol Chem 277:43281-43287.
c. Ranganathan G, Pokrovskaya I, Ranganathan S, **Kern PA**. 2005. Role of A kinase anchor proteins in the tissue-specific regulation of lipoprotein lipase. Mol Endocrinol 19:2527-2534.
d. Ranganathan G, Unal R, Pokrovskaya ID, Tripathi P, Rotter JI, Goodarzi MO, **Kern PA**. 2012. The lipoprotein lipase (LPL) S447X gain of function variant involves increased mRNA translation. Atherosclerosis 221:143-147. PMCID: PMC3288274.

2. **The role of muscle in insulin resistance and metabolism (over 30 publications)**. Many studies have been performed involving the role of muscle in insulin resistance, lipotoxicity and the response to physical activity and weight loss. Recent studies have examined changes in muscle with exercise, with a particular emphasis on inflammatory cells, satellite cells and vascularity. Current funding involves the repurposing of metformin as a drug which may augment the muscle response to resistance training in the elderly.

a. Saghizadeh M, Ong JM, Garvey WT, Henry RR, **Kern PA**. 1996. The expression of TNFα by human muscle: relationship to insulin resistance. *J. Clin. Invest*. (Rapid Publication) 97:1111-1116. PMCID: PMC507159
b. Varma V, Yao-Borengasser A, Rasouli N, Nolen GT, Phanavanh B, Starks T, Gurley C, Simpson P, McGehee RE, Jr, **Kern PA**, Peterson CA. 2009. Muscle inflammatory response and insulin resistance: synergistic interaction between macrophages and fatty acids leads to impaired insulin action. *Am. J. Physiol. Endocrinol. Metab*. 296:E1300-1310. PMCID: PMC2692398
c. Fry CS, Noehren B, Mula J, Ubele MF, Westgate PM, **Kern PA**, Peterson CA. 2014. Fiber type-specific satellite cell response to aerobic training in sedentary adults. J. Physiol. 592:2625-35. PMCID: 4080942
d. Walton RG, Finlin BS, Mula J, Long DE, Zhu B, Fry CS, Westgate PM, Lee JD, Bennett T, **Kern PA**, Peterson CA. 2015. Insulin resistant subjects have normal angiogenic response to aerobic exercise training in skeletal muscle, but not in adipose tissue. Physiological Reports 3: e12415. PMCID 4510621

3. **Mechanisms of insulin resistance: pioglitazone and metformin (over 20 publications).** Thiazolidinediones (TZDs) improve peripheral insulin sensitivity through targeting of adipocyte gene, PPARγ. We examined mechanisms underlying the effectiveness of PPARγ agonists on improving insulin resistance in both clinical and *in vitro* studies. Pioglitazone attenuated lipotoxicity with a shift of lipid into subcutaneous adipose tissue and away from skeletal muscle. Other studies demonstrated a reduction in adipose macrophages and mast cells following pioglitazone treatment and an induction of adipose macrophage apoptosis along with an induction of adiponectin posttranslational processing. A recent clinical trial with metformin demonstrated blunting of muscle hypertrophy in response to resistance exercise in elderly non-diabetic sujects.

a. Rasouli N, Raue U, Miles LM, Lu T, Di Gregorio GB, Elbein SC, **Kern PA**. 2005. Pioglitazone improves insulin sensitivity through reduction in muscle lipid and redistribution of lipid into adipose tissue. Am J Physiol Endocrinol Metab 288:E930-934.
b. Bodles AM, Varma V, Yao-Borengasser A, Phanavanh B, Peterson CA, McGehee RE, Jr., Rasouli N, Wabitsch M, **Kern PA**. 2006. Pioglitazone induces apoptosis of macrophages in human adipose tissue. J Lipid Res 47:2080-2088.
c. Rasouli N, **Kern PA**, Elbein SC, Sharma NK, Das SK. 2012. Improved insulin sensitivity after treatment with PPARγ and PPARα ligands is mediated by genetically modulated transcripts. Pharmacogenet Genomics 22:484-497. PMCID: PMC3376224
d. Walton RG, Dungan CM, Long DE, Tuggle SC, Kosmac K, Peck BD, Bush HM, Villasante Tezanos AG, McGwin G, Windham ST, Ovalle F, Bamman MM, **Kern PA**, Peterson CA. 2019. Metformin blunts muscle hypertrophy in response to progressive resistance exercise training in older adults: a randomized, double-blind, placebo-controlled, multi-center trial. Aging Cell. 18(6):e13039. PMC6826125.4.

4. **Mechanisms of insulin resistance: adipose tissue inflammation and extracellular matrix (over 20 publications).** Many studies have examined the adipose tissue extracellular matrix to increase understanding of fibrosis, adipose vascularity and inflammation. In both clinical studies, clinical trials and cell culture models, these studies identified thrombospondin, elastin, collagen V, and other important components of the extracelluar matrix that contribute to the adipose tissue dysfunction, along with changes in macrophage polarization. These studies determined that human adipose is quite different from changes observed in rodent models, involving more fibrosis and different macrophage phenotypes.
   a. Di Gregorio GB, Yao-Borengasser A, Rasouli N, Varma V, Lu T, Miles LM, Ranganathan G, Peterson CA, McGehee RE, Kern PA. 2005. Expression of CD68 and macrophage chemoattractant protein-1 genes in human adipose and muscle tissue: Association with cytokine expression, insulin resistance, and reduction by pioglitazone. Diabetes. 54:2305-13.
   b. Spencer M, Yao-Borengasser A, Unal R, Rasouli N, Gurley CM, Zhu B, Peterson CA, **Kern PA**. 2010. Adipose tissue macrophages in insulin-resistant subjects are associated with collagen VI and fibrosis and demonstrate alternative activation. Am J Physiol Endocrinol Metab 299:E1016-1027. PMCID: PMC3006260
   c. Spencer M, Unal R, Zhu B, Rasouli N, McGehee RE, Jr., Peterson CA, **Kern PA**. 2011. Adipose tissue extracellular matrix and vascular abnormalities in obesity and insulin resistance. J Clin Endocrinol Metab 96:E1990-1998. PMCID: PMC3232606
   d. Spencer M, Finlin BS, Unal R, Zhu B, Morris AJ, Shipp LR, Lee J, Walton RG, Adu A, Erfani R, Campbell M, McGehee, Jr. RE, Peterson CA, **Kern PA**. 2013. Omega-3 fatty acids reduce adipose tissue macrophages in human subjects with insulin resistance. Diabetes 62:1709-1717. PMCID: 3636648
5. **Novel physiologic and mechanistic studies to improve insulin resistance: adipose beiging**. For my entire career, I have tried to translate basic findings into clinical research, and have taken clinical observations on human obesity/metabolic syndrome back to the lab for mechanistic studies. In recent studies, we have, for the first time, demonstrated that human white adipose tissue can undergo beiging in response to seasons, cold exposure and the β3 adrenergic receptor agonist mirabegron, with an increase in UCP1 mRNA and protein. Adipose beiging from both cold and mirabegron result in remodeling of adipose tissue and changes in the inflammatory profile, and experiments with mirabegron show beneficial changes in other tissues, including skeletal muscle and β-cells. Recent studies have identified a role of adipose tissue mast cells, which increase in response to cold and degranulate, releasing histamine to active beige adipose tissue.
   a. Finlin BS, Zhu B, Confides AL, Westgate PM, Harfmann BD, Dupont-Versteegden EE, **Kern PA**. 2017. Mast Cells Promote Seasonal White Adipose Beiging in Humans. Diabetes 66:1237-46. PMCID: PMC5399616.
   b. Finlin BS, Memetimin H, Confides AL, Kasza I, Zhu B, Vekaria HJ, Harfmann B, Jones KA, Johnson ZR, Westgate PM, Alexander CM, Sullivan PG, Dupont-Versteegden EE, **Kern PA**. 2018. Human Adipose Beiging in Response to Cold and Mirabegron. J. Clin Invest Insight 3(15):e121510. PMCID: PMC6129119.
   c. Finlin BS, Confides AL, Zhu B, Boulanger MC, Memetimin H, Taylor KW, Johnson ZR, Westgate PM, Dupont-Versteegden EE, **Kern PA**. 2019. Adipose Tissue Mast Cells Promote Human Adipose Beiging in Response to Cold. Sci. Rep. 9:8658. PMC6572779
   d. Finlin BS, Memetimin H, Zhu B, Confides AL, Vekaria HJ, El Khouli RH , Johnson ZR, Westgate PM, Chen J, Morris AJ, Sullivan PG, Dupont-Versteegden EE, **Kern PA**. 2020. The β3-adrenergic receptor agonist mirabegron improves glucose homeostasis in obese humans. J. Clin Invest. In press. PMID:31961829

Complete bibliography: http://www.ncbi.nlm.nih.gov/pubmed/?term=kern+pa

D. Additional Information: Research Support

Ongoing

R01 DK107646 (PI: Kern, P) 09/21/15 – 07/31/20 NCE
NIH/NIDDK
"Cold induced changes in human subcutaneous white adipose"

Goals: To examine the extent to which subcutaneous white adipose can become brownish or beige. We will determine whether this process is inhibited by inflammation and characterize the effects of cold on adipose triglyceride turnover.

R01 DK112282 (PI: Kern, P) 09/15/16 – 08/31/20 NCE
NIH/NIDDK
"The activation of brown and beige fat and role in insulin sensitivity"
Goals: This study will evaluate the activation of beige fat through biopsies, and brown fat through PET-CT scans, in response to B3 agonist and PPARγ agonist drugs. Study subjects will be insulin resistant and changes in insulin sensitivity will be measured.

R01 AG046920 (MPI: Bamman, M; Kern P; Peterson C) 09/30/14-5/31/20 NCE
NIH/NIA
"Novel actions of metformin to augment resistance training adaptation in older adults"
Goals: To assess potential benefits of metformin on muscle function in elderly subjects in resistance training.
Role: MPI

UL1 TR001998 (PI: Kern, P) 08/15/16 – 06/06/20
"Kentucky Center for Clinical and Translational Science (NCATS)"
Goals: This CTSA award to UK involves numerous Cores, including the clinical research center, a trial innovation network, team science, a pilot grant program, informatics, workforce development and community engagement. A TL1 Training Program (PI: S. Smyth) and a KL2 Career Development Program (PI: T. Kelly) are part of this overall effort, and are funded separately, and Dr. Kern is Co-I of each.

P30 DK020579 (PI: Schaffer, J) 12/01/18 – 11/30/22
NIH/NIDDK
"Diabetes Research Center" at Washington University, St. Louis
Goals: This subcontract involves pilot grants and other interactions between Washington University and UK.
Role: UK Site PI

R01 DK108056 (PI: Nikolajczyk, B) 04/01/18 – 03/31/20
NIH/NIDDK
"Inflammation in Human Obesity and Type 2 Diabetes"
This project tests the hypothesis that a T cell signature distinguishes T2D from non-T2D subjects and is a predictive biomarker for T2D. Dr. Kern is involved establishing and designing the clinical recruitment and characterization.
Role: Co-I

R01 DK119619 (MPI: McCarthy, Peterson) 09/01/18 – 08/31/23
NIH/NIDDK
"Exercise-induced Skeletal Muscle Exosomes Promote Adipocyte Lipolysis"
This proposal hypothesizes that resistance exercise promotes the release of miR-1 containing exosomes from muscle which then are taken up by adipose tissue to promote increase lipolysis and beta adrenergic receptor activity, in both mice and humans.
Role: Co-I

R01 AG062550 (Johnson, LA PI). 04/01/19 – 03/31/24.
NIH/NIA.
"Changing the energy substrate balance: Does APOE2 promote glucose usage to protect from Alzheimer's Disease?"
This proposal hypothesizes that apoE2 affords neuroprotection of Alzheimer's disease by shifting metabolism from fatty acid oxidation to glucose utilization. Studies are performed in both mice and humans.
Role: Co-I.

P20 GM121299 (PI: Sundaram, S) 02/15/18 – 01/31/23
Marshall University Research Co.
"Appalachian Center for Cellular transport in Obesity Related Disorders (ACCORD)"
This COBRE program will develop cores and support projects conducted by Marshall junior investigators. Dr. Kern is a mentor to the trainees and an advisor on the overall project.
Role: UK Consortium PI

OMB No. 0925-0001 and 0925-0002 (Rev. 09/17 Approved Through 03/31/2020)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Charlotte A. Peterson

eRA COMMONS USER NAME (credential, e.g., agency login): capeterson

POSITION TITLE: Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
University of Notre Dame, Notre Dame, IN	B.S.	05/1978	Biology
University of Virginia, Charlottesville, VA	Ph.D.	08/1984	Developmental Biology
National Institutes of Health, Bethesda, MD	Postdoc	12/1987	Molecular Biology
Stanford University School of Medicine, Stanford, CA	Postdoc	12/1990	Mol. Dev. Biology

A. Personal Statement

My research focuses on elucidation of cellular and molecular mechanisms controlling skeletal muscle structure and function. I am funded by the NIH to study satellite cell activity in muscle repair and adaptation, and human muscle response to exercise, with a focus on changes associated with aging and obesity. There is currently a great deal of interest in understanding muscle-bone interactions and her approach is novel, mechanistic and translatable. We meet monthly to discuss research design, progress and interpretation of findings. I will continue to provide expertise and resources to her for the proposed analyses through the Center for Muscle Biology. As a translational researcher leading exercise studies in humans, a muscle molecular and cellular biologist with extensive experience in analysis of the response of rodent and human muscle tissue and cells to environmental stimuli, I bridge multiple disciplines, integrating physicians, physical therapists, and bioengineers with basic researchers. I actively mentor many young postdoctoral fellows and junior faculty and am committed to the development of the next generation of research scientists. Working closely with Dr. Kalaitzoglou on muscle analyses in her project exploring the role of muscle-derived myostatin in diabetic bone disease, I am very committed to her research program, which has the potential to lead to development of new approaches to treat diabetic bone disease. Below are four publications demonstrating my expertise and commitment to our long-term goal of maintaining muscle during aging and in the face of chronic disease, thereby maintaining mobility and functional independence.

1. Srikuea, R., T.B. Symons, D.E. Long, J.D. Lee, Y. Shang, P.J. Chomentowski, G. Yu, L.J. Crofford, **C.A. Peterson** (2012). Fibromyalgia is associated with altered skeletal muscle characteristics which may contribute to post-exertional fatigue in post-menopausal women. *Arthritis & Rheumatism* 65:519-528. [PMCID: PMC3558634]
2. White, S.H., M.M. McDermott, R.L. Sufit, K. Kosmac, A.W. Bugg, M.Gonzalez-Freire, L. Ferrucci, L. Tian, L. Zhao, Y. Gao, M.R. Kibbe, M.H. Criqui, C. Leeuwenburgh, **C.A. Peterson** (2016). Walking performance is positively correlated to calf muscle fiber size in peripheral artery disease subjects, but fibers show aberrant mitophagy: an observational study. *J. Translational Medicine* 14:284-299. [PMCID: PMC5043620]

3. Noehren, B., K. Kosmac, R.G. Walton, K.A. Murach, M.F. Lyles, R.F. Loeser, **C.A. Peterson**, S.P. Messier (2018). Alterations in quadriceps muscle cellular and molecular properties in adults with moderate knee osteoarthritis. *Osteoarthritis & Cartilage* 26 (10), 1359-1368. [PMCID: PMC7050996]

4. Walton, R.G., C.M. Dungan, D.E. Long, S.C. Tuggle, K. Kosmac, Peck, B.D., H.M. Bush, A.G. Villasante-Tezanos, G. McGwin, M.M. Bamman, P.A. Kern, **C.A. Peterson** (2019). Metformin blunts muscle hypertrophy in response to progressive resistance exercise training in older adults: a randomized, double-blind, placebo-controlled, multi-center trial: The MASTERS Trial. *Aging Cell* 18(6), e13039. [PMCID: PMC6826125]

B. Positions and Honors

Positions

1990-96	Assistant Professor, Depts of Medicine and Biochemistry and Molecular Biology, University of Arkansas for Medical Sciences (UAMS), Little Rock, AR.
1996-06	Health Research Scientist, Central Arkansas Veterans Health Care System, Little Rock, AR
1996-03	Associate Professor with tenure, Depts of Medicine, Geriatrics and Physiology, UAMS.
2003-06	Professor, Depts of Geriatrics and Physiology, UAMS.
2006-	Professor, College of Health Sciences, University of Kentucky (UK), Lexington, KY.
2006-16	Associate Dean for Research, College of Health Sciences, UK, Lexington, KY.
2008-15	Co-Director, UK Center for Muscle Biology
2010-16	Associate Director, UK Center for Clinical and Translational Research
2016-	Director, UK Center for Muscle Biology
2016-18	Senior Scientific Advisor, National Institute of Arthritis, Musculoskeletal & Skin Diseases, Bethesda, MD for the Molecular Transducers of Physical Activity Consortium (MoTrPAC)

Honors

1992	Recipient, David Pryor Research Award, American Heart Association (AR Affiliate)
1993-96	Ad Hoc member NIH Respiratory and Applied Physiology Study Section
1995-00	Recipient, NIH Research Career Development Award
1996-01	Member, NIH/NIA Biological and Clinical Aging Study Section
2003	Elected Organizer of the FASEB Conference on “Muscle Stem Cells”
2003-06	Chair, NIH Cellular Mechanisms of Aging and Development Study Section
2004-16	Associate Editor, *Journals of Gerontology: Biological Sciences*
2006-	Joseph Hamburg Endowed Chair, University of Kentucky
2007-11	Member, NIH Skeletal Muscle and Exercise Physiology Study Section
2011	Elected Fellow, Gerontological Society of America
2012-16	Editorial Board, *Aging Cell*
2012-	External Advisory Board, Centre for Integrated Musculoskeletal Aging, United Kingdom
2012	Ad Hoc member, NIH Aging Systems and Geriatrics Study Section
2013	Ad Hoc member, NIH Cellular Mechanisms of Aging and Development Study Section
2014-	External Advisory Boards, NIA Nathan Shock Centers on Aging
2013-14	Chair, Gerontological Society of America, Biological Sciences Section
2014-16	Board of Scientific Counselors, National Institute on Aging
2016-	American Federation for Aging Research “Breakthroughs in Gerontology’ Review Panel (Chair since 2018)
2019-	Ad Hoc member, Muscular Dystrophy Association Review Panel
2019-	Ad Hoc member, Veterans Administration Chronic Medical Conditions & Aging Review Panel

C. Contribution to Science

Identifying new roles for satellite cells in muscle adaptation.

Our work has contributed to a paradigm shift in our understanding of the role of satellite cells in muscle adaptation. Using a newly developed genetic mouse model that enables the inducible depletion of satellite cells in adult muscle, we reported the surprising finding that satellite cells are not necessary for muscle hypertrophy in response to mechanical overload, or for regrowth following atrophy. More recently we established a novel role for activated satellite cells in regulation of the muscle microenvironment. Our findings

demonstrate that in the absence of satellite cells there is an eventual attenuation of growth in response to chronic overload, in addition to reduced force production of the muscle. The reduction in specific force is likely due to an accumulation of extracellular matrix (ECM) in the muscle, accompanied by an expansion of the fibroblast pool. Satellite cells communicate with fibroblasts through exosome-mediated delivery of microRNAs (miRs). These findings demonstrate for the first time, a role for satellite cells beyond providing a nucleus to the growing muscle fiber. Numerous studies have demonstrated a role for the muscle niche in the regulation of satellite cell activity; our studies provide evidence for the novel concept that activated satellite cells regulate the muscle fiber niche, facilitating muscle remodeling to maximum hypertrophy.

1. McCarthy, J.J., J. Mula, M. Miyazaki, R. Erfani, K. Garrison, A.B. Farooqui, R. Srikuea, B.A. Lawson, B. Grimes, C. Keller, G. Van Zant, K.S. Campbell, K.A. Esser, E.E. Dupont-Versteegden, and **C.A. Peterson** (2011). Effective fiber hypertrophy in satellite cell-depleted skeletal muscle. *Development* 138:3657-3666. [PMCID: PMC3152923]
2. Fry, C.S., J.D. Lee, J.R. Jackson, T.J. Kirby, S.A. Stasko, H. Liu, E.E. Dupont-Versteegden, J.J. McCarthy, **C.A. Peterson** (2014). Regulation of the muscle fiber microenvironment by activated satellite cells during hypertrophy. *FASEB J.* 28:1654-65. [PMCID: PMC3963024]
3. Kirby, T.J., R.M. Patel, T.S. McClintock, E.E. Dupont-Versteegden, **C.A. Peterson**, J.J. McCarthy (2016) Myonuclear transcription is responsive to mechanical load and DNA content but uncoupled from cell size during hypertrophy. *Mol. Biol. Cell.* 27:788-798. [PMCID: PMC4803305]
4. Fry, C.S., T.J. Kirby, K. Kosmac, J.J. McCarthy, **C.A. Peterson** (2017). Myogenic progenitor cells regulate extracellular matrix production by fibroblasts during skeletal muscle adaptation. *Cell Stem Cell* 20:1-14. [PMCID: PMC5218963]

Satellite cells and aging.

Numerous studies from our lab and many others in both human and rodent skeletal muscle have demonstrated that muscle aging is associated both with dysfunction of satellite cells and with loss of muscle mass and strength (sarcopenia). Based on this correlation, coupled with the fact that regeneration is compromised in aged muscle, the field has made the reasonable assumption that loss of satellite cell activity with aging *causes* sarcopenia, but this has never been directly tested. We used our established genetic mouse model to determine whether loss of satellite cells results in muscle wasting as the mice age. Much to our surprise, significant depletion of satellite cells throughout adulthood (much greater than that observed with normal aging) did not cause premature sarcopenia or exacerbate the magnitude of sarcopenia; however, consistent with other studies, muscle regeneration was still significantly impaired. On the other hand, we did observe an enhanced accumulation of the ECM in satellite cell-depleted muscle with age, consistent with emerging evidence that stem cells are required for mainntenance of the niche environment. Our findings have clinical importance as they draw a clear distinction between appropriate therapeutic approaches to treat degenerative pathologies involving satellite cell activation and regeneration (dystrophies and cachexia), and sarcopenia.

1. Beggs, M.L., R. Nagarajan, J.M. Taylor-Jones, G. Nolen, M. Macnicol, and **C.A. Peterson** (2004). Alterations in the TGFβ signaling pathway in myogenic progenitors with age. *Aging Cell* 3:353-361. [PMCID: PMC15569352]
2. Fry, C.S., J.D. Lee, J. Mula, T.J. Kirby, J.R. Jackson, F. Liu, L.Yang, C.L. Mendias, E.E. Dupont-Versteegden, J.J. McCarthy, **C.A. Peterson** (2015). Inducible depletion of satellite cells in adult, sedentary mice impairs muscle regenerative capacity without affecting sarcopenia. *Nature Medicine* 21:76-80. [PMCID: PMC4289085]
3. Lee, J.D., C.S. Fr y, J. Mula, T.J. Kirby, J.R. Jackson, F. Liu, L. Yang, E.E. Dupont-Versteegden, J.J. McCarthy, **C.A. Peterson** (2016). Aged muscle demonstrates fiber-type adaptations in response to mechanical overload, in the absence of myofiber hypertrophy, independent of satellite cell abundance. *J. Gerontol.* 71:461-467. [PMCID: PMC5175449]
4. Murach, K.A., S.H. White, A. Ho, E.E. Dupont-Versteegden, J.J. McCarthy, **C.A. Peterson** (2017). Differential requirement for satellite cells during overload-induced muscle hypertrophy in growing versus mature mice. *Skeletal Muscle* 7:14-27. [PMCID: PMC5504676]

Mechanisms underlying response to exercise in humans.

Loss of skeletal muscle mass and strength with advancing age reduces quality of life and is a major factor limiting an elderly person's chance of living independently. Resistance exercise training is the most effective intervention identified to increase muscular strength and combat muscle atrophy of aging; however, overall the

muscle response to exercise is blunted and highly variable in the elderly. During the last several years we have identified potential mechanisms related to inflammation and satellite cell function that may influence the response to exercise in the elderly. Our current funded work is focused on testing the hypothesis that adjuvant metformin may improve responses to resistance exercise training in the elderly by altering the resident muscle macrophage phenotype, thereby enhancing mechanisms that drive exercise-induced myofiber hypertrophy. Prospective identification of individuals likely to be refractory to routine exercise programs may contribute to development of personalized approaches to maintain or restore skeletal muscle mass and strength in the elderly.

1. Dennis, R.A., H. Zhu, P.M. Kortebein, H.M. Bush, J.F. Harvey, D.H. Sullivan, and **C.A. Peterson**. (2009). Skeletal muscle expression of genes associated with inflammation, growth, and remodeling is strongly correlated in older adults with resistance training outcomes. *Physiol. Genomics* 38:169-175. [PMCID: PMC2712223]
2. Fry, C.S., B. Noehren, J. Mula, M.F. Ubele, P.M. Westgate, P.A. Kern, **C.A. Peterson** (2014). Fiber type-specific satellite cell response to aerobic training in sedentary adults. *J. Physiol.* 592:2625-2636. [PMCID: PMC4080942]
3. Walton, R.G., B.S. Finlin, J. Mula, D.E. Long, B. Zhu, C.S. Fry, P.M. Westgate, J.D. Lee, T. Bennett, P.A. Kern, **C.A. Peterson** (2015). Insulin resistant subjects have normal angiogenic response to aerobic exercise training in skeletal muscle, but not in adipose tissue. *Physiologic Reports* 3:e12415. [PMCID: PMC4510621]
4. Murach, K.A., R.G. Walton, C.S. Fry, S.L. Michaelis, J.S. Groshong, B.S. Finlin, P.A. Kern, **C.A. Peterson** (2016). Cycle training modulates satellite cell and transcriptional responses to a bout of resistance exercise. *Physiologic Reports* 4:e12973. [PMCID: PMC5037921]

Role of resident muscle macrophages in muscle adaptation.

