DIABETES RESEARCH CENTERS

2018 Meeting of the Diabetes Centers' Directors

March 28, 2018

John Edward Porter Neuroscience Research Center (PNRC II)

Building 35A (main NIH campus)

Bethesda, MD

DIABETES RESEARCH CENTERS

2018 Meeting of the Diabetes Research Centers' Directors

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    f. University of Alabama at Birmingham
    g. UCSD/UCLA
    h. UCSF
    i. University of Chicago
    j. University of Michigan
    k. University of Pennsylvania
    l. University of Washington
    m. Vanderbilt University
    n. Washington University in St. Louis
    o. Yale University

2018 NIDDK Diabetes Center Directors' Meeting

March 28, 2018

John Edward Porter Neuroscience Research Center (PNRC II)
Building 35A (main NIH campus)
Bethesda, MD 20892

Agenda

7:30-8:00 am Meeting Check-in

Session 1 - General

8:00 - 8:15 am Welcome and Opening Remarks (Dr. Germino)

8:15 - 9:00 am The view from NIDDK
- Perspectives & Opportunities (J. Fradkin)
- Brief Introduction (J. Hyde)

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Session 2 – Biomedical Research Cores

9:00 - 9:15 am Albert Einstein-Mount Sinai Diabetes Research Center

9:15 - 9:30 am Boston Area Diabetes Endocrinology Research Center

9:30 - 9:45 am Columbia University

9:45 – 10:00 am Indiana University

10:00 – 10:15 am Joslin Diabetes Center

10:15-10:30 am BREAK

10:30 – 10:45 am Stanford University

10:45 – 11:00 am UCSD/UCLA

11:00 - 11:15 am UCSF

11:15 – 11:30 am University of Alabama at Birmingham

11:30 – 11:45 am University of Chicago

11:45 – Noon	University of Michigan
Noon – 1:00 pm	LUNCH, on your own

****

Session 2 – Biomedical Research Cores (continued)

1:00-1:15 pm	University of Pennsylvania
1:15 – 1:30 pm	University of Washington
1:30 – 1:45 pm	Vanderbilt University
1:45 – 2:00 pm	Washington University in Saint Louis
2:00 – 2:15 pm	Yale University

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Session 3 – Informational Items

2:15 – 2:30 pm	NIDDK Medical Student Summer Program (A. Powers)
2:30 – 2:40 pm	NIDDK STEP-UP Program (T. Garvey)
2:40 – 3:00 pm	Diabetes Centers and Research Resource Identifiers (K. Abraham)
3:00 – 3:30 pm	Update: NIH Changes Related to Clinical Trials (B. Linder)
3:30 – 4:00 pm	Updates: Next RFA, Interim RPPR (J. Hyde)
4:00 pm	Adjourn

2018 NIDDK Diabetes Research Center Directors' Annual Meeting

John Edward Porter Neuroscience Research Center
Building 35A, National Institutes of Health
Bethesda, MD
March 28, 2018

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DIABETES RESEARCH CENTERS

UPCOMING NIH/NIDDK MEETINGS & WORKSHOPS

Network of Minority Health Research Investigators — April 11-13, 2018
NMRI 16th Annual Workshop
Double Tree Hotel, Bethesda, MD

Towards a Functional Understanding of the Diabetic Genome — April 23-24, 2018
Lister Hill Auditorium, Bethesda, MD

Obesity and Fat Metabolism in HIV Infected Individuals — May 22-23, 2018
5601 Fishers Lane, Rockville, MD

NIDDK New Principal Investigator Workshop — September 13-14, 2018
Bethesda North Marriott Hotel and Conference Center, Rockville, MD

Autonomic Nervous System: Role in the Regulation of Peripheral Metabolism — September 20-21, 2018
Bethesda North Marriott Hotel and Conference Center, Rockville, MD

"March Madness"

Diabetes Research Center Directors' Meeting

Gregory G. Germino, M.D.

Deputy Director

National Institute of Diabetes and Digestive and Kidney Diseases

March 28, 2018

National Institute of Diabetes and Digestive and Kidney Diseases

Update on NIDDK Budget

Diabetes Research Center Directors' Meeting

Gregory G. Germino, M.D.

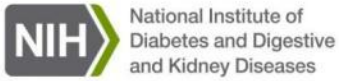
Deputy Director

National Institute of Diabetes and Digestive and Kidney Diseases

March 28, 2018

National Institute of Diabetes and Digestive and Kidney Diseases

2017 Budget Re-cap



FY 2017 Budget

NIDDK

	FY 2017	Over 2016	% Increase
Total	$1.871B*	$52.24MM	2.9

NIH

	FY 2017	Over 2016	% Increase
Total	$34.084B*	$2.0B	6.2

*Excludes Special Diabetes Program funds, $139 MM

National Institute of Diabetes and Digestive and Kidney Diseases

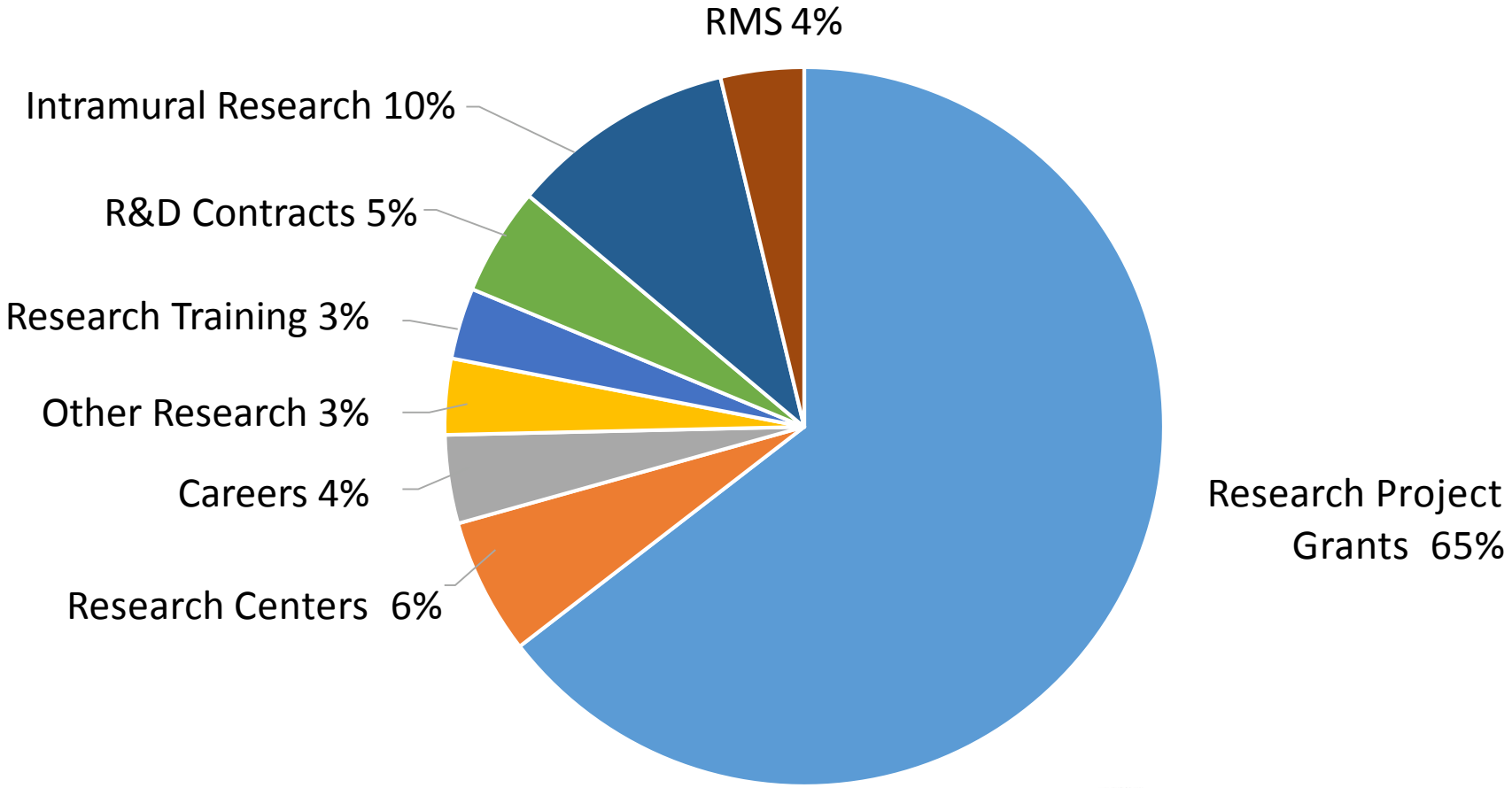
NIH Targeted Increases

	$ in millions	Institute
Alzheimer's disease	**400**	**NIA**
Zika Response and Preparedness	**152**	**NIAID**
Antibiotic resistance research	**50**	**NIAID**

National Institute of Diabetes and Digestive and Kidney Diseases

NIDDK FY 2017 Actual Obligations by Mechanism

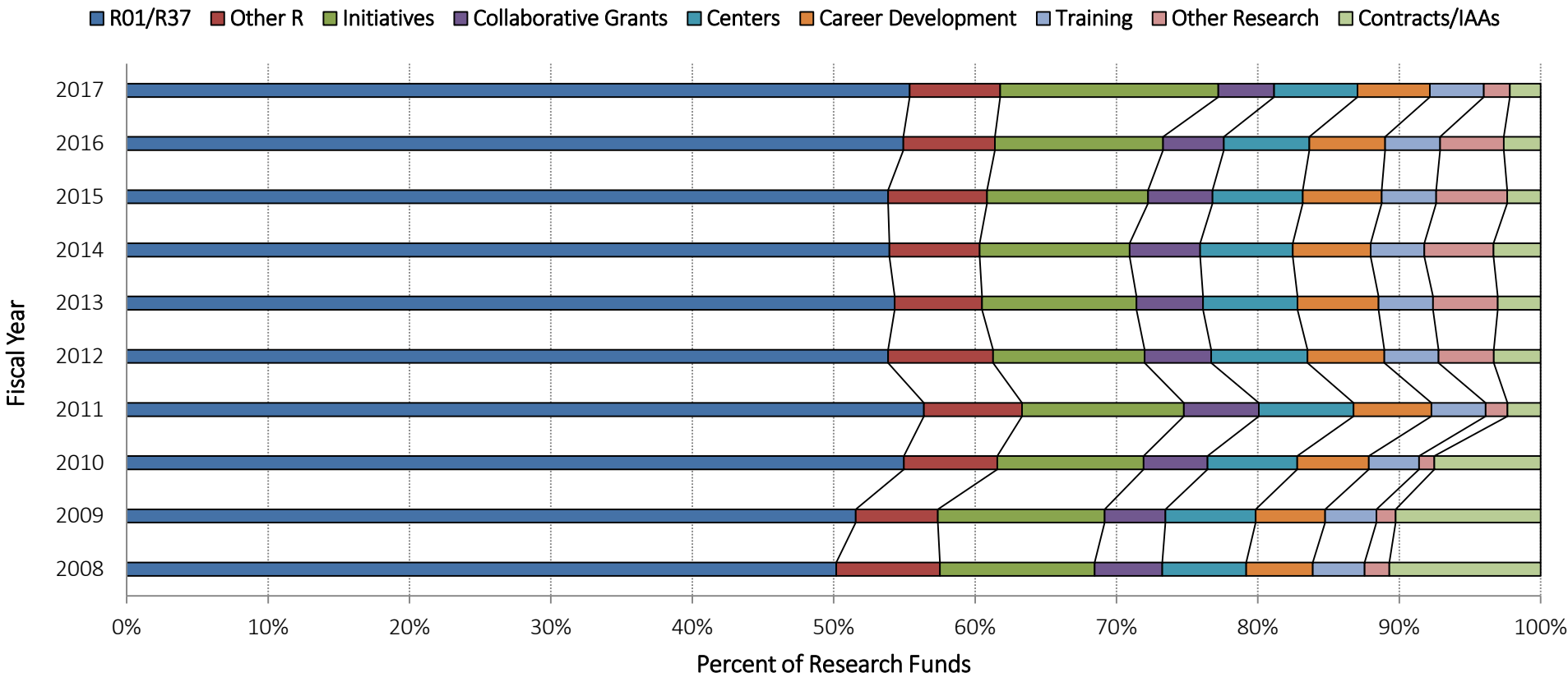
$1,869,798,000*



* Does not include Type 1 Diabetes Special Statutory Authority

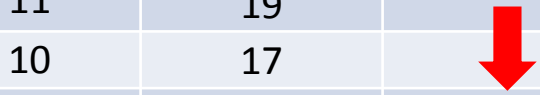
National Institute of Diabetes and Digestive and Kidney Diseases

NIDDK Extramural Research Funding by Category: Competing and Non-Competing



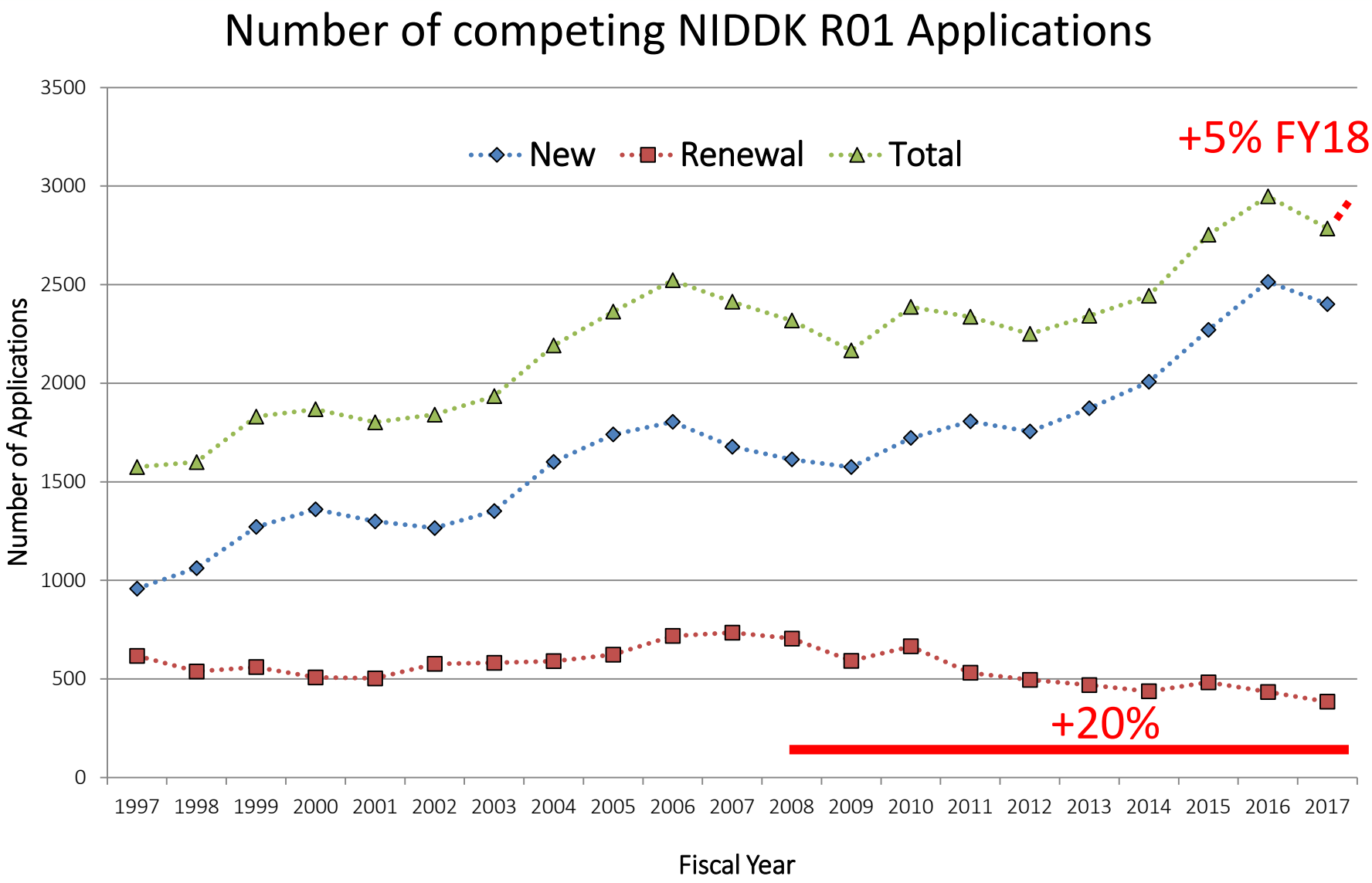
NIDDK Payline Trends 2008-2017

Fiscal Year	General Payline	$>500K Payline	New Investigator Payline	Early Stage Investigator (ESI) Payline	ESI First Competitive Renewal Payline
2008	17	11	19		
2009	17	11	19		
2010	17	11	19		
2011	15	10	17		
2012	13	9	13	18	
2013	11	7	11	16	
2014	13	8	13	18	
2015	13	8	13	18	15
2016	13	8	13	18	15
2017	12	7	12	17	15

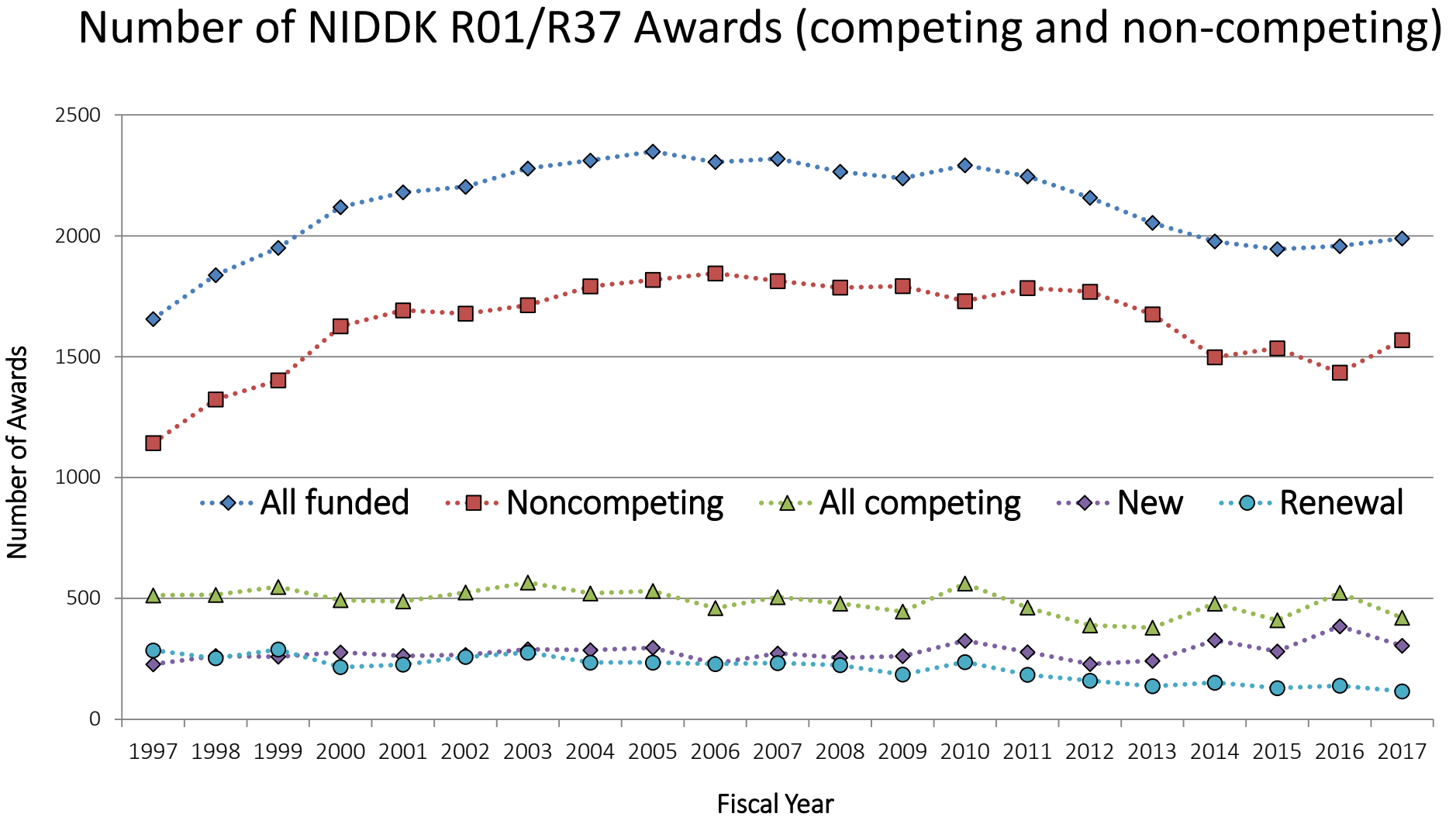


If the 2017 budget went up $59M, why did the 2017 payline go **DOWN?**

Trends in Application Numbers (2017)

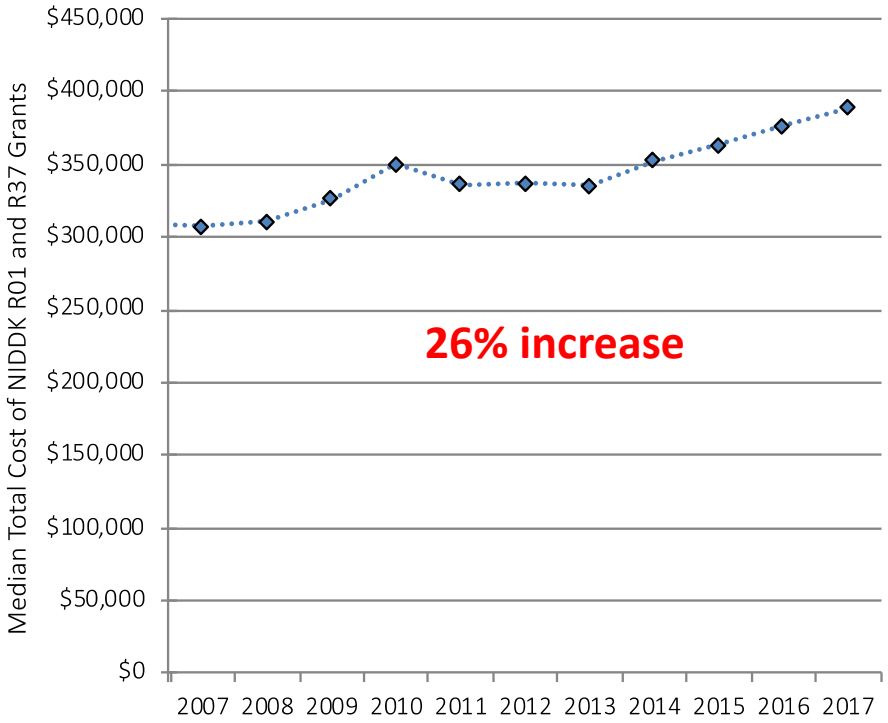


R01 Award Trends (2017)

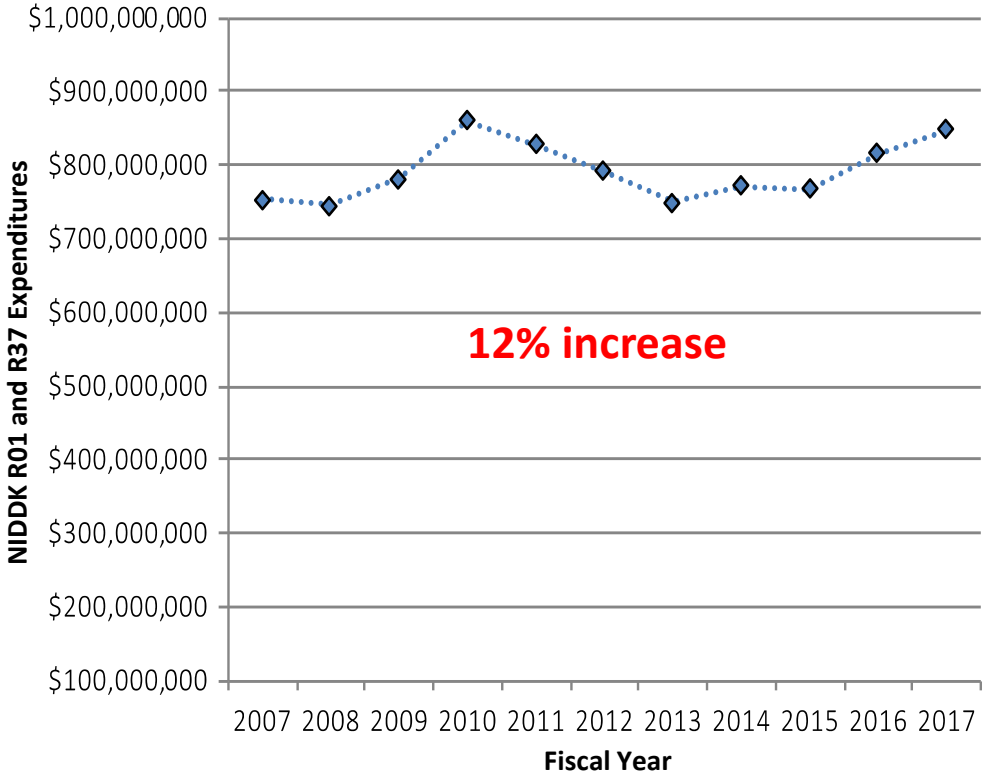


Trends in Award Cost (2017)

Cost per Grant Has Risen More than Total R01 Budget



Median total costs of R01 grants

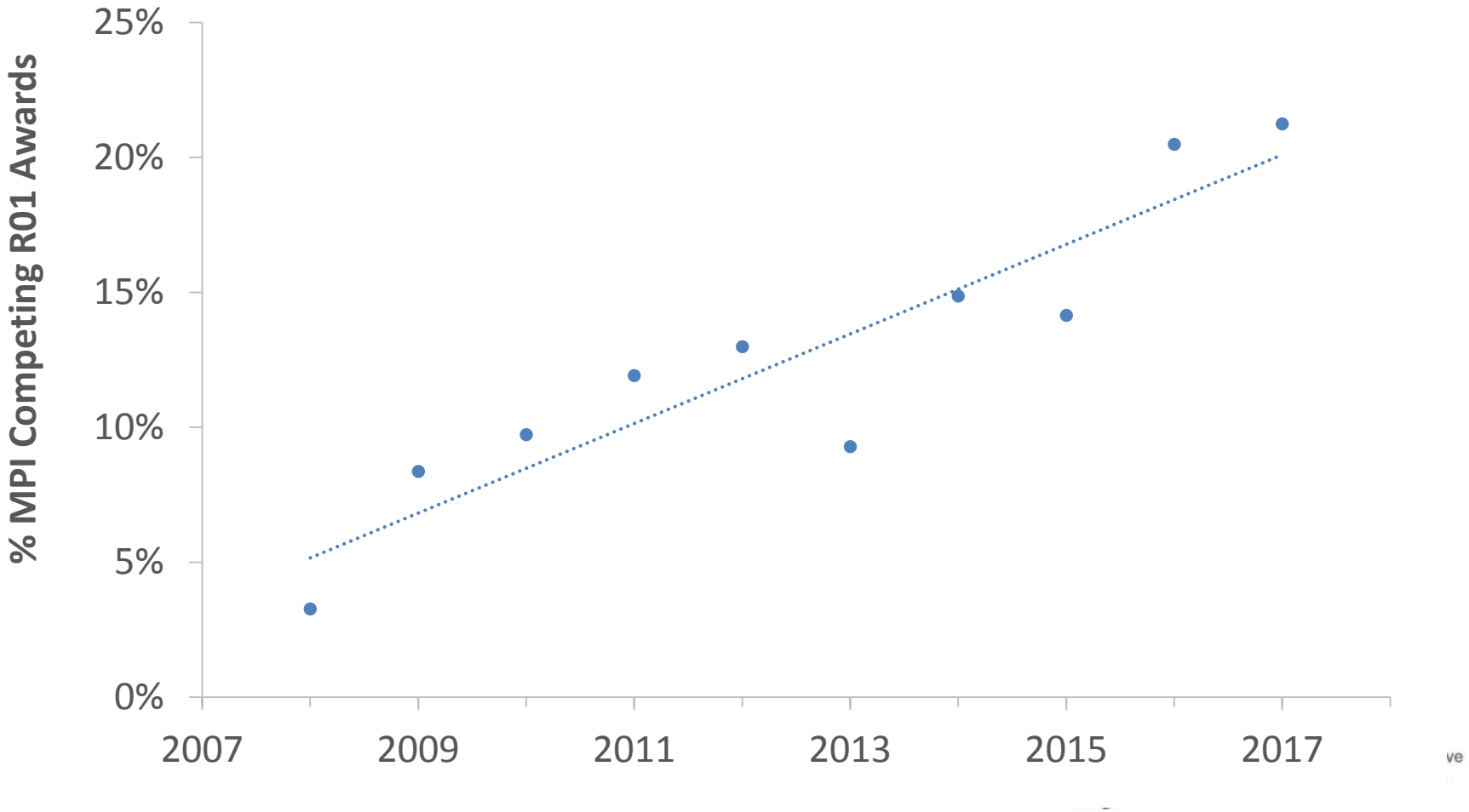


Overall NIDDK Expenditures on R01s

National Institute of Diabetes and Digestive and Kidney Diseases

MPI make up an increasing proportion of competing R01 awards

And they cost on average about $90K more/award



NIH Appropriation 2018

"What a long strange trip it's been!"

CR Number	Date CR Passed	Expiration Date (duration)
1	October 1, 2017	December 9, 2017 (~10 weeks)

NIH Appropriation 2018

"What a long strange trip it's been!"

CR Number	Date CR Passed	Expiration Date (duration)
1	October 1, 2017	December 9, 2017 (~10 weeks)
2	December 9, 2017	December 22, 2017 (3 weeks)

NIH Appropriation 2018

CR Number	Date CR Passed	Expiration Date (duration)
1	October 1, 2017	December 9, 2017 (~10 weeks)
2	December 9, 2017	December 22, 2017 (3 weeks)
3	December 22, 2017	January 19, 2018 (4 weeks) (T1D Funding Part 1)
4	January 22, 2018	February 8, 2018 (~2 weeks)
5	February 9, 2018	March 23, 2018 (~6 weeks) (T1D Funding Part 2)

FY 2018 Consolidated Appropriations Act (H.R. 1625)

signed March 23, 2018

NIH

	FY 2018	Over 2017	% Increase
Total	$37.084B*#	$3.0B	8.8!

NIDDK

	FY 2018	Over 2017	% Increase
Total	$1.971B*	$100.2M	5.4!!

*Excludes Special Diabetes Program funds, $150 M/y FY2018, FY2019

Includes $496M authorized in 21st Century Cures Act

National Institute of Diabetes and Digestive and Kidney Diseases

NIH Targeted Increases

Research Area	$$ in Millions
Opioid Addiction	500
Alzheimer's Disease	414
Brain	140
All of Us	60
Antibiotic Resistance Research	50
Universal Influenza Vaccine	40
Regenerative Medicine	8

National Institute of
Diabetes and Digestive
and Kidney Diseases

National Institute of
Diabetes and Digestive
and Kidney Diseases

Perspectives and Opportunities

Directors' Meeting
Diabetes Research Centers

Judith Fradkin, M.D.

Director, Division of Diabetes, Endocrinology,

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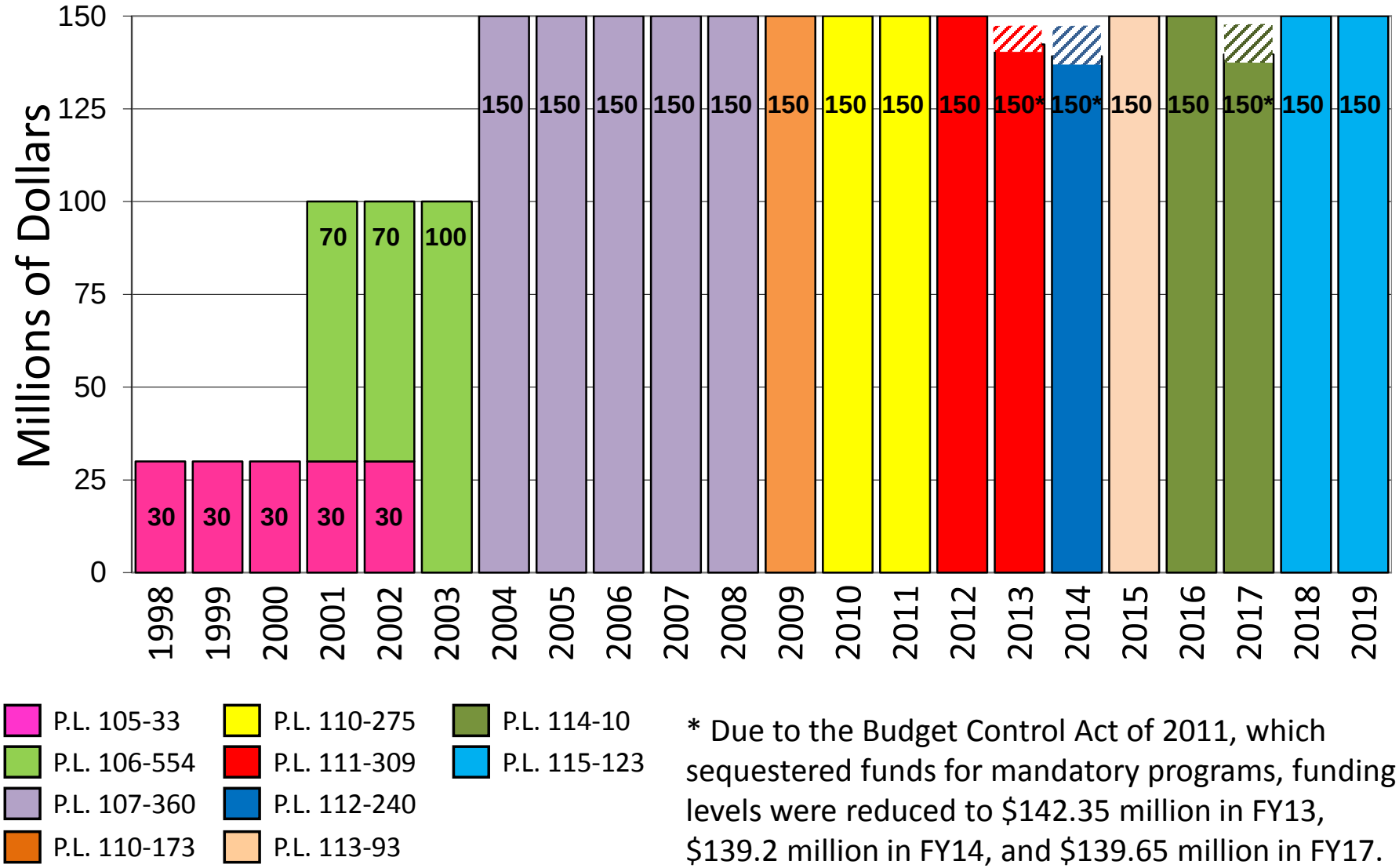
March 27, 2018

Outline

- Special Diabetes Program
- Other Funding Opportunities
- Upcoming Meetings

Laws Provide $2.76 Billion over 22 years*



* Due to the Budget Control Act of 2011, which sequestered funds for mandatory programs, funding levels were reduced to $142.35 million in FY13, $139.2 million in FY14, and $139.65 million in FY17.

“No Year” Funds

Optimize Receipt Dates

DP3 → R01

UC4 → U01

Type 1 Diabetes Special Statutory Funding Program

- About the Program +
- Funding Opportunities −
  - Funding Opportunities Archive
- Research Resources
- Clinical Trials Recruiting Patients & Families
- Consortia, Networks & Centers
- Reports & Planning

Special Diabetes Program: Funding Opportunities

Below are current funding opportunities supported by the Special Diabetes Program. For other NIH funding opportunities, please see the NIH Guide for Grants and Contracts NIH.

View archived research funding opportunities.

Posted Date	Title	Open Date (Earliest Submission Date)	Letter of Intent Due Date	Full Announcement
2/15/2018	Development of New Technologies and Bioengineering Solutions for the Advancement of Cell Replacement Therapies for Type 1 Diabetes (T1D) (R01 Clinical Trial Optional)	April 10, 2018	April 10, 2018	RFA-DK-18-004 NIH
2/15/2018	Impact of the Use of Glucose Monitoring and Control Technologies on Health Outcomes and Quality of Life in Older Adults with Type 1 Diabetes (T1D) (R01 Clinical Trial Required)	March 26, 2018	March 26, 2018 and November 6, 2018	RFA-DK-17-024 NIH

Artificial Pancreas

- Development and Integration of Novel Components for Open- and Closed-loop Hormone Replacement Platforms for T1D Therapy
- Support for Small Business Innovation Research to Develop New Open- and Closed-loop Automated Technologies for Better T1D Therapy and Monitoring
- Clinical, Behavioral and Physiological Research Testing Current and Novel Closed Loop Systems
- Impact of the Use of Glucose Monitoring and Control Technologies on Health Outcomes and Quality of Life in Older Adults with T1D
- Elucidating the Effect of Glycemic Excursions on Patient Well-being and Cognitive Status in People with T1D

Patient Well-Being

- Treating Diabetes Distress to Improve Glycemic Outcomes in Type 1 Diabetes

- Incorporating Patient-Reported Outcomes into Clinical Care for Type 1 Diabetes

Autoimmunity and the Beta Cell

- The Characterization and Discovery of Novel Autoantigens and Epitopes in Type 1 Diabetes
- Immune System Engineering for Targeted Tolerance in Type 1 Diabetes
- Discovery of Early Type 1 Diabetes Disease Biomarkers in the Human Pancreas (HIRN Consortium on Beta Cell Death and Survival)
- High-Resolution Exploration of the Human Islet Tissue Environment (HIRN Human Pancreas Analysis Consortium)
- Development of New Technologies and Bioengineering Solutions for the Advancement of Cell Replacement Therapies for Type 1 Diabetes
- Cell Replacement Therapies for Type 1 Diabetes (SBIR)
- Mass Spectrometric Assays for the Reliable and Reproducible Detection of Proteins/Peptides of Importance in Type 1 Diabetes Research
- Limited Competition for the Continuation of the Clinical Centers for The Environmental Determinants of Diabetes in the Young (TEDDY) Study

Building on Previous Initiatives

- Career Development Programs in Diabetes Research for Endocrinologists (K12)
- Funding for Collaborative Clinical Research in Type 1 Diabetes: Living Biobank
- Some continuing programs don’t require FOAs in FY18-19 (SEARCH, ITN, DRCR, islet distribution, assay standardization)

Outline

- Special Diabetes Program
- **Other Funding Opportunities**
- Upcoming Meetings

High Impact Research and Research Infrastructure Program (RC2)

- RC2 activity code could support
  - a specific research question or
  - the creation of a unique infrastructure/resource designed to accelerate scientific progress in the future

- RC2 Requirements
  - Accelerate critical breakthroughs in biomedical research relevant to NIDDK
  - Novel approaches to specific knowledge gaps, scientific opportunities, new technologies, data generation, or research methods
  - Interdisciplinary team –provide an integrated plan of working together
  - Sharing Plan-a sustainable plan to share data and other resources with the broader scientific community

High Impact, Interdisciplinary Science in NIDDK Research Areas (RC2)

- PAR-18-111 issued in November, 2017
- Receipt dates (June and November, 2018)
- Large budget (>500K)
- Pre-Approval process through Divisions
- NIDDK Review Branch

Editorial Board Review

- First level of review by content experts
  - Minimum of 3 per application
  - Mail in reviews
  - Detailed reviews of each category
- Second level of review by Editorial Board
  - 8-10 members (3 year commitment)
  - Provided comments from first level
  - Provide broad view as it relates to DK mission
  - Overall impact score

Catalyst Award in Diabetes, Endocrinology and Metabolic Diseases (DP1)

- RFA DK-17-011
- Modeled on NIH Director's Pioneer Award
- Individual scientists who propose pioneering and possibly transforming studies with the potential to produce an unusually high impact
- $500K DC
- Receipt date Feb 2, 2018

Translational Research

- Pragmatic Research in Healthcare Settings to Improve Diabetes and Obesity Prevention and Care
  - R18 Clinical Trial Required PAR-18-106
  - R34 Planning Grants PAR 18-107
- Evaluating Natural Experiments in Healthcare to Improve Diabetes Prevention and Treatment
  - R18 PAR-17-178
  - Evaluate large scale policies or programs related to healthcare delivery that are expected to influence diabetes prevention and care

Neurocognition and Diabetes

- **Potential Opportunity**:
- Research on Alzheimer's disease increases $414M
- BRAIN initiative increases by $140M

Workshops and Conferences

- Towards a Functional Understanding of the Diabetic Genome 2018
  April 23 - 24, 2018 Bethesda, Maryland
- Obesity and Fat Metabolism in HIV Infected Individuals
  May 22 - 23, 2018 Rockville, Maryland
- New Principal Investigator Workshop
  Sept. 13 - 14, 2018 Rockville, Maryland
- Autonomic Nervous System: Role in the Regulation of Peripheral Metabolism and Pathophysiology of Metabolic Disease
  Sept. 20 - 21, 2018 Rockville, Maryland

National Institute of
Diabetes and Digestive
and Kidney Diseases

National Institute of
Diabetes and Digestive
and Kidney Diseases

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Diabetes and Digestive
and Kidney Diseases

National Institute of Diabetes and Digestive and Kidney Diseases

2018 Diabetes Center Directors' Meeting

March 28, 2018
Bethesda, MD

National Institute of Diabetes and Digestive and Kidney Diseases

2018 Diabetes Center Directors' Meeting

- Brief Overview of Agenda:
  - 8:00 – 10:15: presentations
  - 10:15 – 10:30: break
  - 10:30 – noon: presentations
  - Noon – 1:00: Lunch (on your own)
  - 1:00 – 4:00: presentations (no scheduled break unless time allows)

2018 Diabetes Center Directors' Meeting

- Center presentations: Biomedical Research Cores
  - Describe 1 or 2 (no more than two!) unique or innovative core services offered through your NIDDK-supported biomedical research core(s).
  - Other than providing access to the institutional core, provide an example describing how NIDDK Diabetes Research Center (P30) funding has significantly contributed to one of your 'institutional' cores.
  - What core service do you most wish was available at your Diabetes Research Center, but is currently not available? Why would this service be important for your research base?
  - If NIDDK was able to provide additional 'core development' funds to support a new service(s) in one of your existing cores (or as a new core), what would that new service/core be? This should not simply be importing a core service that is available at another institution, but instead this would be developing a new core service that would require some R&D investment before it could be offered as a service.

National Institute of
Diabetes and Digestive
and Kidney Diseases

Albert Einstein-Mount Sinai Diabetes Research Center (ES-DRC)

Biomedical Cores

Core A- Animal Physiology – Gary Schwartz

Core B- Biomarker & Analytic Research – Dan Stein

Core C- Metabolomics – Irwin Kurland

Core D- Human Islet Biology – Adolfo Garcia-Ocana

Core E- Translational Research Core – Jill Crandall

Innovative Services

Core A- Animal Physiology

- Specialized surgeries – CNS, gastric, denervation
- Gnotobiotic housing – immunologic and microbiome
- Energy balance – 4 environmental chambers, 2 with dedicated indirect calorimetry units
- Continuous glucose monitoring – implantable chips

Innovative Services

Core D- Human Islet Biology

- FACS-Sorted human and rodent beta cells
- Human and Rodent islet perifusion studies

Institutional Core Collaboration

Core B- Biomarker & Analytic Research

- **Robotic high-throughput RIA and ELISA analyses**

New services for existing cores

- Continuous glucose monitoring coupled with optogenetics
- Mitochondria respiration states, chemical potential, metabolic flux, protein turnover
- Next generation islet CyTOF technology

New core facilities

- Spatial single cell analyses
- High-throughput proteomics
- Dedicated metabolism bioinformatics

Boston Area Diabetes Research Center

Boston Area Diabetes Research Center-Cores:

1- Metabolic Physiology (director Barbara Kahn M.D.)

2-Pancreatic Islet (director James Markmann M.D. Ph.D.)

(MGH Pancreas/Islet Transplant program, a member of the Clinical Islet Transplantation Consortium)

3-Transgenic services (director Bradley Lowell M.D. Ph.D.)

(joint with Boston Nutrition Obesity Research Center)

4-Cell Biology and Morphology (director Dennis Brown Ph.D.)