In our human exercise studies, resident muscle macrophages and their secretory products are emerging as important regulators of muscle adaptation. We developed and validated a method for identifying, characterizing and quantifying human skeletal muscle macrophages, which we have extended to mouse muscle. Our current and future studies focus on understanding mechanisms whereby macrophages regulate muscle hypertrophy in response to exercise, and the influence of aging, obesity and chronic disease on macrophage phenotype and function. We are also actively studying adipose-muscle interactions.

1. Varma, V., A.Yao-Borengasser, N. Rasouli, G.T. Nolen, B. Phanavanh, T. Starks, P. Simpson, R. E. McGehee, Jr, P.A. Kern and **C.A. Peterson**. (2009). Muscle inflammatory response and insulin resistance: synergistic interaction between macrophages and fatty acids leads to impaired insulin action. *Am. J. Physiol.* 296:E1300-1310. [PMCID: PMC2692398]
2. Finlin, B.S., V. Varma, G.T. Nolen, J. Dubé, C.P. Starnes, P.A. Kern, and **C.A. Peterson** (2011). DHA reduces the atrophy-associated Fn14 protein in differentiated myotubes during co-culture with macrophages. *J. Nutr. Biochem.* 23:885-891. [PMCID: PMC3223324]
3. Kosmac, K., B. Peck, R.G. Walton, J. Mula, P.A. Kern, M.M. Bamman, R.A. Dennis, C.A. Jacobs, C. Lattermann, D.L. Johnson, **C.A. Peterson** (2018). Immunohistochemical identification of human skeletal muscle macrophages. *Bio-Protocols* 8(12). [PMCID: PMC6105281]
4. Walton, R.G., K. Kosmac, J. Mula, C.S. Fry, B.D. Peck, J. Groshong, B.S. Finlin, B. Zhu, P.A. Kern, **C.A. Peterson** (2019). Human skeletal muscle macrophages increase following cycle training and are associated with adaptations that may facilitate growth. *Scientific Reports* 9(1), 69. [PMCID: PMC6353900]

Complete List of Published Work in MyBibliography:

http://www.ncbi.nlm.nih.gov/sites/myncbi/charlotte.peterson.1/bibliography/40583705/public/?sort=date&direction=descending

D. Research Support

Ongoing Research Support

R01AR60701-10 (Peterson & McCarthy, MPI) 10/2010 - 09/2020
NIH/NIAMS

Novel roles for satellite cells in adult skeletal muscle adaptation
Satellite cells will be specifically ablated in genetically modified mice and the influence on muscle adaptability determined.

R01AG046920-06 (Peterson & Kern, MPI) 10/2014 - 05/2020 (NCE)
NIH/NIA
Novel actions of metformin to augment resistance training adaptation in older adults
We propose to determine the effectiveness of metformin in improving exercise response in the elderly through alterations in muscle macrophage content.

R01AG049806-04 (Peterson & McCarthy, MPI) 12/2016 – 11/2021
NIH/NIA
The effects of exercise on satellite cell dynamics during aging
The purpose of this grant is to use a newly developed mouse model to track satellite cell dynamics with aging and in response to a hypertrophic stimulus.

W81XWH-16-2-0058 (McDiarmid, PI) 09/2016 – 08/2021
DoD/CDMRP
Biomarkers for Assessing Return-to-Duty Potential of Personnel with Embedded Metal-Fragment Wounds
The goal is to develop a rat model of embedded metal in hind limb muscle to provide an evidence base to refine the clinical management of the Veteran/Service Member with retained embedded metal fragments.
Role: Project 2 Leader

R01HL126117-05 (McDermott, PI) 10/2015 - 09/2020
NIH/NHLBI
TELmisartan plus EXercise to improve functioning in PAD: The TELEX trial
This is an interventional study conducted at Northwestern University on patients with peripheral artery disease to analyze the effects of exercise and telmisartan on gastrocnemius muscle morphology, performed by Dr. Peterson on muscle biopsies shipped from NU.
Role: Co-I

R01 HL131771-04 (McDermott, PI) 12/2016 – 11/2021
NIH/NHLBI
Improve PAD Performance with METformin. The PERMET Trial
This is a placebo controlled double-blinded randomized clinical trial to establish whether metformin (2000 mgs daily) improves and/or prevents decline in walking performance and muscle morphology in people with PAD.
Role: Co-I

R01DK119619-02 (McCarthy & Peterson, MPI) 09/2018 - 08/2023
NIH/NIDDK
Exercise-induced skeletal muscle exosomes promote adipocyte lipolysis
The goal of this study is to study muscle-adipose crosstalk to identify mechanisms underlying the beneficial effects of resistance training on adipose tissue metabolism.

R01 DK119619-01S (McCarthy & Peterson, MPI) 09/2019 - 07/2020
NIA/NIDDK
AD Supplement to Exercise-induced skeletal muscle exosomes promote adipocyte lipolysis
The goal of this project to determine if exercise-induced exosomes can offset the deleterious effects of obesity on AD progression.

OMB No. 0925-0001 and 0925-0002 (Rev. 09/17 Approved Through 03/31/2020)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Jeffry S. Nyman

eRA COMMONS USER NAME (credential, e.g., agency login): nymanjs

POSITION TITLE: Associate Professor of Orthopaedic Surgery

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
The University of Memphis, Memphis, TN	B.S.	1996	Mechanical Engineering
The University of Memphis, Memphis, TN	M.S.	1998	Mechanical Engineering
University of California, Davis, CA	Ph.D.	2003	Biomedical Engineering
University of Texas at San Antonio, TX	Post-doc	2006	Bone Mechanics

A. Personal Statement

The ultimate goal of my research is to lower the number of bone fractures associated with **diabetes**, aging, osteoporosis, cancer, and genetic diseases. Building from my post-doctoral research on identifying determinants of bone toughness, my research program involves the assessment of structural, architectural, compositional, and biomechanical properties of bone. I have 10+ years of experience training students, post-doctoral fellows, and surgical residents in the use of both dynamic material testing systems and micro-computed tomography (μCT) scanners. Assessing the fracture resistance of bone in my research includes flexural tests (three-point bending), fatigue (axial and flexural), cyclic reference point indentation (cRPI) and impact micro-indentation (OsteoProbe), ^{1}H nuclear magnetic resonance (NMR) relaxometry which underpins magnetic resonance imaging (MRI), Raman spectroscopy (RS), high performance liquid chromatography, and finite element analysis (FEA). Since 2006, I have been a faculty member of the Vanderbilt Center for Bone Biology where I gained experience with animal models of bone diseases. Now, I have begun translating my research into the clinic assessment of bone. Dr. John Fowlkes and I have been collaborating on bone projects since 2009 (9 co-author publications to date). Our recent collaborative work with Dr. Eva Kalaitzoglou assessed effects of insulin therapy on fracture resistance of bone in a mouse model of type 1 diabetes. In summary, I have a demonstrated a record of successful and productive research projects to conduct studies involving assessment of bone's resistance to fracture.

B. Positions and Honors

Professional Experience

1995-1996	Intern, Orthopaedic Research, Wright Medical Technology.
1997-1998	Research Assistant, Department of Mechanical Engineering, The University of Memphis.
1998-2001	Teaching Assistant, Materials Science, University of California, Davis.
1999-2003	Research Assistant, Orthopaedic Research Laboratory, University of California, Davis.
2003-2006	Post-doctoral Fellow, Department of Mechanical Engineering and Biomechanics, The University of Texas at San Antonio.
2006-2007	Research Instructor, Center for Bone Biology, Vanderbilt University Medical Center (VUMC).
2007-2011	Research Assistant Professor of Orthopaedics & Rehabilitation, VUMC.
2008-2016	Research Assistant Professor of Biomedical Engineering, Vanderbilt University (VU).
2009-2016	Research Health Scientist, Department of Veterans Affairs, Tennessee Valley Healthcare System (TVHS).

2011-2016 Assistant Professor of Orthopaedic Surgery and Rehabilitation, VU.
2016-present Research Associate Professor of Biomedical Engineering, VU.
2016-present Associate Professor of Orthopaedic Surgery and Rehabilitation, VUMC.
2019-present Research Health Scientist, Department of Veterans Affairs, TVHS.

Honors and Award

1996 Participant in the Research Experience for Undergraduates Program, sponsored by the National Science Foundation, at Worcester Polytechnic Institute
1996 Magna Cum Laude at The University of Memphis
1997-1998 Regents Scholar and Herff Fellow at The University of Memphis
2001 Recipient of a Floyd and Mary Schwall Dissertation Year Fellowship
2002 Recipient of the Achievement Reward for College Scientists given by the ARCS Foundation
2006 Recipient of Alice L. Jee Memorial Young Investigator Award at the 2006 Sun Valley Workshop on Skeletal Tissue Biology
2009-2011 A Top Reviewer for *Bone*

Other Experience and Professional Memberships

2004-present Member, Orthopaedic Research Society
2006-present Member, American Society for Bone and Mineral Research
2006-present Member, Vanderbilt Center for Bone Biology
2011-2015 Chair, Vanderbilt Orthopaedic Institute Pilot Project Review Committee
2012-2015 Member, International Bone & Mineral Society
2013-2016 Member, ASBMR Ethics Advisory Committee
2014-2016 Member, Advisory Committee for the ORS Sun Valley Workshop: Musculoskeletal Biology
2014 Co-section Editor for Bone Quality in Osteoporosis in *Current Osteoporosis Reports*
2014-2018 Member, Skeletal Biology Structure & Regeneration Study Section
2017-2018 Member, Society for Applied Spectroscopy
2018-2019 Member, The International Society for Clinical Densitometry

Editorial Appointments

Bone, Clinical Reviews in Bone and Mineral Metabolism, Journal of Bone and Mineral Research

C. Contribution to Science

1. *Effect of diabetes on bone* – Those with diabetes are at a greater risk of fracture than those without diabetes, and that risk is even greater for individuals with type 1 diabetes. My research in collaboration with Dr. Fowlkes (Director of Barnstable Brown Diabetes and Obesity Center, University of Kentucky) and Dr. Kathryn Thrailkill (University of Kentucky) indicates that a decrease in insulin signaling has two dominant effects on bone: loss of bone accrual and deficits in material properties of bone. In addition to the structural difference between normal and mice with type 1 diabetes (induced by streptozotocin), our first publication reported that the bones become brittle as the duration of diabetes progresses. In follow-up work in which my research team measured the biomechanical properties of bone, we reported that delayed insulin therapy partially rescued the deleterious effects of type 1 diabetes on bone. More recently, my lab published how fracture resistance decreases as type 2 diabetes progresses in the ZDSD rat model. Then my former graduate student and I found that the fracture resistance of bone does not progressively worsen as TallyHO mice (juvenile mode of type 2 diabetes) age. Our work strongly indicates that prevention of diabetic bone disease will require a multifactorial approach addressing the anabolic nature of insulin and contribution of poor glycemic control to the material properties of bone.

a. Nyman, J.S., Even, J.L., Jo, C-H, Herbert, E.G., Murry, M.R., Cockrell, G.E., Wahl, E.C., Bunn, R.C., Lumpkin, Jr., C.K., Fowlkes, J.L., and K.M. Thrailkill. Increasing duration of type 1 diabetes perturbs the strength-structure relationship and increases brittleness of bone. *Bone*. 48:733-40, 2011. PMCID: PMC3062641
b. Nyman J.S., Kalaitzoglou E., Bunn R.C., Uppuganti S., Thrailkill K.M., and J.L. Fowlkes. Preserving and restoring bone with continuous insulin infusion therapy in a mouse model of type 1 diabetes. *Bone Reports*. 7:1-8, 2017. PMCID:PMC5508511

c. Creecy A., Uppuganti S., Merkel A.R., O'Neal D., Makowski A.J., Granke M., Voziyan P., and J.S. Nyman. Changes in the fracture resistance of bone with the progression of type 2 diabetes in the ZDSD rat. *Calcified Tissue International*. 99:289-301, 2016. PMCID: PMC4961536
d. Creecy A., Uppuganti S., Unal M., Bunn R.C., Voziyan P., and J.S. Nyman. Low bone toughness in the TallyHO model of juvenile type 2 diabetes does not worsen with age. *Bone*. 110:204-14, 2018. PMCID:PMC5878744.

2. *Regulators of bone material properties* – While there are numerous studies reporting on factors that affect bone mass or volume, little is known about regulators of material properties. Initially supported by a Career Development Award from the VA, I characterized the bone phenotype of several genetic mouse models. In one of my first publications as an independent investigator, I reported that loss of MMP-2 and MMP-9, two similar matrix-associated genes, caused a loss in material strength and a loss in toughness of bone, respectively. More recently, my research team and I found that a transcription factor (ATF4) important to osteoblast differentiation is also important to bone toughness. In collaboration with my colleagues at the Vanderbilt Center for Bone Biology, I've been involved in several important studies. Bringing my expertise to biomechanics, I helped my colleagues show that inhibiting transforming growth factor beta (TGF-β) improves material properties and tissue-level properties of cortical bone and that loss of neurofibromatosis type 1 affects structural and material strength of bone through its regulation of mineralization. All these findings indicate that material properties, not just bone mass, can be a target for preventing fractures.

a) Edwards, J.R., Nyman, J.S., Lwin, S.T., Moore, M.M, Esparza, J., O'Quinn, E.C.; Hart, A.J., Biswas, S.; Patil, C.; Lonning, S.; Mahadevan-Jansen, A., and G.R. Mundy. Inhibition of TGF-β signaling by 1D11 antibody treatment increases bone mass and quality in vivo. *Journal of Bone and Mineral Research*. 25:2419-26, 2010.
b) Nyman, J.S., Lynch, C.C., Perrien, D.S., Thiolloy, S., O'Quinn, E.C., Patil, C.A., Bi, X., Pharr, G.M., Mahadevan-Jansen, A., and G.R. Mundy. Differential effects between the loss of MMP-2 and MMP-9 on structural and tissue-level properties of bone. *Journal of Bone and Mineral Research*. 26: 1252-60, 2011. PMCID: PMC3312757
c) Makowski A.J., Uppuganti S., Waader S.A., Whitehead J.M., Rowland B.J., Granke M., and Mahadevan-Jansen A., Yang X., and J.S. Nyman. The loss of activating transcription factor 4 (ATF4) reduces bone toughness and fracture toughness. *Bone*. 62: 1-9, 2014. PMCID: PMC3992706
d) de la Croix Ndong J., Makowski A.J., Uppuganti S., Vignaux G., Ono K., Perrien D.S. Joubert S., Baglio S.R., Granchi D., Stevenson D.A., Rios J.J. Nyman J.S., and F. Elefteriou. Asfotase-α improves bone growth, mineralization and strength in mouse models of neurofibromatosis type-1. *Nature Medicine*. 20:904-10, 2014. PMCID: PMC4126855

3. *Age-related changes in the fracture resistance of bone* – While toughness and strength of bone have been known to decrease with age for some time, clinically viable methods for assessing these material properties are lacking. During my post-doctoral training, I learned to quantify non-enzymatic collagen crosslinks, namely pentosidine, in bone, and published a paper with my mentor reporting how pentosidine partially explains the variance in post-yield toughness of human cortical bone. Then, when I became an independent investigator, I set out to find collaborators with the goal of developing novel ways to assess the effect of aging on fracture resistance (beyond strength). One example is the use of ^{1}H nuclear magnetic resonance (NMR) to quantify bound water and pore water in bone. My collaborator, Professor Mark Does, and I have ten papers on the role of water in bone mechanics, and recently, we reported that the combination of bound water and pore water helped explain the age-related variance in fracture toughness of human cortical bone. In addition, we found that i) resistance of human bone tissue to micro-indentation using reference point indentation (RPI) is anisotropic, ii) age combined with bound water are potential predictors of fracture toughness, and iii) bound water in rat cortical bone decreases while pentosidine increases with advanced aging. Clinical assessment of bound water and pore water using ultra-short echo-time MRI is now an active area of research, partly due to our research as well as others.

a. Nyman, J.S., Roy, A., Tyler, J.H., Acuna, R.L., Gayle, H.J., and X. Wang. Age-related factors affecting the post-yield energy dissipation of human cortical bone. *Journal of Orthopaedic Researc*h. 25:646-655, 2007. PMCID: PMC1994146

b. Granke M., Coulmier A., Uppuganti S., Gaddy J.A., Does M.D, and J.S. Nyman. Insights into Reference Point Indentation involving human cortical bone: sensitivity to tissue anisotropy and mechanical behavior. *Journal of the Mechanical Behavior of Biomedical Materials*. 37: 174-185, 2014. PMCID: PMC4112765
c. Granke M., Makowski A.J., Uppuganti S., Does M.D., and J.S. Nyman. Identifying novel clinical surrogates to assess human bone fracture toughness. *Journal of Bone and Mineral Research*. 30:1290-300, 2015. PMCID: PMC4478129
d. Uppuganti S., Granke M., Makowski A.J., Does M.D., and J.S. Nyman. Age-related changes in the fracture resistance of male Fischer F344 Rat Bone. *Bone*. 83:220-32, 2016. PMCID: PMC4724327

4. *The application of Raman spectroscopy to bone* – Because Raman spectroscopy (RS) is non-destructive, requires minimal sample preparation, and is sensitive to collagen, it has been used to analyze bone in numerous studies. While RS has given insight into how various proteins, signaling pathways, and diseases affect the matrix of bone, the best way to analyze the Raman spectrum of bone has not been established when the goal is to assess the contribution of matrix to the fracture resistance of bone. After initially reporting that nu1 Phosphate peak per Proline peak (v_1PO_4/Proline) was more effective than the traditional nu1 phosphate peak per Amide I peak (v_1PO_4/AmI) in differentiating osteonal tissue from the more mineralized interstitial tissue, my former graduate student and I, along with my collaborator Professor Mahadevan-Jansen, performed a comprehensive assessment of polarization bias within a standard Raman microscope and established that the out-of-phase v_1PO_4/AmI is sensitive to organization and composition of bone tissue while the nearly in-phase v_1PO_4/Proline was primarily sensitive to composition of bone tissue. We subsequently demonstrated the usefulness of analyzing the entire spectra from bone, especially the Amide I band, to predict fracture toughness of cortical bone. There is a growing recognition that multiple peaks should be analyzed when assessing compositional properties of bone with RS, partly due to our publications.

a. Nyman, J.S., Makowski, A.J., Patil, C.A., Masui, T.P., O'Quinn, E.C., Bi, X., Guelcher, S.A., Nicollela, D.P. and A. Mahadevan-Jansen. Measuring differences in compositional properties of bone tissue by confocal Raman Spectroscopy. *Calcified Tissue International*. 89:111-22, 2011. PMCID: PMC4471954
b. Makowski, A.J., Patil, C.A., Mahadevan-Jansen, A., and J.S. Nyman. Polarization control of Raman spectroscopy optimizes the assessment of bone tissue. *Journal of Biomedical Optics*. 18: 055005, 2013. PMCID: PMC3662990
c. Makowski A.J., Granke M., Ayala O., Uppuganti S., Mahadevan-Jansen A. and J.S. Nyman. Applying full spectrum analysis in the Raman spectroscopic assessment of fracture toughness of human cortical bone. *Applied Spectroscopy*. 71:2385-94, 2017. PMCID: PMC5561524
d. Unal M., Uppuganti S., Timur S., Mahadevan-Jansen A., Akkus O., and **J.S. Nyman***. Assessing matrix quality by Raman spectroscopy helps predict fracture toughness of human cortical bone. *Scientific Reports*. 9(1), 7195. PMCID: PMC6510799

URL to a list of published work:
https://www.ncbi.nlm.nih.gov/myncbi/jeffry.nyman.1/bibliography/public/

D. Additional Information: Research Support

Ongoing Research Support

VA Merit Award I01 BX004297 Nyman 10/01/2018 – 09/30/2022
Diabetes-Related Changes Affecting Bone Quality
The objectives of this project are to determine how type 2 diabetes (T2D) decreases the fracture resistance of bone, identify post-translational mechanisms that negatively alter bone matrix properties, and assess the ability of matrix-sensitive tools to detect differences in bone quality between non-diabetic and T2D patients.

NIH/NIBIB 2R01 EB014308 Does 09/01/2017 – 10/31/2021
Bone Fracture Risk Assessment Through Bound- and Pore-Water MRI
The objective of this project is to develop, optimize, and quantitatively evaluate MRI methods for assessing bone fracture risk.
Role: Site PI

NIH/NIAMS 1R21 AR072483-01A1 Nyman/Elefteriou 03/01/2018 – 02/29/2020 (NCE)
Matrix-Sensitive Tools for Detecting NF1-Related Changes in Bone Quality
The objective of this project is to compare matrix-sensitive methods for their ability to predict NF1-related deficits in bone quality and assess the response of bone to NF1-specific treatments.

NIH/NIAMS 1R21 AR073133-01A1 Nyman 07/30/2018 – 05/31/2020
Advancing Raman spectroscopy toward the clinical assessment of bone quality
The overarching goal of this project is to assess the ability of Raman spectroscopy to predict the mechanical properties of human cortical bone using different strategies for spectral acquisition and identify matrix factors that influence Raman spectroscopic properties of bone quality.

Completed Research Support

NIH/NIAMS 1R21 AR070620-01 Thraillkill/Nyman 07/01/2016 – 06/30/2019 (NCE)
Effects of Sodium-dependent Glucose Co-transporter 2 Inhibition on Bone
The objective of this project is to utilize several relevant rodent models (slc5a2-functional mutants, STZ-induced diabetes, TallyHo) to investigate potential mechanisms contributing to the adverse effects of SGLT2-inhibitor therapy on the skeleton.
Role: Site PI

NIH/NIAMS 1R01 AR063157-01 Nyman 09/01/2012 – 08/31/2018 (NCE)
Roles of Collagen and Water in the Fracture Resistance of Bone
The overarching goal of this proposal is to determine whether the functional state of water in bone explains the disproportionate increase in fracture risk relative to age- and diabetes-related changes in bone mineral density (BMD).
Role: PI

NIH/NIAMS 1R21 AR067871 Nyman 07/01/2016 – 06/30/2018
The Role of Tissue Matrix in the Fracture Resistance of Diabetic Bone
The objective of this project is to identify pathogenic changes in the bone tissue matrix that contribute to bone fragility as diabetes progresses and determine how well clinically translatable diagnostic tools (sensitive to the matrix and the mineral of the bone) reflect the diabetes-related changes in fracture resistance.
Role: PI

DOD/CDMRP NF140017 Elefteriou 09/30/2015 – 09/29/2018
Targeting RAF1 with C-type Natriuretic Peptide to Promote Bone Union in NF1
The objective of this project is to determine whether a CNP analog promotes fracture healing in mice lacking neurofibromatosis type 1.
Role: Site PI

NIH/NIDDK 1R01 DK084045 Fowlkes 7/1/2012 – 06/30/2017
The Insulin/GF-I Axis in Diabetic Osteopathy
This proposed project aims to elucidate the mechanism of how diabetes affects bone using conditional knock-out mouse models.
Role: Site PI

OMB No. 0925-0001 and 0925-0002 (Rev. 09/17 Approved Through 03/31/2020)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: HAWKE, Thomas James

eRA COMMONS USER NAME (credential, e.g., agency login):

POSITION TITLE: Professor of Pathology and Molecular Medicine, McMaster University (Hamilton Canada)

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
University of Guelph, Ontario, Canada	BSc	05/1994	Human Kinetics
University of Guelph, Ontario, Canada	MSc	08/1996	Human Kinetics
University of Guelph, Ontario, Canada	PhD	05/2000	Biophysics- Electrolyte physiology
University of Texas-Southwestern Medical Center, Dallas, TX	Postdoctoral	05/2003	Muscle Stem Cell Biology

A. Personal Statement

I have built a successful laboratory with the knowledge and expertise to fully analyze skeletal muscle health with demonstrated expertise in electron microscopy, histology/immunohistochemistry, primary and cell line muscle cultures, morphometrics, human muscle biopsies, functional testing in rodents and humans, and therapeutic interventions. As a PI, I have maintained consistent funding from Natural Sciences and Engineering Research Council of Canada since 2005 and have also held other government, foundation and industry-sponsored research funds. In addition, my research has also been recognized with the prestigious Alexander von Humboldt Fellowship (2011-2013) for my research investigating novel mediators of muscle regeneration. Given my laboratory's established expertise in skeletal muscle biology, our team is uniquely positioned to contribute significantly to the proposed research project. Also, with my expertise in skeletal muscle biology and mentoring, I am uniquely positioned to collaborate significantly with Dr. Kalaitzoglou on her NIH K08 proposed research project. As can be seen from my publication record, I have a well-established track record of international collaborations and am aware of the importance of clear communication and respected timelines for deliverables.