(no institutional support; NIDDK P30 support via BADRC and the Center for the Study of IBD)

[BADRC-Broad Alliance]

BADERC-Broad program

- Subcontract to Broad Institute to support proposals that aim to utilize technological capabilities at Broad not easily available at home institutions
- Broad Platforms: Technology Labs, Genetic Perturbation, Genomics, Imaging, Metabolomics, and Proteomics
- Scientific collaboration between BADERC and Broad scientists
- Evaluation in parallel and with same standards as P&F program

PI	Institution	Title	Year
Welt, Corrine	MGH	Coading variants associated with ris of PCOS	2012
Ruderman, Neil	BU	RNA seq in adipose tissue of obese humans	2012
Walford, Geoffrey	MGH	Pharmacometabolite signatures of the acute response to metformin	2013
Lowell, Brad	MGH	Single-neuron RNA Seq	2013
Nikolajczyk, Barbara	BU	ChIPseq of T cells in weight cycling	2013
Rosen, Evan	BIDMC	Transcriptional signature of obesity and insulin resistance in isolated adipocytes	2014
Herman, Mark	BIDMC	The epigenomics of fructose sensitivity in mice	2104
Isganaitis, Elvira	Joslin	Novel mediators of excessive fetal growth in type 1 diabetes pregnancy	2015
Bentley-Lewis, Rhonda	MGH	Metabolomic profiling and diabetes incidence in women with history of GDM	2017
Jennifer Lee	BIDMC	Beneficial effects of novel lipids on the gut microbiome for treating type 2 diabetes	2017

Unique or Innovative core services:

1. Islet related services:

a. Research Human Islet Isolation to provide normal, type I/II diabetic pancreatic islets

b. Large Animal Islet Isolation non-human primate (NHP) and porcine islet isolation

c. Small Animal Islet Isolation mouse and rat islet isolation of any strains and age

d. Experimental Islet Transplant Models NHP and small animal transplantation models

2. New Transgenic Services- *Easi*-CRISPR: concept to mouse in 3 months

Rapid, one-stage generation of gene knockout or gene knock-in mice, including targeted insertion of Cre, Flp, GFP, epitope tags, loxed exons, etc.

- Select a sgRNA that cuts insertion site and design targeting construct *in silico*.
- Company synthesizes construct as ssDNA.
- Co-injection of sgRNA, ssDNA and Cas9 protein into 1 cell embryos.
- Offspring are genotyped with PCR. Targeted founders are ready for use.

(Based on Quadros RM, Genome Biology, 2017, PMID 28511701)

How has DRC (P30) funding has significantly contributed an 'institutional' core?

The islet Core at MGH is part of clinical Pancreas/Islet transplantation program, which encompasses Pancreas transplantation, islet autotransplantation and allotransplantation. Islet isolation is a demanding process and a team of experienced individuals is required for success. Supporting such a team requires matrixed support from grants, clinical revenue and the institution. Allogeneic islet transplantation is considered investigational for the treatment of type 1 diabetes and is not reimbursable by Insurance, and by CMS only in the setting of a clinical trial; funding for clinical islet trials is limited.
The Islet core receives nominal institutional support.

The funding of the Islet core provided by the BADRC has been essential in keeping a trained islet isolation team intact, thereby enabling the ongoing provision of human, non-human primate and rodent islets for research.

What core service do you most wish was available at your Diabetes Research Center, but is currently not available?

1-Glucose clamp in the Conscious mouse.

2-Pancreatic islet secretory function by perifusion.

3. Mouse Imaging.

4-Indirect Calorimetry and Determination of physical activity and wheel running. (operative w/o DRC support)

what would that new service or core would you create if additional NIDDK core development funds were available?

Sequential single molecule Fluorescence In Situ hybridization-

A technique that enables the quantification and localization of endogenous RNAs, DNA regions and (potentially) proteins in tissue sections; an advance beyond single cell RNAseq.

RNA-e.g.:
RNA Imaging with Multiplexed Error –Robust Fluorescenc In Situ Hybridization (MERFISH)
Moffitt JR & Zhuang X. Methods Enzymol. 2016, 572:1-49
Profiling the Transcriptome with RNA SPOTs. Eng et.al., Nat Methods 2017 14:1153-1155.

DNA regions:
Multiplexed Dynamic Imaging of Genomic Loci by Combined CRISPR Imaging and DNA sequential FISH. Takei et.al., Biophys J. 2017,112:1773-1776

Protein/PO4-Protein:
Awaits development

Slide 1: Columbia University DRC

Slide 2: Current NIDDK-supported biomedical research cores

- Translational Biomarker Analytical Core (B)
- Advanced Tissue Pathology and Imaging (E)
- Mouse Metabolic Function & Phenotyping (F)
- Cytometry & Cell Sorting Core (G)

Slide 3: Unique or innovative services offered through our NIDDK-supported biomedical research cores

- Bile Acid qualitative analysis
- Isothermal titration calorimetry (ITC) to measure cellular and tissue thermogenesis

Slide 4: Example of NIDDK Diabetes Research Center funding contributing to 'institutional' core

- Development of methods to image live mouse and human islets using 2-photon microscopy to measure β-cell stress
- Specialized flow cytometry analysis of gut, islet, adipose immune cells, and neurons

Slide 5: Core services we wish were available

- Quantification of exosomes & other extracellular vesicles
  - NanoSight (Malvern, Inc)
  - ViewSizer 3000 (Manta Instruments Inc)
- Expand available fluorochromes for Aurora instrument from 28 to more than 50 parameters
- DRC bioinformaticians

Slide 6: Additional ‘core development’ funds to support new services and why

- Creation and manipulation of human stem cells and their differentiation into islets, beta, gut, and hypothalamic cells
- We have a large research base interested in using these cells and a large institutional initiative in this area. It leverages directly our clinical practice
  - These cells can be created from patient-specific somatic cells and/or specific sequence variants can be introduced/corrected using crispr to generate allelic series
  - Cells can be interrogated using physiological and pharmacological manipulation, electrophysiology, RNA sequencing, transplantation, and drug discovery

Indiana Diabetes Research Center

Raghu G Mirmira, MD, PhD
Departments of Pediatrics and Medicine
Indiana University School of Medicine
Indianapolis, IN

CENTER FOR DIABETES
AND METABOLIC DISEASES
INDIANA UNIVERSITY

Biomedical Research Cores at Indiana

Islet and Physiology

Carmella
Evans-Molina, MD, PhD

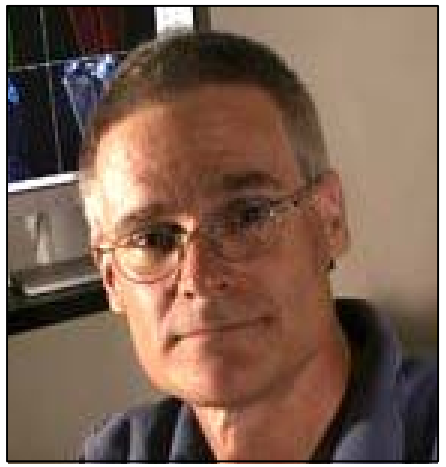
Islet isolation/perifusion
β cell isolation
Rodent metabolic studies
Zebrafish screening
Histology services/archiving
mRNA Translation Analysis

Translation

Kieren Mather, MD

Analyte measurements
Human in vivo physiology
Living Biobank

Microscopy



Kenneth Dunn, PhD

Intravital Multiphoton microscopy
-biosensors/physiology
FRET/FLIM
Equipment access
P30 O'Brien Kidney Center

Swine

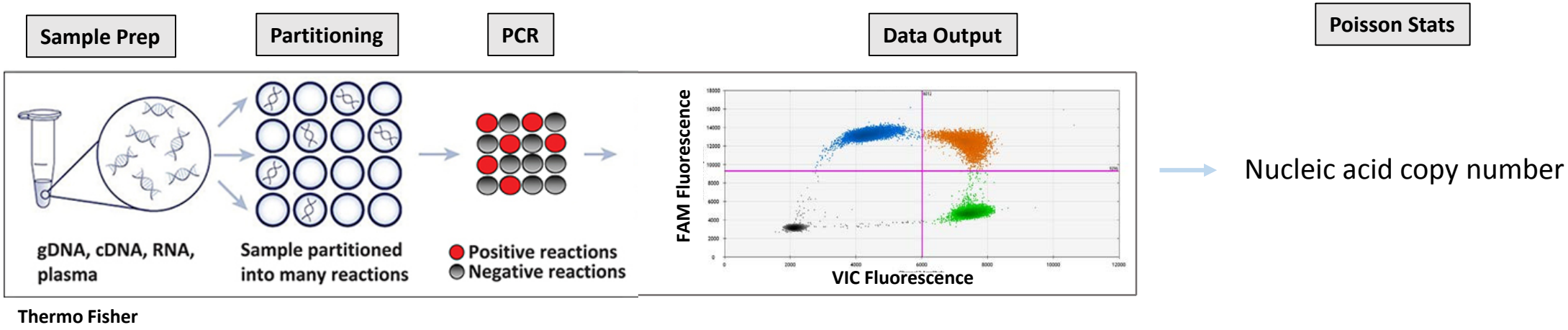
Michael Sturek, PhD

MetS/MetS-D pigs
Tissue biobank
In vivo physiology

Translation Core—Digital PCR

Functions: Analyte measurements (hormones and other analytes in rodent, swine, humans), human physiology studies (clamp studies, biopsies), biostatistical support, biobank (blood, tissues).

Digital PCR: Liquid Biopsy, Copy number variation, Rare sequence detection, Gene expression, Single cell analysis, Pathogen detection, NGS

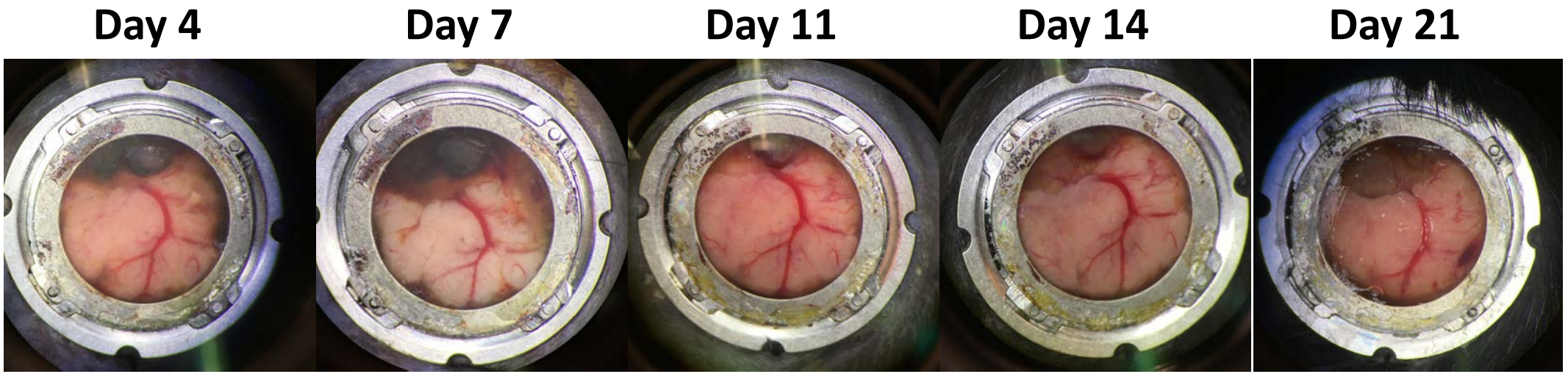


Thermo Fisher

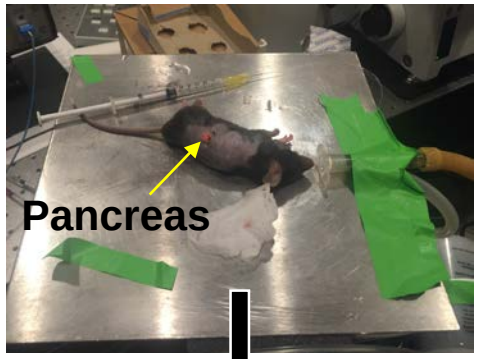
Microscopy Core: Abdominal Imaging Window for Diabetes-specific applications

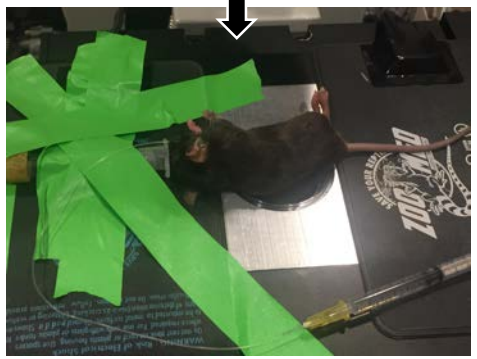
Advanced Microscopy Core at IUSM is also funded by the O'Brien Kidney Centers P30 award (P30DK079312-11).

The goal of this Core is to develop new optical methodologies for investigators in Diabetes Research. Approaches include intravital multiphoton microscopy, 3-dimensional imaging, and quantitative microscopic analyses.



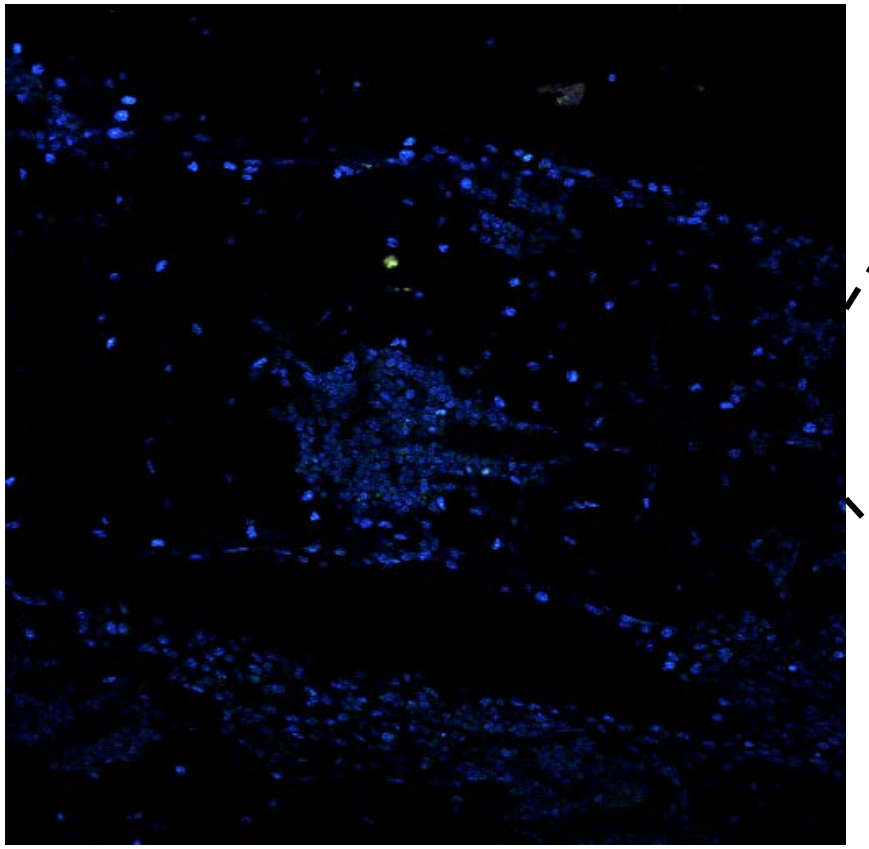
Analysis of endogenous islets in vivo using 2-photon intravital microscopy

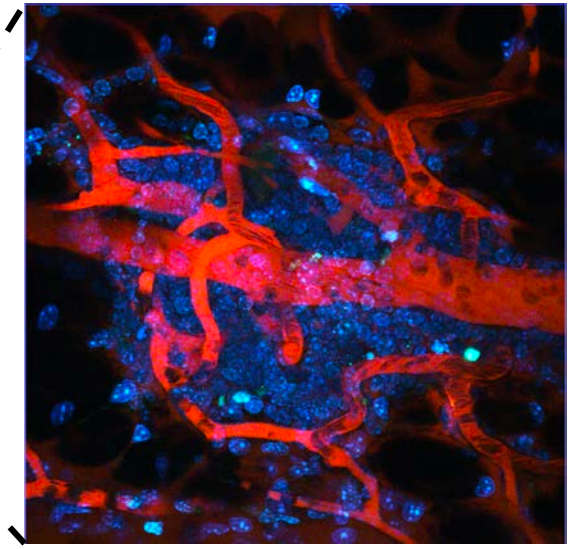




Inverted 2-photon microscope

Inject:
Hoechst
Dextran





60x

New Capabilities

Single Cell Transcriptomics

Dedicated CyTOF

Metabolomics/Lipidomics

Bioinformatics

NIDDK-Diabetes Research Center
Joslin Diabetes Center

George L. King MD

DRC Directors Meeting

March 28, 2018

Cores at Joslin Diabetes Center

DRC supported cores

Animal Physiology Core
Clinical Translational Research Core
Flow Cytometry Core
Genome Editing Core
Molecular Phenotyping and Genotyping Core
Enrichment Program

Joslin Institutional Cores

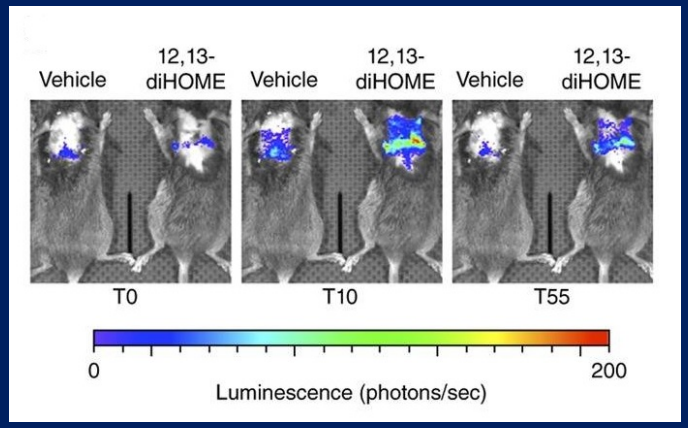
Advanced Microscopy Core*
Biostatistics and Bioinformatics Core*
Clinical Research Center
Islet Isolation Core
Media Core*
Small Animal Facility

*= previous DRC supported cores

Unique offering-Animal Physiology Core
IVIS Spectrum CT In Vivo Imaging System

- Integrated optical and microCT technology
- 2D & 3D optical tomography imaging
- Applications include:
  - Bioluminescence
  - Multispectral fluorescence and
  - Cerenkov imaging for optical radiotracer imaging
  - Low dose and ultra fast microCT

12,13-diHOME enhances cold tolerance and facilitates fatty acid uptake by BAT



Lynes, M. etal. Nat Med 2017; 23(11); 1284.

Supported by NIDDK

3D Localization of stem cells in adipose tissue.

Unique Offering: Genome Editing Core in NOD Mice

Hypothesis

Microbiome

↓

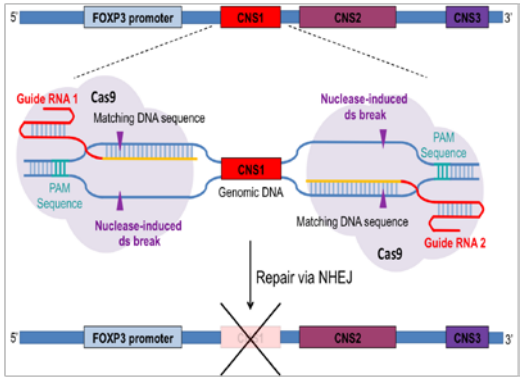
Induction of regulatory T cells

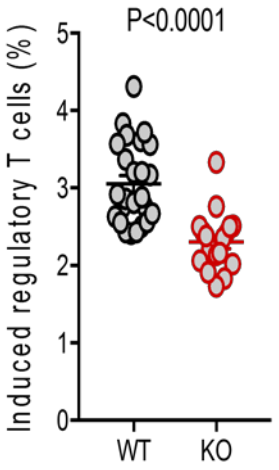
⊥ ?

Type 1 diabetes

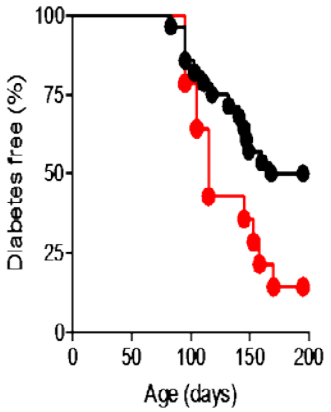
Approach

CRISPR deletion to prevent induction of regulatory T cells in NOD mice





Conclusion



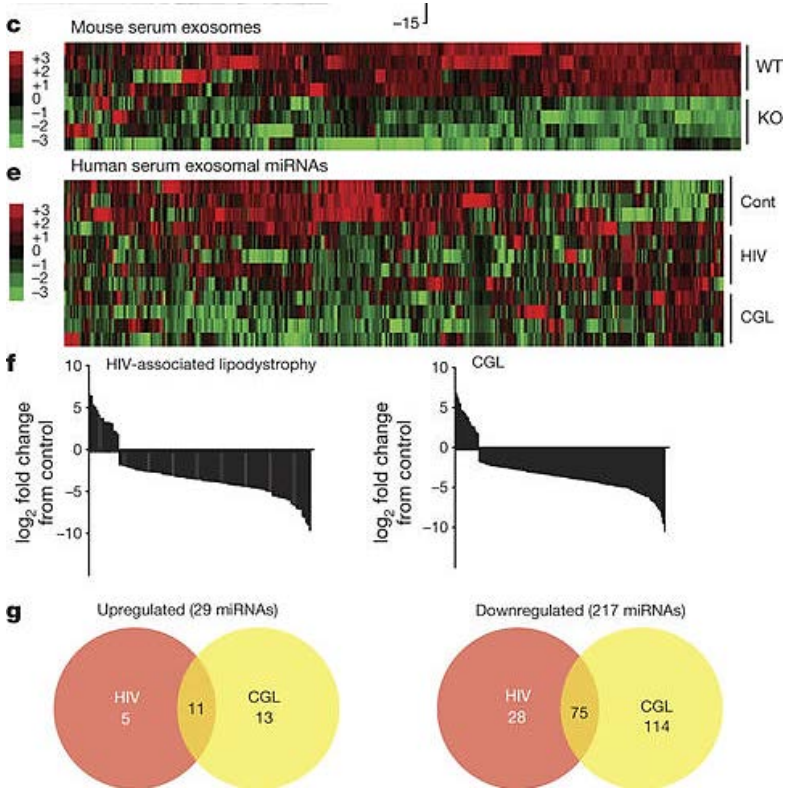
Induction of regulatory T cells decreases the risk of T1D In NOD mice

Schuster et al. 2018
R21 AI122110 (In press)

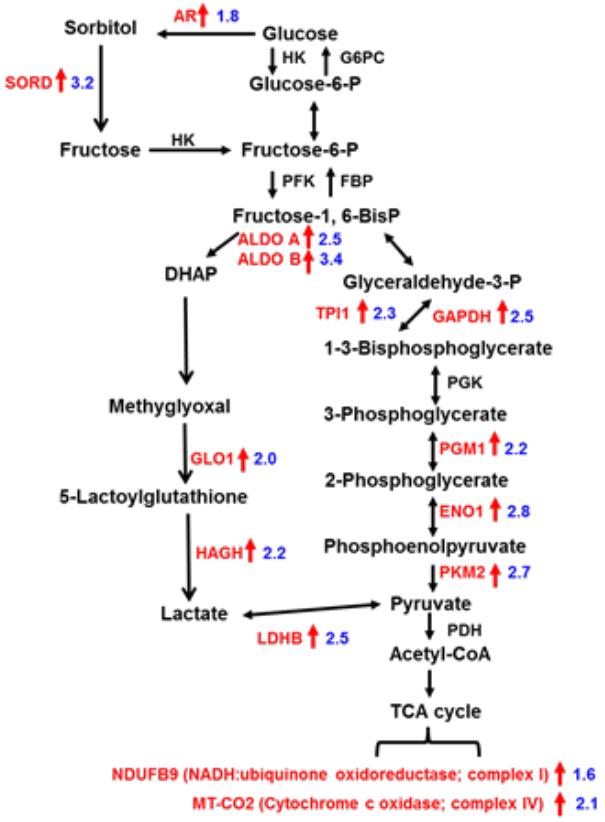
Enhancement of An Institutional Core

Joslin Diabetes Center

Bioinformatics and Biostatistics Core



Adipose-derived circulating exosomal miRNAs in mouse and human lipodystrophy. Thomou, et al, Nature, 2017



Proteomic analysis of renal glomeruli from The Medalist Study Nature Med. 2017

Supported by NIDDK

CRC/CTRC/Joslin Clinical Exercise Research Laboratory

Six currently active exercise protocols

Examples of NIH-funded studies:

R01 PI: Goodyear **Exercise Regulation of Human Adipose Tissue**

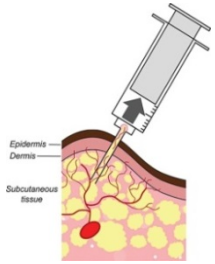
Goals of the project are to determine if: 1) exercise training causes beiging of white adipose tissue; 2) people with type 2 diabetes have defects in exercise-induced adaptations to adipose tissue; and 3) exercise training-induced changes in adipose tissue contribute to improved metabolic health.

DRC P&F PI: Lessard **Mechanisms of Exercise Resistance in Metabolic Disease**

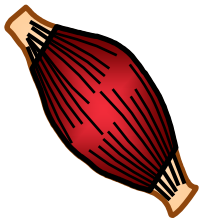
To determine whether increased angiogenic potential in response to exercise is blunted in people with impaired glucose tolerance and the role of glycemic control in the molecular response to exercise in skeletal muscle.

K23 PI: Middelbeek **Mechanisms of Exercise Effects in Obese Humans: Sex-Specific Regulation of Novel Adipose Tissue Function**

To determine if there are sex-specific adaptations to adipose tissue in response to exercise training in women and men with and without obesity, and to determine the mechanisms for these effects.



Adipose Tissue Biopsy



Skeletal Muscle Biopsy

Core Services That Are Needed

Biobanks-Specialized populations with diabetes

Examples: Type 1 with very young onset

Joslin Medalist Study (>60 years DM)

Type 2 diabetes with low BMI

Germ-free small animal facility for microbiome studies

Building of facility and initiation of operation

Support For Developing a New Core Single Cell Analysis for Molecular Phenotyping

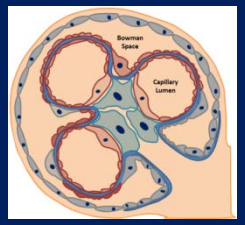
Complex Tissues

Islet

Microfluidics-Based Separation →

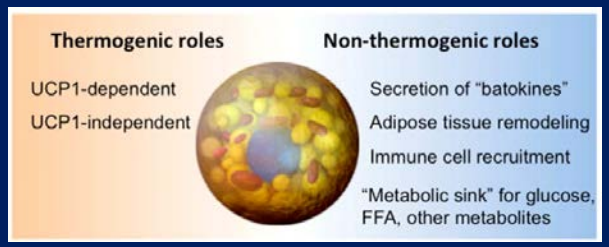
Blastocysts

Gametes



Glomerulus

Adipose tissue



DNA Sequencing

- whole genome amplification
- detection of somatic cell mutations

Transcriptomics

- Identify cell types within complex mix
- Heterogeneity of cell type transcriptional patterns
- Stem cell identity & lineage
- Identify steps in developmental trajectories or differentiation
- Splice isoform regulation

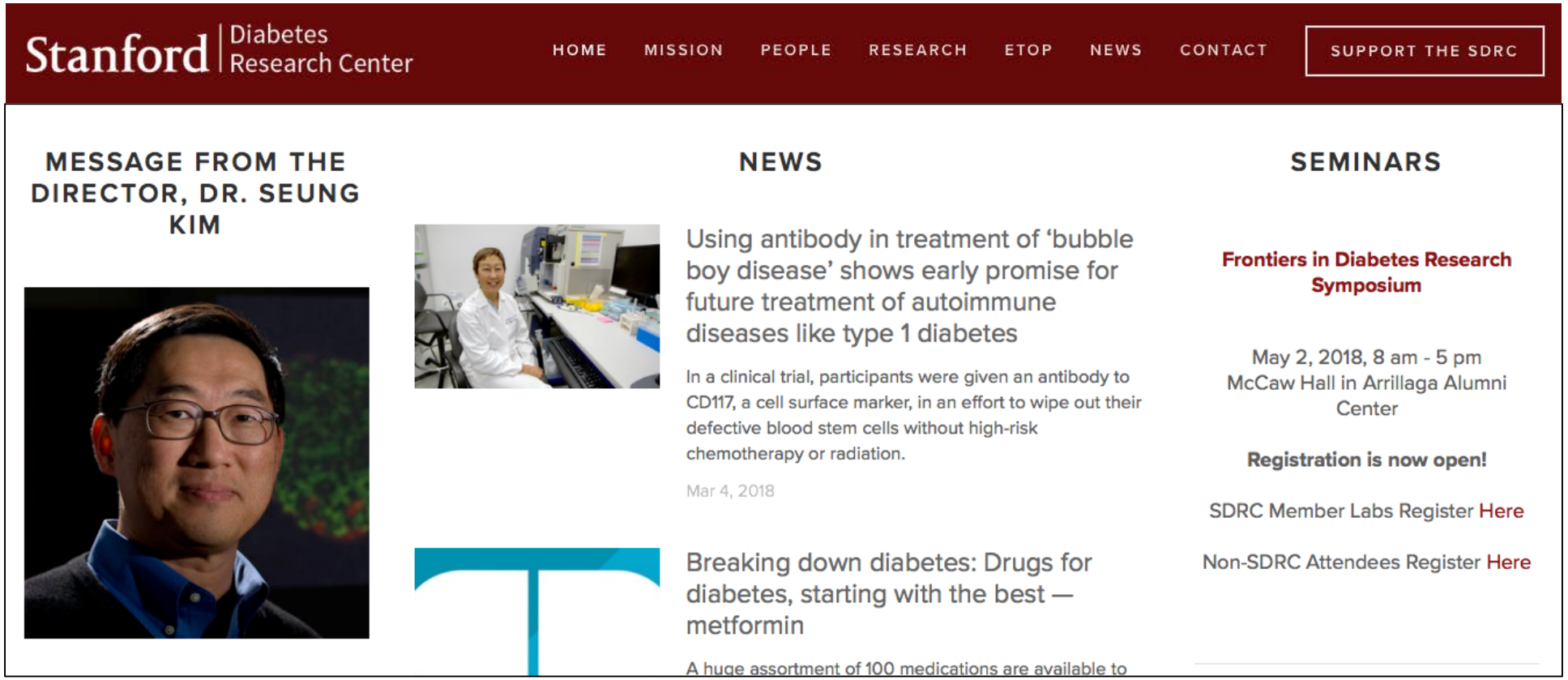
Epigenomics

- DNA methylation
- Histone modification
- Chromatin organization

Proteomics

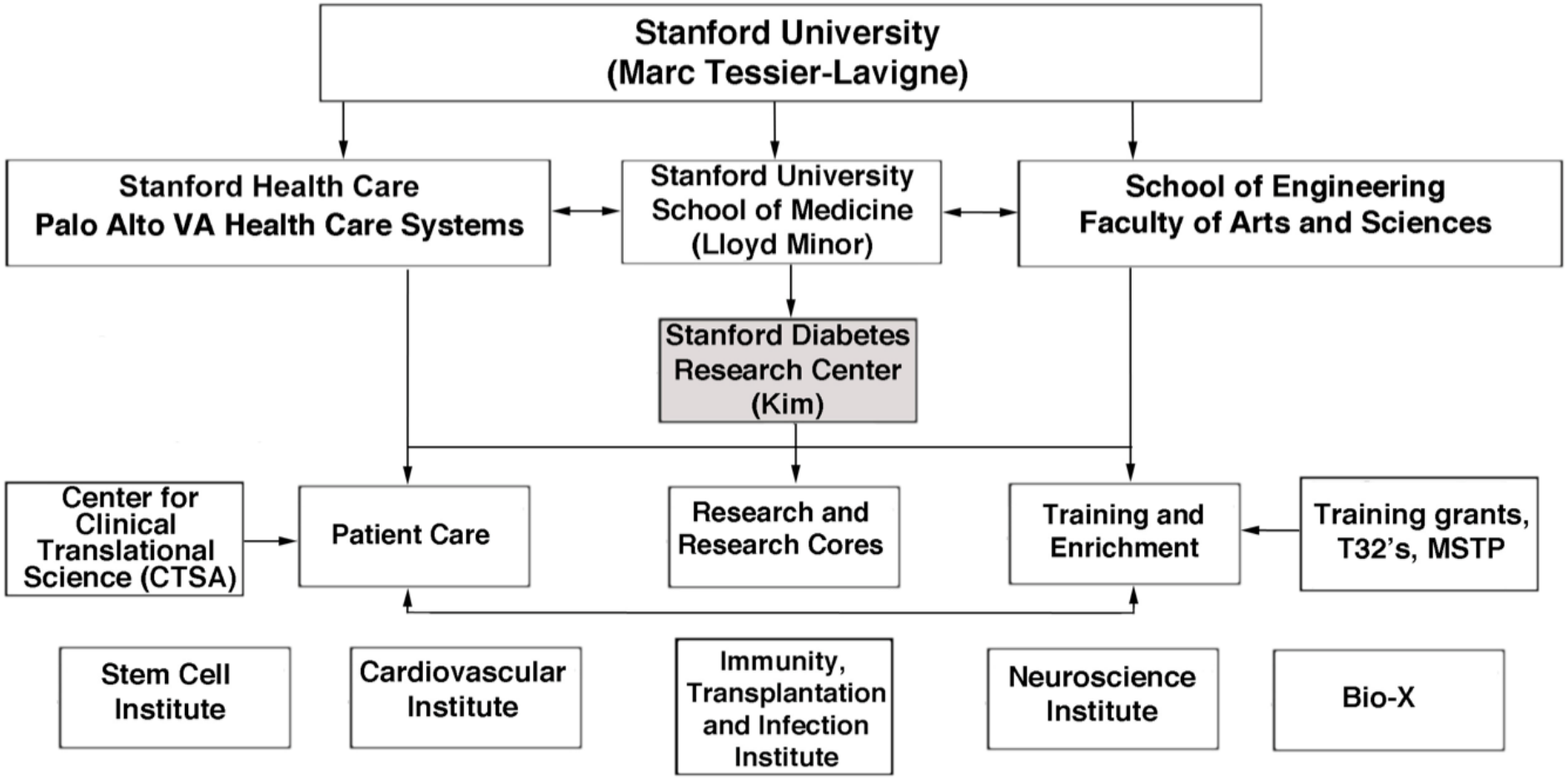
Metabolomics

https://sdrc.stanford.edu



- There are 96 **Stanford Diabetes Research Center** members
- 3 Stanford Schools: Medicine, Engineering, Arts & Sciences
- Stanford Health Care and Children's Hospital
- Dr. David Maahs, Professor of Pediatrics,
  Chief Pediatric Endocrinology, SDRC Associate Director
- Dr. Kiran Kocherlakota, SDRC Program Manager
- SDRC supports Research Cores, Pilot & Feasibility Grants, and
  Education and Training Outreach Programs

SDRC Oversight



Stanford DRC Research Base Investigators

Pancreas and islet research group

Justin Annes *
Mike Bassik
Carolyn Bertozzi
Howard Chang
Jerry Crabtree
Karl Deisseroth
Will Greenleaf
Aida Habtezion
Mark Kay
Kyle Loh
Seung Kim *
Brian Kobilka
Roel Nusse
Lucy O'Brien
Walter Park
Steve Quake
Mike Snyder
Avnesh Thakor
Monte Winslow
Richard Zare

Metabolism and signaling in diabetes

Fahim Abbasi
Tim Assimes
Salman Azhar
Vivek Bhalla
Tara Chang
Mike Cherry
Glenn Chertow
Jennifer Cochran
Ron Dalman
Manisha Desai
Brian Feldman
Chris Gardner
Kevin Grimes
Eric Gross
Geoff Gurtner
Francois Haddad
Mark Hlatky
Andrew Hoffman
John Ioannidis
Erik Ingelsson *
Sun H Kim *
Joshua Knowles *
Rick Kraemer
Won Hee Lee
Mary Leonard
Joyce Liao
Jingwen Liu
Jonathan Long
Michael Longaker
Ken Mahaffey
Tracey McLaughlin
John Morton
Tom Quertermous
Vittorio Sebastiano
Randall Stafford
Marcia Stefanick
Katrin Svensson
Marilyn Tan
Mary Teruel
Phil Tsao
Sean Wu
Joy Wu
Joe Wu

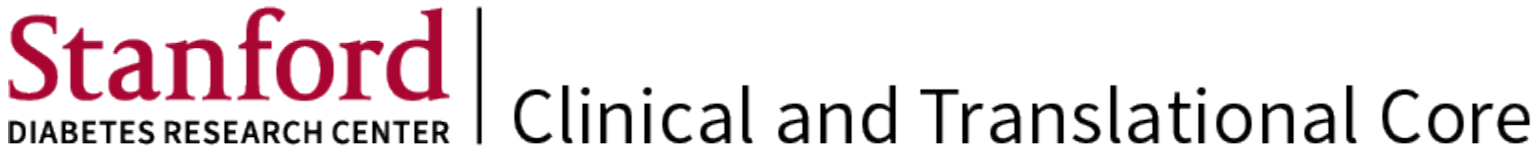
Diabetes Immunology & Transplantation

Marina Basina
Paul Bollyky *
Stephan Busque
Mark Davis
Dave DiGiusto
Ed Engleman
Gary Fathman
Holden Maecker
Everett Meyer *
Sara Michie
Nadine Nagy
Garry Nolan
Maria Grazia-Roncarolo
Judy Shizuru
Sam Strober
Brendan Visser
Irving Weissman

Diabetes Bioengineering and Behavioral Sciences

Eric Appel
Tandy Aye
Bruce Buckingham
Korey Hood *
Ngan Huang
Abby King
David Maahs *
Diana Naranjo
Latha Palaniappan
Anisha Patel Alan Reiss
Alan Reiss
Thomas Robinson
Manpreet Singh
Tom Soh
Darrell Wilson
Xiaolin Zheng


Stanford
Diabetes
Research Center


Stanford
DIABETES RESEARCH CENTER
Clinical and Translational Core


Stanford
DIABETES RESEARCH CENTER
Islet Research Core


Stanford
DIABETES RESEARCH CENTER
Immune Monitoring Core


Stanford
DIABETES RESEARCH CENTER
Genomics and Analysis Core

Stanford
DIABETES RESEARCH CENTER

Clinical and Translational Core

Biobank update

Active SDRC sample procurement, processing, and banking using central resources

Short Title	Value Type	Buffy Coat	Cryopreserved...	DNA	EDTA Plasma	Feces	Fresh Tissue	Not Specified	PBMC	Plasma	RNA	Serum	Urine	Whole Blood	Total
Grand Total	*Total Participa...*	279	127	413	7	143	146	12	285	793	255	482	245	376	3563
	Aliquot Count	990	8798	7599	35	2121	3698	23	3392	40546	4362	10250	4845	3827	90486
1-RobinsonGO...	*Total Participa...*	0	0	254	0	0	0	0	0	255	255	0	0	0	764
	Aliquot Count	0	0	6378	0	0	0	0	0	6450	4362	0	0	0	17190
1636-69-Snyd...	*Total Participa...*	70	72	0	0	65	72	0	72	72	0	71	72	69	635
	Aliquot Count	447	5765	0	0	1187	2241	0	1378	10163	0	4727	2366	622	28896
1636-70-Snyd...	*Total Participa...*	12	12	0	0	11	12	0	12	12	0	12	12	12	107
	Aliquot Count	100	1215	0	0	271	400	0	270	1891	0	977	497	119	5740
1649-Gardner	*Total Participa...*	92	0	0	0	0	0	0	0	94	0	94	0	0	280
	Aliquot Count	184	0	0	0	0	0	0	0	1857	0	340	0	0	2381
1726 Kim S D2d	*Total Participa...*	0	0	0	5	0	0	0	0	22	0	17	13	0	57
	Aliquot Count	0	0	0	5	0	0	0	0	47	0	35	24	0	111
1792-McLaugh...	*Total Participa...*	15	3	0	0	0	10	0	10	17	0	16	0	2	73
	Aliquot Count	48	36	0	0	0	120	0	23	824	0	198	0	4	1253
1941-Bucking...	*Total Participa...*	0	0	0	0	0	0	0	0	3	0	0	0	0	3
	Aliquot Count	0	0	0	0	0	0	0	0	48	0	0	0	0	48
1957 McLaughlin	*Total Participa...*	0	0	0	0	0	13	0	9	40	0	40	0	0	102
	Aliquot Count	0	0	0	0	0	165	0	63	2124	0	490	0	0	2842
1963-iPOP OG...	*Total Participa...*	38	0	0	0	0	0	12	0	47	0	38	0	0	135
	Aliquot Count	130	0	0	0	0	0	23	0	4800	0	128	0	0	5081
2-RobinsonCH...	*Total Participa...*	0	0	159	0	0	0	0	0	0	0	0	0	159	318
	Aliquot Count	0	0	1221	0	0	0	0	0	0	0	0	0	1628	2849
2093 Kim Y	*Total Participa...*	1	0	0	0	0	0	0	0	4	0	3	3	0	11
	Aliquot Count	4	0	0	0	0	0	0	0	33	0	14	15	0	66

Erik Ingelsson, MD, PhD
Cardiovascular Medicine
Cytokines

Tracey McLaughlin MD
Endocrinology/ Fat Biopsies

Research

JAMA | Original Investigation

2018;319(7):667-679.

Effect of Low-Fat vs Low-Carbohydrate Diet on 12-Month Weight Loss in Overweight Adults and the Association With Genotype Pattern or Insulin Secretion
The DIETFITS Randomized Clinical Trial

Christopher D. Gardner, PhD; John F. Trepanowski, PhD; Liana C. Del Gobbo, PhD; Michelle E. Hauser, MD; Joseph Rigdon, PhD; John P. A. Ioannidis, MD, DSc; Manisha Desai, PhD; Abby C. King, PhD

Holden Maecker, PhD
DIMC

Christopher Gardner, PhD
Nutrition Science
SDRC CTC Director

Manisha Desai, PhD
Statistics

Latha Palaniappan, MD
GIM/ Ayurvedic Body Types

Justin & Erica Sonnenburg, PhDs
Immunology/ Microbiome

Joshua Knowles, PhD
Genetic Cardiology/ SNPs

Themistocles Assimes, MD, PhD
Genetics/ SNPs

Michael Snyder, PhD
Genetics/DNA Samples

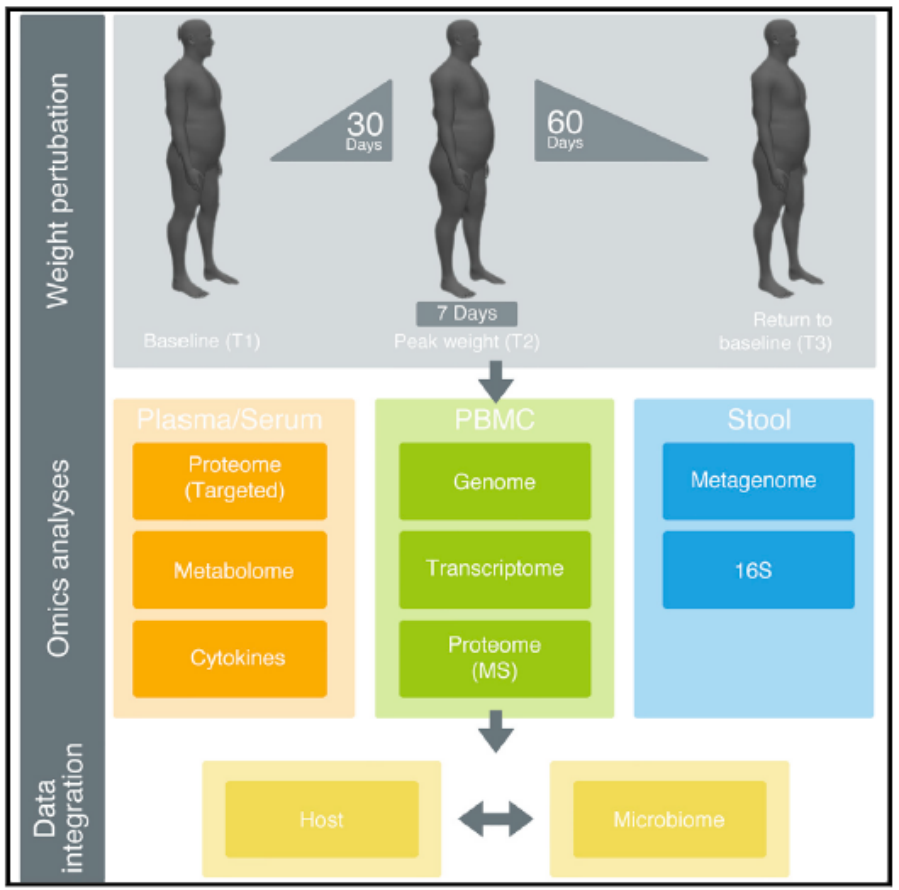
Rohit Gupta
CTRU/ Biobank

Article

Cell Systems

Integrative Personal Omics Profiles during Periods of Weight Gain and Loss

Graphical Abstract



Authors

Brian D. Piening, Wenyu Zhou, Kévin Contrepois, ..., Tracey L. McLaughlin, George M. Weinstock, Michael P. Snyder

Correspondence

tmclaugh@stanford.edu (T.L.M.), george.weinstock@jax.org (G.M.W.), mpsnyder@stanford.edu (M.P.S.)