B. Positions and Honors

Positions and Employment

2000-2003	Postdoctoral Fellow, Division of Cardiology; UT Southwestern Medical Center. Dallas TX
2003-2007	Assistant Professor, School of Kinesiology and Health Sciences. York University. Toronto ON Canada
2007-2009	Associate Professor, School of Kinesiology and Health Sciences. York University. Toronto ON Canada
2009-2018	Associate Professor, Department of Pathology and Molecular Medicine, McMaster University. Hamilton ON Canada
2018-present	Full Professor, Department of Pathology and Molecular Medicine, McMaster University. Hamilton ON Canada

Other Experience and Professional Memberships

1997- present	Member, American Physiological Association
2003- present	Member, Canadian Society for Exercise Physiology
2014-2016	Canadian Society of Exercise Physiology-Director Academic
2015- present	Review Editor, Frontiers in Stem Cell Research
2016-2018	Canadian Society of Exercise Physiology-Vice Chair Research
2016- present	Associate Editor, Journal of Diabetes Research
2017- present	Associate Editor, American Journal of Physiology- Cell Physiology

Honors

2011-2013	Alexander von Humboldt Visiting Scholar Fellowship- Experienced Researcher, Germany. Host- Universtät Bonn, Institute for Cell Biology.

C. Contributions to Science

My lab has developed an international reputation for our studies investigating the growth, adaptation and repair of skeletal muscle in health and in response to disease (with a focus on diabetes). Collectively, this work (funded by NSERC, CIHR, CFI/ORF and Sick Kids Foundation) has resulted in 60+ publications, citations exceeding 5290 and an H-index of 31 (Google Scholar, TJ Hawke: accessed 05/22/19). I have presented this work locally, nationally and internationally in a variety of forums including invited lectures, keynote speeches and as conference presentations. Below I have summarized the primary pillars of my work and highlighted some of our selected publications:

1. Advances in our Understanding of Skeletal Muscle Growth and Repair

My lab has made great strides in our understanding of factors regulating muscle growth and repair, as well as the muscle stem cell population. Our reputation for investigating and defining myopathic conditions has led to numerous collaborative endeavors both locally and internationally. Specifically, Xin (Cmya-1; cardiomyopathy-associated-1) is an adapter protein containing novel actin-binding repeats which is involved in the skeletal muscle remodeling. Our currents studies into Xin are investigating its function in skeletal muscle regeneration and the effects of modulating Xin expression levels during the regenerative process.

Liu C, Gersch RP, Hawke TJ, Hadjiargyrou M. Silencing of Mustn1 inhibits myogenic fusion and differentiation. Am J Physiol Cell Physiol. 2010. 298: C1100-C1108. PMCID: PMC2867393

Nissar AA, Zemanke B, Labatia R, Atkinson DJ, van der Ven PF, Furst DO, Hawke TJ. Skeletal muscle regeneration is delayed by reduction in Xin expression: consequence of impaired satellite cell activation? Am J Physiol Cell Physiol. 2012. 302: C220-C227.

Nilsson MI, Nassar AA, Al-Sajee D, Tarnoplosky MA, Parise G, Lach B, Furst DO, van der Ven PFM, Kley RA, Hawke TJ. Xin is a marker of skeletal muscle damage severity in myopathies. Am J Pathol. 2013. 183: 1703-1709.

Bujak AL, Crane JD, Lally JS, Ford RJ, Kang SJ, Rebalka IA, Green AE, Kemp BE, Hawke TJ. AMPK activation of muscle autophagy prevents fasting-induced hypoglycemia and myopathy during aging. Cell Metab. 2015. 21: 883-890. PMCID: PMC5233441

2. Defining the Impact of Diabetes on the Health of Skeletal Muscle

My research in this field has convincingly demonstrated that type 1 diabetes and diet-induced obesity (pre-diabetes) can negatively affect the physical, metabolic & regenerative capacities of skeletal muscle- a condition we have termed diabetic myopathy. My group has made novel findings demonstrating that the growth and regenerative capacity of skeletal muscles is dramatically reduced in the presence of T1D and diet-induced obesity. These defects in repair are due in part to diabetes-specific effects on the satellite cell population and to altered extracellular matrix remodeling a pathology which we recently demonstrated also occurs in young adults with T1D, regardless of glycemic control.

Krause MP, Riddell MC, Gordon CS, Imam SA, Cafarelli E, Hawke TJ. Diabetic myopathy differs between Ins2Akita+/- and streptozotocin-induced type 1 diabetic models. J Appl Physiol. 2009. 106: 1650-1659.

Gordon CS, Serino AS, Krause MP, Campbell JE, Cafarelli E, Adegoke OA, Hawke TJ, Riddell MC. Impaired growth and force production in skeletal muscles of young partially pancreatectomized rats: a model of adolescent type 1 diabetic myopathy? PLoS One. 2010. 5: e14032. PMCID: PMC2984438

Thomas MM, Trajcevik KE, Coleman SK, Jiang M, Di Michele J, O'Neill HM, Lally JS, Steinberg GR, Hawke TJ. Early oxidative shifts in mouse skeletal muscle morphology with high-fat diet consumption do not lead to functional improvements. Physiol Rep. 2014. 2: e12149. PMCIS: PMC4270228

D'Souza DM, Zhou S, Rebalka IA, MacDonald B, Moradi J, Krause MP, Al-Sajee D, Punthakee Z, Tarnopolsky MA, Hawke TJ. Decreased satellite cell number and function in humans and mice with type 1 diabetes is the result of altered notch signaling. Diabetes. 2016. 65: 3053-3061.

3. Therapeutic Advances to Treat Diabetic Complications

My work into diabetic myopathy naturally led us to the development of therapeutic strategies to reduce the regenerative (muscle, dermal) defects that characterize diabetes. These studies uncovered novel therapeutic targets to reduce the negative impact of type 1 diabetes on skeletal muscle and dermal wound healing. An area of focus has been on delineating the importance of modulating key fibrotic pathway proteins and inhibiting circulating factors which impair muscle growth.

Krause MP, Moradi J, Nissar AA, Riddell MC, Hawke TJ. Inhibition of plasminogen activator inhibitor-1 restores skeletal muscle regeneration in untreated type 1 diabetic mice. Diabetes. 2011. 60: 1964-1972. PMCID: PMC3121432

Krause MP, Al-Sajee D, D'Souza DM, Rebalka IA, Moradi J, Riddell MC, Hawke TJ. Impaired macrophage and satellite cell infiltration occurs in a muscle-specific fashion following injury in diabetic skeletal muscle. PLosOne. 2013. 8: e70971. PMCID: PMC3741394

Rebalka IA, Raleigh MJ, D'Souza DM, Coleman SK, Rebalka AN, Hawke TJ. Inhibition of PAI-1 Via PAI-1 improves dermal wound closure in diabetes. Diabetes 2015. 64: 2593-2602.

Coleman SK, Rebalka IA, D'Souza DM, Deodhare N, Desjardins EM, Hawke TJ. Myostatin inhibition therapy for insulin-deficient type 1 diabetes. Sci Rep. 2016. 6:32495. PMCID: PMC5007491

Complete list of publications: https://www.ncbi.nlm.nih.gov/pubmed/?term=hawke+tj

D. Additional Information: Research Support

Current:

NSERC Discovery Grant	Hawke-Sole PI	04/2018 - 03/2023
Impact of Adipokines of Skeletal Muscle Regeneration		
NSERC Discovery Accelerator Supplement	Hawke-Sole PI	04/2018 - 03/2021
Impact of Adipokines of Skeletal Muscle Regeneration		
Industrial partnership	Hawke- Sole PI	04/2018 - 04/2021
Ventech Solutions		
Development of a muscle pain biosensor.		

Completed:

NSERC RTI (Equipment)	Hawke- Co-I	04/2017 - 032018
Research Tools & Instruments I		
Research Personnel Grant	Hawke-Sole PI	01/2017 - 12/2017
Exerkine Corporation		

For New and Renewal Applications (PHS 398) – DO NOT SUBMIT UNLESS REQUESTED

PHS 398 OTHER SUPPORT

Provide active and pending support for all senior/key personnel. **Other Support includes all financial resources, whether Federal, non-Federal, commercial or institutional, available in direct support of an individual's research endeavors, including but not limited to research grants, cooperative agreements, contracts, and/or institutional awards.** Training awards, prizes, or gifts do not need to be included.

There is no "form page" for other support. Information on other support should be provided in the *format* shown below, using continuation pages as necessary. The sample below is intended to provide guidance regarding the type and extent of information requested.
For instructions and information pertaining to the use of and policy for other support, see Other Support in the Supplemental Instructions, Part III, Policies, Assurances, Definitions, and Other Information.
Effort devoted to projects must be measured using person months. Indicate calendar, academic, and/or summer months associated with each project.

Format

NAME OF INDIVIDUAL
ACTIVE/PENDING

Project Number (Principal Investigator) Source Title of Project *(or Subproject)* The major goals of this project are…	Dates of Approved/Proposed Project Annual Direct Costs	Person Months (Cal/Academic/ Summer)

OVERLAP *(summarized for each individual)*

FOWLKES, J.L.

ACTIVE

No Sponsor ID (Fowlkes, J.) 02/15/2018 – 01/31/2023 0.60 calendar
Marshall University (P20 GM121299-01A1) $49,108
"Appalachian Center for Cellular Transport in Obesity Related Disorders (ACCORD)"
Goals: This COBRE will identify novel targets that can potentially be modeled as new therapeutics to ameliorate and/or prevent obesity related disorders not only in WV/CA and across the country.
Role: Co-Investigator/Subproject Mentor

P30 ES026529-03 (Hahn, E.) 05/01/2017 – 03/31/2022 0.40 calendar
NIH/NIEHS $994,327
"Center for Appalachian Research in Environmental Sciences"
Goals: The goal of this project is to examine environmental influences on health and wellbeing.
Role: Metabolic Disorders Research Interest Group Leader

R56 DK084045-05A1 (Fowlkes, J.) 05/23/2012 – 03/31/2020 0.12 calendar
NIH/NIDDK $100,786 (NCE)
The Insulin/IGF-I Axis in Diabetic Osteopathy
Goals: The major goal of this project is to investigate the contributions of the insulin and IGF-1 axis to skeletal physiology and specifically, how disruptions of this axis impact the pathophysiology of diabetic osteopathy.
Role: PD/PI

SC 728 1900000105 (Day, S.) 07/01/2018 – 06/30/2020 0.12 calendar
Cabinet for Health and Family Services $312,000
FY'19-20 Metabolic Newborn Screening
Goals: Advisor to Kentucky State NBS for endocrine related screens
Role: Co-Investigator

5UL1TR001998-04 (Kern, P.) 08/15/2016 – 05/31/2020 0.60 calendar
NIH/NCATS $2,437,463
“Kentucky Center for Clinical and Translational Science”
The Kentucky Center for Clinical and Translational Science (CCTS) provides infrastructure, services, and programs to support clinical and translational investigators, to foster collaborations between basic and clinical scientists to facilitate research translation, to train the clinical and translational workforce of the future, and to enhance community engagement pathways to confront chronic health issues in rural Appalachia.
Role: Metabolic Disorders Research Interest Group Leader (as of 11/01/2018)

PENDING

U54 UL1TR001998-05 (Kern, P.) 06/01/2020 – 05/31/2025 0.60 calendar
NIH/NIDDK $3,507,934
Kentucky Center for Clinical and Translational Sciences
Goals: The University of Kentucky (UK) Center for Clinical and Translational Science (CCTS) is an integrated home for clinical and translational research to promote scientific progress and discoveries at every phase of the translational continuum.
Role: Co-I and Director of Translational Research Development Team (TREE).

OVERLAP

There is no scientific or fiscal overlap with the project under consideration for funding.

Philip A. Kern, M.D.

Active

5R01DK107646 (Kern) 09/21/15 – 07/31/20 0.60 Cal Mnths
NIH/NIDDK $338,141
"Cold Induced Changed in Human Subcutaneous White Adipose"
This project examines the effects of cold temperatures on the beiging of human white adipose tissue.
Role: PI

5R01DK112282 (Kern) 09/15/16 - 08/31/20 2.40 Cal Mnths
NIH/NIDDK $361,559
"The activation of brown and beige fat and role in insulin sensitivity"
This study will evaluate the activation of beige fat through biopsies, and brown fat through PET-CT scans, in response to B3 agonist and PPARγ agonist drugs. Study subjects will be insulin resistant and changes in insulin sensitivity will be measured.
Role: PI

5R01AG046920 (Peterson/Kern/Bamman Multi-PI) 09/30/14 – 05/31/20 0.60 Cal Mnths
NIH/NIA $437,650
"Novel Actions of Metformin to Augment Resistance Training Adaptations in Older Adults"
This project will recruit older subjects who will engage in an exercise program. The overall aim will be to determine whether metformin treatment will improve the response of muscle to exercise. There is no overlap with other projects.
Role: Co-PI

5UL1TR001998 (Kern) 08/15/16-05/31/20 3.60 Cal Mnths
NIH/NCATS $2,437,463
"Kentucky Center for Clinical and Translational Science"
The Kentucky Center for Clinical and Translational Science (CCTS) provides infrastructure, services, and programs to support clinical and translational investigators, to foster collaborations between basic and clinical scientists to facilitate research translation, to train the clinical and translational workforce of the future, and to enhance community engagement pathways to confront chronic health issues in rural Appalachia.
Role: PI

2P30DK020579-41/ WU-13-238 (Kern) 12/01/17 – 11/30/22 0.24 Cal Mnths
NIH Federal Flow-through of Diabetes Research Center, PI: Jean E. Schaffer Washington University – St. Louis $75,914
"University of Kentucky Pilot and Feasibility Research Program"
This is a Diabetes Research Center, awarded to Washington University, and with a small subcontract to UK. The goal of the overall Center is to provide infrastructure to diabetes research and to stimulate innovative new ideas. The University of Kentucky has a partnership with Washington University in St. Louis related to diabetes/cardiovascular research. This partnership involves a subcontract on the Wash U Diabetes Research Center (PI: Jean Schaffer) for pilot grants to eligible investigators at UK, and Dr. Kern is the PI of the University of Kentucky subcontract.
Role: PI of UK subcontract

7R01DK108056 (Nikolajczyk) 04/01/18 – 03/31/20 0.60 Cal Mnths
NIH/NIDDK $482,808
"Inflammation in Human Obesity and Type 2 Diabetes"
This project tests the hypothesis that a T cell signature distinguishes T2D from non-T2D subjects and is a predictive biomarker for T2D. This signature may include multiple T cell cytokines, many of which are preferentially produced by the Th17 or Th1 T cell subsets. This human T cell inflammatory signature may identify diabetogenic inflammation and better characterize T2D pathogenesis. Dr. Kern is a Co-I involved establishing and designing the clinical recruitment and characterization.

Role: Co-I

P20GM121299 (Sundaram) 02/15/18 – 01/31/23 0.60 Cal Mnths
Marshall University Research Co. $48,662
"Appalachian Center for Cellular transport in Obesity Related Disorders (ACCORD)"
The COBRE program, "Appalachian Center for Cellular transport in Obesity Related Disorders (ACCORD)," will develop two research cores, two pre-cores, and support five projects conducted by Marshall junior investigators. These projects will be thematically focused on cellular transport issues and their effect on obesity-related disorders. Dr. Kern is a mentor to the trainees and an advisor on the overall project.
Role: UK Consortium PI

1R01DK119619 (McCarthy, Peterson, Co-PI) 09/19/18 – 07/31/23 0.60 Cal Mnths
NIH/NIDDK $264,684
"Exercise-induced Skeletal Muscle Exosomes Promote Adipocyte Lipolysis"
This proposal hypothesizes that resistance exercise promotes the release of miR-1 containing exosomes from muscle which then are taken up by adipose tissue to promote increase lipolysis and beta adrenergic receptor activity, in both mice and humans.
Role: Co-I

R01 AG062550 (Johnson, LA PI). 04/01/2019 – 03/31/2024 0.60 Cal Mnths
NIH/NIA $264,111
"Changing the energy substrate balance: Does APOE2 promote glucose usage to protect from Alzheimer's disease?"
This proposal hypothesizes that apoE2 affords neuroprotection of Alzheimer's disease by virtue of shifting metabolism from fatty acid oxidation to glucose utilization. Studies in both mice and humans will examine the respiratory exchange ratio in subjects with different apoE isoforms.
Role: Co-I.

1UT2GM130174 (Willmot) 09/01/18 – 08/31/21 0.12 Cal Mnths
XLerate Health LLC $49,280
"STTR: Southeast XLerator Network"
The Southeast XLerator Network proposes to create a networked and easily accessible regional technology transfer accelerator hub ("XLerator Hub" or "Hub") to share best practices, disseminate education content, and offer products, services, facilities and other resources connected through both physical and online platforms for innovators and trainees in the Southeast IDeA states.
Role: Co-I of UK Subcontract

Pending

R01 DK124626-01A1 (Kern) 09/01/20 – 08/31/25 2.4 Cal mnths
NIH/NIDDK $457,600
"Mechanisms for activation of beige adipose tissue in humans"
This project extends studies on beige adipose tissue by determining whether chronic treatment with mirabegron, a β3 adrenergic receptor agonist approved for treatment of over active bladder, improves metabolic homeostasis in prediabetic, obese, research participants. It also seeks to investigate the underlying mechanisms for improved β-cell function and insulin sensitivity by mirabegron treatment
Role: PI

R01 DK127039-01 (Kern & Pendergast) 09/01/20 – 08/31/25 2.4 Cal mnths
NIH/NIDDK $440,850
"Impact of chronotype on the metabolic benefits of timed exercise"
This project seeks to determine whether the circadian-personalized timing of exercise enhances the efficacy of exercise in improving metabolic health. This research could elucidate strategies that make exercise programs more tenable (such as short duration bouts at a specific time of day) and thus increase long-term adherence to these regimens for the treatment of metabolic disorders.

Role: Contact PI

R01 AG069467-01 (Nikolajczyk/Kern/Lauffenburger) 07/01/20 – 06/30/25 1.8 Cal mnths
NIH/NIA $580,013
"The impact of metformin on mechanisms that drive inflammation in older adults"
This project seeks to test the possibility that metformin promotes healthspan by ameliorating a newly identified inflammaging profile.
Role: non-contact PI

Overlap

None

PETERSON, CHARLOTTE A.

CURRENT

R01AR060701-10 (Peterson & McCarthy, MPI) 09/17/10-06/30/20 1.65 academic
NIH/NIAMS $220,000
Novel roles for satellite cells in adult skeletal muscle adaptation
Satellite cells will be specifically ablated in genetically modified mice and the influence on muscle adaptability determined.

R01AG046920-05 (Peterson & Kern, MPI) 09/30/14-05/31/20 (NCE) 1.1 academic
NIH/NIA $437,650
Novel actions of metformin to augment resistance training adaptation in older adults
Resistance exercise training is the most effective intervention identified to combat loss of skeletal muscle mass and strength with advancing age; however, the response to exercise is blunted and highly variable in the elderly. We propose to prospective identify individuals likely to be refractory to routine exercise programs by characterizing macrophage content in muscle, and determining the effectiveness of metformin in improving the muscle growth response.

R01HL126117-05 (McDermott, PI) 08/17/15-04/30/20 0.44 academic
NIH/NHLBI $26,758
TELmisartan plus EXercise to improve functioning in PAD: The TELEX trial
This is an interventional study conducted at Northwestern University on patients with peripheral artery disease. Analysis of the effects of exercise and telmisartan on gastrocnemius muscle morphology and composition will be performed by Dr. Peterson at the University of Kentucky on muscle biopsies shipped from NWU.
Role: Co-I

R01AG049806-04 (Peterson & McCarthy, MPI) 01/01/16-11/30/21 1.65 academic
NIH/NIA $182,274
The effects of exercise on satellite cell dynamics during aging
The purpose of this grant is to use a newly developed mouse model to track satellite cell dynamics with aging and in response to a hypertrophic stimulus.

W81XWH-16-2-0058 (McDiarmid, PI) 09/30/16-9/29/21 1.1 academic
DoD/CDMRP $291,127
Biomarkers for Assessing Return-to-Duty Potential of Personnel with Embedded Metal-Fragment Wounds
The goal of this proposal is to develop a rat model of embedded metal in hindlimb muscle to provide an evidence base to refine the clinical management of the Veteran or Service Member with retained embedded metal fragments.
Role: Co-I, Project 2 Leader

R01 HL131771-03 (McDermott, PI) 12/09/16–11/30/21 0.22 academic
NIH/NHLBI $19,045
Improve PAD Performance with METformin. The PERMET Trial
The purpose of this study is to propose a placebo controlled double-blinded randomized clinical trial to establish whether metformin (2000 mgs daily) improves and/or prevents decline in walking performance in people with PAD at six-month follow-up.
Role: Co-I

R21AG056903-02 (McDermott, PI) 09/15/17–05/31/20 (NCE) 0.11 academic
NIH/NIA $10,789
Hepatocyte growth factor to improve walking performance in PAD: the HI-PAD Study
The goal of this project is to identify biologic mechanisms to help identify new therapies, with similar biologic actions, that improve walking performance in people with peripheral artery disease.
Role: Co-I

R01AG057693-02 (McDermott) 08/01/18-04/30/23 0.11 academic
NIH/NIA $2,401
Intermittent pneumatic compression for disability reversal in PAD: The INTERCEDE Trial
In people with PAD, this study will determine whether treatment with intermittent pneumatic compressive augments the benefits of exercise, whether intermittent pneumatic compression alone improves walking performance compared to control, and whether the benefits of intermittent pneumatic compression are durable.
Role: Co-I

R01DK119619-02 (McCarthy & Peterson, MPI) 09/19/18-07/31/23 1.1 academic
NIH/NIDDK $264,684
Exercise-induced skeletal muscle exosomes promote adipocyte lipolysis
The purpose of the study is to investigate the mechanism through which resistance exercise causes skeletal muscle to communicate with adipose tissue to promote the burning of fat.

R01 DK119619-02S1 (McCarthy & Peterson, MPI) 06/01/19-05/31/20 0.55 academic
NIH/NIDDK $250,000
AD Supplement to Exercise-induced skeletal muscle exosomes promote adipocyte lipolysis
The goal of this project is to determine if exercise-induced exosomes can offset the deleterious effects of obesity on AD progression.

PENDING

R01AG069909 (Peterson & McCarthy) 09/01/2020-8/31/2025 1.1 academic
NIH $326,141
Role of Satellite Cells in Skeletal Muscle Hypertrophy with Aging
The goal of this project is to provide a more comprehensive understanding of the effects of age on satellite cell function during muscle hypertrophy to effectively target this stem cell population to prevent and/or restore the loss of muscle mass associated with aging, prolonged inactivity and muscle wasting diseases.

R21AG06605 (McCarthy & Peterson, MPI) 07/01/2020 – 06/30/2022 .6 academic
NIH $150,000
The Role of the Gut Microbiome in the Etiology of Sarcopenia
The purpose of this exploratory project is to test the hypothesis that changes in the composition of the gut microbiome with age promotes sarcopenia by altering anabolic metabolism of skeletal muscle.

R01 AG066724 (Peterson & Kosmac, MPI) 10/01/2020 – 09/30/2025 .55 academic
NIH $250,000
Novel gastrocnemius muscle characteristics in peripheral artery disease patients associated with impaired functional performance
The purpose of this study is to define specific characteristics of muscle in PAD associated with impaired walking performance through detailed immunohistochemical analyses of approximately 300 baseline gastrocnemius muscle biopsies stored in the Northwestern biorepository collected from 12 different clinical trials.

No ID (Peterson) 03/01/2020 – 02/28/2022 .49 academic
Chan Zuckerberg Foundation $157,460
Muscle Macrophage Inflammation in Sarcopenia and Impaired Adaptability
The purpose of this study is quantify resident macrophage populations in human skeletal muscle and identify inflammatory properties via single-cell RNA-sequencing that may contribute to muscle wasting and resistance to the beneficial effects of exercise associated with aging.