In Brief

Extensive multi-omic profiling of the blood and microbiomes of healthy and insulin-resistant humans as they gain and lose weight reveals insights into the systemic impacts of weight gain.

P30 CONTRIBUTION TO CLINICAL CORE ACTIVITIES TOWARDS T1D SAMPLE BANKING

- IRB process in place to establish protocol with SDRC funding so that antibodies can be measured in new onset patients and samples stored for future research. (Meyer, Jensen, Wilson)
  - Two different NIH supported cores have come together in a unique interaction to help with new onset patient sample collection
  - DIMC support to Dr. Meyer and Dr. Jensen
  - CTC support to Dr. Gardner and CRC Ms. Petlura

Core service wish list
Importance for research base

GTP grade enclosed rooms will be available for clinical grade islet cell processing.

→ SDRC can contribute to clinical islet processing.

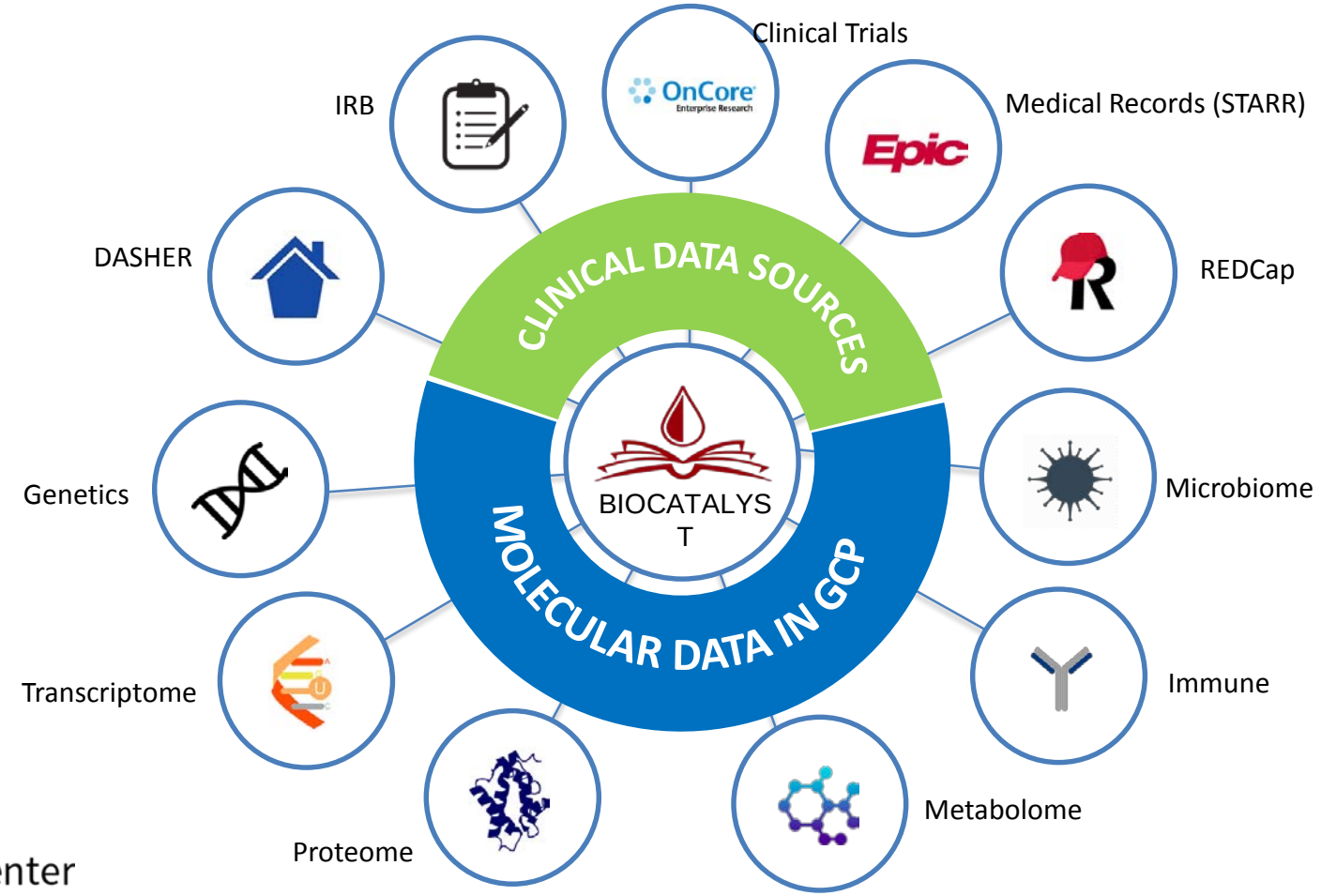
→ SDRC supported islet transplantation will be incorporated into ongoing organ/tissue and hematopoietic stem cell transplantation immune tolerance protocols.

Stanford Cell Therapy Facility Renovations Complete 6-2018

ADDITIONAL CORE DEVELOPMENT FUNDS TO SUPPORT A NEW SERVICE IN AN EXISTING CORE

BIOCATALYST

BioCatalyst is a first-of-its-kind search engine aiming to accelerate precision health by connecting clinically-annotated specimens and associated molecular data to existing inventory in a central ecosystem.



Effort support for a software developer

Stanford | Diabetes Research Center

Stanford | Diabetes Research Center

Goals

1. Promote basic and clinical diabetes research

- Research Cores, Internal grants, Education/Training Outreach Programs
- Resources for faculty recruiting and development

2. Foster SDRC-industry partnerships

3. Enhance interactions with stakeholders in the community: patients and their families, physicians, clinical care providers

4. Improved focus for philanthropy efforts

SDRC Enrichment Programs

The SDRC promotes and enhances enrichment programs that nurture interactions within Stanford and with the local, national and international community. **Leader: Justin Annes**

1. Multiple seminar series highlighting advances and challenges of diabetes research. SDRC Research Symposia, SDRC Research Forum, Medicine and Pediatric Endocrine Grand Rounds

2. Monthly Research Affinity Group sessions-devoted to data sharing or journal article discussions.

3. SDRC professional development for junior faculty

4. The SDRC has nurtured research education opportunities for patients

P&F Awards 2017 (Director: Dr. Rick Kraemer)

PI	Affinity	Designation/Dept	Title
Annes, Justin	Pancreas and islet biology	Asst Prof DoM (Endocrinology)	Mitochondrial Dysfunction Promotes b-Cell Failure and diabetes via a Previously Unrecognized Mechanism: Protein Hyper-Succinylation
Appel, Eric	Bioengineering and behavioral	Asst Prof Materials Science and Engg	Novel ultra-fast insulin formulations for diabetes treatment
Lee, Jennifer	Metabolism and signaling	Associate Prof Medicine (Endocrinology)	Diabetes Precision Phenotyping & Outcomes Prediction in Health Care
Meyer, Everett	Immunology and transplantation	Asst Prof DoM (Bone Marrow Transplantation)	Therapeutic targeting of islet-infiltrating invariant NKT cells in diabetes to promote islet tolerance
Palaniappan, Latha	Metabolism and signaling	Clinical Prof DoM (General Medicine)	Elucidating the mechanisms of Diabetic Cardiomyopathy through Exosome Profiling, A Diabetes Research Center Collaborative Initiative
Soh, H. Tom	Bioengineering and behavioral	Prof Electrical Engineering	Real-time biosensor for continuous in vivo detection of glucose
Thakor, Avnesh	Pancreas and islet biology	Asst Prof Radiology	A novel collagen based cryogel bioscaffold that generates oxygen and promotes angiogenesis for islet transplantation
Zheng, Xiaolin	Bioengineering and behavioral	Associate Prof Mech Engg	Breath Acetone Sensor towards Non-invasive Diabetic Monitoring

SDRC Outreach Programs

The SDRC promotes and enhances outreach programs that help educate and provide support for local patient community.

Leader: Marina Basina

Class	Date	Time	Location
Diabetes Wellness Group	2nd Wednesday of every Month	6:00 – 8:00 pm	Stanford Health Library Main Branch- 2nd Floor
Diabetes and Insulin; Tips and Tools	4th Thurs of Jan, April, July, Oct	3:00 – 5:00 pm	Stanford Family Medicine Clinic 4th Floor Conference Room
Insulin Pump and Sensor Workshop	4th Thurs of Feb, May, Aug, Nov	3:00 – 5:00 pm	Stanford Family Medicine Clinic 4th Floor Conference Room
Advanced Pump and Sensor Workshop	4th Thurs of Mar, June, Sept	3:00 – 5:00 pm	Stanford Family Medicine Clinic 4th Floor Conference Room

Stanford HEALTH CARE
STANFORD MEDICINE

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Strategic Research Initiatives in the SDRC Linking Basic and Clinical Research

1. Development of an islet transplantation and tolerance program at SHC and Packard Children's with support from the Stanford DRC

2. Engineering practical interventions to maximize artificial pancreas benefits in T1D

3. JDRF-funded study on new platforms to measure T1D biomarkers

4. Human pancreas atlas (with CZI and CZ BioHub)

5. Development of specific therapeutics for insulin resistance, an unmet clinical need

6. Investigating mechanistic links between diabetes, chronic pancreatitis and pancreatic cancer. Development of a NCI-supported center

Stanford | Diabetes Research Center

How are clinic and research working together?

- IRB process in place to establish protocol with SDRC funding so that antibodies can be measured in new onset patients and samples stored for future research. (Meyer, Jensen, Wilson, etc)

- Clinical trials in AP in Pediatrics and Adult clinic population (Buckingham, Basina, etc)

- Pilot & Feasibility awards that include clinical research (Naranjo)

- Weekly seminar series in 4 thematic areas to bring together clinicians and basic and clinical researchers


Stanford Diabetes Research Center Presents
"Frontiers in Diabetes Research Symposium"
8am-5pm | May 2nd, 2018
McCaw Hall, Arrillaga Alumni Center, Stanford University
Stanford | Diabetes Research Center


UCSD/UCLA
Diabetes Research Center

Biomedical Research Cores

- Transgenic, CRISPR Mutagenesis, and Knock-Out Mouse Core
- Genomics and Epigenetics Core
- Metabolic and Molecular Physiology Core
- Targeted Pathway Analysis Core
- Human Genetics Core

Targeted Pathway Analysis Core

Julian Whitelegge, PhD, UCLA, Core Director
Oswald Quehenberger, PhD, UCSD, Core Co-Director

- The overall premise is to steer investigators through introductory "discovery" lipidomics and proteomics experiments toward statistically powered, targeted experiments aimed at measuring biological pathways relevant to diabetes and metabolism.

- This structure is designed to leverage state-of-the-art approaches to these omics technologies at UCLA and UCSD, while addressing the NIH's guidelines for improved rigor and transparency. The TPAC consolidates mass spectrometry – omics experiments within a single core, with lipidomics led by Oswald Quehenberger at UCSD and proteomics led by Julian Whitelegge at UCLA.

- Well integrated with other cores.

Genomics and Epigenetics Core

- Director Chris Glass, MD PhD
- Operates under the auspices of the Institute for Genomic Medicine (IGM) at UCSD
- UCSD has made major investments in instrumentation to support next gen sequencing, data acquisition and analysis
- DRC funds support salaries, supplies and reagents for DRC investigators, leveraging university investment in instrumentation and technology development.

Additional Core Services

- High end microscopy
- High content/ high throughput screening for chemical genomics, siRNA, CRISPR/Cas9
- Untargeted metabolomics

THE UNIVERSITY OF ALABAMA AT BIRMINGHAM

Knowledge that will change your world

UAB Diabetes Research Center

UAB Diabetes Research Center

DRC Cores Integrate Metabolic and Vascular Technologies

CORE A
New Institutional Core

CORE B has subcores that support NORC, CCC, Shock Aging Center,

CORE C has subcores that support:
NORC, CTSA

CORE D
Serves DRC only

Biomedical Research Cores

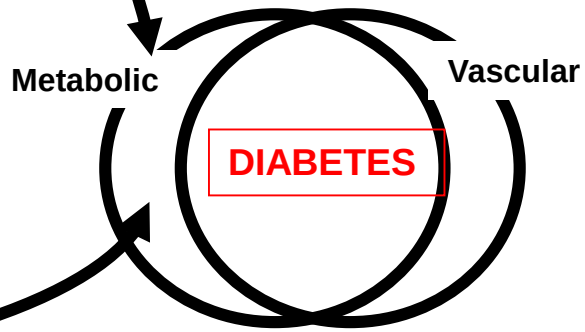
BIO-ANALYTIC REDOX BIOLOGY CORE
Scott Ballinger, Doug Moellering, Steve Barnes, Victor Darley-Usmar

ANIMAL PHYSIOLOGY CORE
Tim Nagy, Bob Kesterson

HUMAN PHYSIOLOGY CORE
Barbara Gower, David Calhoun

Translational Research Core

INTERVENTIONS & TRANSLATION CORE
Andrea Cherrington, Beth Lewis, Mona Fouad

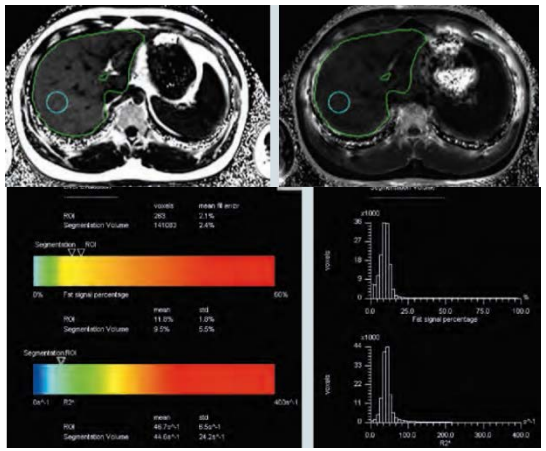


Bioanalytic Redox Biology Core

1. *In Situ/Ex Vivo* analysis of mitochondrial function in live & intact tissue
   A. Permeabilized skeletal muscle fibers using high resolution respirometry
   B. Aortic rings using Seahorse Extracellular Flux (XF) analyses
   C. Whole white adipose tissue using Seahorse XF analyses
   D. Precision Cut Tissue Slices (liver, hippocampus, retina)

Human Physiology Core

1. MRI/MRS of tissues and organs for accurate quantification of tissue composition using state-of-the art software and combined MRI/MRS automated output.
2. 24-hour whole room indirect calorimetry for total, resting, sleeping, meal-related, and activity-related energy expenditure and substrate utilization.



LiverLab images and sample MRS report.

Animal Physiology Core

1. In vivo assessment of brown adipose tissue in rodent models using MRI/MRS.
2. Creating gene knockouts using TALEN and CRISPR/CAS9 nucleases in mice, rats, and zebrafish.

Interventions & Translation Core (ITC)

1. Spatial analysis techniques / Geographic Information Systems (GIS) that allow us to examine the relationship between built and contextual environment, health behaviors, and outcomes by providing geographically linked data.
2. Maintains a ITC Community Advisory Board and a Primary Care Diabetes Coalition that provide platforms for researchers to engage communities of patients and primary care professionals.

UAB Diabetes Research Center

Desired Services

Bioanalytic Redox Biology Core
Enhanced metabolomics platforms specifically designed to assess oxidized lipids and oxidative stress

Human Physiology Core
- Comprehensive analyses of adipose tissue and adipocytes involving mRNA, protein, macrophage infiltration/polarization, histology, immunohistochemistry, crown-like structures, and cell size.
- Lab for human circadian biology: sleep studies, time-feeding studies

Animal Physiology Core
The ability to quantify energy expenditure and circadian clock mechanisms in rat models. Numerous diabetes researchers are moving to rat models and having a rat indirect calorimeter is becoming necessary.

Interventions & Translation Core (ITC)
Electronic and mobile health technology exemplified by emtHealth – (electronic/mobile/telemedicine Health). The goal would be to provide assistance and guidance for technology development and creative approached to assessment and interventions.

New Core Development Requiring R&D

Near-infrared spectroscopy (NIRS). Allows non-invasive continuous monitoring of the local oxygen status. This method measures light absorbance to calculate oxy-hemoglobin and deoxy-hemoglobin, which provides an indirect measure of mitochondrial activity.

Methods for assessing hepatic versus muscle contributions to glucose homeostasis without clamps or intravenous tracers (e.g., data mine OGTT, IVGTT, Clamp studies, or use of oral tracers), and an approach to quickly assess insulin sensitivity (combine impedance spectroscopy, fasting insulin, FFA, metabolomics)

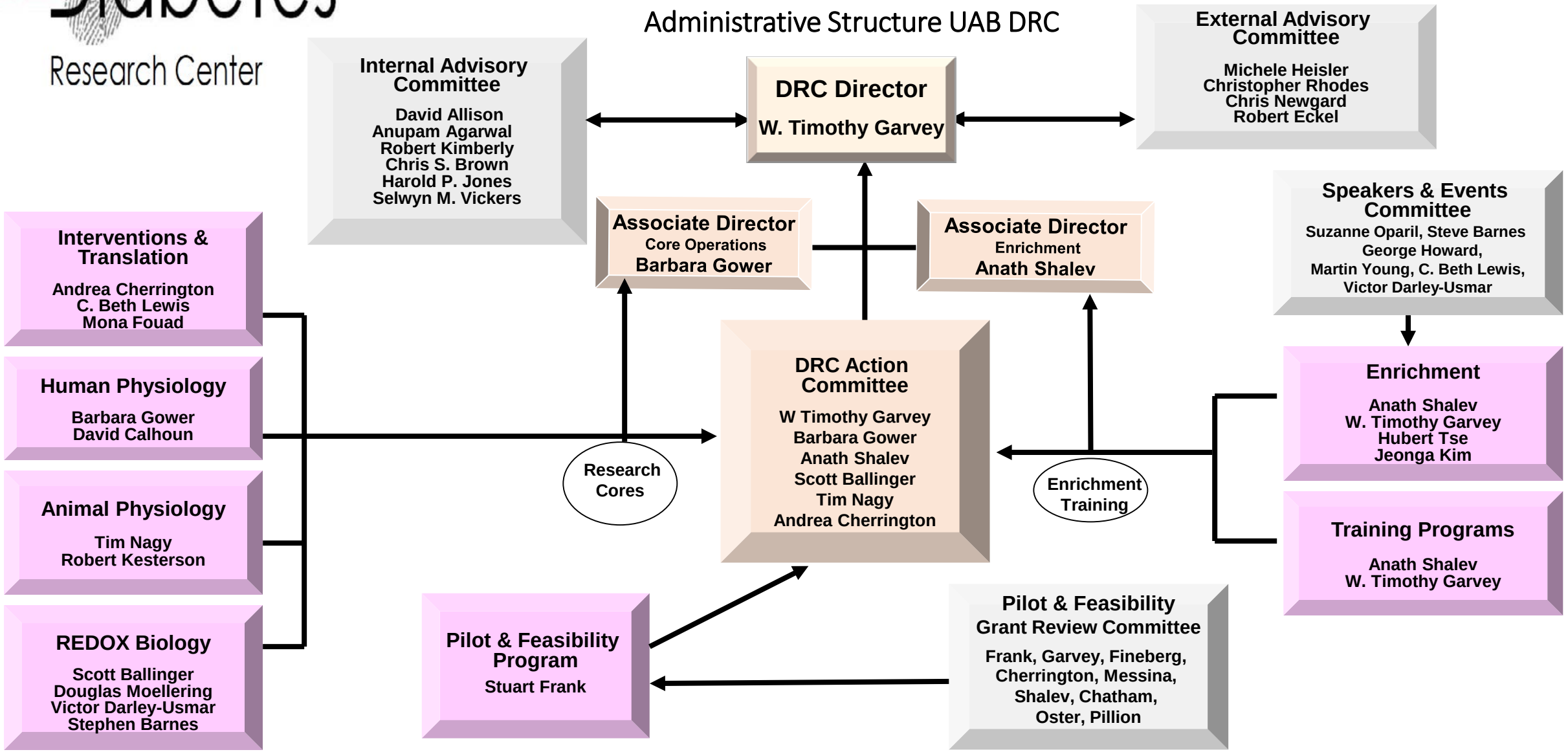
Given inaccuracy of food records, biomarkers of food intake (e.g., metabolomics, cell membranes, blood fractions) to assess dietary intake.

Quantification and characterization of cell free RNA species and exosomes as mediators of cross-organ communication

Overall Center

UAB Diabetes Research Center

Administrative Structure UAB DRC



THE UNIVERSITY OF ALABAMA AT BIRMINGHAM

Contributions to Institutional Cores

Bioanalytic Redox Biology Core

DRC services attracted other investigators studying intrinsic lung disease, immunology, cancer, and neonatal medicine, resulting in recent institutional support to develop subcores tailored to other research communities and “to continue your growth and promotion of interdisciplinary research, education, and service”

Human Physiology Core

1. Help sustain experienced faculty with methodological expertise, evolution of technologies, and access to high quality services
2. Attract faculty for recruitment and new users at UAB to diabetes-related research

Animal Physiology Core

Dr. Nagy received a DRC P&F grant to develop MRI/MRS methods for the *in vivo* determination of brown adipose tissue in rodents. Once the method was proven and published, the core began offering the assessment as a service.