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 1

ORGANIZATIONAL DUNS*: 939017877

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: University of Kentucky Research Foundation

Start Date*: 09-01-2020 **End Date*:** 08-31-2021 **Budget Period: 1**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	
Additional Equipment: File Name:	

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	
Total Travel Cost	

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 1

ORGANIZATIONAL DUNS*: 939017877

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: University of Kentucky Research Foundation

Start Date*: 09-01-2020 **End Date*:** 08-31-2021 **Budget Period: 1**

F. Other Direct Costs	**Funds Requested ($)***
1. Materials and Supplies	25,000.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**25,000.00**

G. Direct Costs	**Funds Requested ($)***
Total Direct Costs (A thru F)	**153,551.00**

H. Indirect Costs

Indirect Cost Type	**Indirect Cost Rate (%)**	**Indirect Cost Base ($)**	**Funds Requested ($)***
1 . On Campus Research	8	153,551.00	12,284.00
		Total Indirect Costs	**12,284.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number) DHHS, Arif Karim, 214-767-3261

I. Total Direct and Indirect Costs	**Funds Requested ($)***
Total Direct and Indirect Institutional Costs (G + H)	**165,835.00**

J. Fee	**Funds Requested ($)***

K. Total Costs and Fee	**Funds Requested ($)***
	165,835.00

L. Budget Justification*	File Name: K08_Resub_Budget_Justification_SC1009017468.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 2

ORGANIZATIONAL DUNS*: 939017877

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: University of Kentucky Research Foundation

Start Date*: 09-01-2021 **End Date*:** 08-31-2022 **Budget Period: 2**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	
Additional Equipment: File Name:	

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	
Total Travel Cost	

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 2

ORGANIZATIONAL DUNS*: 939017877

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: University of Kentucky Research Foundation

Start Date*: 09-01-2021 **End Date*:** 08-31-2022 **Budget Period: 2**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	25,000.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**25,000.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**153,636.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . On Campus Research	8	153,636.00	12,291.00
		Total Indirect Costs	**12,291.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number) DHHS, Arif Karim, 214-767-3261

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**165,927.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	165,927.00

L. Budget Justification*	File Name: K08_Resub_Budget_Justification_SC1009017468.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 3

ORGANIZATIONAL DUNS*: 939017877

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: University of Kentucky Research Foundation

Start Date*: 09-01-2022 **End Date*:** 08-31-2023 **Budget Period: 3**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	
Additional Equipment: File Name:	

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	
Total Travel Cost	

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 3

ORGANIZATIONAL DUNS*: 939017877

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: University of Kentucky Research Foundation

Start Date*: 09-01-2022 **End Date*:** 08-31-2023 **Budget Period: 3**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	25,000.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**25,000.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**153,722.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . On Campus Research	8	153,722.00	12,298.00
		Total Indirect Costs	**12,298.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number) DHHS, Arif Karim, 214-767-3261

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**166,020.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	166,020.00

L. Budget Justification*	File Name: K08_Resub_Budget_Justification_SC1009017468.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 4

ORGANIZATIONAL DUNS*: 939017877

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: University of Kentucky Research Foundation

Start Date*: 09-01-2023 **End Date*:** 08-31-2024 **Budget Period: 4**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	
Additional Equipment: File Name:	

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	
Total Travel Cost	

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 4

ORGANIZATIONAL DUNS*: 939017877

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: University of Kentucky Research Foundation

Start Date*: 09-01-2023 **End Date*:** 08-31-2024 **Budget Period: 4**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	25,000.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**25,000.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**153,808.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . On Campus Research	8	153,808.00	12,305.00
		Total Indirect Costs	**12,305.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number) DHHS, Arif Karim, 214-767-3261

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**166,113.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	166,113.00

L. Budget Justification*	File Name: K08_Resub_Budget_Justification_SC1009017468.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 5

ORGANIZATIONAL DUNS*: 939017877

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: University of Kentucky Research Foundation

Start Date*: 09-01-2024 **End Date*:** 08-31-2025 **Budget Period: 5**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	
Additional Equipment: File Name:	

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	
Total Travel Cost	

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 5

ORGANIZATIONAL DUNS*: 939017877

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: University of Kentucky Research Foundation

Start Date*: 09-01-2024 **End Date*:** 08-31-2025 **Budget Period: 5**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	25,000.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**25,000.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**153,895.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . On Campus Research	8	153,895.00	12,312.00
		Total Indirect Costs	**12,312.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): DHHS, Arif Karim, 214-767-3261

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**166,207.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	166,207.00

L. Budget Justification* File Name: K08_Resub_Budget_Justification_SC1009017468.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Budget justification

Senior/Key person

Evangelia Kalaitzoglou, MD, an Assistant Professor of Pediatrics in the Division of Pediatric Endocrinology at the University of Kentucky, School of Medicine is the Principal Investigator of the Career Development Award. She will commit a minimum of 9 calendar months (75% effort) per year to conduct all work associated with this study as proposed for the duration of this project. Salary support of $100,000 plus fringe, is requested for Dr. Kalaitzoglou in years 1-5.

Mentors, co-mentors and collaborators:

There is no salary support requested for primary mentors (**John Fowlkes, MD and Charlotte Peterson, PhD**), co-mentors (**Philip Kern, MD**) and collaborators (**Thomas Hawke, PhD and Jeffry Nyman, PhD**).

Other Direct Costs

Project support funds will be used to cover the costs to conduct the proposed study inclusive of (but not limited to):

- Core facility user fees for CCTS and Magnetic Resonance Imaging (MRI) services
- Research subject compensation for study participants
- Materials and supplies
- Animal purchase and per diem charges for housing of mice
- Insulin and control LinBit implants
- Reagents for PCR
- Antibodies for Western Blot and immunohistochemistry
- Serum marker kits for bone and skeletal muscle biomarkers
- Cell culture supplies and recombinant myostatin protein
- Travel costs to cover related expenses to conferences such as the NIDDK K awardee's workshop, the Musculoskeletal Biology workshop and the American Society for Bone and Mineral Research annual meeting.
- Publication costs to cover page charges and other related expenses to cover publication/dissemination of the findings in a recognized scholarly journal

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals ($)	
Section A, Senior/Key Person		643,612.00
Section B, Other Personnel		
Total Number Other Personnel		
Total Salary, Wages and Fringe Benefits (A+B)		643,612.00
Section C, Equipment		
Section D, Travel		
1. Domestic		
2. Foreign		
Section E, Participant/Trainee Support Costs		
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other		
6. Number of Participants/Trainees		
Section F, Other Direct Costs		125,000.00
1. Materials and Supplies	125,000.00	
2. Publication Costs		
3. Consultant Services		
4. ADP/Computer Services		
5. Subawards/Consortium/Contractual Costs		
6. Equipment or Facility Rental/User Fees		
7. Alterations and Renovations		
8. Other 1		
9. Other 2		
10. Other 3		
Section G, Direct Costs (A thru F)		768,612.00
Section H, Indirect Costs		61,490.00
Section I, Total Direct and Indirect Costs (G + H)		830,102.00
Section J, Fee		
Section K, Total Costs and Fee (I + J)		830,102.00

PHS 398 Cover Page Supplement

OMB Number: 0925-0001

Expiration Date: 03/31/2020

1. Vertebrate Animals Section

Are vertebrate animals euthanized? ● Yes ○ No

If "Yes" to euthanasia

Is the method consistent with American Veterinary Medical Association (AVMA) guidelines?

● Yes ○ No

If "No" to AVMA guidelines, describe method and provide scientific justification

2. *Program Income Section

*Is program income anticipated during the periods for which the grant support is requested?

○ Yes ● No

If you checked "yes" above (indicating that program income is anticipated), then use the format below to reflect the amount and source(s). Otherwise, leave this section blank.

*Budget Period	*Anticipated Amount ($)	*Source(s)

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3. Human Embryonic Stem Cells Section

*Does the proposed project involve human embryonic stem cells? ❍ Yes ● No

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s) from the following list: http://grants.nih.gov/stem_cells/registry/current.htm. Or, if a specific stem cell line cannot be referenced at this time, check the box indicating that one from the registry will be used:

Specific stem cell line cannot be referenced at this time. One from the registry will be used.

Cell Line(s) (Example: 0004):

4. Inventions and Patents Section (Renewal applications)

*Inventions and Patents: ❍ Yes ❍ No

If the answer is "Yes" then please answer the following:

*Previously Reported: ❍ Yes ❍ No

5. Change of Investigator/Change of Institution Section

❑ Change of Project Director/Principal Investigator

Name of former Project Director/Principal Investigator

Prefix:

*First Name:

Middle Name:

*Last Name:

Suffix:

❑ Change of Grantee Institution

*Name of former institution:

Tracking Number: GRANT13045904 Funding Opportunity Number: PA-19-117 . Received Date: 2020-03-11T14:42:33.000-04:00

PHS 398 Career Development Award Supplemental Form

OMB Number: 0925-0001
Expiration Date: 03/31/2020

Introduction	
1. Introduction to Application (for Resubmission and Revision applications)	K08_Introduction_page__RK08_0305201009017487.pdf
Candidate Section	
2. Candidate Information and Goals for Career Development	Candidate_Info___Goals_for_Career_De1009017519.pdf
Research Plan Section	
3. Specific Aims	SPECIFIC_AIMS_RK08_0305201009017501.pdf
4. Research Strategy*	Approach_030220_RK08_v41009017520.pdf
5. Progress Report Publication List (for Renewal applications)	
6. Training in the Responsible Conduct of Research	Training_in_the_RCR_RK08_0304201009017503.pdf
Other Candidate Information Section	
7. Candidate's Plan to Provide Mentoring	
Mentor, Co-Mentor, Consultant, Collaborators Section	
8. Plans and Statements of Mentor and Co-Mentor(s)	Mentor_letter_RK08_0304201009017508.pdf
9. Letters of Support from Collaborators, Contributors, and Consultants	Letters_of_Collaboration_All1009017517.pdf
Environment and Institutional Commitment to Candidate Section	
10. Description of Institutional Environment	Desc__of_Institutional_Env_0227201009017515.pdf
11. Institutional Commitment to Candidate's Research Career Development	Chair_letter_of_support_RK08_0224201009017516.pdf
Other Research Plan Section	
12. Vertebrate Animals	K08_VERTEBRATE_ANIMALS_0304201009017507.pdf
13. Select Agent Research	
14. Consortium/Contractual Arrangements	
15. Resource Sharing	Resource_Sharing_Plan_RK08_0227201009017506.pdf
16. Authentication of Key Biological and/or Chemical Resources	Authentication_of_resources_0304201009017505.pdf
Appendix	
17. Appendix	

Funding Opportunity Number: PA-19-117. Received Date: 2020-03-11T14:42:33.000-04:00

PHS 398 Career Development Award Supplemental Form

Citizenship*:

18. U.S. Citizen or Non-Citizen National?* ● Yes ○ No

If no, select most appropriate Non-U.S. Citizen option

- ❑ With a Permanent U.S. Resident Visa
- ❑ With a Temporary U.S. Visa
- ❑ Not Residing in the U.S.

If you are a non-U.S. citizen with a temporary visa applying for an award that requires permanent residency status, and expect to be granted a permanent resident visa by the start date of the award, check here: ❑

We would like to thank the reviewers for their very thoughtful critiques, which greatly strengthen this revised proposal**.** In addition, we thank the reviewers for recognizing the excellent environment and commitment to training and research at the University of Kentucky (UK) for the applicant and for recognizing that she is a promising clinician scientist dedicated to a long-term career in research.

In the initial application, there was some concern about the novelty of the proposal. In this revised application we have highlighted not only the gaps in knowledge on the role of myostatin and downstream signaling pathways in bone in general, but also specifically **in the context of the pathogenesis of diabetic bone disease (DBD) associated with Type 1 Diabetes (T1D)**. There is no published information on whether myostatin is dysregulated in humans with T1D, and our preliminary data are the first to show a connection between this myokine and bone in T1D (Aim 1). Furthermore, most animal studies showing positive effects of inhibiting myostatin on bone have used only non-specific inhibitors, which also inhibit other activin receptor 2b ligands that may affect bone, such as activin. Therefore, we will use a specific myostatin inhibitory antibody to unequivocally clarify whether myostatin inhibition in T1D affects DBD in mice (Aim 3, previously Aim 2B). Additionally, we have modified our *in vitro* studies to be more mechanistic in Aim 4 (previously Aim 3), to address the reviewers' comments on experiments being too observational.

Our *specific responses* to other comments *related to the* **Research Plan:** 1) *Insufficient rationale for duration of blockade with myostatin antibody in Aim 2 (now Aim 3):* Eight weeks of treatment is based on our previous publications and our preliminary data showing quantifiable changes in bone with T1D at this time point. The myostatin antibody will be given as soon as mice are confirmed diabetic and therefore would be utilized to preserve bone and prevent DBD. 2) *Aim 1 is in humans and Aim 2A (now Aim 2) is in mice, but the scientific question is the same, showing possible duplication.* For these studies to be translational, it is vital, to compare findings regarding myostatin from animal models to humans with T1D, as there is no published evidence that myostatin is dysregulated in humans with T1D. These studies will also lay the groundwork and establish the infrastructure for future studies and interventional clinical trials. 3) *No clear central hypothesis.* Our central hypothesis is now clearly stated in the specific aims page. 4) *Myostatin floxed mice can be used instead of antibody injection (Aim 2B, now Aim 3).* This is a constructive suggestion we have entertained in alternative approaches. In our studies, we have prioritized studies using the myostatin inhibitory antibody instead of a myostatin knockout model because it has the translational potential for the use in humans. 5) *Not clear if metabolic effects of myostatin will or can be uncoupled from proposed direct effects on bone.* We appreciate this concern. We will account for any metabolic effects of myostatin on glucose homeostasis by intraperitoneal glucose tolerance testing and body composition assessment by EchoMRI. 6) *Data is not very robust with regard to strength of effects and amount of data.* We have included additional data from in vitro effects of myostatin on mRNA of osteogenic gene expression (Aim 4). We have collected additional human specimens as the interval enrollment has increased threefold from the original submission.

For the **Career Development Plan,** we describe the training activities more comprehensively and the unique responsibilities of each mentor in the training plan. We also describe the path towards independence in more detail in the career development plan and the mentors' letter- including future plans for independent research (R01 application). Lastly, we have included the mentoring track record for all mentors. *Our response to specific comments*: **Candidate:** 1) *Modest level of productivity in terms of limited research first authorship- journals are average.* During my first three years at UK I was heavily involved in expansion and relocation of the pediatric endocrinology clinic, which limited my research productivity. However, my research effort only recently (07/2018) increased to 75%. Since the previous submission, I have co-authored a new publication, submitted a manuscript, and will submit two additional manuscripts as primary or senior author by the time this grant is reviewed. 2) *No publication link provided.* An updated URL link has been added to my Biosketch. **Career Development:** 1) *No novel techniques are proposed and training is standard and routine*. Although most of the techniques described in our proposal might not be novel, I have very limited experience in obtaining human muscle, skeletal muscle analysis and bone cell culture, and therefore would require additional training to become proficient in these techniques. Furthermore, all techniques will be used in novel approaches to understand muscle-bone interactions in T1D. **Mentors:** 1) *Expertise of mentor and co-mentors is similar or overlapping.* The mentors in this application bring together *complementary* expertise. Dr. Fowlkes is an expert in the bone field, Dr. Peterson has extensive expertise in skeletal muscle biology, and Dr. Kern has extensive skill sets in clinical research and muscle biopsy techniques. 2) *Additional expertise in cellular and molecular biology.* Dr. Charlotte Peterson is a well-established expert in cellular and molecular biology.3) *Evidence of independent investigator is not in letter from mentor.* The mentor letter as well as the career development plan now describes the pathway to independence in detail.

We hope that reviewers' concerns have been addressed and now a more competitive proposal is presented.

Candidate's Background, Professional Training and Previous Clinical/Research Experience

I have been fascinated by the area of endocrinology since my early years in medical school, which led to my decision to pursue a career in Pediatric Endocrinology. I have completed my clinical training in Pediatric Diabetes and Endocrinology and have a specific research interest in musculoskeletal complications of Type 1 Diabetes (T1D). My career goal as a Pediatric Endocrinologist is to become an independent physician scientist focused on understanding and modifying aspects of diabetic muscle and bone disease, with the long-term goal of developing and testing interventions to prevent these complications, as they can be devastating over the lifetime of individuals with T1D.

I received my medical degree (MD) with honors from the National and Kapodistrian University Medical School, in Athens, Greece. I completed my residency in Pediatrics at Penn State Hershey Medical Center, followed by a fellowship in Pediatric Endocrinology at Oklahoma University Health Sciences Center (OUHSC). It was during my second year of fellowship, when I worked with Dr. Mary Beth Humphrey, an NIH funded researcher, that I developed the motivation to further my training in the field of musculoskeletal disease. My fellowship research project, which focused on the role of the innate immune system in development of osteoarthritis, was funded by the Children's Hospital Foundation. The training I received while working with Dr. Humphrey was essential in learning laboratory skills required to perform bench and animal research focused on glucose metabolism and the musculoskeletal system. This training allowed me a greater understanding of the basic principles of animal research. In addition, this fellowship extended my scientific writing and presentation skills as this work resulted in a national presentation at ASBMR in 2014, two manuscripts and an ORS/OREF travel grant in Orthopaedic Research Translation in 2016.

The combination of clinical and basic science experience in bone metabolism and diabetes placed me in a unique position to obtain employment as a tenure-track Assistant Professor of Pediatrics at the University of Kentucky (UK) and to work on diabetic bone disease (DBD) with my Primary Mentor, Dr. John Fowlkes, Director of the Barnstable Brown Diabetes Center (BBDC). The Department of Pediatrics and the BBDC have been supportive of my research efforts as they have provided me with start-up funds and protected time to complete an animal study on the effects of insulin treatment on bone in an animal model of T1D. This work resulted in obtaining the Helmsley Charitable Trust Award in T1D, presenting at the Endocrine Society meeting in 2017, and co-authoring the manuscript containing these results, titled "Preserving and Restoring Bone with Continuous Insulin Infusion Therapy in a Mouse Model of Type 1 Diabetes". Furthermore, I have obtained a Children's Miracle Network grant (2017) through the Department of Pediatrics and a SChoLAR grant (2018) through the UK College of Medicine and Center for Clinical and Translation Science (CCTS) to secure funding for equipment/supplies and a technician, as well as further training in Clinical and Translational Science. Through the CCTS I have obtained direct mentorship from Dr. Philip Kern, Director of the CCTS, who has a long track record in clinical research related to obesity and diabetes. Additionally, I was fortunate enough to meet Dr. Charlotte Peterson, who is an internationally known muscle molecular and cellular biologist and the Director of the UK Center for Muscle Biology. She and Dr. Kern are long-time collaborators on both human and mouse studies, having recently completed a clinical trial examining the effects of metformin on response to exercise in the elderly. Together, my mentors' many years of experience in training and mentoring doctoral, post-doctoral and junior faculty members will be very beneficial to me as I transition into an independent research career.

Towards achieving my short-term career goals, I intend to maintain close working relations with my mentors and colleagues to achieve a multidisciplinary approach in the field of diabetic bone and muscle complications. A mentored career development award would provide me with valuable training and experience, which would allow me to further my research and my career as an independent investigator.

Candidate's Goals for Career Development

My long-term career goal is to be an independent physician scientist and a leader in the field of musculoskeletal complications in diabetes. I plan to study interactions between muscle and bone in the context of T1D and to apply this knowledge to clinical studies and translational practices for patient care. My short-term career goal, as a junior faculty member, is to establish myself as a translational researcher focused on muscle and bone interactions and to understand their relative contributions in diabetic complications. This would be the first such comprehensive research approach to focus **on both muscle and bone and their interactions** in patients with T1D. Furthermore, this focus would not only be unique within the muscle, bone and diabetes research communities, but very distinct from my mentors' research areas. My immediate goal is to complete the proposed studies that will serve as the foundation for future grants.

My career objectives for this K08 award are:

1. To develop all the requisite background and skills to create, develop and operationalize a broad-based research project, “bench-to-bedside”, in order to establish myself as a competitive translational researcher.
2. To develop expertise in obtaining muscle biopsies, processing and analyzing muscle samples.
3. To master bone cell culture and stimulation and gene expression techniques.
4. To enhance my development as an independent investigator, through acquiring new skills in grantsmanship and manuscript writing, project design, and statistical tools and methodologies.
5. To develop collaborative relationships and network with researchers in the field of musculoskeletal research inside and outside the UK campus.

Candidate’s Plan for Career Development/Training Activities During Award (Table 1)

As a junior investigator at UK, I am uniquely positioned to proceed with this proposed research. Since arriving at UK, I have been provided independent laboratory and office space, as well as access to all resources housed in the BBDC Laboratories. I have been fortunate enough to have a team of experienced mentors in the fields of diabetes and the musculoskeletal system with an excellent track record in basic, translational and clinical research. This interdisciplinary team of mentors and collaborators has assisted me in developing a comprehensive training program, which includes research and didactic components, which will facilitate my development as an independent investigator in the field of muscle-bone interaction in diabetes. Protected time for this K08 award will enable me to dedicate 75% of my time to this proposed research and training plan, 15% towards clinical efforts, 5% towards career development and 5% towards teaching activities.

My mentors, Drs. John Fowlkes, Charlotte Peterson, and Philip Kern are committed to promote my career development and support my research efforts during this award. If awarded, this application will provide important training in muscle physiology, which is a new area to me. In addition, the proposed research will provide me with additional training in different animal models of insulin-deficient diabetes (Dr. Fowlkes), training in clinical research (Drs. Kern and Peterson), muscle biopsy technique (Dr. Kern), molecular and cellular analyses of skeletal muscle (Dr. Peterson) and bone cell stimulation and bone morphology (Dr. Fowlkes). Training in these new areas of study and new methodologies is essential to my success as a muscle-bone interaction researcher in the field of diabetes. My mentors will support and foster my path to become an independent investigator. As training is completed and competency milestones are reached (see **Table 2**), I will develop my own independent research identity. While my mentors have expertise in the fields of *either* muscle or bone biology, I plan to develop a new area of research expertise in understating and investigating muscle-bone interactions in chronic disease states. During this career development award (year 3) I will apply for an R01 grant that will focus on pathways involved in muscle-bone interactions in chronic illness (like diabetes) with particular interest on skeletal muscle derived factors that negatively impact bone to increase fracture risk – which is now a well-recognized comorbidity of many chronic medical conditions. In doing so, I will not only be exploring novel pathways that will enhance our scientific knowledge, but I will also clearly differentiate my scientific future from that of my mentors.

Mentors and Collaborators and respective roles in training activities (Table 2)

John L Fowlkes, MD is a Professor of Pediatrics and Director of BBDC at UK. He is an NIH-funded and experienced investigator with expertise in DBD. As my Primary Mentor he will provide mentorship in study design, data analysis and interpretation of results related to DBD, as well as guidance in grantsmanship. His background in the field of bone and diabetes research will be essential to the success of this project and his mentorship in this area has already influenced my interest and productivity. Scientists in his laboratory and our collaborators (**Dr. Jeffry S. Nyman**, see letter of collaboration) have extensive experience in diabetic animal models and the necessary techniques required to study bone morphology and function. The mentored training plan that Dr. Fowlkes and I have developed will consist of direct laboratory instruction on the techniques required to evaluate bone quality, as well as direct laboratory instruction in cell culture, stimulation and subsequent gene expression analysis (qPCR). This laboratory instruction by Dr. Fowlkes and his trained lab personnel will assist me in analysis of data derived from the skeletal system in future studies and assist me with the laboratory skills required to delineate signaling pathways. Dr. Fowlkes and I will work closely and meet on a weekly basis to discuss the progress of the study and data analysis as well as the training progression.

Charlotte Peterson, PhD is Director of the Center for Muscle Biology and a Professor in the Department of Rehabilitation Sciences at UK. She is an internationally renowned, NIH-funded scientist with extensive expertise in extramural funding on muscle structure and function in both humans and animal models, and has served as a mentor for numerous successful scientists. As my Co-mentor, she will be involved in study design, data analysis and interpretation in proposal components that relate to skeletal muscle. Her laboratory is

focused on muscle adaptation, muscle regeneration and the effects of aging and chronic disease on muscle function. Her expertise on muscle physiology and cellular and molecular biology will assist me in designing future studies focused on the musculoskeletal system and her mentorship will assist me in achieving the goal of studying musculoskeletal disease in patients with diabetes. Dr. Peterson has extensively used immunohistochemical techniques and has worked with muscle atrophy and myostatin in previous studies. The mentored training plan that Dr. Peterson and I have developed will consist of direct laboratory instruction on the techniques of immunohistochemistry (IHC) (in the Muscle Immunohistochemistry and Molecular Imaging Core Laboratory-MIMIC) required to evaluate muscle morphology and structure (such as fiber typing), as well as techniques of measuring myostatin activity and using it for cell stimulation. This instruction will improve my laboratory skills and assist me in future processing and analyses of muscle tissues. In addition, Dr. Peterson will provide mentorship in laboratory and personnel management, grant writing, networking and strategies for success in academia. Dr. Peterson and I will meet monthly to discuss the progress of the study and the training milestones.

Philip Kern, MD is a well-established NIH-funded investigator, who is a Professor of Medicine and Director of the UK CCTS. He will serve as a Co-mentor in the clinical component of this proposal. Dr. Kern has been studying muscle and adipose tissue biology for many years and he has been engaged in both basic and clinical studies. The mentored training plan that Dr. Kern and I have developed will consist of instruction in human muscle biopsy, as well as development of clinical research skills. Dr. Kern has performed numerous muscle biopsies and he will directly supervise and instruct me with the goal of becoming proficient in harvesting human muscle tissue. Since the previous submission of this proposal, I have successfully completed 5 muscle biopsies in humans under Dr. Kern's supervision. This skill will be very useful in future clinical or translational studies. Additionally, his extensive experience in clinical and translational research will assist me in developing the technical and academic skills necessary to design and complete translational studies. These techniques will include best practices in recruitment, retention, data management and data safety monitoring. Dr. Kern has designed many pharmacologic interventional studies in obesity and metabolic syndrome and he will assist me in designing future studies and developing a clinical research team. Dr. Kern and I will meet monthly or on as needed basis to discuss the progress of training milestones during this award.

Thomas Hawke, PhD is an Associate Professor of Pathology and Molecular Medicine at McMaster University, and has served as a valuable collaborator for this project. His expertise in diabetic myopathy in patients with T1D has been very useful in helping me to generate preliminary data for this proposed project. This collaboration will assist me in furthering my knowledge in muscle physiology in T1D. During this award Dr. Hawke and I plan to meet by teleconference biannually to discuss the progress, as well as results from his studies in diabetic myopathy. Additionally, Dr. Hawke has offered to share resources, including the myostatin antibody that will be used for myostatin quantification in skeletal muscle (see his letter of collaboration).

We have developed a comprehensive training plan that includes hands on technical training (summarized above), as well as didactic course work (summarized below), presentations, training in grant writing and research ethics **(Table 1).** Completion of these career development activities will enable me to achieve my goals (**Table 2**) which will facilitate my transition to a successful physician scientist with an independently funded research program.

Didactic Training

Instruction in Muscle Physiology: I will attend the Muscle Forum Series weekly and plan to present my research annually in this setting. Both internal and external speakers in the muscle field share their research, present journal club or their proposals for critique at the Forum. This course will develop my critical evaluation skills through participation and contribution to research seminars in the field of muscle biology.

Instruction in Clinical and Translational Research: I have attended two courses offered by the Department of Behavioral Science ("Methods and Technologies in Clinical and Translational Science" and "Seminar in Clinical and Translational Science") towards the completion of the Certificate Program in Clinical and Translational Science offered at UK. During the first two years of this award I plan to attend additional courses *such as "Interdisciplinary Protocol Development"* (BSC 732) and "*Ethics in Scientific Research*" (TOX 600) that will provide me with scientific knowledge on designing and conducting clinical and translational research and will also count towards this certificate program. My goal is to complete the certificate within the next 2 years.

Grantsmanship: To enhance my grant and manuscript writing skills, I plan to attend Workshops and Seminars that are offered through the Proposal Development Office at UK that are related to grantsmanship (*"Basic Grantsmanship: A Framework for Success and Navigating the NIH Process"*).

Statistical Training: I will continue working closely with biostatistician, Aric Schadler (supported by the Department of Pediatrics), during this career development award period and for guidance with future project design. To improve my knowledge in biostatistics and data analysis skills, I will complete the *"Fundamentals of Biostatistics for Clinical and Translational Research"* course (BSC 625).

Professional Development/Networking: Additionally, during this award, I plan to attend national meetings such as the *American Diabetes Association (ADA)* and the *American Society for Bone and Mineral Research (ASBMR)* meetings and the *Musculoskeletal Biology Workshop* organized by the *Orthopaedic Research Society (ORS)* in Sun Valley, which is a scientific meeting focused on the field of skeletal biology. At these meetings, I plan to present my research to a diverse audience and network with junior and senior colleagues. Attendance and networking during these meetings will assist me in building collaborations and expanding my research interests, while gaining visibility in my area of expertise.

Table 1. Timeline of Career Development Activities

Training Activity	Year 1	Year 2	Year 3	Year 4	Year 5
Instruction in Musculoskeletal Techniques and Muscle Physiology					
Muscle forum (weekly)					
Techniques on bone cell culture, qPCR, animal models of diabetes					
Weekly sessions in MIMIC lab (IHC), skeletal muscle analysis					
Instruction in muscle biopsy (human), skills for clinical research					
Clinical and Translational Science Courses					
Interdisciplinary Protocol Development (BSC 732)					
Research in Medical Behavioral Science (BSC 790)					
Responsible Conduct of Research and Ethics					
Ethics in Scientific Research (TOX 600)					
Responsible Conduct of Research and CITI					
Grantsmanship Instruction					
Basic Grantsmanship: A Framework for Success and Navigating the NIH Process					
Biostatistics					
Fundamentals of Biostatistics for Clinical and Translational Research (BSC 625)					
Conference Attendance					
American Society for Bone and Mineral Research					
American Diabetes Association					
Musculoskeletal Biology Workshop					
NIDDK K Awardees' Workshop					
Manuscript and NIH Grant Submissions					
Manuscripts for Aims 1, 2, 3 and 4					
R01 submission and resubmission if needed					

Table 2. Training goals and milestones

Training Activity	Mentor and Aim	Training skills and competency milestones
Animal models of diabetes	Dr. Fowlkes, Aims 2,3	Gain expertise in multiple models of insulin-deficient diabetes (Akita new model to my training) and their bone phenotype
Bone cell culture techniques	Dr. Fowlkes, Aim 4	Become proficient in harvesting primary osteoblasts and master stimulation and gene expression techniques (qPCR)
Skeletal muscle analysis	Dr. Peterson, Aims 1, 2, 3	Gain expertise in immunohistochemistry techniques (fiber type staining, SDH staining etc.), and fibrosis quantification
Bone cell stimulation	Drs. Fowlkes and Peterson, Aim 4	Become proficient in cell stimulation with myostatin and in determination of myostatin activity
Muscle biopsy technique	Dr. Kern, Aim 1	Exhibit independence in harvesting human skeletal muscle
Clinical research training	Dr. Kern, Aim 1	Become familiar with strategies for subject recruitment and retention, data management and data safety monitoring

SPECIFIC AIMS

A serious complication of Type 1 Diabetes (T1D) is diabetic bone disease (DBD), manifesting as increased risk for fracture throughout life (1). The prevalence of T1D in those younger than 20 years of age has risen significantly (2); therefore, prevention of and treatment for diabetic complications is of high importance to improve clinical outcomes and complication-associated costs. Population based studies reveal T1D is among the top 10 factors associated with the highest risk of fracture (3). Large prospective clinical studies have demonstrated that T1D is associated with an increased risk of hip and upper extremity fracture, with relative risks ranging from 5.81 (4) to 12.25 (5). The Nurses' Health Study has confirmed these findings for incident hip fracture in T1D (RR = 6.4) (6). Those with T1D also exhibit impaired skeletal muscle function characterized by lower muscle mass, weakness and overall reduced physical capacity (7). We hypothesize that skeletal muscle is an important mediator of DBD.

In addition to mechanical interactions between muscle and bone, paracrine and endocrine signals contribute to muscle-bone interactions; muscle-derived trophic factors, collectively termed myokines, have recently been shown to impact bone (8, 9). However, the role of myokines in contributing to DBD is largely unexplored. Myostatin (GDF-8), a myokine secreted by skeletal muscle, negatively regulates muscle mass. There is limited information to suggest that myostatin may have a negative impact on bone. Specifically, it negatively affects differentiation and function of bone marrow stem cells in vitro (10), osteocyte function in vitro (11) and fracture healing in vivo (12). Myostatin is elevated in muscle in mouse models of T1D (i.e., insulin-deficient diabetes) (13-16); however, it is unknown if myostatin is dysregulated in humans with T1D. Furthermore, how myostatin specifically, or myokines in general, may be involved in mediating muscle-bone interactions has not been explored in mouse models or in humans with T1D. Our preliminary data are the first to indicate that systemic concentrations of myostatin are higher in individuals with T1D compared to those without T1D, supporting the idea that targeting muscle-derived myokines, such as myostatin, could prove beneficial for the prevention or treatment of DBD in T1D.

The overall hypothesis of the proposed research is that myostatin negatively affects bone in T1D, contributing to DBD, particularly through its effects on osteoblasts in a diabetogenic environment. We propose the novel hypothesis that myostatin contributes to DBD in humans and mice with T1D through uncoupling of bone turnover and decrease in bone mass (Aims 1 and 2). We further hypothesize that inhibition of myostatin will result in prevention or improvement in the diabetic bone phenotype in animal models of T1D known to exhibit DBD (Aim 3). Finally, we propose that myostatin exerts negative, direct effects on bone forming cells through the activin receptor 2b (AcvR2b), which are amplified by a diabetic environment (Aim 4).

Aim 1: Quantify the relationship between myostatin levels in serum and skeletal muscle, and bone parameters of humans with and without T1D. On young adults with and without T1D, we will obtain serum samples and muscle biopsies and perform bone imaging (Dual X-ray absorptiometry (DXA) and trabecular bone score (TBS)). We will measure myostatin in the serum and myostatin gene expression and protein content in skeletal muscle and relate these levels to bone mineral density and to serum bone turnover markers.

Aim 2: Quantify the relationship between myostatin levels in serum and muscle and the bone phenotype of mice with insulin-deficient diabetes. We will use two well characterized animal models of insulin-deficient diabetes (T1D) and evaluate myostatin in serum and muscle as it relates to bone parameters (micro (μ)CT and bone formation and resorption markers) and skeletal muscle parameters to discern the relationship between myostatin and bone and muscle phenotype of mice with T1D.

Aim 3: Evaluate whether inhibition of myostatin is beneficial for the prevention of DBD in insulin-deficient diabetes. We will evaluate if pharmacologic myostatin inhibition with an inhibitory myostatin antibody improves the bone and skeletal muscle phenotype of T1D mice known to exhibit DBD.

Aim 4: Determine the mechanism of action of myostatin on osteoblastic bone cells under normoglycemic and hyperglycemic conditions. We will assess if myostatin has direct effects on osteoblasts by evaluating differentiation and mineralization of a murine osteoblast cell line (MC3T3) and of primary osteoblasts in a diabetes-like environment. Specifically, we will determine how signaling pathways (e.g., Smad, Akt/mTOR and MAPK/ERK) and genes involved in osteoblast differentiation (Runx2, Osx, osteocalcin, etc.) are affected by myostatin in the presence of hyperglycemia. Additionally, we will evaluate whether those effects are blocked in the presence of inhibitory antibodies against myostatin or its receptor, AcvR2b.

Clinical Significance. *Results from this study will contribute to our knowledge about the role of myostatin and its associated pathways in muscle-bone interactions in T1D and may lead to pharmacologic interventions to prevent and/or treat musculoskeletal complications in T1D. Additionally, they will provide the foundation for future studies evaluating the effects of the skeletal muscle secretome on bone in T1D.*

SIGNIFICANCE

The prevalence of T1D in those younger than 20 years of age has risen significantly (2), therefore, prevention and therapy of diabetic complications is of high importance to improve clinical outcomes and complication-associated costs. Diabetic bone disease (DBD), considered now a serious complication associated with T1D, is characterized by a marked increase in risk of fracture. Specifically, women have a fourfold increase in risk for fracture, whereas men have a twofold increase compared to the general population (17) and this risk is evident soon after diagnosis of T1D (1). The overall fracture incidence is evident throughout the lifespan and as early as childhood (1). Epidemiologic studies have shown that T1D is among the top 10 factors associated with the highest risk of fracture (3).

Bone fragility in T1D is associated with decreased bone mass and mineral density (18, 19), low bone turnover (20), abnormal bone microarchitecture and bone quality (21, 22). Although several factors likely contribute to DBD in T1D, including poor glycemic control, microvascular complications and lack of endogenous insulin and insulin-like growth factor 1 (IGF-1) production (22), patients with T1D continue to exhibit fractures, despite improvements in glycemic control and advancements in insulin therapy. Furthermore, despite the well-established risk of fracture associated with T1D, there are no clinical trials to evaluate anti-fracture therapy in patients with T1D (23), in large part due to the lack of information about underlying mechanisms and potential therapeutic targets. Therefore, currently there is no prevention or treatment of DBD in humans with T1D (24, 25), although certain bone anabolic agents have shown some promise in animal models of T1D (24).

In addition to DBD, T1D is associated with deficits in skeletal muscle mass and function (7, 26). Muscle mass and bone mass are closely related during development and growth, and muscle and bone interact through mechanical, endocrine and paracrine factors in physiologic and disease states (9). Myokines, secreted by skeletal muscle, contribute to muscle-bone communication and have even been associated with direct effects on bone and bone cells (8). However, there is a significant gap in knowledge regarding muscle-bone interactions mediated through myokines in T1D and their potential to contribute to DBD.

One myokine, myostatin, or Growth and Differentiation Factor 8 (GDF-8), a member of the TGF-β family that is primarily expressed by skeletal muscle cells, is a known negative regulator of muscle mass (8). Myostatin primarily binds to and signals through the Activin Receptor 2b (AcvR2b). Some studies have shown direct negative effects of myostatin on proliferation of bone marrow stromal cells (10) and primary osteoblasts (12). Other studies have demonstrated an indirect negative effect of myostatin on osteoblast differentiation (11) and a negative effect on bone fracture healing (27, 28). Often, animal studies that report on the effects of this pathway on bone use follistatin or target the Acvr2b (29-31), potentially inhibiting the action of other AcvR2b ligands besides myostatin (such as activin or GDF-11). Therefore, it can be difficult to differentiate whether the reported effects seen on bone with these studies are due to myostatin inhibition alone. Additionally, there is evidence to support that a ligand of the AcvR2b other than myostatin regulates bone mass (32) indicating that multiple molecules that bind the AcvR2b could have effects on bone. Finally, a study utilizing myostatin propeptide as a myostatin inhibitor did not result in improvements in bone of aged mice (33), raising some concerns about the role of myostatin in bone homeostasis. Only a handful of studies that utilize either a specific antibody against myostatin (12) or genetic deletion of myostatin (34-36) have demonstrated effects on bone. None of these studies have been done on animal models involving diabetes.

Recent studies have implicated myostatin signaling in diabetic skeletal muscle dysfunction (14); however, it is unknown if muscle-derived myostatin may affect bone in T1D. Due to its potential negative effects on osteoblasts (10, 12), we hypothesize that myostatin, which is elevated in insulin-deficient rodent models of T1D (13, 14) may play a critical role in the development and progression of DBD. In non-diabetic models of aging where myostatin is also believed to be elevated (10), myostatin inhibition has ameliorated or prevented myostatin-mediated negative effects on muscle (37) and bone tissue (12). Thus, specific inhibition of myostatin might offer an opportunity for pharmacologic intervention to prevent both DBD and diabetic skeletal muscle dysfunction. We will investigate myostatin function on diabetic muscle and bone in mouse models of T1D and evaluate whether myostatin inhibition will result in improvements in the musculoskeletal phenotype of T1D.

In addition to delineating the role of myostatin in the origins of DBD, our findings will expand what is the current knowledge regarding its role on bone cell growth and differentiation. There are only limited studies describing the effects of myostatin on bone cells. A few descriptive studies support a direct action of myostatin on osteoblast differentiation and mineralization (10, 12); whereas, other studies suggest indirect effects of myostatin on osteoblasts through osteocyte-derived exosome miRNA (11). Furthermore, we plan to investigate whether myostatin has direct effects on Akt/mTOR and MAPK/ERK pathways, which mediate insulin and IGF-1 anabolic signaling in osteoblasts. Our studies will unequivocally establish if myostatin has direct effects on

osteogenic cells, and if these effects are modulated in hyperglycemic and/or hyperosmotic environments, as would be expected in T1D. These studies will provide essential knowledge about the interplay between diabetes and myostatin specifically on bone cell health.

INNOVATION

To our knowledge, this is one of the first studies that has proposed that a muscle-bone interaction is involved in development of DBD in humans, providing a new thought paradigm to explain what tissue-to-tissue cross-talk mechanisms may be involved in the evolution of DBD. Specifically, these studies will be the first to explore the relationships between myostatin (in the circulation and at the muscle level) and skeletal outcomes (by Dual X-ray absorptiometry (DXA)) in humans with T1D. While other animal studies have used exercise or follistatin to inhibit myostatin activity in diabetes, our study is the first translational study to utilize specific pharmacologic myostatin inhibition in animal models of T1D aiming to prevent DBD and provide proof-of-concept information essential for consideration for its use in future human studies. Furthermore, we will be the first to investigate whether myostatin has effects on insulin signaling in osteoblasts and whether it interferes with its anabolic effects on these cells. While our proposed intervention is specifically targeted towards myostatin, we will in future studies be capable of exploring if other myokines or myokine receptors are also involved in muscle-bone interactions in T1D. With expertise from the Center of Muscle Biology and the Barnstable Brown Diabetes Center (BBDC) in muscle function and DBD, respectively, this composite of basic, translational and human studies will provide a unique opportunity to gain critical new knowledge necessary to proceed with future research proposals focused on treatment of musculoskeletal disease in humans. These findings will function as the basis for future translational and clinical studies, focusing on the role of muscle-derived molecules and their effects on bone, and may provide essential and novel information to advance future guidelines for screening, preventing and treating musculoskeletal disease.

APPROACH

Aim 1: Quantify the relationship between myostatin concentrations in serum and within skeletal muscle, and bone parameters of human subjects with and without T1D.

Rationale and preliminary data: DBD ultimately manifests as fragility fractures. The Fracture Risk Assessment Tool (FRAX), bone mineral density (BMD) and the trabecular bone score (TBS) – all derived from DXA assessment - are clinical tools commonly used to predict bone fragility and fracture risk. Nevertheless, these tools underestimate the overall risk for fracture in diabetes (23). Therefore, adding additional indicators or biomarkers to a fracture risk-assessment model may be useful in describing and predicting DBD and fracture risk in T1D. These studies will provide new information about myostatin and how it relates to skeletal phenotypes in individuals with and without diabetes. These studies will lay the groundwork to understand if myostatin serves as a clinically useful biomarker in assessing DBD and fracture risk in T1D.

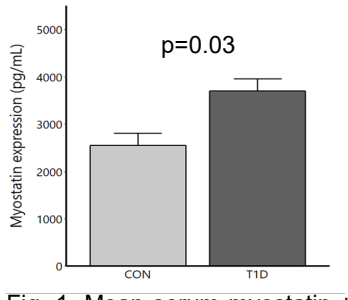


Fig. 1. Mean serum myostatin ± SE measured by ELISA in controls (n= 15) and individuals with T1D (n= 29).

A few studies suggest that myostatin may be involved in regulation of bone mass and bone formation in animal models (12, 34). However, very few studies have considered a potential role for myostatin in bone physiology in humans. One correlative study showed that myostatin was negatively associated with bone mineral density in humans (38). One clinical trial showed that myostatin inhibition in healthy volunteers was associated with improvement in bone turnover (39). Although myostatin has been shown to be elevated in animal models of insulin deficiency and T1D, to our knowledge, there are no published studies evaluating serum levels and skeletal muscle gene and protein expression of myostatin in patients with T1D and how these levels may relate to critical bone parameters that are predictive of DBD (e.g., bone mineral density and TBS). In collaboration with Dr. Thomas Hawke (see letter of collaboration), serum myostatin was measured by ELISA in young adults with T1D and in control individuals (Fig. 1). In these young (mean age 24 vs 26 years for CON and T1D respectively), primarily female individuals (CON = 4 male, 11 female, T1D = 4 male, 25 female) mean myostatin serum concentrations were significantly higher in the presence of T1D (2551± 998- T1D vs 3702 ± 1365- control, p=0.03). We hypothesize that myostatin levels will be high in serum and skeletal muscle of humans with T1D, associated with deficits in bone mineral density and decreased bone formation, both of which are characteristics of DBD (22). Further, we hypothesize that myostatin will be associated with muscle atrophy and decreased muscle function in T1D. In Aim 1 we will quantify the relationship between myostatin and bone parameters, and muscle fiber size in those with T1D compared to healthy aged-matched controls. If myostatin is upregulated in humans with T1D, it may be a potential mechanism for low bone mineral density

and increased risk of fracture observed in T1D. Such associations may introduce myostatin as a useful surrogate to assess DBD risk. Furthermore, relational studies could support the consideration of inhibiting myostatin in T1D to improve overall musculoskeletal health.

Experimental design

Study overview, study sites and recruitment, study participants: We will recruit 35 subjects with and 35 subjects without T1D who are otherwise healthy between the ages of 18-45 years (matched for age, sex and BMI). Baseline labs and consent will be obtained at first visit at the Barnstable Brown Diabetes Center (BBDC) research unit. The following procedures will be performed during a second visit at the University of Kentucky Center for Clinical and Translational Science (CCTS) outpatient unit: a. muscle biopsy to evaluate mRNA and protein expression of myostatin and its primary receptor, AcvR2b, and fiber size and fiber type composition by immunohistochemistry (IHC); b. DXA scan to evaluate BMD and body composition; and c. peripheral blood collection for serum myostatin levels, and serum markers of bone formation (amino-terminal propeptide of type I procollagen-P1NP) and resorption (C-terminal telopeptide-CTX). Additionally, urine collection will be performed to exclude pregnancy in women and ketosis in all subjects. Participants will be asked to complete a physical activity questionnaire at their second visit. Subjects will be recruited primarily through the BBDC or online through the UK clinical research websites and registries. Obese individuals will be excluded due to myostatin levels being affected by obesity. Exclusion criteria will include HbA1c ≥12%, any unstable medical condition, use of oral hypoglycemic agents, diabetes complications (including diabetic neuropathy), recent use of anticoagulants, obesity (BMI>30), pregnancy, evidence of ketosis, co-existing disorders that can affect bone (celiac disease, corticosteroid use etc.) and vigorous exercise prior to the procedures.

Procedures and Methods

Muscle biopsy: Muscle will be obtained under local anesthesia from the anterior thigh (vastus lateralis) of the subject, under sterile conditions using a Bergstrom needle. Dr. Kalaitzoglou is being trained and credentialed in this method by Dr. Philip Kern, who has been performing muscle biopsies routinely for research for more than 25 years. Approximately 200 mg of muscle tissue is obtained with half mounted on a cork in tragacanth gum and frozen in liquid nitrogen-cooled isopentane for immunohistochemical (IHC) analyses. The other half is snap frozen directly in liquid nitrogen for RNA and protein; all are stored at -80^{o}C until analysis.

Muscle gene expression analysis by qRT-PCR and protein quantification: Skeletal muscle will be processed for RNA (Zymo-Spin Column) and protein isolation. Genes of interest including myostatin, AcvR2b, irisin and other myokines (Il-6 and IGF-1) will be measured using a Skeletal Muscle Myogenesis and Myopathy array (RT^{2} Profiler™ PCR Arrays, SABiosciences). Protein expression of active (C-dimer) myostatin and myostatin propeptide by Western blot [using a custom made myostatin antibody provided by our collaborator, Dr. Hawke (see letter of collaboration)] and AcvR2b (Abcam, ab 76940) will be determined.

Muscle IHC: Fiber type distribution will be determined using a battery of mouse monoclonal primary antibodies directed against myosin heavy chain (MyHC) isoforms (40). All three MyHC antibodies (type I, IIA, and IIX) are of different isotypes, allowing primary antibodies to be added to sections simultaneously, followed by isotype specific secondary antibodies conjugated to different fluorescent tags. Additionally, a rabbit polyclonal antibody directed against laminin will identify muscle fiber borders. Digital images of entire biopsy cross-sections will be analyzed for fiber type composition and fiber type-specific fiber size using our automated image quantification platform, MyoVision (41). Fibrosis will also be quantified as described in detail (42).

DXA scan: At the time of muscle biopsy, DXA will be performed to measure BMD of the spine (L1-L4) and hip compartments (T-score and Z-scores) and trabecular bone score (TBS) of the spine. Body composition from total body DXA will provide mineral free lean and fat-free mass measurements in different regions of the body. Further analysis for skeletal muscle index (SMI) will also be performed by DXA (43).

Blood collection: Serum will be collected, processed, and subsequently stored at --80^{o}C. Baseline labs at screening (visit 1) will include: hemogram (CBC), Thyroid Stimulating Hormone (TSH), glycated hemoglobin (HbA1c) and comprehensive metabolic panel (CMP). Samples obtained during visit 2 will be analyzed for myostatin (Myostatin Quantikine ELISA Kit, R&D Systems), bone formation marker P1NP and bone resorption marker CTX levels with ELISA.

Power calculation and statistical analysis: Where necessary for parametric assumptions, appropriate transformations will be employed with justification for their use. Significance will be determined at the 5% level. As there is no published data on myostatin levels in human subjects with T1D, based on the difference observed in serum myostatin between healthy controls and subjects with T1D in our preliminary data, a sample size of 11 subjects per group would be required to detect significant differences between groups with alpha=0.05 and power=0.8, using a matched pairs analysis. Studies evaluating men and women with T1D of

similar ages to those being studied report an effect size ~0.5 (44, 45), which would require 33-34 subjects per group to establish significance differences between groups with alpha=0.05 and power=0.8. We will include 35 subjects per group (matched for age, sex and BMI). Bivariate analyses will include *t*-tests to test for significant differences of the means of parameters of interest between groups, and Pearson's correlation will be used to test whether there are significant linear associations between continuous parameters of interest. We will define a dichotomous fracture risk variable based on bone properties derived from DXA and bone markers and using a multivariate logistic regression model we will evaluate whether variables, such as serum and muscle-derived myostatin can predict fracture risk in the controls or subjects with T1D.

Expected outcomes, pitfalls and alternative approaches: We anticipate that serum myostatin levels will be elevated in those with T1D compared to healthy controls. In addition, we anticipate increased gene expression of myostatin in muscle tissue from subjects with T1D compared to controls. Although we expect elevated myostatin in muscle to be associated with smaller fiber size, we predict that muscle size will not necessarily be correlated to BMD, suggesting that muscle-bone interaction extends beyond mechanical factors. As myostatin is associated with fibrosis in muscle, this will be quantified (46). We anticipate that circulating and muscle myostatin levels will relate to each other. We anticipate P1NP to be decreased, CTX to be elevated, and BMD to be lower in T1D subjects compared to healthy controls. We expect that these parameters will negatively correlate with myostatin measures. In the event that myostatin is not associated with bone and muscle parameters, we will proceed with quantification of other markers in serum and muscle that act on the AcvR2b, such as activin A and GDF-11, and other myokines such as irisin (47), and evaluate their relationship to muscle and bone parameters.