Interventions & Translation Core (ITC)

No involvement in institutional cores

UC San Francisco
Diabetes Research Center

UCSF Diabetes Research Center
Biomedical Research Cores

- Islet Production Core
- Microscopy Core
- Mouse Genetics Core
- Cytometry & Cell Sorting Core

- Regional Core: Islet Production

UCSF Diabetes Research Center
Biomedical Research Cores

- Islet Production Core
- Regional Core: Islet Production

Human islets

Juvenile islets

T2DM islets

Surgical islets

Collaboration with Vanderbilt and Stanford DRCs

UCSF Diabetes Research Center Biomedical Research Cores

- Microscopy Core

The Biological Imaging Development Center is an interdisciplinary center configured to assemble, test, and apply emerging light microscopy techniques and technologies.

Developed practical whole pancreas imaging techniques with DRC development and equipment funds.

UCSF Diabetes Research Center Biomedical Research Cores

- New service for Islet Core

Exosome/extracellular particle purification/characterization

UCSF Diabetes Research Center Biomedical Research Cores

- Development funds

Collaboration with the Chan Zuckerberg Medical Sciences Biohub at UCSF Mission Bay

UC San Francisco
Diabetes Research Center

UNIVERSITY OF CHICAGO DIABETES RESEARCH CENTER "THE DRTC" AND NIDDK-SUPPORTED BIOMEDICAL RESEARCH CORES

Graeme Bell

March 28, 2018

Bethesda, MD

NIDDK-Supported Biomedical Research Cores

- Cell Biology Core
- Genetics and Genomics Core
- Physiology Core
- Circadian Rhythms Core – Proposed National Core

NIDDK-Supported Biomedical Research Cores

- Cell Biology Core
  - Provide pancreatic islets, adipocytes and immune cells from normal and diabetic humans and mice
  - implement new experimental approaches that provide better physiological and pathophysiological context for discovery
- Genetics and Genomics Core
  - Implement genetic studies in diabetes research
  - Maintain the Monogenic Diabetes Registry as a clinical and genetic resource and living biobank for diabetes research locally and nationally
- Physiology Core
  - Provide support for clinical studies of insulin secretion and action
  - Large-scale analyses of panceatic islet mass, size, distribution in human and mouse
  - Metabolic phenotyping of mice including metabolic cage studies and body composition by DEXA
- Circadian Rhythms Core – Proposed National Core
  - Builds on resources available in the Bass Laboratory at Northwestern University
  - Analyze behavioral and clock function in vivo and in vitro

Unique or Innovative Core Services

- Monogenic Diabetes Registry as a clinical and genetic resource and living biobank for diabetes research
- Detailed quantitative and qualitatve analyses of human pancreatic islets *in situ*

NIDDK Diabetes Research Center (P30) Funding

- Allowed us to develop and maintain the Monogenic Diabetes Registry which is a national and international resource for research of patients with primary genetic forms of diabetes

What Core Service do you Most Wish was Available in the DRTC

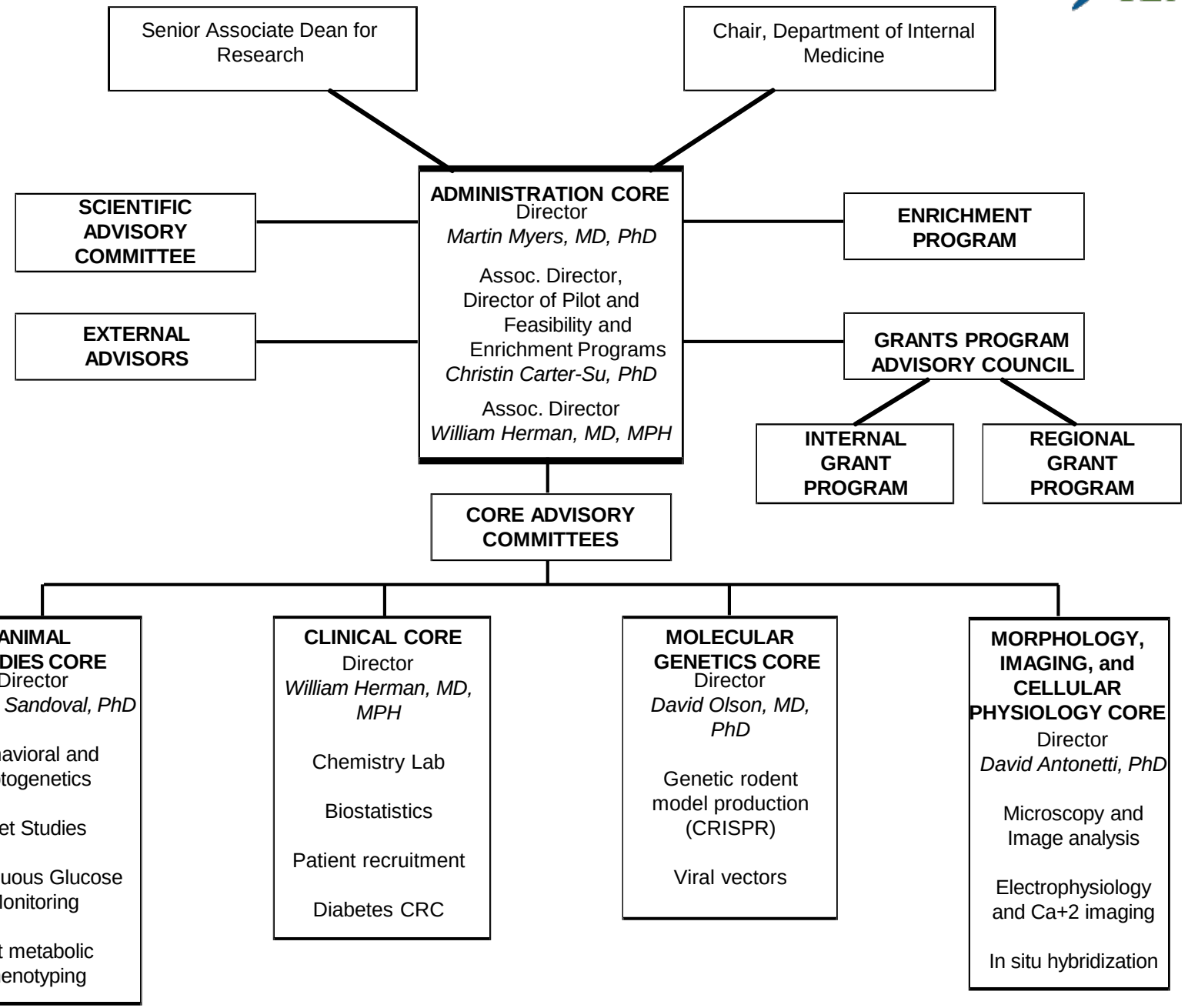
- The DRTC works closely two other NIDDK-funded P30 programs, the Chicago Center for Diabetes Translation Research and the Digestive Disease Research Center Core. They allow investigators to access a wide range of additional services including cost-effective analyses of diabetes therapies and microbiome studies. The three programs are closely integrated so that we are able to provide new services efficiently and cost-effectively.

Core Development

- Epitranscriptomics
- Post-translational modifications of proteins

- The University of Chicago has exceptional local expertise in both areas and could expand both into national cores

MICHIGAN DIABETES RESEARCH CENTER



MICHIGAN MEDICINE
UNIVERSITY OF MICHIGAN

DIABETES RESEARCH CENTERS

Michigan Diabetes Research Center

MICHIGAN DIABETES RESEARCH CENTER CORES (AND INTERACTIONS WITH INSTITUTIONAL CORES)

ANIMAL STUDIES CORE
Director
Darleen Sandoval, PhD

Behavioral and Optogenetics

Islet Studies

Continuous Glucose Monitoring

Rat metabolic phenotyping

CLINICAL CORE
Director
William Herman, MD, MPH

Chemistry Lab

Biostatistics

Patient recruitment

Diabetes CRC

MOLECULAR GENETICS CORE
Director
David Olson, MD, PhD

Genetic rodent model production (CRISPR)

Viral vectors

MORPHOLOGY, IMAGING, and CELLULAR PHYSIOLOGY CORE
Director
David Antonetti, PhD

Microscopy and Image analysis

Electrophysiology and Ca+2 imaging

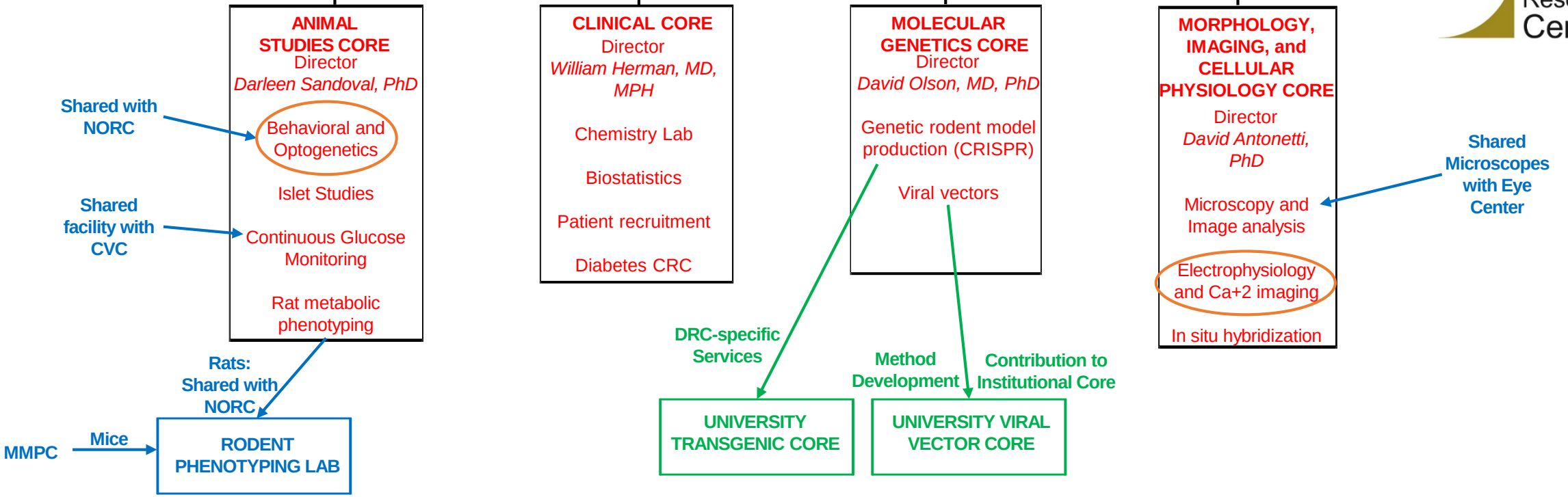
In situ hybridization

MICHIGAN MEDICINE
UNIVERSITY OF MICHIGAN

DIABETES RESEARCH CENTERS

Michigan Diabetes Research Center

MICHIGAN DIABETES RESEARCH CENTER CORES (AND INTERACTIONS WITH INSTITUTIONAL CORES)

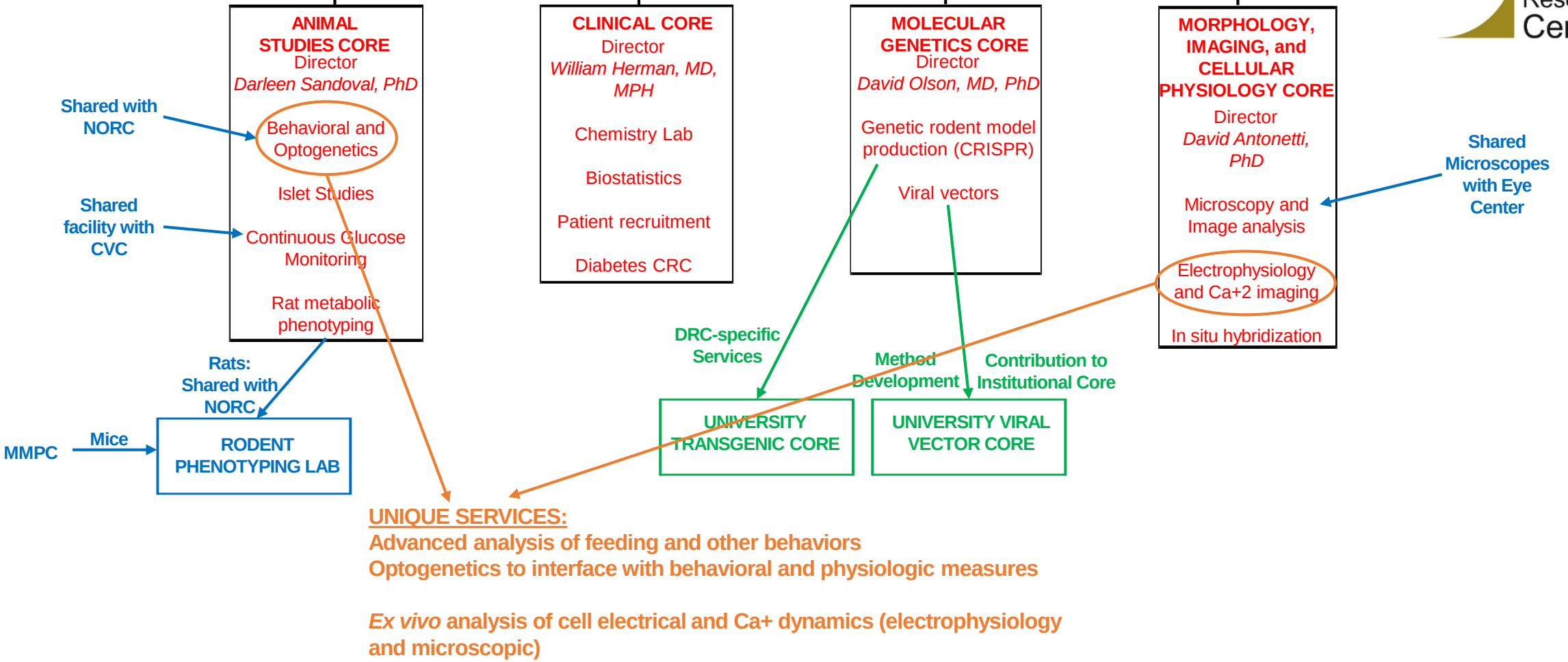


MICHIGAN MEDICINE
UNIVERSITY OF MICHIGAN

DIABETES RESEARCH CENTERS

Michigan Diabetes Research Center

MICHIGAN DIABETES RESEARCH CENTER CORES (AND INTERACTIONS WITH INSTITUTIONAL CORES)



UNIQUE SERVICES:

Advanced analysis of feeding and other behaviors

Optogenetics to interface with behavioral and physiologic measures

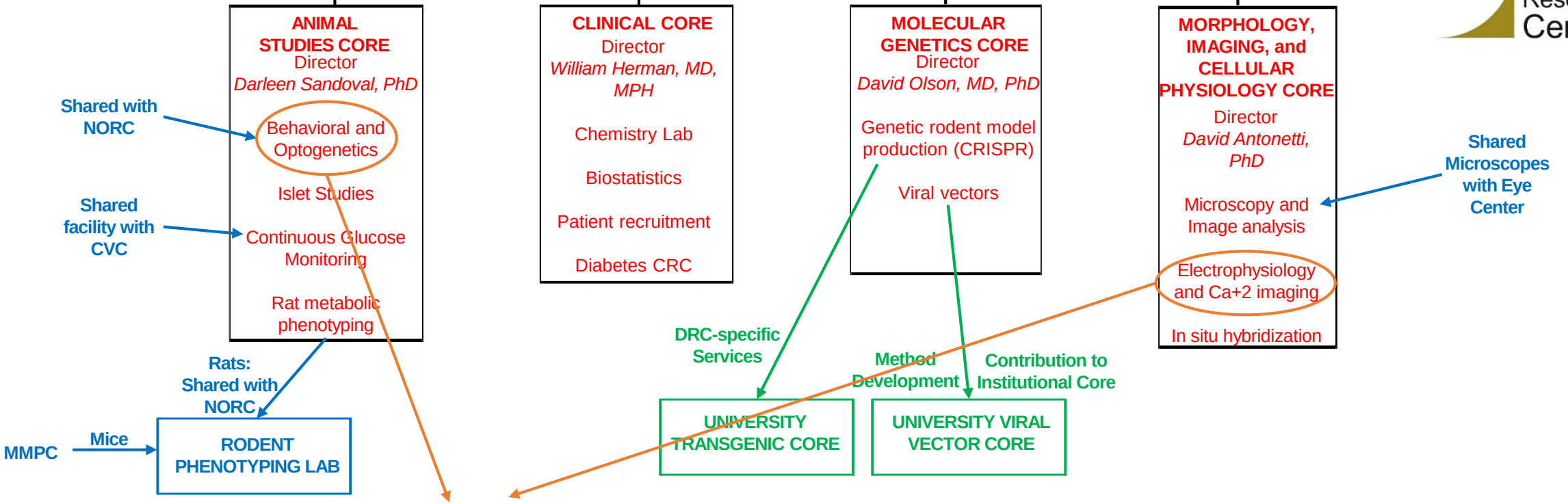
***Ex vivo* analysis of cell electrical and Ca+ dynamics (electrophysiology and microscopic)**

MICHIGAN MEDICINE
UNIVERSITY OF MICHIGAN

DIABETES RESEARCH CENTERS

Michigan Diabetes Research Center

MICHIGAN DIABETES RESEARCH CENTER CORES (AND INTERACTIONS WITH INSTITUTIONAL CORES)



New services that we want to develop (will take $ investment):

Additional assays of in vivo function using imaging or electrical readings- this includes in vivo insulin secretion from individual islets, in vivo Ca+2 imaging in the CNS and elsewhere, and in vivo reporter activity for gene expression, etc.

Also, development of less-invasive continuous glucose monitoring technology

Penn Diabetes Research Center

Mitch Lazar, M.D., Ph.D.

Klaus Kaestner, Ph.D.

Penn Diabetes
Institute for Diabetes, Obesity & Metabolism
IDOM
Home of the PENN Diabetes Research Center NIH DK 19525

http://www.med.upenn.edu/idom/

NIDDK Diabetes Research Center Directors' Meeting

March 28, 2018

NIDDK-Supported Research Cores in the Penn DRC

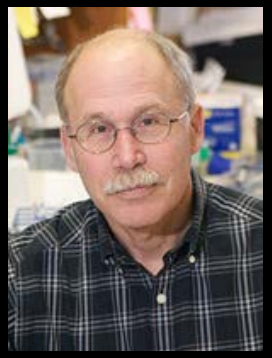
Islet Cell Biology
Doris Stoffers

Mouse Phenotyping, Physiology, and Metabolism
Joe Baur

RIA/Biomarkers
Mike Rickels

Metabolomics
Josh Rabinowitz
Princeton

Functional Genomics
Klaus Kaestner



Transgenic & Chimeric Mouse
Steve Liebhaber **Jorge Henao-Mejia**

Viral Vector
Julie Johnston

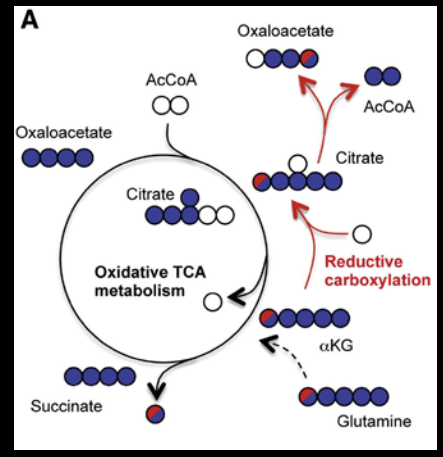
- White indicates DRC-specific cores
- Red designates ‘institutional’ cores

Unique and Innovative Core Services

1) Metabolic flux studies in mice.

Mouse Phenotyping, Physiology & Metabolism Core collaboration with Metabolomics Core (Princeton)

- Mouse core puts in the catheter, gives tracer, and collects samples which are sent to the Metabolomics Core for analysis.



2) Single cell and single nucleus RNA-seq.

Functional Genomics Core

- Single cell: Fluidigm and Drop-seq are available with technical support.

- Single nucleus: Powerful technique that allows high throughput analysis of transcription in single cells that cannot be preserved or lose critical properties when dissociated (adipose, brain, liver, pancreas).

Penn DRC Contribution to an Institutional Core

Functional Genomics Core

The FGC was originally a DRC-specific core.

It purchased the first next generation sequencer on campus (with philanthropic support and funds from the School of Medicine to the IDOM/DRC), initially offered it only to DRC members.

Because of its success, the School of Medicine contributed $4.8 million in new equipment to expand to a school-wide Next Gen Sequencing core.

This also dramatically increased the capabilities of the core for DRC users, and allowed DRC funding to be earmarked for bioinformatic services.

A Core Service That Would Help the Research Base

Proteomics

Many DRC members are doing proteomics.

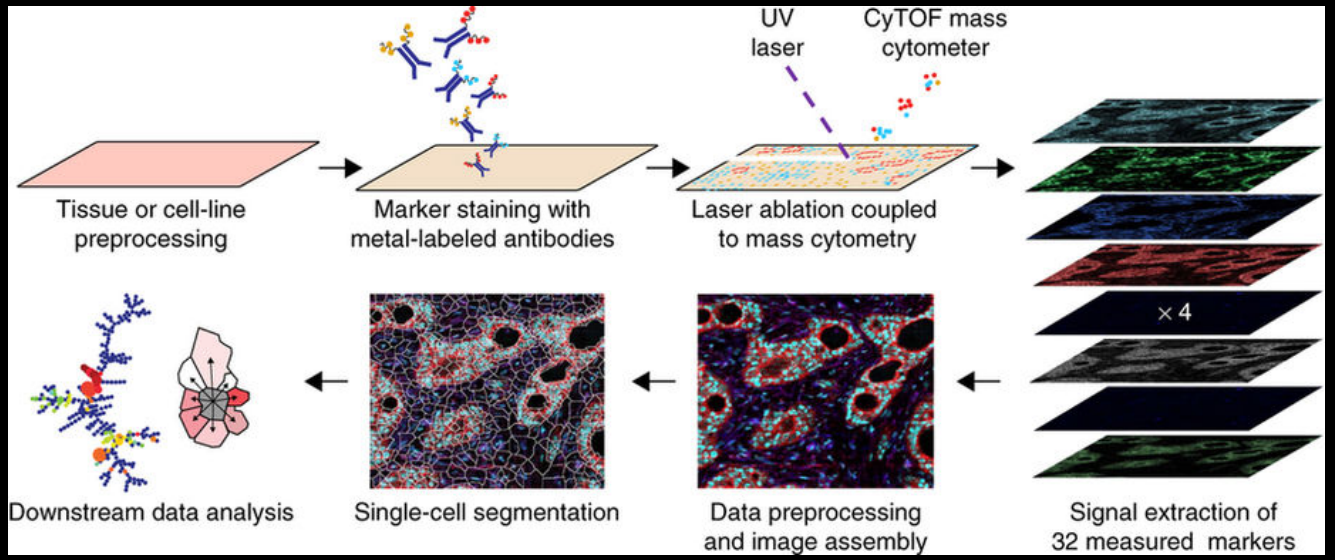
Currently it is performed at an institutional proteomics core, directed by Ben Garcia.

DRC members use this core, but it is costly and the queue is long.

DRC members would benefit from subsidized prices and preferential access, which they enjoy at other DRC-supported institutional cores.

An Ideal Expansion Area

Imaging Mass Cytometry



Powerful technology allowing simultaneous imaging of dozens of molecules in the same tissue section.

Currently available at Penn for study of human islets, via NIDDK-funded Human Pancreas Analysis Program.

Ideally could leverage expertise and antibody panels to expand with a second machine for DRC investigators intersted in mouse, as well as in other important tissues including liver, pancreas, adipose, brain.

Penn Diabetes Research Center

NIDDK Diabetes Research Center Directors' Meeting
Bethesda, MD
March 28, 2018

Steven E. Kahn
VA Puget Sound Health Care System
University of Washington
Seattle, WA

List of Biomedical Cores

A. Cell Function Analysis Core

B. Cellular and Molecular Imaging Core

- CFRTC (P30) and VA Puget Sound

C. Quantitative and Functional Proteomics Core

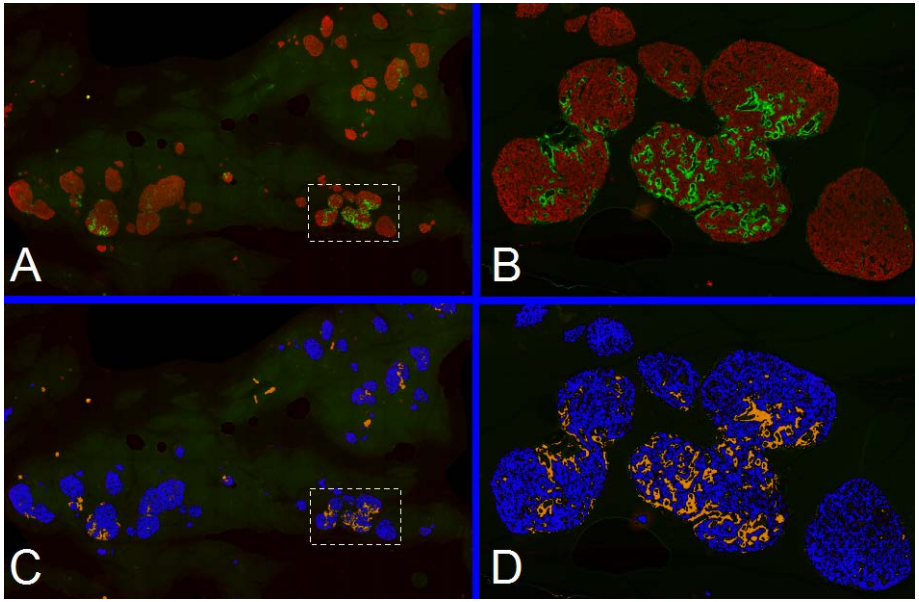
D. Vector and Transgenic Mouse Core

Unique or Innovative Services

1. Test panels of GFP-expressing adeno-associated virus (AAV) serotypes that allow affiliates to test and determine the optimal AAV before using it in their diabetes-related models
2. CRISPR/Cas9 services in stem cells

Contribution of NIDDK DRC Funding to an “Institutional” Core

1. Cellular and Molecular Imaging Core – rapid large area scanning and quantitative analysis on multichannel fluorescence images



“Wish List” - Core Services Available at DRC that are not Currently Available

Bioinformatics and Biostatistics

a) Analysis and integration of RNA-Seq (organ and single cell), ChIP-Seq, metabolomic, proteomic and lipidomic data
b) Biostatistics related to metabolism (diabetes, obesity) projects

Use of Core Development Funds to Support New Services

1. Establish Bioinformatics and Biostatistics Core

   R&D investment would be in support of:

   a) Training faculty and personnel in required sample preparation and analyses

   b) Required equipment

2. Fluorescence In Situ Hybridization (FISH)

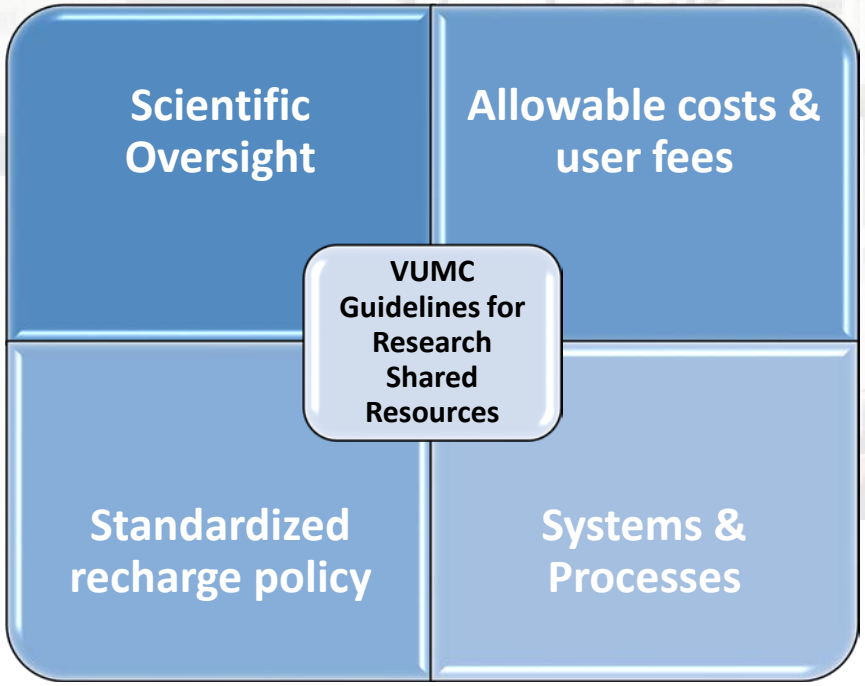
Vanderbilt Diabetes Research Center

SCHOOL OF MEDICINE
VANDERBILT UNIVERSITY

Vanderbilt Model
Integrated Approach to Shared Resources

Consistent policies across all cores or shared resources.

- Dedicated institutional and administrative support
- Business plan development
- Compliance and training
- Guiding principle: One service = one price for federal grants



VANDERBILT UNIVERSITY MEDICAL CENTER

Vanderbilt Cores

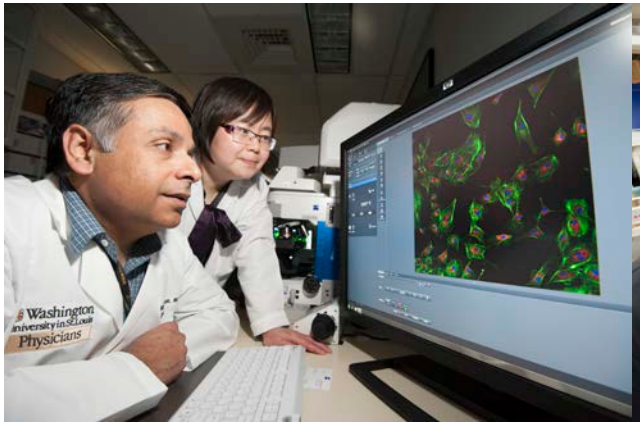
- **Islet Procurement and Analysis Core**
- **Hormone Assay and Analytical Services Core**
- **Metabolic Physiology Shared Resource**
- **Cell Imaging Shared Resource**
- **Transgenic Mouse/ESC Shared Resource**

Wish/Challenge List

- **Combine metabolomics and tracers to probe cellular metabolic flux**
  - **Mouse, human in vivo and ex vivo**
- **Human tissue collection/processing from phenotyped individuals for multi-modal analysis**
  - **Liver, muscle, fat (pancreas)**
  - **Post-intervention (exercise, diet, etc.)**
  - **Personalized metabolism**

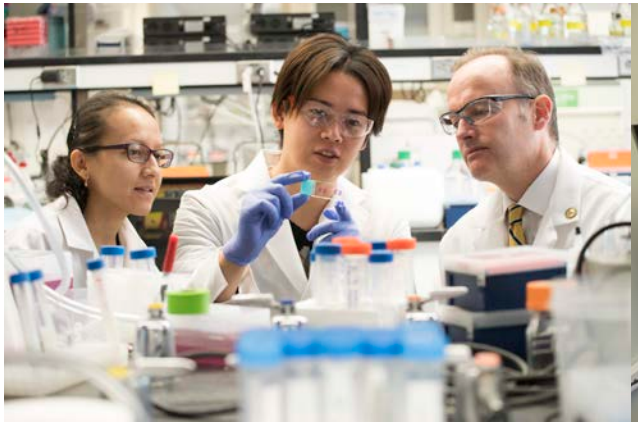
Thoughts

- **Support innovation and new core services provided by DRC-supported cores**
- **Incentivize and facilitate introduction and adaptation of new technologies by DRC investigators (lower the activation bar)**
- **Provide consultative services for diabetes-specific experimental approaches**
- **Multi-center initiatives to support diabetes-related research**




Diabetes Research Center

Washington University in St. Louis



Biomedical Research Cores

- Cell and Tissue Imaging—new, integrated with recently established state-of-the-art institutional resource
- Diabetes Models Phenotyping
- Metabolic Tissue Function
- Metabolomics
- Translational Diagnostics
- Transgenic and ES Cell

Innovative Core Services

Metabolomics Core

Integrated services for biomarker development from untargeted/targeted methods for discovery to development of FDA-compliant clinical assays

- Novel C24:0/C16:0 ceramide assay for cardiovascular risk (Linda Peterson); utility as predictor of cardiovascular mortality established in ~6000 subjects

Cell and Tissue Imaging Core

Correlative Imaging with light and electron microscopy workflows

- Fluorescence labeling to spatially target cilia in isolated mouse islets for subsequent three-dimensional ultrastructural imaging using serial block face Focused Ion Beam-SEM (Dave Piston and Jing Hughes)

Cryo EM

- Single particle cryo EM to generate atomic-level structures of the KATP channel (Colin Nichols)

DRC Contributions to Institutional Cores

- Translational Diagnostics Core → major stakeholder in Washington University Core Laboratory for Clinical Studies
  - Supported purchase of Erenna instrument and personnel time to develop digital single molecule counting approach for insulin (and other analyte) assays in small volumes (facilitating small model organism experiments)
  - Pays a portion of chargebacks for diabetes testing

- DRC Cell and Tissue Imaging Core → major stakeholder in the Washington University Center for Cellular Imaging
  - Representation on Advisory Board (David Piston Co-Chair, Jean Schaffer, Member) enables significant input into management and programming
  - DRC member scholarships enhance access for diabetes research

Core Wish:
Clinical Diabetes Resource

Need

- >350 approved diabetes studies
- ~1/2 WU DRC members actively recruiting
- 2/3 clinical researchers face recruitment challenges

Goal

- Enhance diabetes-related clinical research studies
- Facilitate translation of new knowledge to improved care of patients with diabetes

Proposed Development

- Pursue new resources specifically related to clinical translation in diabetes, beyond what exists in highly effective CTSA
  - Applications for P30 supplement funds from NIDDK
  - Leverage institutional support

- Enhance identification of potential research subjects in the complex academic medical center environment
  - Study tree model for all ongoing approved diabetes human studies
  - Dedicated coordinator with clinical trials expertise to develop and maintain tree, interface with study PIs and providers (including those in the community) at multiple points of care, leverage EMR, initiate contacts with potential subjects

- Assist investigators initiating clinical studies
  - Pool of experienced study coordinators tasked with part-time coordination for individual DRC members (e.g., community outreach, enrollment, scheduling, visits)
  - Scholarships for early stage investigators

DIABETES RESEARCH CENTER

Yale

The Yale Diabetes Research Center was initially funded in 1993.

It is 1 of 16 DRCs supported by NIDDK, one of the few DRCs with a major engagement in Immunology, Imaging and Neuroscience.

It currently has 110 members from 22 departments in the School of Medicine, Schools of Public Health and Nursing as well as Yale College, .

Annual DRC budget $999,993

Institutional Support: YARC facilities, HRU Support, and CTSA Resources for Clinical Trials.

Funding of Current DRC Members:

Total DRC Member NIH Funding = $336.8 million

Administrative Core
Director- Robert S. Sherwin, MD

Yale

Services-

- 1) Administrative and financial management of the DRC
- 2) Informational services and coordinate pilot review committee
- 3) Maintenance of Yale DRC web page
- 4) Financial oversight
- 5) Education and training for new DRC investigators
- 6) Management of the Pilot and Feasibility Program
- 7) Administration of enrichment program

Pilot Project Program – For the period 1993 to 2016, 96 pilots awarded

60 pilots received peer-reviewed funding (35 from NIH).

Enrichment Program.

- Weekly seminars, mainly presentations from external scientists
- Annual retreat featuring junior faculty and postdoc fellow presentations
- Annual DRC Symposium

DRC Cores

Yale

Clinical Metabolism Core: Director Gerald Shulman, MD, PhD

Provides assays that require the use of mass spectrometers to determine isotopic enrichments and concentrations of multiple metabolites in limited amounts of tissues.

Molecular Genetics Mouse Core: Director Richard Flavell, PhD & Li Wen, MD, PhD

The core offers CRISPR to rapidly generate mice on a pure B6 background. It also generates transgenic, knock-out and knock-in mice on NOD backgrounds. In addition, the core provides mouse breeding services as well as mice expressing human genes.

Physiology Core: Director Raimund Herzog, MD

This core performs vascular and brain surgeries and glucose clamp studies examining CNS and peripheral metabolism as well as insulin secretion studies in conscious, freely moving rat & mouse models. The core also provides diabetes-related hormone assays.

Cell Biology Core: Director Jonathan Bogan, MD

This core offers unique expertise in cell imaging and state-of-the-art methodologies for live cell fluorescence microscopy for metabolic studies.

Translational Core: Director William Tamborlane, MD

Provides research nurses and training of new investigators to conduct cutting-edge, patient-based research in diabetes. The core also provides biostatistics & study design support as well as an extensive registry of diabetes patients for clinical trials.

Black indicates institutional Core

Red indicates diabetes-specific Core

DRC Clinical Metabolism Core – Director – Gerald I. Shulman, MD, PhD

Yale

- **Services:** Provides a centralized facility for analysis of metabolite concentrations and isotopic enrichments to assist researchers conducting human studies of diabetes and other related metabolic diseases.
  - GC-MS Stable isotope analysis of glucose, glycerol, acetate, palmitate for calculation of whole body and tissue specific substrate fluxes (unique to lab)
  - Liquid chromatography tandem mass spectrometry: mass isotopomer analysis of cytosolic and mitochondrial intermediates of metabolism for calculation of cellular metabolic fluxes (unique to lab)
  - LC/MS/MS of Lipid Metabolites (DAG, Acetyl-Carnitines & ceramindes) obtained from tissue biopsy using API-4000 (unique to lab)

- **Recent Developments:**

1. Lipid and apolipoprotein turnover: Optimized isotope-labeling strategies and GC-MS methodologies for ^{13}C-leucine, d-palmitate, d-water).
2. Glycolytic and mitochondrial metabolites in human biopsy material: optimized LC/MS/MS analyses.
3. Mitochondrial-cytosolic substrate cycles of pyruvate and phospho-enol-pyruvate: developed isotope-labeling strategy and LC/MS/MS analyses for quantitation of fluxes.

DRC Cell Biology Core – Director – Jonathan S. Bogan, MD

Yale

Consultation and Assistance:

- Tissue and cell preparation
- Light microscopy immunocytochemistry
- “Standard” and immuno-electron microscopy
- Dynamic analysis of living cells containing fluorescent markers
- Super-resolution microscopy of fixed and living cells
- Post-acquisition image analysis (tracking, 3D reconstruction)
- Quantitative infrared fluorescent imaging of proteins
- Consultation and training in imaging techniques and analysis

Recent Developments:

- Emphasis has been on super-resolution microscopy (”nanoscopy”) and dynamic light microscopy imaging of living cells.
- Development of new fluorescent probes to image specific lipids, cell membranes, and phosphoinositides.
- 3D Electron microscopy tomography
- Application of these tools to studies of insulin secretion and action
- Future use of Seahorse Technology to measure cell metabolic activity

Yale DRC Synergies

Yale

Core	Partners for Synergy
Molecular Genetics	HHMI,
Human Physiology	GCRC/CTSA
Cell Biology	Liver Center, Cellular & Mol. Imaging
Translational	GCRC/CTSA

Other Yale Program Collaborations:

Yale Center for Analytical Science (Biostats.)

Bioinformatics

Yale Imaging and PET Centers

Keck Foundation Lab (genomic, proteomic analyses)

Generation of transgenic, knock-out and knock-in NOD mice.

New developments:

- Cryopreservation of mouse sperm & use them for in vitro fertilization
- Gene targeting of NOD embryonic stem cells
- Generation of mice capable of supporting human innate immune cells that are capable of generating human immune responses.

New Research Services Needed at Yale DRC

Yale

A Human Metabolic Imaging Core:

To support:

a). Human MR Spectroscopy of brain/liver/muscle.

b) Human MR Imaging of brain & liver.

The imaging equipment needed to conduct these studies is already in place at Yale and some DRC members are able utilize these state-of-the-art facilities. However, use of these resources is limited, particularly for junior DRC faculty, since these studies require careful monitoring by experienced nurses to ensure patient safety that is not currently available via the DRC.

Development of a New DRC Research Core

Yale

- Create a Metabolomics Core to provide services for mass spectrometry-based analysis of metabolites & lipids.

- The core facility would be equipped with a high-resolution mass spectrometry system (HRMS) coupled with liquid chromatography (UHPLC) and direct infusion (NanoMate) for separation and detection of various classes of small molecules, metabolites and lipids in mammalian cells

- Further services would include:
  - Global untargeted metabolomic profiling
  - Targeted metabolomic & lipidomic analysis
  - Metabolite and lipid identification

Impact of the DRC

Yale

- For the period 1993 to 2016, 96 pilot grants were awarded. 60 received peer-reviewed funding (including 35 from NIH).
- Nearly 500 publications over the past 5 yrs. were supported by the DRC, most of which appeared in high impact journals, e.g.

N Eng J Med	(5)
Nature	(8)
Nature Med	(4)
Nature Sci.	(2)
Nature Neurosci	(1)
Nature Cell Biol	(3)
Science	(4)
Immunity	(1)
J Clin. Invest.	(21)
J Neurosci.	(4)
PNAS	(41)

Cell	(7)
Cell Metab	(12)
Cell Microbiol	(3)
Diabetes	(45)
Diabetes Care	(8)
JCEM	(4)
Endocrinology	(5)
AJP	(15)
JBC	(13)
J Cell Biology	(17)

The current Yale DRC Director & Deputy Director are recipients of both the Lilly and Banting Award from American Diabetes Assoc.

NIDDK Medical Student Research Program in Diabetes

- Tenth summer (2009-2018)
- 4-10 students/Diabetes Center
- Over 1000 students from >120 medical schools
- Funding
  - Supplement to T32s at Diabetes Centers
  - Diabetic Complications Consortium

DIABETES RESEARCH CENTERS

NIDDK Medical Student Research Program - Summer 2018

November

- Recruit/advertise
  - Deans of all US Medical schools
  - First-year class presidents of all US Medical sc
  - AAMC email (~1500 individuals)
  - National Hispanic Medical Association
  - Association of Native American Medical Studen
  - Electronic/Web site

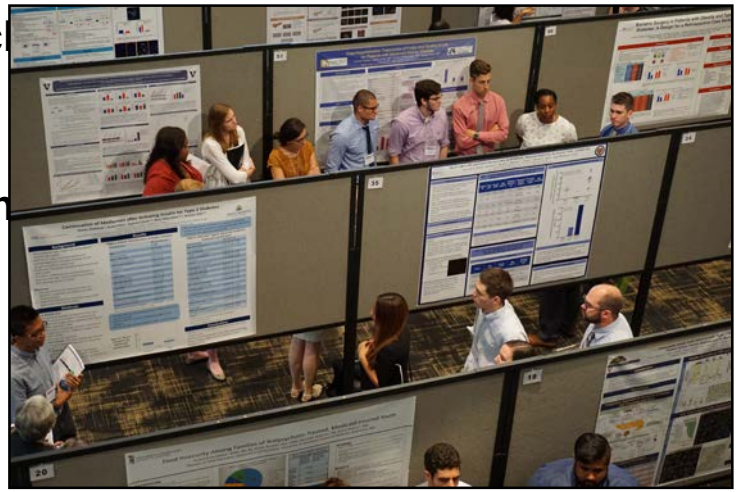
February-March

- Each Center selects students

May - June – July – research!