Aim 2. Quantify the relationship between myostatin levels in serum and muscle and bone phenotype of mice with insulin-deficient diabetes (T1D).

Rationale and preliminary data: We and others have shown that in the streptozotocin (STZ)-induced diabetic mouse (an animal model of T1D), many features of DBD occur, including decreased BMD, decreased cortical bone area (Fig. 2A&C), and thinner trabecular bone (Fig. 2B&D) compared to non-diabetic mice (48). Additionally, in serum, bone formation markers (procollagen type 1 N propeptide (P1NP)) are significantly decreased and bone resorption markers (C-terminal telopeptide of type 1 collagen (RatLaps) are significantly increased (48). This indicates an uncoupling of bone metabolism in insulin-deficient diabetes, with decreased bone formation. Ultimately, these structural changes in conjunction with poor bone formation in this model result in weaker bones that fracture easily, similar to human T1D-related DBD. Apart from the STZ-induced diabetic mouse, the Akita mouse model also exhibits features of DBD (49), however there is a gap in knowledge regarding how myostatin levels relate to DBD and muscle properties in these two animal models of T1D.

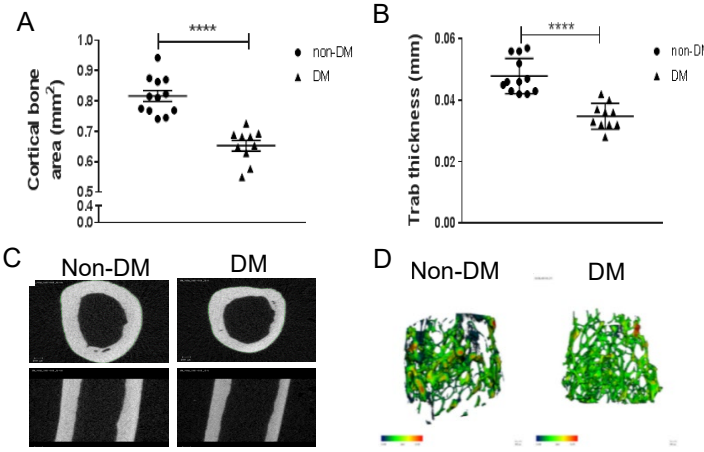


Fig. 2. Micro CT analysis showing decrease in cortical bone area (A, C) and trabecular thickness (B, D) with diabetes (DM), compared to non-diabetes (non-DM). Data in A and B presented as scattered plot with mean ± SEM, **** p<0.0001.

As myostatin is elevated in multiple studies of STZ-induced diabetes in mice (13, 14, 16) and these mice exhibit DBD and muscle dysfunction, we hypothesize that there is a negative association between myostatin levels in the serum and skeletal muscle and, bone mineral volume, bone quality and bone formation markers as well as muscle mass and altered metabolic activity in mouse models of insulin-deficient diabetes (T1D). Because STZ may have some direct, toxic effects on muscle, we will validate our hypothesis in a second mouse model of T1D (the Akita mouse), wherein STZ exposure is not a possible concern. Only male Akita mice will be used, as their diabetic phenotype is more penetrant compared to females.

Experimental design

STZ-induction of diabetes: 10 week-old DBA/2J mice will be treated with either vehicle (n=20; 10 male, 10 female; non-diabetes group) or STZ, 40 mg/kg of STZ x 5 days, i.p. (n=20; 10 male, 10 female; STZ-induced diabetes group), according to previously published studies from our lab (48, 50). After induction of diabetes is confirmed (Blood glucose >300, 7-14 days post-STZ injections) mice will be followed for 8 weeks and then euthanized to quantify myostatin levels in serum and skeletal muscle, and bone turnover markers in serum.

Bone parameters associated with bone microarchitecture and bone quality with micro-computed tomography and biomechanical testing and skeletal muscle parameters will be assessed (see procedures and methods).
Akita mouse model: Male C57BL/6-Ins2Akita/J mice will be monitored for development of diabetes at 4 weeks of age (n=10). Male WT littermates will be used as controls (n=10). After confirmation of diabetes (4-6 weeks of life), mice will be followed for 8-10 weeks and subsequently euthanized for similar analysis as for mice with STZ-induced diabetes to characterize the relationship between myostatin, skeletal muscle composition and mitochondrial function as well as bone parameters and bone strength (see procedures and methods).

Procedures and methods
Weight, blood glucose, skeletal muscle, bone and systemic serum markers: Animal weights and blood glucose will be assessed weekly. At termination, body weight will be measured, and blood will be collected and separated for plasma and serum, and stored at -80°C. Tibiae, vertebrae and femurs will be dissected from soft tissues. Gastrocnemius, soleus, plantaris and tibialis anterior (right and left) will be dissected and weighed. Serum or plasma will be assayed for serum glucose, glycated hemoglobin, myostatin (ELISA kit, R&D), bone formation markers (PINP), and bone resorption markers (RatLAPS) as previously described (51).
Muscle IHC: The gastrocnemius, soleus, plantaris and tibialis anterior hind limb muscles will be mounted with OCT, frozen using liquid nitrogen-cooled isopentane, and subsequently sectioned using a cryostat (7 μm) as previously published (52). Sections will be stained for assessment and quantification of fiber type, fiber type-specific cross sectional area, and other muscle morphological features as described in Aim 1, except that a MyHC type IIB antibody will be included.
Micro-computed tomography analysis and bone biomechanical testing: The three-dimensional microarchitecture of intact femurs and L6 vertebrate will be evaluated by high-resolution micro-computed tomography (μCT50, Scanco Medical AG, Bassersdorf, Switzerland) as we have previously described (51, 53). Cortical strength of the femur will be measured using 3-point bending analyses as described previously (51, 53). If indicated based on initial findings, bone histomorphometry will also be performed.
Gene expression analysis: RNA will be isolated and quantified as previously described for bone (54) and muscle (55). Genes of interest (myostatin, AcvrR2b), and genes critical in osteoblastogenesis (e.g., RUNX2, Osterix, osteocalcin, etc.), or myogenesis (e.g., myogenic differentiation 1, myogenin, etc.) will be assessed by qRT-PCR with Osteogenesis and Skeletal Muscle Myogenesis and Myopathy arrays (RT² Profiler™ PCR Arrays, SABiosciences), respectively.
Protein quantification in skeletal muscle: Skeletal muscle will be processed for western blot analysis and evaluated for protein expression as described in Aim 1.

Power calculations and statistical analysis: Where necessary for parametric assumptions, appropriate transformations will be employed. Significance will be determined at the 5% level. Based on previous power analyses in insulin-deficient diabetic animal models (48, 49) or animal models with myostatin inhibition (56-58) detecting differences in muscle and/or bone parameters requires no less than 10-12 mice per group providing an expected minimum of 8 survivors per treatment. It is possible that measurements will be unavailable and/or mice will die. Wherever possible we will include them in the analysis by either using methods which allow missing data or by the use of multiple imputations for items or for dropout due to death. A one-way or two-way analysis of variance (ANOVA) will test whether the means of parameters of interest are equal between groups. For each parameter, a post hoc comparison using a Bonferroni correction will be used. Pearson's correlation will test whether there are significant linear associations between parameters of interest, such as bone or skeletal muscle phenotype and myostatin and multiple regression analysis will evaluate the relationship between muscle, bone parameters and myostatin.

Expected outcomes, pitfalls and alternative approaches: We anticipate that mice with untreated T1D (STZ-induced diabetes and Akita) will have impaired bone structure and strength, and that this phenotype will relate to serum and skeletal muscle myostatin levels. We also anticipate that diabetic mice will have suppressed RUNX2, Osx and osteocalcin gene expression in bone, while at the same time they will exhibit upregulation of myostatin expression in their muscle and serum. Furthermore, these mice will be informative as to how myostatin functions to regulate myogenic genes in the context of T1D (13, 14, 16). While we anticipate that myostatin will be correlated to bone and muscle parameters in our diabetic mouse models, in the event that it is not, we will assess other ligands that act through the AcvR2b and AcvR2a receptors (e.g., GDF 11, activin A) and AcvR2a levels. GDF11 and activin A have both been implicated in dysfunctional bone metabolism (59, 60), and therefore, may contribute to the observed diabetic bone phenotype.

Aim 3. Evaluate whether inhibition of myostatin is beneficial for the prevention of DBD in insulin-deficient diabetes.

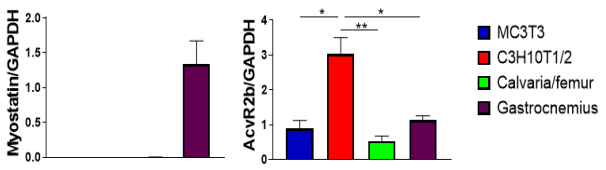


Fig. 3. Myostatin and AcvR2b transcription in bone and skeletal muscle. Myostatin and AcvR2b mRNA were quantified in murine pre-osteoblast cell line (MC3T3), murine fibroblast cell line (C3H10T1/2), murine bone tissue (calvaria/femur) and murine skeletal muscle (gastrocnemius). Data reported as average concentration normalized to GAPDH ± SE. * p <0.05 and ** p <0.01.

Rationale and preliminary data: Myostatin is exclusively produced in skeletal muscle. Our preliminary data show that murine pre-osteoblast cell line (MC3T3), murine fibroblast cell line (C3H10T1/2), as well as bone from mice (calvaria and femur) *do not* express myostatin mRNA (Fig. 3A), supporting that myostatin is not produced by bone cells. However, mRNA of the AcvR2b receptor is present in bone cells and in whole bone (Fig. 3B). In contrast to bone, both myostatin and AcvR2b mRNA are present in skeletal muscle from mice (gastrocnemius; Fig. 3A& B). This suggests that while bone forming cells do not produce myostatin, they are likely responsive to myostatin-mediated signaling events. Furthermore, myostatin downregulation or deletion is beneficial for bone in animal models of several disease processes (12, 28, 35). Taking into consideration that conventional insulin therapy does not completely protect bone (48), there is a need to develop further therapeutic strategies specific to bone in T1D. Specifically, given that myostatin is elevated in STZ-induced diabetes (13, 14) and its downregulation is potentially beneficial for bone, pharmacologic myostatin inhibition could prevent DBD and skeletal dysfunction associated with animal models of T1D.

Experimental design

We will determine whether pharmacologic inhibition of myostatin is beneficial for the bone and skeletal muscle phenotype in (STZ)-induced diabetic mice. 10 week-old DBA/2J mice will be treated with vehicle (n=40; 20 male, 20 female; non-diabetes group) or STZ i.p. (n=80; 40 male, 40 female; STZ-induced diabetes group) as in Aim 2. After induction of diabetes (see Aim 2), 50% of diabetic mice will receive insulin treatment with LinBit – sustained release insulin implants as per manufacturer recommendations (LinShinCanada, Inc) with replacement as needed for the duration of the study (8 weeks). The remaining mice will receive control implants without insulin (LinShinCanada, Inc). In addition, 50% of non-diabetic mice and 50% of diabetic mice (half treated with insulin and half with control implants) will receive weekly i.p. injections of 10 mg/kg of a myostatin monoclonal blocking antibody (REGN647, Regeneron) or an isotype control antibody similarly to previously published studies (57) for 8 weeks. We have chosen this interval of treatment based on previous publications showing quantifiable changes in bone with Type 1 diabetes over 8 weeks (48, 50). The myostatin antibody will be given once mice are confirmed to have diabetes to preserve bone and prevent the bone phenotype of T1D. Mice will be euthanized and tissues will be collected for further analysis as in Aim 2.

Procedures and methods

All procedure and methods for *1. Weight, blood glucose, skeletal muscle, bone and systemic serum markers, 2. Muscle IHC, 3. Micro-computed tomography analysis and bone biomechanical testing, 4. Gene expression analysis and 5. Protein quantification* are identical to those described in Aim 2.

Glucose tolerance test: Mice will be weighed and then fasted for 4-5 hours with free access to water. Fasting BG will be measured via glucometer. For glucose tolerance testing (GTT), 20% glucose in sterile saline will be injected at 1.5-2 mg glucose per gram body weight and BG measurements will be obtained at 0, 15, 30, 45, 60, 90 and 120 minutes following glucose injection, as previously described (61).

Body composition analysis by EchoMRI: Conscious mice will be individually restrained in a clear cylindrical plastic tube (sized by animal weight). The tubes have holes for breathing and are maintained in the horizontal plane during the procedure. Three sequential scans will be conducted (approximately 2 minute/scan). This procedure will precisely measure whole-body composition parameters such as total body fat, lean mass, body fluids, and total body water in live mice.

Power calculations and statistical analysis: Power calculation, statistical analysis and post hoc comparisons, analysis of missing data and significance level will follow statistical methods as described in Aim 2.

Expected outcomes, pitfalls and alternative approaches: We expect that treatment of diabetic mice with the myostatin blocking antibody will prevent the diabetic bone and muscle phenotype. We will discern if the improvements observed with insulin alone may be mediated by down-regulation of myostatin expression and production in skeletal muscle. If insulin and myostatin work through different mechanisms to improve diabetic bone, we anticipate an additive, positive effect of insulin and myostatin blocking antibody towards improvement of DBD in diabetic mice. We also anticipate that diabetic mice will have suppressed RUNX2, Osx and

osteocalcin gene expression in bone, amongst other osteoblastic genes, while at the same time they will exhibit upregulation of myostatin expression in their muscles and serum. However, we don't anticipate these changes with myostatin inhibition, suggesting that elimination or inhibition of myostatin is important in regulating osteogenic gene pathways. Through this approach of combining the STZ-induced diabetic mouse model and the novel use of specific myostatin inhibition as a modifier of muscle mass and bone structure and strength, we anticipate that inhibition of myostatin will be sufficient to improve DBD and skeletal muscle. An alternative approach to the myostatin inhibitor would be to proceed with STZ-induction of diabetes on a genetic myostatin deficient model. Mstn-/- mice are available to us through the same material transfer agreement we have with Regeneron. With this approach we would anticipate that diabetic Mstn -/- mice will have less severe DBD compared to WT diabetic mice; however, these findings may be confounded due to the significant muscle hypertrophy, a major feature of the Mstn -/- mouse.

Additionally, in order to ascertain whether myostatin alone or another ligand of the AcvR2b is responsible for the observed diabetic bone and muscle phenotype, we could use AcvR2b inhibitors (through our Regeneron collaboration) alongside the specific inhibitory myostatin antibody and compare their efficacy. In this proposal, we hypothesize that specific myostatin inhibition will block any of its activities mediated through AcvR2b on bone; however, myostatin inhibition will not be tissue specific and therefore, can inhibit signaling through the AcvR2b in muscle, which might lead to muscle hypertrophy. An alternative approach that we could undertake to solve this issue is to genetically silence AcvR2b expression in skeletal muscle cells, avoiding the possibility that myostatin was indirectly affecting bone through its influence on skeletal muscle. Our labs have successfully knocked-down receptors specifically in osteoprogenitor cells and skeletal muscle cells using inducible Cre-recombinase technology (61, 62), so such studies using tissue-specific ablation of the AcvR2b are well within the expertise of our labs if justified by our results from studies described above.

Aim 4: Determine the mechanism of action of myostatin on osteoblastic bone cells under normoglycemic and hyperglycemic conditions.

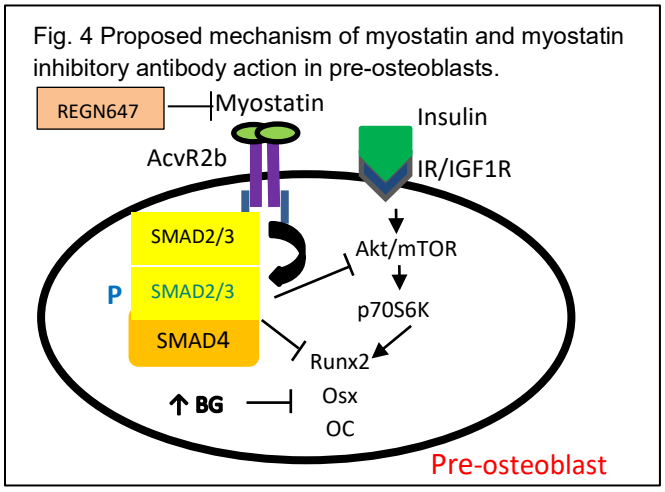


Fig. 4 Proposed mechanism of myostatin and myostatin inhibitory antibody action in pre-osteoblasts.

Rationale and Preliminary data: With these experiments we will: a. assess if myostatin has direct effects on osteoblasts by measuring osteoblast differentiation, mineralization, and osteogenic gene expression, and how these are affected by hyperglycemia, b. explore if and how myostatin alters pathways that mediate insulin signaling in osteoblasts (Akt/mTOR and MAPK/ERK) and finally, c. attempt to inhibit myostatin effects in osteoblasts using myostatin inhibitory antibody in vitro (Fig. 4). We have shown that bone cells express AcvR2b (Fig. 3), so myostatin-mediated signaling occurs in osteoblasts. Furthermore, myostatin signaling events involve the Smad2/3 intracellular pathway. In preliminary data, we also demonstrated that Smad2 phosphorylation in MC3T3 results from myostatin stimulation (Fig. 5), providing clear evidence that myostatin exerts direct effects on bone cells via the AcvR2b/Smad2/3 signaling pathway. The C2C12 myoblast cell line in Fig. 5 serves as a positive control. Because RUNX2, a "master regulator" of osteogenesis, is down-regulated by Smad2/3 activation (59), we explored if myostatin impacted RUNX2 expression in MC3T3 pre-osteoblasts. Under normal and hyperglycemic conditions (Fig. 6) these studies show that RUNX2 is downregulated by myostatin in pre-osteoblasts. Furthermore, hyperosmolar (i.e., mannitol) and hyperglycemic (dextrose) conditions are associated with even lower levels of transcription of RUNX2 and osterix, another important transcription factor of osteoblast development, when cells are treated with myostatin. Based on these preliminary data we propose that myostatin exerts negative effects on osteoblastogenesis through downregulation of essential genes for osteoblast differentiation and activity normally, but more so under hyperglycemic conditions (Fig 4).

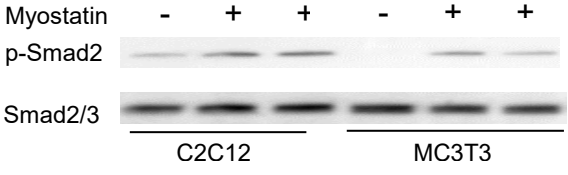


Fig 5. Myostatin stimulation induces phosphorylation of Smad2 in MC3T3 cells. C2C12 (murine myoblast cell line) and MC3T3 cells were stimulated with myostatin 500 ng/ml or vehicle for 45 minutes. They were collected and analyzed with western blot for phospho-Smad 2 and total Smad2/3.

Experimental design

Cell culture and differentiation, RT-PCR and Western Blot: Primary osteoblasts from WT mice will be isolated and cultured as previously described (63). MC3T3 cells

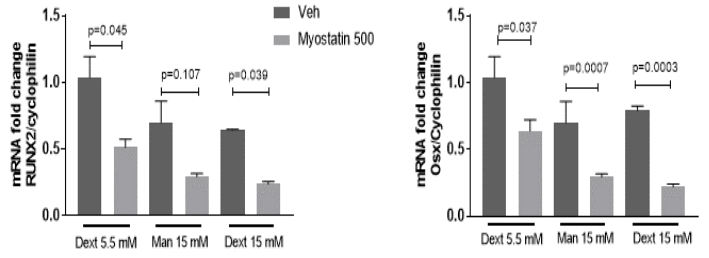


Fig. 6 Effects of myostatin and hyperglycemia on RUNX2 and Osx mRNA in MC3T3 cells. Cells were cultured after confluency in normoglycemic (5.5 mM dextrose), hyperglycemic (15 mM dextrose) and hyperosmolar (15 mM mannitol) media (5 days) and then stimulated with myostatin or vehicle for 24 hours. Data presented as fold change (2^-ΔΔCt) normalized to cyclophilin ± SE after log transformation (One way ANOVA with post-hoc analysis)

and primary osteoblasts will be plated in 6-well plates at a density of 2.5 x 10^5 cells and grown to confluency, then differentiated for 0 to 4 weeks using ascorbic acid and β-glycerol phosphate, as described (63, 64). Cells at each week (0, 1, 2, 3 or 4) of differentiation will be stimulated with or without recombinant myostatin (R&D Systems) at 500 ng/ml under normoglycemic or hyperglycemic conditions (mannitol will be used as osmolar control). In separate experiments, cells will be pretreated with recombinant myostatin and stimulated with insulin at different concentrations to assess whether myostatin affects insulin signaling pathways (Akt/mTOR, MAPK/ERK), as has been reported in myoblasts (65). Cells will be lysed and processed for RNA isolation using a Zymo Research column per manufacturer recommendations. Genes of interest (e.g., myostatin, AcvR2b, AcvR2a, GDF-11, RUNX2, Osx, osteocalcin etc.) will be assessed, as previously described (66). Additionally, cells will be collected and processed as previously described (67), for protein quantification and assessment of phosphorylation of proteins (Western blot analysis), in myostatin-related pathways (Smad2/3, Akt/mTOR and MAPK/ERK).

Mineralization activity: Primary osteoblasts and MC3T3 cells will be grown to confluency and differentiated as per section above, in the presence and absence of recombinant myostatin for 14 days. Cells will be subsequently stained with Alizarin Red S kit (Sigma) per manufacturer's protocol to evaluate mineralization.

Myostatin and Acvr2b inhibition: We will proceed with the above experiments of cell culture and differentiation in the presence of myostatin inhibitory antibody (REGN647, Regeneron) or AcvR2b inhibitory antibody (Regeneron). Myostatin signaling pathways and genes of interest will be analyzed as per above section of cell culture and differentiation.

Expected outcomes, pitfalls and alternative approaches: We hypothesize that our in vitro experiments will demonstrate a direct effect of myostatin on osteoblastogenesis that will be potentiated in hyperglycemic conditions. We anticipate that this effect will be a result of downstream signaling via the Smad 2/3 pathway, resulting in subsequent myostatin-induced RUNX2 suppression and RUNX2-regulated osteogenic genes (Fig 6). Previously published studies have suggested both direct and indirect effects of myostatin on bone marrow stromal cells; however, underlying mechanisms and interactions that involve myostatin signaling in osteoblast precursors and osteoblasts in hyperglycemic conditions are unknown. A recent report suggests that some effects of myostatin on osteoblast cells may be mediated through the osteocyte (11). Additionally, we anticipate that myostatin will inhibit insulin signaling pathways in osteoblasts and blunt insulin's anabolic effects on these cells (Fig 6). If direct effects of myostatin on mineralization, gene expression and/or downstream signaling in osteoblast cell cultures are not observed, we will consider in vitro experiments involving osteocyte and/or osteoclast stimulation with myostatin to evaluate alternative actions of myostatin on other bone cell types. Additionally, we anticipate that myostatin signaling and its effects on osteoblastogenic genes will be reversed in the presence of myostatin or AcvR2b inhibitors. Similar molecules to myostatin, such as GDF-11 and activin A, that act on the same family of activin receptors, have been implicated in bone metabolism and osteoblast signaling; therefore, we will explore these additional pathways and their potential synergy to myostatin if indicated by our initial findings.

FUTURE DIRECTIONS

Training obtained in the proposed project combined with previous clinical training, will allow me to design mechanistic, translational and clinical studies focused on bone-muscle interactions. Once we have established 1) the relationship between myostatin and DBD and skeletal muscle function in animal models of insulin-deficiency and in humans with T1D, 2) the mechanism of action of myostatin on bone cells in hyperglycemic conditions and its effects on insulin signaling in osteoblasts and 3) the effectiveness of myostatin inhibition in preventing DBD in animal models of insulin-deficiency, we will proceed with future studies (R01) targeted towards muscle-bone interactions in chronic conditions (such as diabetes) , in an attempt to improve musculoskeletal function that is often compromised. Additionally, subsequent studies will be based on the results from this study and will further identify how the muscle secretome affects bone and use these novel discoveries to envision and deploy new interventions to prevent or reverse musculoskeletal complications.

Training in the Responsible Conduct of Research

As a faculty member and an investigator at the University of Kentucky my previous instruction in the Responsible Conduct of Research (RCR) has included obtaining certifications in the Biomedical Responsible Conduct of Research and Good Clinical Practice through the Collaborative Institutional Training Initiative (CITI) training, which was renewed in the fall of 2018. Also, I attended a four-hour workshop offered by the Office of Research Integrity titled" Informed Consent Workshop: From Perception to Process" in the summer of 2018. In addition, during the past few years I have completed online training through the American Association for Laboratory Animal Science (AALAS) in animal handling and ethical treatment of animals, including instruction in the Guidelines for Euthanasia of Animals.

Furthermore, I am currently enrolled in a 3 credit hour course "Ethics and Responsibility in Clinical Research" (BSC 534) to continue my training in the Responsible Conduct of Research (RCR). I will also attend the Research Ethics Lecture Series at the University of Kentucky (presented monthly September through November and February through April each year) and enroll in a 1 credit hour course titled "Ethics in Scientific Research" offered by the Department of Toxicology and Cancer Biology during this award period.

Format: Training in the RCR will include didactics, seminars and small face-to-face group discussions with my Mentors during the Advisory Committee meetings and our scheduled monthly meetings. Additionally, I will plan to attend the "Ethics in Scientific Research" course offered by the Department of Toxicology and Cancer Biology during the first year of this award period.

Subject Matter: The objectives and goals for the "Ethics in Scientific Research" course are "to provide overview of good laboratory practices and present them as the basis of good scientific research, along with an overview of quality assurance and appropriate practices in data analysis and data interpretation. The course will then move to the ethics of human and animal experimentation and discuss the concepts of data and intellectual property, their ownership and access to them. The problems of reviewing other workers' intellectual property such as grant applications, research papers and other intellectual property will be addressed". As part of my current training in the course "Ethics and Responsibility in Clinical Research" I will have completed human subject protection training and learn to conduct research in an ethical manner and participate in discussion sessions and out of class learning activities. Additional discussions with my Mentors and Advisory Committee during this award will involve scientific misconduct, peer review and responsible authorship and publication, collaboration in research, including collaborations with industry, live vertebrate animal subjects in research, and safe laboratory practices.

Faculty Participation: During my scheduled meetings with my Mentor, Dr. Fowlkes as well as my Co-Mentors Dr. Peterson and Dr. Kern, I will discuss relevant ethical issues and topics including data acquisition and management, human subject protection and clinical research design. Additionally, I will have discussions pertinent to my research project ethical issues with the instructor of the "Ethics in Scientific Research" course.

Duration: The "Ethics in Scientific Research" course will occur during an entire semester, for ½-1 hour/week. The Research Ethics Lecture Series will involve 6 hours/academic year. Additionally, non-formal instruction will occur periodically, throughout the duration of this award.

Frequency: Training in the RCR will occur throughout the duration of this award. In the first year, training will involve informal as well as formal training as part of the "Ethics in Scientific Research" course. For the subsequent years of this award, I plan to continue to attend the Research Ethics Lecture Series and participate in informal discussions regarding ethical issues related to my research project.

To the K08 committee

We are writing as Primary Mentor (John Fowlkes, MD) and Co-mentors (Charlotte Peterson, PhD; Phil Kern, MD), to provide our strongest commitment to serve as mentors to Dr. Evangelia Kalaitzoglou. Eva completed her medical school training at The Kapodistrian University of Athens in Greece and her pediatric residency at Penn State Milton S. Hershey Medical Center. Eva was recruited from her Pediatric Endocrinology Fellowship training program at the University of Oklahoma to the University of Kentucky, Department of Pediatrics, Division of Pediatric Endocrinology, and the Barnstable Brown Diabetes and Obesity Center in August 2015. It was with great excitement and anticipation for us all when Eva chose to come to our program to pursue a clinician scientist career pathway. She has proven to be a well-versed and highly competent pediatric endocrinologist who has made serious strides in obtaining essential research experience and training in her fellowship and in her early academic career at UK in skeletal biology, molecular biology, animal modeling, and scientific investigations in humans.

Since arriving at UK in 2015, and in recognition of her desire to pursue an academic research career, Eva was initially provided 65% protected research effort supported through the Barnstable Brown Diabetes Center to pursue her research goals. In July, 2018, she was awarded a competitive physician research pipeline award through the University of Kentucky Center for Clinical and Translational Sciences (CCTS), which has increased her protected research time to 75%. Overall, Eva has displayed an excellent work ethic and has demonstrated maturation in her research skills and scientific thought processes. She has been entirely “hands-on” with her research projects, learning and carrying out research in the lab and working collaboratively with research teams in the Barnstable Brown Pediatric Diabetes Laboratory, the Center for Muscle Biology, and the CCTS. Through this process, she has remained true to her desire to pursue a research career with a bench-to-bedside approach. Eva has been very conscientious in leading and managing her research projects. She has become very proficient in writing and in obtaining her own approvals for all her research projects, (both IACUC and IRB approved studies). She has independently navigated all of her own collaborative efforts. She has gained skills in basic biology as well as clinical investigations. She also has presented her work at local and national meetings including the Endocrine Society meeting and the Association for Clinical and Translational Science meeting, and has recently presented her work as a CCTS scholar at Indiana University, where she established new and external academic alliances with researchers that have additional experience in muscle-bone interactions and myostatin biology. She has also supervised a medical student and oversees a project-dedicated technician.

While early in her research experience at UK, Eva began to explore muscle-bone interactions as they relate to diabetes. It is well known, that musculoskeletal complications occur commonly in those who have type 1 diabetes (T1D). Over the last decade, Dr. Fowlkes (Primary Mentor) has developed a strong track record of studying skeletal (bone) complications in diabetes, but has never studied the possibility that muscle, which is also impacted by diabetes, might mediate negative effects of diabetes on bone. Eva then saw this significant gap in knowledge and an opening to gain more insights into how changes in skeletal muscle might drive diabetic bone disease in type 1 diabetes. This area of research has received very little attention in previous models of diabetic bone disease. Furthermore, the overall research focus in skeletal muscle-derived factors that negatively impact bone and increase fracture risk in chronic illness and aging is fertile ground for Eva’s future independent investigations. Thus, Eva’s area of research and her planned studies are and will be uniquely her own as she pursues an independent research career, separate from anything her mentors have

previously studied or plan to study in the future. To this end, Eva will submit an independent investigator (R01) application in year 3 of her K08 award. Shortly after arriving at UK and because of Eva's research interests in skeletal muscle and bone, Dr. Charlotte Peterson agreed to become a Co-mentor to Eva, as Dr. Peterson is a highly accomplished and acclaimed researcher, and an expert in skeletal muscle biology. Going forward, Dr. Fowlkes and Dr. Peterson have met to review and critique her research on a regular basis over the last 2 years. Dr. Phil Kern has also joined the mentoring team once Eva secured the physician research pipeline award from the CCTS. Dr. Kern has had a distinguished career in adipose biology and skeletal muscle/adipocyte physiology. He has also mentored many junior investigators, and as Director of the CCTS, he has a broad-based knowledge of resources that would help advance Eva's research career at UK as a translational researcher. Furthermore, he offers specific technical skills in fat and skeletal muscle biopsy techniques, in which he has been training Eva, as well as in all components of research involving human subjects.

Together, this collaborative mentoring team has been successful in helping advance Eva's scientific goals, as evidenced by the fact that Eva has now developed preliminary data to develop a working hypothesis, which is novel and potentially clinically impactful. She proposes that the muscle myokine, myostatin, which is upregulated in T1D, can directly impair osteogenesis and bone formation in T1D. Her work is indeed novel and translatable to humans. With her collaborator, Dr. Tom Hawke, they have been able to demonstrate that serum myostatin in humans is higher in serum compared to non-diabetic individuals; consistent with what is observed in rodents, making for a strong justification to study myostatin as a target in diabetic bone disease in T1D in humans. She has demonstrated that bone cells express all the needed signaling armamentarium to respond to myostatin and that myostatin can signal in osteoblasts through the Smad2/3 signaling pathway, which then suppresses RUNX2 and other osteogenic genes. In her preliminary data, she has shown that the effects of myostatin are further down-regulated in the presence of hyperglycemic conditions, consistent with poorly controlled diabetes. This new knowledge will be highly relevant and valued in the field of muscle-bone research and the science of diabetic bone disease.

We are all extremely committed to support Eva's career development every step of the way and we think that our combined expertise as her mentoring team places her in a unique situation to explore muscle-bone interactions in type 1 diabetes. Specifically, each mentor brings his or her own research focus and expertise to this mentored project. Dr. Kern is Director of the Center for Clinical and Translational Science, Dr. Peterson is the Director of the Center for Muscle Biology and Dr. Fowlkes is the Director of the Barnstable Brown Diabetes Center. Each mentor has also served as PI on numerous NIH research grants and a robust funding record (see Table 1). Furthermore, the mentor team has an extensive mentoring track record, including students, post-doctoral and research fellows and junior faculty with a high retention rate (see Table 2). Their combined mentoring experience will be instrumental in Eva's

TABLE 1 - NIH funding of Mentors (1985-present)

Administering Institute/Center	Projects	Total Funding	Sub Projects	Sub Project Funding
NIDDK	75	$17,956,620		
NIA	39	$8,784,273		
NIAMS	17	$4,865,804		
NCATS	17	$27,590,220		
NEI	7	$35,579		
NCRR	4	$4,214,798	15	$4,007,026
Total	159	$63,447,294	15	$4,007,026

TABLE 2 – Mentor track records (last 10 years)

Type of mentee	Student	Postdoctoral and research fellow	Junior faculty
Number- All mentors combined	33	25	28
Currently in academia	~70%	80%	100%

training. Dr. Kern is a very well established, translational investigator in adipocyte biology, lipidology, diabetes, and insulin resistance. Dr. Peterson is a highly regarded scientist in the fields of skeletal muscle, exercise, ageing and sarcopenia. Dr. Fowlkes works in the fields of Type 1 diabetes and the complications of diabetes on bone. Their distinct and overlapping research interests illustrate the breadth and depth of the mentors' combined research experience and expertise and show how together they possess all the requisite scientific background to mentor Eva in the research focus areas of muscle-bone interactions in T1D: bench-to-bedside.

In his role as Primary Mentor and Director of the Barnstable Brown Diabetes Center, Dr. Fowlkes will meet with Eva weekly to review the progress of her research, ensure that the training schedule is progressing, and facilitate data collection, analysis and interpretation. Eva has been very fortunate to have recently been awarded a Children's Miracle Network grant to help with her research career and which will allay some of the additional costs of the research outlined. However, the costs of the research described in her K08 award will require additional financial resources beyond what the grant can cover. Therefore, as Director of the Barnstable Brown Diabetes Center, he will commit additional institutional resources to insure that Dr. Kalaitzoglou is able to successfully complete the proposed scope of work. He also will write and provide annual evaluations of the candidate's progress as required in the annual progress report.

Drs. Fowlkes, Peterson and Kern will meet monthly with Eva in a formal process to review progress related to her research goals, help her troubleshoot, and identify any areas of training that require additional attention. Within her overall clinical work schedule (see Dr. Day's LOS), we will ensure that she has the protected time (minimum of 75% protected research effort) needed to attend course work, workshops, and training events that may even be offsite. Furthermore, her mentors will work with Eva on skills needed to write manuscripts and grants, which are critical to a successful research career. Eva will have complete access to the Barnstable Brown Pediatric Diabetes Laboratory for her work. She has an office adjacent to the lab and has access to equipment and personnel in the lab, including the Director of the lab, Dr. Clay Bunn, PhD. She has also been engaged with the research staff in the Muscle Immunohistochemistry and Molecular Imaging Core Laboratory. She also has access to CCTS resources, which include DXA scanning equipment and expertise, as well as experienced CCTS staff that will assist in muscle biopsies. Overall, the mentors have provided Eva with a highly structured, very strong mentoring and resource-rich environment at UK to support her throughout her K08 award and beyond.

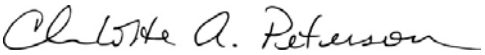
Eva has taken classes in Clinical and Translational Science (CTS), which have enabled her to gain more experience in clinical and translational study design, and she will continue to take classes to obtain a formal Certificate in CTS through the CCTS. Eva has outlined a clear path for career development during this phase of her training. She has chosen future coursework specifically to gain more experience in Statistics and Ethics. Moreover, she will continue to take advantage of the multitude of resources and courses available through the CCTS to make the transition from mentored (K) to independent (R) awards in terms of overall expertise in grantsmanship and critical thinking. She has previously completed training requirements for human subjects' research and the responsible conduct of research and will continue to update as required. She has outlined annual national conferences where she may attend to gain new knowledge and present her work, as well as establish new collaborations. In addition, beyond the resources of the Fowlkes' and Peterson's labs, she will have access to the many resources of Dr. Jeffry Nyman at Vanderbilt University. These include state-of-the-art imaging and biomechanical assessments of bone. Dr. Nyman is an accomplished skeletal researcher and has been a frequent collaborator with the Fowlkes' lab, now over a decade, and has agreed to assist and collaborate with Eva in her proposed work (see letter of support and Biosketch from Dr. Nyman).

We, as Eva's mentoring team, are confident that a five-year period of mentored career development is appropriate for her to become an independent investigator. As mentors, we are fully committed to Eva's maturation and development as a successful physician scientist. As her primary mentors are an MD and a PhD, we recognize the challenges and the rewards of a career that dovetails clinical medicine with scientific

pursuits. During the tenure of the award, we anticipate multiple publications from the work described in Eva's proposal with a minimum of one annually in Years 2, 3 and 4. We expect that Eva will submit an R01 in Year 3. Applications for funding will be written with the oversight and input of her mentoring team. If specific opportunities arise in the interim, she will be encouraged to submit applications for specific RFAs or targeted grant mechanisms. Throughout the K08 timeline, as Eva gains additional expertise, skills and competencies, it is anticipated that she will become a proficient, thoughtful and independent researcher by year 3 of the K08 award, relying on her mentors only for certain resources and scientific advice. Our goals for Eva are that she will continue to take the outcomes of the research described within this proposal and transition her work and career into an independent research focus as a physician scientist. Eva's accomplishments over her brief academic career provide the confidence that she will be successful due to her personal resilience, intellectual fortitude, and ability to work with and learn from others. This K08 award will come at a pivotal time in Eva's career, as she begins to make her transition towards independence in research. With the full support of both labs and the CCTS, we are confident of Eva's success. Overall, we think that Eva is a superior candidate for the K08 award. We are convinced that Eva has great potential to be a superior clinician scientist, one who will make seminal contributions to the research community and improve the lives of others. The protected research effort, training and research resources supported by the NIH through the K08 mechanism will afford Eva the additional research training necessary to facilitate and promote her future independent research career, and her goals and aspirations of becoming an outstanding clinician scientist.

Sincerely,

John L. Fowlkes, MD
Professor of Pediatrics, Medicine and Pharmacology and Nutritional Sciences
Director, Barnstable Brown Diabetes and Obesity Center



Charlotte Peterson, PhD
Joseph Hamburg Endowed Professor
Director, Center for Muscle Biology

Philip Kern, MD
Professor, Internal Medicine, Division of Endocrinology
Director, Center for Clinical and Translational Sciences

To: Evagelia Kalaitzoglou, MD

From: Thomas Hawke, PhD

Date: Feb 27, 2020

Re: K08 Career Development Award Support Letter

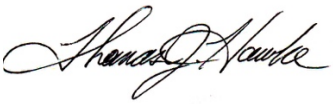
Dear Evangelia,

I am writing this letter to express my interest and support in collaborating on this proposed research application for your K08 Career Development Award entitled "Muscle-bone interaction and its role in diabetic bone disease of Type 1 diabetes".

My recent work has focused on the underlying mechanisms of diabetic myopathy. My laboratory has published extensively on diabetic myopathy in rodent models of Type 1 diabetes (STZ-induced and Akita mouse models- both insulinopenic diabetic models) and, more recently, in humans with Type 1 diabetes. With this background, I am in a unique position to offer my guidance in the analysis and interpretation of your findings and assist in future study design. Furthermore, I would be willing to share resources with you, such as our myostatin antibodies. The research plan you have proposed is novel and potentially highly translatable. I am particularly intrigued by your ideas that relate to muscle-bone interactions and the pathophysiologic mechanism related to increased fracture risk in those with Type 1 diabetes. I am looking forward to collaborating with you on this project and offering any resources (technical or otherwise) to ensure the success of this project.

I would also be happy to serve as a member of your scientific advisory committee and bring my expertise in diabetic myopathy and participate in your committee meetings every 6 months during the duration of this award to discuss your research progress.

Sincerely,



Thomas Hawke, PhD
Professor
Dept. of Pathology & Molecular Medicine
McMaster University
email: hawke@mcmaster.ca
webpage: www.hawkelabca.com
phone: 905-525-9140 ext 22372

VANDERBILT UNIVERSITY MEDICAL CENTER

Vanderbilt Orthopaedic Institute

February 26, 2020

Evangelia Kalaitzoglou, MD
Assistant Professor of Pediatrics
Division of Pediatric Endocrinology

Re: K08 Career Development Award Support Letter

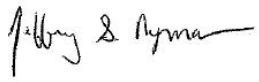
Dear Evangelia:

I write to express my interest in collaborating with you on your proposed research application for the K08 Career Development Award entitled “Muscle-bone interaction and its role in diabetic bone disease of Type 1 diabetes”. As you know, I have a long-standing collaboration with the Barnstable Brown Diabetes Center Faculty, namely on the effect of type 1 diabetes on skeletal physiology and bone strength involving Drs. Fowlkes, Thrailkill, Bunn, and yourself. Specifically, we have published a number of papers over the last several years related to diabetic bone disease. With this experience, I find your new work to be very novel and highly translatable as it relates to pathophysiologic mechanisms causing increased fracture risk in type 1 diabetes.

As part of our collaboration, I will provide micro-structural/architectural, compositional, and biomechanical measurements of the bones from your proposed studies using mouse models. I also will help with data analysis and interpretation of the findings. Being less than 3 hours away by car, I will have no problem visiting the University of Kentucky to review data, discuss results, and offer suggestions on the direction of the project. I am also more than happy to be involved in manuscript writing and any future projects that come from this Career Development Award.

I am excited by the prospect of working with you on these novel studies and examining heretofore under-investigated interactions between muscle and bone in the evolution of musculoskeletal complications of type 1 diabetes.

Kindest regards,



Jeffry S. Nyman, PhD
Associate Professor of Orthopaedic Surgery, Biomedical Engineering

Medical Center East, South Tower
Suite 4200
Nashville, TN 37232-8774

Description of Institutional Environment

My training environment includes state-of-the-art facilities and mentorship in the areas of musculoskeletal research and diabetes throughout the University of Kentucky (UK) campus. The Charles T. Wethington (CTW) building houses the laboratories of Dr. John Fowlkes (primary mentor), Dr. Charlotte Peterson (co- mentor) and Dr. Philip Kern (co-mentor). Dr. Peterson and Dr. Kern have office space in this same building, whereas Dr. Fowlkes' primary office is located in the Barnstable Brown Diabetes Center (BBDC). The BBDC, a nationally recognized center and leader in diabetes prevention, education, research and comprehensive care, is directed by Dr. Fowlkes. In this setting my colleagues and I practice medicine. With assistance from a research team, this is the primary recruiting site for subjects in Aim 1 of my proposal. The CTW building and the BBDC, along with the Center for Clinical and Translational Science located at the UK campus, is the primary environment where my training and research project will take place.

The CTW building houses the Barnstable Brown Pediatric Diabetes Research (BBPDR) laboratory, run by Dr. Fowlkes and the Center for Muscle Biology (CMB) core laboratory, run by Dr. Peterson. Both laboratories are located in the 4th floor of the CTW building. The BBPDR lab is part of the Barnstable Brown Diabetes Center (BBDC), whose mission is to provide a comprehensive approach to diabetes treatment, education and research. The CMB core lab is an integral component of the Center for Muscle Biology (CMB), whose primary mission is to support world-class basic, clinical and translational research relating to striated and smooth muscle, providing space, equipment and expertise for investigators to perform a wide variety of muscle analyses. In addition to laboratory meetings, our monthly meetings with Drs. Fowlkes, Peterson and Kern for me to present my research progress will be held in this location.

The BBDC is located in a new state-of-the-art facility where diabetes care delivery is provided across the lifespan; the center treats more than 7,500 adults and 2,500 pediatric patients annually. Both adult and pediatric endocrinology providers working with a team of diabetes educators and social work support practice in this setting, in very close proximity. This is the setting where my Pediatric Endocrinology practice is located, and where endocrinologists treating pediatric and adult patients interact in a common space to provide clinical care. Additionally, the Division of Pediatric Endocrinology holds journal clubs and clinical meetings quarterly in this location, where providers discuss about research advances and incorporation into practice. BBDC also has a dedicated research coordinator with whom I will be working closely to enroll subjects in my study from the BBDC clinics. During this project, I will also work closely with the NIH-funded Center for Clinical and Translational Science (CCTS), whose director is Dr. Kern (my co-mentor). The CCTS supports clinical and translational science for accelerating discoveries to improve health. The Center offers many services which I will take advantage of during this project, including the Biostatistics, Epidemiology and Research Design (BERD) group for study consultation, Recruitment Services for research subjects and Clinical Research Unit, which will provide expert support for sample collection. In the outpatient area of the CCTS, the proposed procedures in our human subjects will be performed either by myself under Dr. Kern's instruction (muscle biopsy) or by highly trained CCTS staff (blood draw and Dual X-ray Absorptiometry-DXA).

In addition, other facilities and resources that are available to me during this award include Division of Laboratory Animal Resources (DLAR), which is the centralized core facility responsible for housing biomedical research animals at UK, the Immunohistochemistry (IHC) Lab and the Imaging Facility both of which are part of the CMB, a Flow Cytometry Facility and other core labs that belong to the CCTS, such as the Biostatistics, Epidemiology and Research Design Core as well as the Metabolic Core facility which is a part of the Center of Research on Obesity and Cardiovascular Disease (COCVD). Furthermore, I will work closely with the Office of Research Integrity (ORI) and the Proposal Development Office (PDO), by participating in grant and manuscript workshops particularly as I get closer to applying for further funding (R01). To strengthen my training in Clinical and Translational Science (CTS), courses offered by the Department of Behavioral Science along with UK Graduate School are available to me for completing a Certificate in Clinical Translational Science during this award period.

All the resources available at the University of Kentucky, including BBPDR and CMB labs, BBDOC and CCTS, combined with expertise from my mentors and co-mentor and institutional support by the Department of Pediatrics will be essential to my successful transition towards establishing myself as an independent investigator.

HealthCare

Pediatrics

University of Kentucky
138 Leader Avenue
Lexington, KY 40506
O: 859-323-1432
F: 859-323-3499
ukhealthcare.uky.edu

February 24, 2020

Re: Evangelia Kalaitzoglou, MD: Statement of Institutional Commitment

To K08 review committee:

It is my pleasure to write this letter of support for Dr. Evangelia Kalaitzoglou's K08 application "Muscle-bone interaction and its role in diabetic bone disease of Type 1 diabetes." She currently serves as an Assistant Professor at University of Kentucky's (UK) Department of Pediatrics with an appointment in both the Division of Pediatric Endocrinology and the Barnstable Brown Diabetes Center (BBDC). Dr. Kalaitzoglou joined UK in 2015 after completing her fellowship in Pediatric Endocrinology. Her recruitment and research efforts were sponsored by the BBDC (65% research effort). In July 2018, she competitively obtained her own intramural funding through the SChoLAR physician-scientist program to support her research effort (75% effort). Her independent research efforts involve unique approaches to understanding how muscle-derived factors may impact bone in the context of diabetes. These interactions may be important in explaining why muscle and bone functions are impaired in diabetes. As a result of her research efforts, she has published 4 manuscripts and presented 4 abstracts at the regional/national levels.

Dr. Kalaitzoglou's position is not dependent upon her obtaining funding though this mechanism, as she continues to have intramural support and is an active clinician. As Chair of the Department, I will protect Dr. Kalaitzoglou's research career at a minimum of 75% effort. Her clinical efforts will be maintained at no more than 15% during the duration of the award. I will work with her mentors to ensure she has adequate research space and resources. Currently she has office space adjacent to the research labs, dedicated lab space and access to all equipment, staff and resources within the lab.

As Dr. Kalaitzoglou's research project intersects at bone and skeletal muscle biology in diabetes, her primary mentors are Drs. John Fowlkes, MD and Charlotte Peterson, PhD. Dr. Fowlkes is a physician scientist and the Barnstable Brown Kentucky Diabetes and Obesity Center Endowed Chair and Director. He is an established investigator in the fields of diabetes, growth factors and bone biology and has been well funded through NIH. Dr. Peterson is a well-known and accomplished scientist in the field of skeletal muscle biology and is the Joseph Hamburg Endowed Professor and Director of the Center for Muscle Biology at UK, with extensive NIH funding. Together they bring complementary expertise to study muscle-bone interactions in diabetes. Dr. Kalaitzoglou's progress will be overseen by a scientific advisory committee, which will include Dr. Philip Kern (Co-Mentor), Professor of Medicine and the Director of the CCTS; and Dr. Thomas Hawke (collaborator), Professor of Pathology and Molecular Medicine at McMaster University. The scientific advisory committee will meet with Dr. Kalaitzoglou and her primary mentors every 6 months where she will present her updated work and discuss future research and career development.

Dr. Kalaitzoglou has proven herself to be an outstanding clinician-scientist, with great potential towards an independent career in the field of musculoskeletal disease and diabetes; therefore, I am very pleased to provide her the institutional support for her career plan as outlined in this application.

Sincerely,

Scottie B. Day, M.D., F.A.A.P.
Chair, UK Department of Pediatrics
Physician in Chief, Kentucky Children's Hospital
Jacqueline A. Noonan-CMN Research Chair in Pediatrics

PHS Human Subjects and Clinical Trials Information

OMB Number: 0925-0001 and 0925-0002

Expiration Date: 03/31/2020

Are Human Subjects Involved	● Yes ❍ No
Is the Project Exempt from Federal regulations?	❍ Yes ● No
Exemption Number	❑ 1 ❑ 2 ❑ 3 ❑ 4 ❑ 5 ❑ 6 ❑ 7 ❑ 8
Other Requested Information	

Human Subject Studies

Study#	Study Title	Clinical Trial?
1	Muscle-bone interaction and its role in diabetic bone disease of Type 1 diabetes	No

Section 1 - Basic Information (Study 1)

OMB Number: 0925-0001 and 0925-0002

Expiration Date: 03/31/2020

1.1. Study Title *

Muscle-bone interaction and its role in diabetic bone disease of Type 1 diabetes

1.2. Is this study exempt from Federal Regulations * ❍ Yes ● No

1.3. Exemption Number ❑ 1 ❑ 2 ❑ 3 ❑ 4 ❑ 5 ❑ 6 ❑ 7 ❑ 8

1.4. Clinical Trial Questionnaire *

1.4.a. Does the study involve human participants?	● Yes	❍ No
1.4.b. Are the participants prospectively assigned to an intervention?	❍ Yes	● No
1.4.c. Is the study designed to evaluate the effect of the intervention on the participants?	❍ Yes	● No
1.4.d. Is the effect that will be evaluated a health-related biomedical or behavioral outcome?	❍ Yes	● No

1.5. Provide the ClinicalTrials.gov Identifier (e.g. NCT87654321) for this trial, if applicable

Section 2 - Study Population Characteristics (Study 1)

2.1. Conditions or Focus of Study

- Type 1 diabetes
- Diabetic bone disease
- Diabetic skeletal muscle dysfunction

2.2. Eligibility Criteria

Inclusion criteria: Ages 18-45, Type 1 diabetes or healthy subjects. Exclusion criteria: glycated hemoglobin≥12%, any unstable medical condition, use of oral hypoglycemic agents, diabetes complications (including diabetic neuropathy), recent use of anticoagulants, obesity (BMI>30), pregnancy, evidence of ketosis, co-existing disorders that can affect bone (celiac disease, corticosteroid use etc.) and vigorous exercise prior to the procedures.

2.3. Age Limits	Min Age: 18 Years	Max Age: 45 Years
2.4. Inclusion of Women, Minorities, and Children	Inclusion_of_w_c_m_RK08_0227201009017469.pdf	
2.5. Recruitment and Retention Plan	Recruitment_Plan_RK08_0227201009017470.pdf	
2.6. Recruitment Status	Not yet recruiting	
2.7. Study Timeline	Timeline_of_study_RK08_0227201009017485.pdf	
2.8. Enrollment of First Subject	12/01/2020	Anticipated

Inclusion of women and minorities

Eligible adult males and females from all ethnic backgrounds will be included in this study and we expect our population to be representative of the ethnic composition of Central Kentucky. Approximately 10% of the population is African American, whereas the Hispanic, Asian and Native American population of Kentucky is <1%, with the rest being Caucasian. During our recruitment we will try to match the ethnic background of the diabetic subjects and the control group. Women who are pregnant will not be eligible due to effects of radiation from DXA to fetus.

Inclusion of children

Only adults over the age of 18 will be eligible for participation in this study, as growth and puberty during childhood affect the musculoskeletal system. Additionally, we will not include any children due to the nature of the study requiring skeletal muscle biopsies.

Recruitment Plan

Subjects with diabetes will be recruited primarily through the Barnstable Brown Diabetes Center, and with the assistance of the Center for Clinical and Translational Sciences (CCTS) Participant Recruitment Services through print advertisements, research participant registries, and clinic visits. Control subjects will be recruited either through the Barnstable Brown Diabetes Center or through volunteers (ResearchMatch). Additionally, this study will be advertised on recruitment internet webpages in digital or video form (e.g., UKclinicalresearch.com, ResearchMatch.org, CenterWatch.com, CISCRP, UK, CCTS and may utilize Google Adwords). The study will be promoted via social media, including Facebook boost ads, UK_CCTS Facebook, UK_CCTS Twitter, UK and UKHC social media, and departmental/lab pages. If advertised on UKClinicalresearch.com, the study flyer will include an option for interested individuals to enter and submit their contact information so that principal investigators or research coordinators can contact potential volunteers about participating, and CCTS will ask 'How did you learn about the study?" Internet and social media recruitment will follow the terms of use for each site utilized. The study will also be promoted through UK HC monitor screens. We do not anticipate difficulties with recruiting as there are approximately 2500 patients with Type 1 diabetes between the ages of 18 and 45 followed in the BBDC.

To retain subjects, we will use several strategies: obtain several contact phone numbers (cell, home, work) from participants, a reminder phone call from the study coordinator 1- 2 days prior to the 2nd visit, and compensation to the subjects after completion of the 2nd study. Additionally, we will ensure that the interval between the 1st and 2nd visits will be 2-4 weeks, to improve retention of subjects that have been screened at the 1st visit but have yet to complete the 2nd visit.

Timeline of study

The plan is to recruit during the first and second years of the proposed career development award (Year 1 and Year 2). Sample analysis will be completed in Year 3 of the study.

There will be 2-4 weeks between screening visit (Visit 1) and study visit (Visit 2). 1st visit will be conducted at the Barnstable Brown Diabetes Center-BBDC and 2nd visit at the Center for Clinical and Translational Science-CCTS. Monitoring for adverse events will be performed after visit 2 with phone call follow up by the study coordinator (*).

	Enrollment	Study
TIMEPOINT	1st visit	2nd visit
Eligibility screen	X	
Informed consent	X	
TESTS		
Safety Labs	X	X
Study Labs		X
Vitals (Blood pressure, pulse, etc.)	X	X
Anthropometric measures (height, weight)	X	X
Muscle biopsy		X
DXA scan		X
Activity questionnaire		X
Monitoring for adverse events		X*

Inclusion Enrollment Reports

IER ID#	Enrollment Location Type	Enrollment Location
Study 1, IER 1	Domestic	university hospital/clinics

Inclusion Enrollment Report 1

Using an Existing Dataset or Resource* : ❍ Yes ● No

Enrollment Location Type* : ● Domestic ❍ Foreign

Enrollment Country(ies): USA: UNITED STATES

Enrollment Location(s): university hospital/clinics

Comments:

Planned

Racial Categories	Ethnic Categories				Total
	Not Hispanic or Latino		Hispanic or Latino		
	Female	Male	Female	Male	
American Indian/ Alaska Native	0	0	0	0	0
Asian	0	0	0	0	0
Native Hawaiian or Other Pacific Islander	0	0	0	0	0
Black or African American	3	3	0	0	6
White	29	29	1	1	60
More than One Race	1	1	1	1	4
Total	33	33	2	2	70

Cumulative (Actual)

Racial Categories	Ethnic Categories									Total
	Not Hispanic or Latino			Hispanic or Latino			Unknown/Not Reported Ethnicity			
	Female	Male	Unknown/ Not Reported	Female	Male	Unknown/ Not Reported	Female	Male	Unknown/ Not Reported	
American Indian/ Alaska Native	0	0	0	0	0	0	0	0	0	0
Asian	0	0	0	0	0	0	0	0	0	0
Native Hawaiian or Other Pacific Islander	0	0	0	0	0	0	0	0	0	0
Black or African American	0	0	0	0	0	0	0	0	0	0
White	0	0	0	0	0	0	0	0	0	0
More than One Race	0	0	0	0	0	0	0	0	0	0
Unknown or Not Reported	0	0	0	0	0	0	0	0	0	0
Total	0	0	0	0	0	0	0	0	0	0

Section 3 - Protection and Monitoring Plans (Study 1)

3.1. Protection of Human Subjects	Prot_of_Human_Subjects_RK08_0227201009017483.pdf
3.2. Is this a multi-site study that will use the same protocol to conduct non-exempt human subjects research at more than one domestic site?	❍ Yes ● No ❍ N/A
If yes, describe the single IRB plan	
3.3. Data and Safety Monitoring Plan	DSMP_RK08_0227201009017504.pdf
3.4. Will a Data and Safety Monitoring Board be appointed for this study?	❍ Yes ● No
3.5. Overall structure of the study team	

Protection of Human Subjects

Risks to the Subjects

a. Human Subjects Involvement and Characteristics

All human subject work will be performed at the University of Kentucky and the outpatient unit of the Center for Clinical and Translational Science (CCTS). The subjects recruited include individuals with or without clinically confirmed T1D but otherwise healthy between the ages of 18 and 45. They will be matched for age, sex and BMI.

Interventions: Baseline labs at screening include hemogram (CBC), thyroid stimulating hormone (TSH), glycated hemoglobin (HbA1c) and comprehensive metabolic panel (CMP). We will recruit subjects without regard to ethnicity, and will study no vulnerable populations. This study involves a blood collection, a muscle biopsy and a Dual X-ray Absorptiometry (DXA) scan as well as urine collection to exclude pregnancy and ketosis. These studies will take place in the Barnstable Brown Diabetes Center and/or the CCTS outpatient area, where skilled research nurses are involved with all the procedures. Muscle biopsies will be performed under local anesthesia by the principal investigator who is currently in the process of training by Dr. Kern, who has completed hundreds of such biopsies. No patient will have a biopsy if it represents an unacceptable risk, such as platelets <75,000, evidence of moderate platelet dysfunction, chronic aspirin use, or hematocrit < 30. In addition, body composition and bone density will be measured by DXA.

b. Sources of Materials

Skeletal muscle and blood samples will be obtained from subjects, and these tissues will be used exclusively for research purposes. DXA reports will be sources of data. Under no circumstances will patients' clinical care be compromised.

c. Potential Risks

Patients will be informed of the risks associated with skeletal muscle biopsy include bleeding, infection, and pain. Rarely (<1%) have oral antibiotics been needed following a biopsy. Risks associated with blood collection through venipuncture might include a sense of pressure while the arm is being prepared for the needle stick, the needle stick may cause temporary discomfort, and there could be bruising or tenderness for about a week after the needle stick. The subject could have discomfort, soreness and/or a bruise after the blood draw. It is rare, but some people may get an infection, a small blood clot, swelling of the vein and surrounding tissue or bleeding where the needle enters the skin. From both procedures, subjects sometimes have a vasovagal reaction. The DXA involves a small amount of radiation, but the amount is within the reasonable range of exposure, and subjects are apprised of this. The consent form will clearly inform the participant of these risks.

The alternatives to this study are to not participate. The care of the patient will never be compromised by nonparticipation.

Adequacy of Protection against Risks

a. Recruitment and Informed Consent

Patients will be compensated $150 for completion of the full study. We will explain the potential risks and benefits, and the patient will be asked to sign a consent form. Consent for this study will be obtained either by Dr. Kalaitzoglou or her designate research coordinator for all procedures, where the witness for the consent is usually a nurse. Patients will be informed of the voluntary nature of the study, and that their clinical care will not be compromised. We do not anticipate difficulties with recruiting as there are approximately 2500 patients with Type 1 diabetes between the ages of 18 and 45 followed in the BBDC. This protocol and a consent form have been reviewed by the University of Kentucky institutional IRB (45363).

b. Protection against Risk

All procedures will be performed in the outpatient unit of the Center for Clinical and Translational Sciences and/or the Barnstable Brown Diabetes Center, using skilled nursing, a hospital environment, and sterile

procedures. No provision is made to compensate patients for any research related injury, and this is stated in the consent form. To protect confidentiality, all subjects are assigned a unique number, and this number will be used for labeling of all samples and the identification of all laboratory material obtained from subjects, and there is no public release of the name of the subject from whom the material was derived. All data will be kept in a password protected electronic file and/or in double-locked filing cabinets/dedicated clinical research space.

Potential Benefits of the Proposed Research to the Subjects and Others

Routine blood tests of CBC, TSH and comprehensive panel and DXA results will be shared with the patient. The knowledge gained from these studies will likely have no clinically relevant importance to the subject. The subject will be compensated up to $150 for his/her participation.

Data & Safety Monitoring Plan

All research personnel who work with subjects, subject data, or subjects' research samples in this project will have completed training in the protection of human research participants. All aspects of this protocol will receive final approval by the institutional IRB. The research team will meet on a monthly basis to review the progress of the study and address any human subject issues that occur. These discussions may involve adverse event prevention measures, subject accrual issues, research staff training on protection of human subjects, as well as discussion of occurrence of adverse events. This protocol is monitored by the principal investigator and study coordinator for adverse events (AEs), as described above. The research coordinator will contact subjects within 48 hours of each procedure to assess for pain, infection, and other symptoms indicating possible post-procedure complications. Subjects are discharged with specific self-monitoring guidelines and instructed to call immediately for any concerning signs or symptoms.

AEs will be graded according to intensity. Mild: Discomfort noticed but no disruption of normal daily activity. Moderate: Discomfort sufficient to reduce or affect normal daily activity. Severe: Incapacitating with inability to work or perform normal daily activity.

The attribution scale for AE reporting will be as follows. Probable: AE is related to the procedure (e.g. infection from an IV). Possible: AE follows the procedure within a reasonable period (within 7 days), but may have been produced by some other factors (e.g. local rash 5 days following the procedure). Remote: AE does not follow the procedure or drug within a reasonable period and could readily have been produced by the subject's clinical state or other factors. Unrelated: AE is judged to be clearly due to extraneous causes and does not meet the above criteria.

Plan for unexpected Adverse Event (AE) reporting: Serious AEs will be reported to Human Subjects/IRB within 48 hours. Unanticipated events will be reported to the study coordinator real time and to the IRB no later than 15 days after the event. Annual reporting of adverse events will be conducted with the Human Subjects annual review/renewal according to their guidelines.

Monitoring of adverse events. Adverse events will be monitored via review of subject's medical chart and direct reporting from subjects, etc. and documented. No provision is made to compensate patients for any research related injury, and this is stated in the consent form.

Section 4 - Protocol Synopsis (Study 1)

4.1. Brief Summary

4.2. Study Design

4.2.a. Narrative Study Description

4.2.b. Primary Purpose

4.2.c. Interventions

Type	Name	Description

4.2.d. Study Phase

Is this an NIH-defined Phase III Clinical Trial? ❍ Yes ❍ No

4.2.e. Intervention Model

4.2.f. Masking ❍ Yes ❍ No

❑ Participant ❑ Care Provider ❑ Investigator ❑ Outcomes Assessor

4.2.g. Allocation

4.3. Outcome Measures

Type	Name	Time Frame	Brief Description

4.4. Statistical Design and Power

4.5. Subject Participation Duration

4.6. Will the study use an FDA-regulated intervention? ❍ Yes ❍ No

4.6.a. If yes, describe the availability of Investigational Product (IP) and Investigational New Drug (IND)/ Investigational Device Exemption (IDE) status

4.7. Dissemination Plan

Delayed Onset Studies

Delayed Onset Study#	Study Title	Anticipated Clinical Trial?	Justification
The form does not have any delayed onset studies			

VERTEBRATE ANIMALS SECTION

1. Description of animals and procedures:

All procedures are or will be approved by the Institutional Animal Care and Use Committee at the University of Kentucky before animal experiments begin.

General considerations for all animal subjects

Mice are acclimatized to the UK facility for at least one week before any procedures are performed. All mice are provided ad libitum access to water and food; and, also monitored for any signs of distress or pain. Mice will be housed ≤5 per cage and subjected to a standard 12-hour light, 12-hour dark cycle. Weekly animal assessments will include individual body weight, and blood glucose (BG), as measured via glucometer. Blood will be collected at the time of euthanasia for assessment of bone and muscle biomarkers. All bones for skeletal phenotyping (femurs, tibiae and L1-5 vertebrae) and the gastrocnemius, soleus and tibialis anterior muscles (right and left) will be harvested following euthanasia. Longer-term glycemic control will be assessed using HbA1c and intraperitoneal (ip)GTT. At conclusion of the study, mice are euthanized in accordance to the methods approved by the AVMA guidelines.

Ten week old mice are required for completion of this Project:

Aim	**Genotype**	**Source**	**Male**	**Female**
2	DBA/2J	Jackson	20	20
	C57BL/6-Ins2Akita/J		10	
	C57BL/6		10	
3	DBA/2J		60	60
Total by sex			**100**	**80**
Total			**180**	

Procedures:

Streptozotocin (STZ)-induction of diabetes: STZ injected into mice at 40 mg/kg/day for five consecutive days leads to pancreatic beta cell destruction and diabetes within 7-10 days. STZ is prepared with sterile technique in a Class II biosafety cabinet at 7.5 mg/ml in citrate buffer, pH 4.5 and used within 10 minutes of preparation. Intraperitoneal injections (40 mg/kg) will be performed in a biosafety cabinet once daily for five days.

LinBit Implant Surgery (LinShin Canada): Surgical area, instruments and gloves are disinfected prior to use with Hibiclens or autoclave, respectively. Inhalational isoflurane, 1-4% in O_2, is used for intraoperative anesthesia. Reflex responses (tail, paw, and eye), breathing and skin color are monitored during implant placement. After induction of anesthesia, ophthalmic ointment is applied to both eyes, and the mid-scapular site is shaved with clippers on a designated table separate from surgical table. The shaved site is gently scrubbed 2-3 times with Povidone iodine or Chlorhexidine solution. The mouse's body temperature is maintained with heating pads or lamps as necessary. The surgeon wears a clean lab coat, hair covering, and face mask. After scrubbing in with Hibiclens, the surgeon will don a pair of sterile surgical gloves. Instruments are steam autoclaved prior to use and sterilized between use by dipping in Povidone iodine. The mid-scapular skin is pierced with a 16G disposable hypodermic needle which is then withdrawn. A 12-gauge trocar provided by the manufacturer of the implants is briefly immersed in a diluted solution of Povidone Iodine and pushed through the skin orifice. The trocar guides the implant underneath the skin after it is also immersed in Povidone Iodine. The number of implants is determined based on the animal's weight per manufacturer instructions. Once all implants have been placed, the wound is closed with a drop of 10% Povidone Iodine. Mouse is then placed into a clean, dry cage for monitoring by surgery team members. Mice are monitored every 5-10 minutes following surgery until they have self-righting behavior and are breathing with normal rhythm.

Blood collection: Blood for glucose (BG) measurements will be obtained by tail prick weekly. After the tail is cleaned with alcohol and pricked with a 22-gauge needle, a drop of blood will be tested with an AlphaTrack II glucometer. BG readings above 300 will be deemed evidence of overt diabetes.

Myostatin monoclonal antibody treatment: Mice will receive weekly subcutaneous or IP injections of 10 mg/kg of a myostatin monoclonal blocking antibody (REGN647, 54.9 mg/ml, Regeneron) or an isotype control (REGN1945, 52.2 mg/ml, Regeneron) with a 1 ml syringe-25 gauge needle (sterile solution) for the duration of 8 weeks.

Glucose tolerance test: Mice will be weighed and then fasted for 4-5 hours with free access to water. Fasting BG will be measured via glucometer. For glucose tolerance testing (GTT), 20% glucose in sterile saline will be given by injected intraperitoneally at 1.5 mg glucose per gram body weight and BG measurements will be obtained at 15, 30, 45, 60, 90, and 120 minutes following glucose injection. This will require only approximately 5 microliters for measurement of blood glucose from nick of tail. The total volume of blood collected during the ipGTT procedure (for blood glucose measurement) will not exceed approximately 40 microliters.

EchoMRI: Conscious mice will be individually restrained in a clear cylindrical plastic tube (sized by animal weight). The tubes have holes for breathing and are maintained in the horizontal plane during the procedure. Three sequential scans are conducted (approximately 2 minute/scan, 6 minutes total procedure). Following scanning, the tubes are cleaned with soap (Steris Acute-Kare®, 1% chlorxylenol) and water.

2. Justification for use of animals, species and numbers

The use of mice is necessary to study effects of diabetes and various pharmacological therapies on the skeleton. No *in vitro* or computational model would adequately reproduce the complex physiological changes inherent in these situations, nor allow for complex analyses of relevant bone and skeletal muscle specimens. Moreover, *in vivo* treatment with a myostatin antibody is essential in studying the effects of myostatin on diabetic bone disease, since bone is a complex tissue that cannot be effectively mimicked in cell culture systems or in silico simulations. Additionally, experiments described in this application are considered preclinical studies; hence, the use of animal models is appropriate.

3. Provisions to minimize discomfort, distress, pain and injury

All personnel handling laboratory animals will successfully complete a basic training program (Education and Training in the Care and Use of Laboratory Animals: A Guide for Developing Institutional Programs, National Academy Press, 1991). Additional species and procedure specific training programs are provided through the DLAR training program and the AALAS Learning Library is available to all University of Kentucky personnel using vertebrate animals in research and teaching.

Untreated diabetic mice typically exhibit polyuria beginning 2-3 weeks after induction of diabetes. Therefore, cage changes will occur once per week or more frequently if needed to minimize discomfort.

For LinBit implantation surgeries, mice are monitored daily for the first week after implant placement and the wound is healed. If signs of pain are present, Carprofen will be administered 10 mg/kg SQ every 12-24 hours until signs of pain have subsided. If signs of pain persist longer than 24 hours post-op or if signs of wound infection develop, such as redness and swelling, veterinary staff are consulted for advice on treatment options.

For all studies, monitoring of animal well-being include: food and water intake, defecation, and signs of pain or distress including reduced activity, hunched appearance, poor grooming and vocalizations. If during the course of these experiments, mice demonstrate signs of obvious distress, failure to groom (ruffed fur), difficulty in breathing, loss of reflexes (foot pad pressure), become cachexic (lack of feeding, weight loss > 20% of total body weight etc.), they will be euthanized immediately. Euthanasia will be performed in accordance with American Veterinary Medical Association (AVMA) Guidelines for the Euthanasia of Animals.

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Resource Sharing Plan

This proposal will not generate renewable materials or unique data set therefore material transfers are not applicable. For the myostatin inhibitory antibody (REGN647), the isotype control antibody (REGN1945) and the myostatin knock-out mice, we have a material transfer agreement in place with Regeneron (Dr. John Fowlkes). Therefore, we do not intend to share any of these mice or reagents, so any interested third party would have to contact Regeneron directly to obtain access to them. All other mice and materials are commercially available and therefore accessible to the scientific community. Genome Wide Association Studies (GWAS) are not planned for this proposed study.

I plan to publish the data in peer reviewed journals and present my research findings in conferences and/or workshops in a timely manner. Final research data from this study will be made available for use after the main findings have been accepted for publication.

Authentication of Key Resources

Mice: All mouse models will be obtained from The Jackson Laboratories (Bar Harbor, ME), except the Myostatin KO mice (alternative approach), which have been provided by Regeneron through a material transfer agreement (MTA). Breeding mice and resultant progeny will be genotyped by PCR using genomic DNA from ear tissue. At termination, PCR of genomic DNA from study mice will confirm genotype. Genotyping and back crossing of all mice will be confirmed by our laboratory on a routine basis.

Cells: Murine cell lines are commercially available and periodically evaluated in the BBPDR laboratory to verify they maintain their reported characteristics. MC3T3 cells have been purchased from RIKEN cell bank and will be used from passage 3-5 for all experiments. Cells will be periodically tested for Mycoplasma by PCR. Primary osteoblast cells will be isolated from mice as described in the Approach section using standard procedures, as previously published by the BBPDR laboratory and are validated based on their morphology under microscopy, and their ability to differentiate and mineralize. Primary osteoblast cells will be used from passage 3-7 for all experiments. All cells will be grown using sterile procedures.

Antibodies: The inhibitory myostatin antibody (REGN647) provided through the MTA with Regeneron has been validated and reported to be bioequivalent to REGN1033, which has been included in a previous publication by Regeneron reporting increased muscularity in mice (Salzler at al., Proteomics 2016). We will evaluate the muscle phenotype of mice receiving REGN467 and also evaluate the inhibitory effects of REGN647 on myostatin signaling in cultured cells in the presence of recombinant myostatin to validate the antibody. The myostatin antibody used for western blotting will be provided by our collaborator (Dr. Thomas Hawke). It is a rabbit antibody that detects mature myostatin (25 kDa) as well as its precursor (50 kDa). It will be used for protein quantification in skeletal muscle and will be validated by testing it on wild-type and myostatin KO mice.

Biochemical and Molecular Assays: All biochemical assays will be performed using commercially available products that have been validated for the analyte of interest. Results will be verified against what are expected for each assay's inter- and intra-assay coefficients of variance as well as negative and positive controls.

RT-qPCR arrays, routinely performed in our laboratory, will be performed according to manufacturer's instructions and will be analyzed using commercial software specifically designed for the array of interest. Negative and positive controls (such as skeletal muscle tissue/cells or bone/bone cells) will be used to validate arrays.

Rigor and Reproducibility: To decrease bias, enhance reproducibility, and ensure objectivity, all direct skeletal measures will be performed in Dr. Nyman's laboratories on samples blinded to the operator. In Dr. Fowlkes' and Dr. Peterson's lab, all samples will be harvested, processed and coded before analyses. This is currently our standard operating procedure.