August

- Research symposium
- Poster presentations



DIABETES RESEARCH CENTERS

2017 NIDDK Medical Student Research Symposium

- Visiting professors
  - Arshiya Baig, MD, MPH (Chicago)
  - Rodica Pop-Busui, MD, PhD (Michigan)
  - Jose Florez (BADERC)
  - Gordon Weir, MD (Joslin)
  - Susan Bonner-Weir, PhD (Joslin)

- Visitors to symposium
  - Kristin Abraham, PhD (NIH/NIDDK)
  - Pamela Thornton, PhD (NIH/NIDDK)
  - Richard McIndoe, PhD (DiaComp)
- Career pathways/advice
  - Art Castle, PhD (NIH/NIDDK)
  - Residency program directors and physician-scientist program directors from Med and Peds

Program Oversight

- **Advisory Committee**
  - **Art Castle (NIDDK)**
  - **Steven Kahn (University of Washington)**
  - **Mary Elizabeth Patti (Joslin)**
  - **Louis Philipson (University of Chicago)**
  - **Mike Rickels (Penn)**

Demographics in 2017

Student Participant Demographics 2017		US Medical School Enrollment 2016-17		US Census 2010	
Race		Race		Race	
African American	13 (10%)	African American	5856 (7%)	African American	38.9 million (13%)
American Indian	0	American Indian	223 (<1%)	American Indian	2.9 million (0.9%)
Asian	43 (31%)	Asian	18430 (21%)	Asian	14.7 million (5%)
Caucasian	61 (45%)	Caucasian	46841 (54%)	Caucasian	173.1 million (56%)
Hispanic	13 (10%)	Hispanic	5344 (6%)	Hispanic	50.5 million (16%)
Native Hawaiian or Pacific Islander	0	Native Hawaiian or Pacific Islander	96 (<1%)	Native Hawaiian or Pacific Islander	0.5 million (0.2%)
Other/ multiple race/ unknown/ no answer	6 (4%)	Other/ multiple race/ unknown/ no answer	10139 (12%)	Other/ multiple race/ unknown/ no answer	28.1 million (9%)
Gender		**Gender**		**Gender**	
Female	82 (60%)	Female	42089 (48%)	Female	156,964,000 (50.8%)
Male	54 (40%)	Male	46207 (52%)	Male	151,781,000 (49.2%)

DIABETES RESEARCH CENTERS

Demographics in 2017

2017 Program			
Center	# Participants	# Participants from medical schools associated with a DERC/DRTC	# applicants listing Center as #1 or 2 choice
BADERC	7*	0	61
Columbia	7*	0	127
Indiana	6*	5	31
JHopkins/Univ MD	8*	0	79
Joslin	6	0	62
UAB	7*	6	20
UCLA/UCSD	1	0	107
Univ Chicago	4	0	100
Univ Colorado	6*	0	42
Univ Michigan	9*	2	31
Univ Pennsylvania	4	0	50
Univ Washington	8*	0	59
Vanderbilt - NIDDK	6	0	73
Washington Univ	4	0	22
Yale	5*	1	54
Sub-total	88	14	

Medical Students Also Participating in the Summer Sympsoium		
Vanderbilt T35 grant	32	1
Vanderbilt O'Brien NIDDK Renal Ctr	7	0
SPORT	9**	0
TOTAL	**136**	**15**

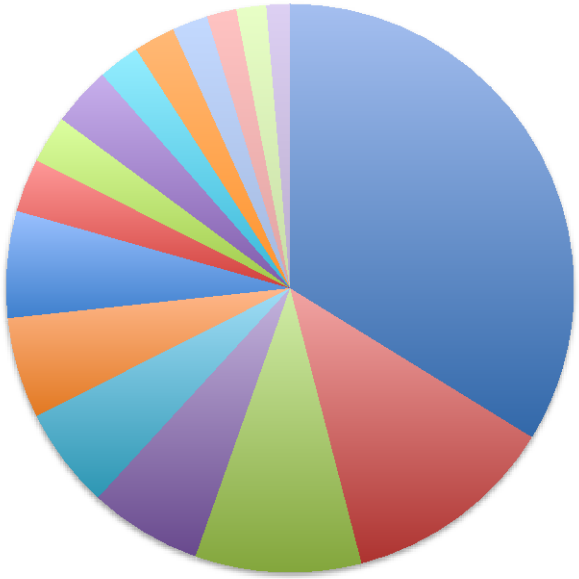
DIABETES RESEARCH CENTERS

Challenges for Discussion

- **Follow up of student participants**

Residency Choices of NIDDK Participants 2009-2014

37% Internal Medicine
12% Pediatrics
9% Emergency Medicine



Internal Medicine | Pediatrics | Emergency Medicine | Anesthesiology
Family Medicine | Ob/Gyn | Surgery (General) | Neurology
Psychiatry | Radiology | Dermatology | Ophthalmology
Pathology | Physical Med & Rehab | Radiation Oncology | Otolaryngology

DIABETES RESEARCH CENTERS

Interactions / Follow up

- Interactive web-based platform for the lectures
  - Fosters better interactions across centers
- Enhanced assessment and evaluation
  - Assessment of students – take-home points, quiztime
  - Evaluation –immediate feedback for improvement
- Database to follow students and aid with next steps in their career development
  - FERPA compliant SPARK platform
  - Unique identifiers of students (ORCID ID, PMID)
  - Alumni association

Challenges for Discussion

- Follow up of student participants
- **Medical school curriculum revisions**

Challenges for Discussion

- Follow up of student participants
- Medical school curriculum revisions
- **New US medical schools**
- **Increased applicants from osteopathic schools**
- **New Osteopathic schools**

340B Simplified

A new video explains how the 340B Drug Pricing Program benefits teaching hospitals, patients, and communities at no cost to taxpayers.

More on *AAMCNews*

Medical Schools, Teaching Hospitals

AAMC Members

- Medical Schools

Students, Applicants, Residents

Pre-Medical Students

- MCAT®
- AMCAS®

News and Advocacy

Newsroom

- AAMC Statement on Senate Passage of Omnibus

What is Osteopathic Medicine?

- About Osteopathic Medicine
  - U.S. Colleges of Osteopathic Medicine
  - Osteopathic Specialty Colleges
- How To Apply To Osteopathic Medical College
- Financial Aid and Scholarships
- Fellowships and Internships
  - International Student Fact Sheet
  - Osteopathic Medical Student Profiles

Osteopathic medicine is a distinct form of medical practice in the United States.

Osteopathic medicine provides all of the benefits of modern medicine including prescription drugs, surgery, and the use of technology to diagnose disease and evaluate injury. It also offers the added benefit of hands-on diagnosis and treatment through a system of treatment known as osteopathic manipulative medicine. Osteopathic medicine emphasizes helping each person achieve a high level of wellness by focusing on .... Read more

Osteopathic Medicine and Medical Education in Brief

- The nation faces a critical physician workforce shortage. According to a report by the American Association of Medical Colleges (AAMC), by 2025, the gap between our physician supply and demand will range from 34,500 to 88,000.
- Colleges of osteopathic medicine are graduating more and more students each year. More than 5,400 new osteopathic physicians enter the workforce each year.

Learn About U.S. Osteopathic Medical Schools

Osteopathic Medicine Overview

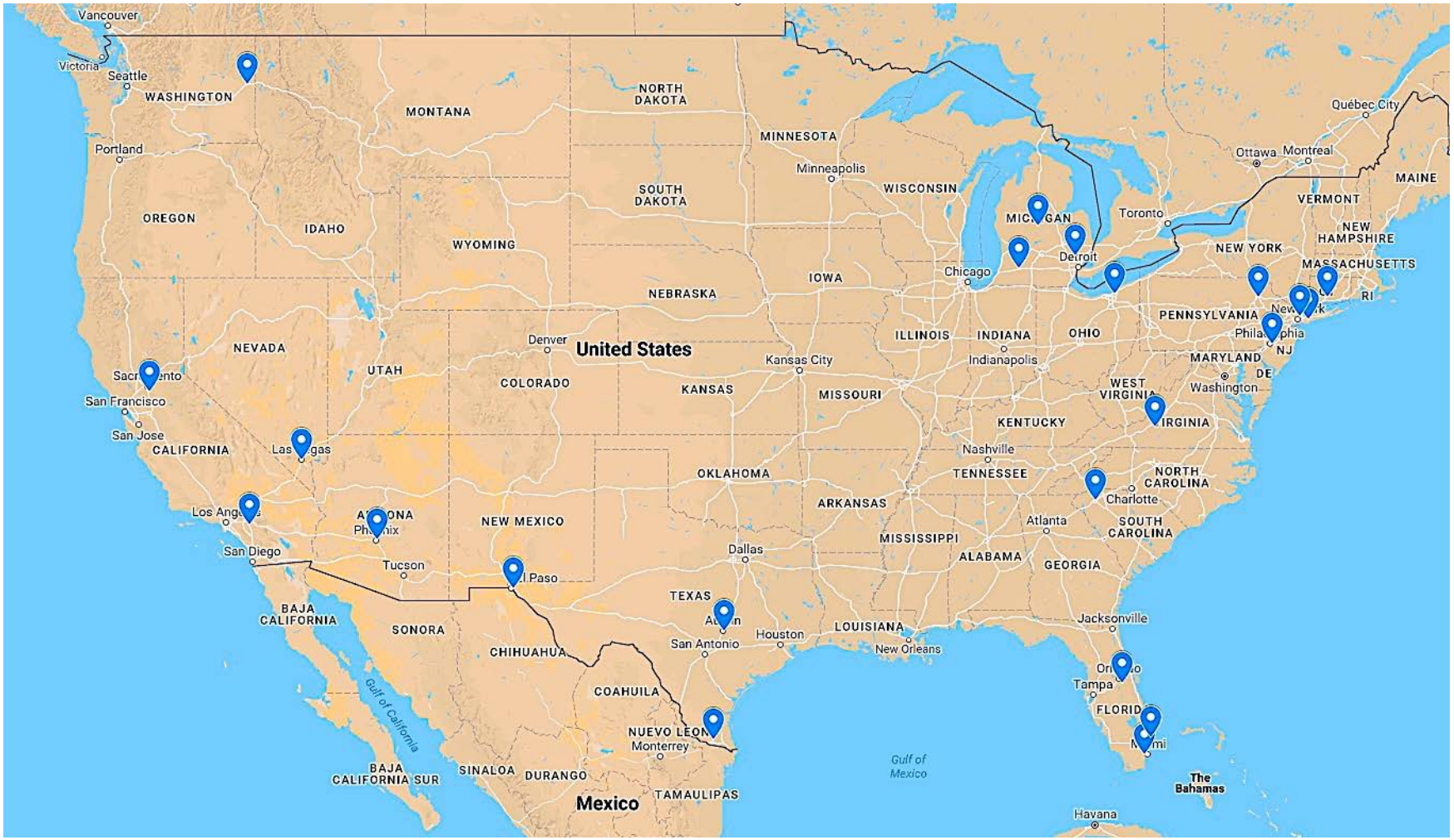
Some NIDDK Program Stats

	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018
# Applicants	190	425	485	394	564	550	685	574	501	469
# from Medical Schools	183	411	470	379	536	522	656	520	437	376
# from MD schools <10 yrs	1	5	9	10	17	11	31	37	39	34
# from DO schools	7	15	15	15	28	28	29	54	64	93
# from DO schools <10 yrs	4	8	5	2	3	2	8	11	7	16

	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018
# Participants	52	67	76	78	87	87	97	98	89	85
# from Medical Schools	52	67	76	75	85	87	93	95	80	
# from MD schools <10 yrs	0	0	2	4	4	1	7	4	7	
# from DO schools	0	0	0	3	2	0	4	3	9	
# from DO schools <10 yrs	0	0	0	0	0	0	3	1	2	

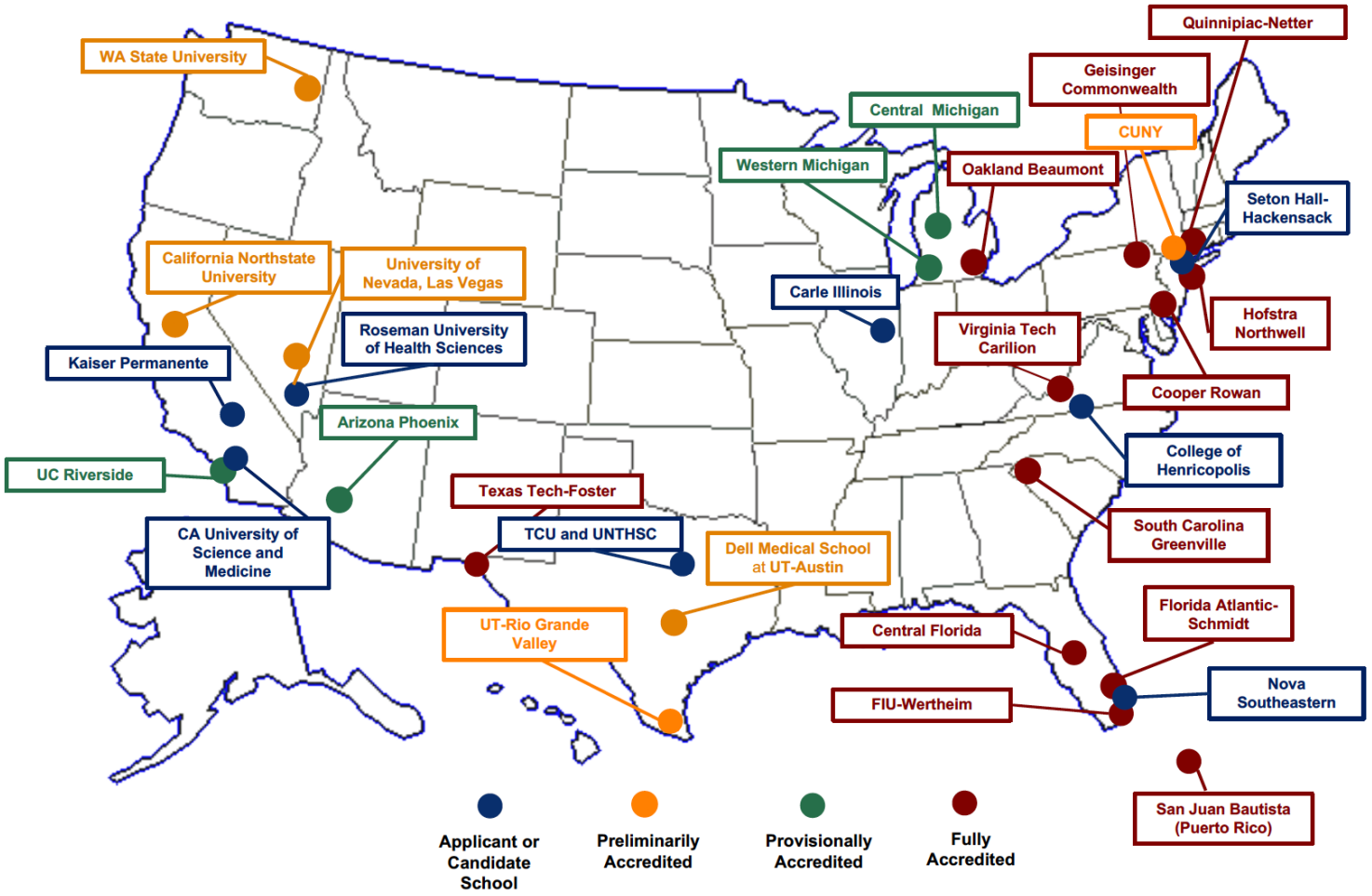
^ Includes students supported by Diabetes Complications Consortium

New MD Schools Starting Enrollment in Last 10 Years



DIABETES RESEARCH CENTERS

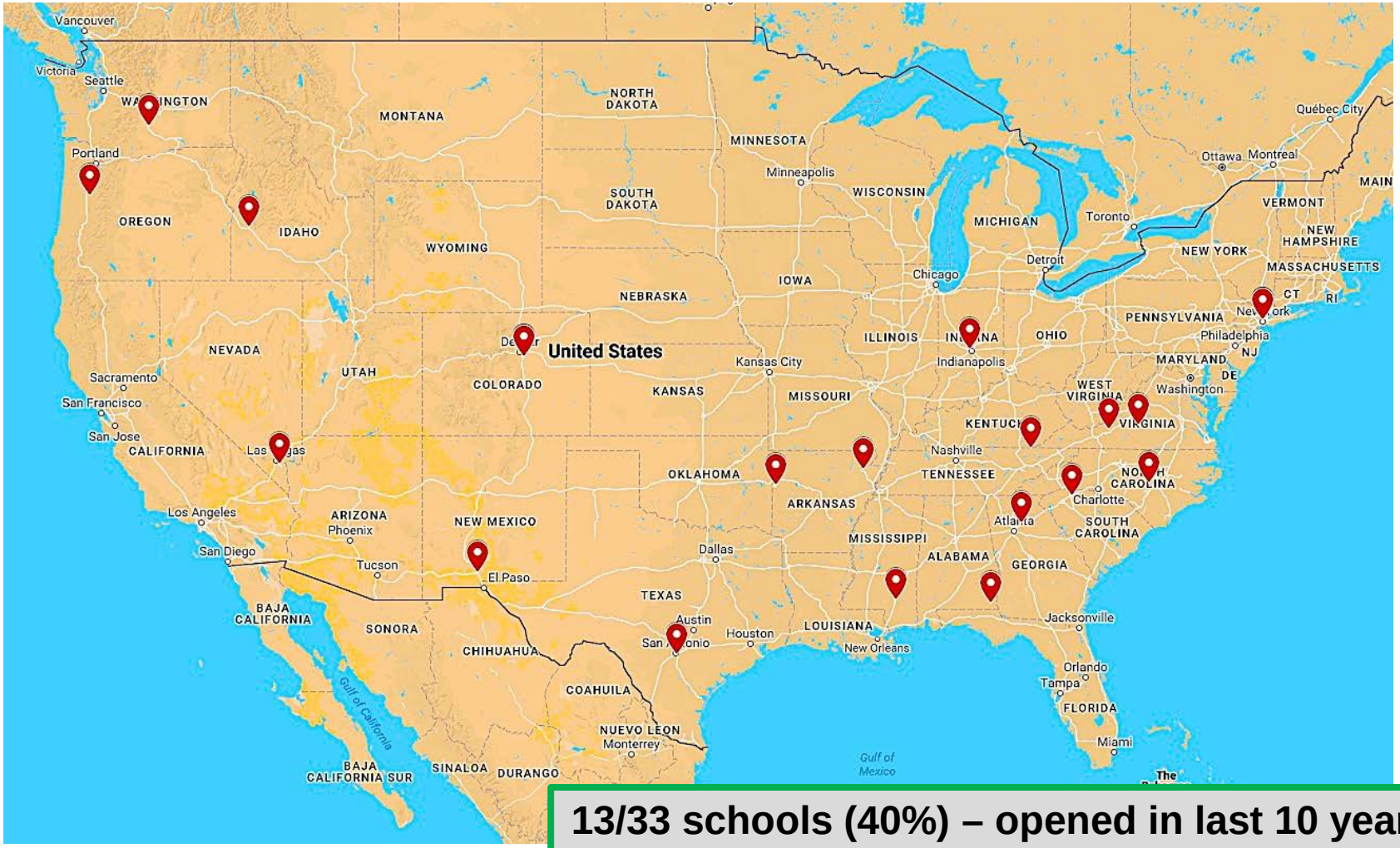
Figure 1. New MD-granting medical schools accredited since 2002 or in the LCME accreditation process (as of March 2017).



22/147 schools (15%) – opened in last 10 years (8 more in pre-certification phase)

AAMC, 2017

New DO Schools Starting Enrollment in Last 10 Years



13/33 schools (40%) – opened in last 10 years
(14 more in pre-certification phase)

DIABETES RESEARCH CENTERS

MD and DO School Enrollment Has Increased (and will continue)

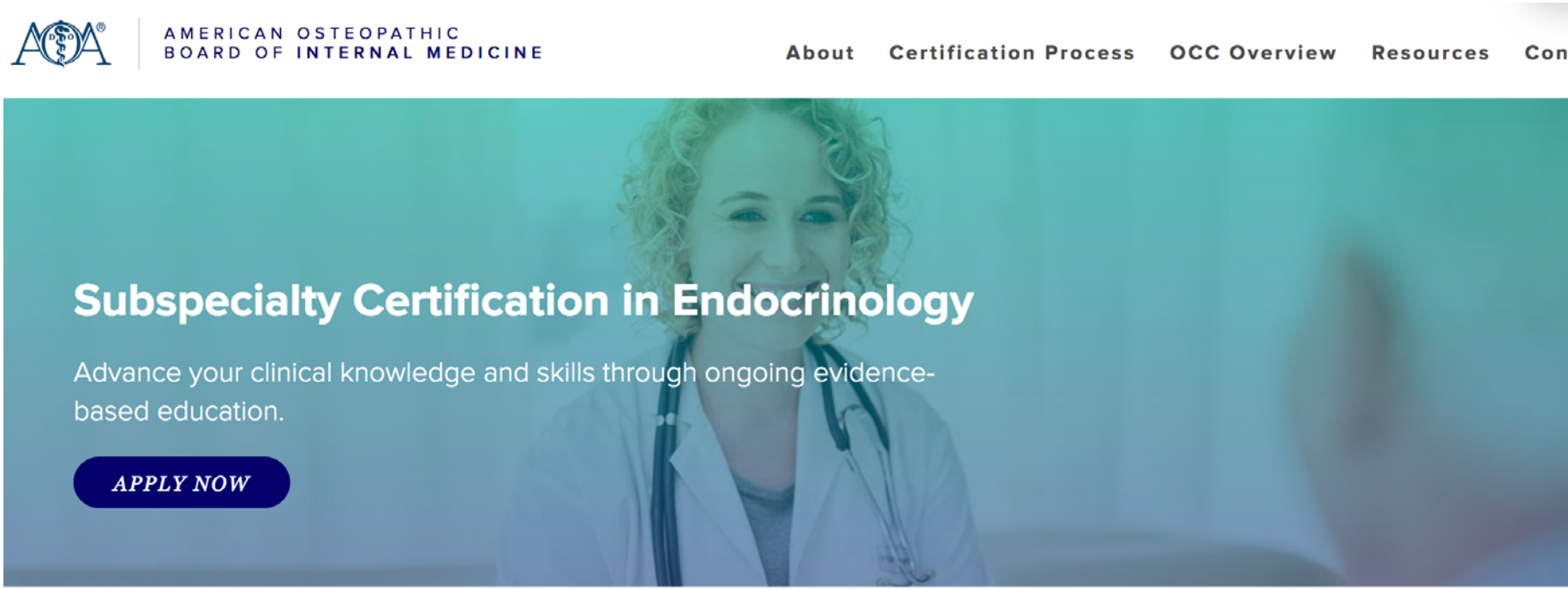
Table 3. MD and DO Enrollment Growth Since 2002

	2002 Base Enrollment	2016 Current			2021 Projected		
		Enrollment	**# Increase**	**% Increase**	**Enrollment**	**# Increase**	**% Increase**
MD	16,488	21,030	4,542	28%	22,225	5,737	35%
DO	2,968	7,369	4,401	148%	8,800	5,832	196%
Total	19,456	28,399	8,943	46%	31,025	11,569	59%

DIABETES RESEARCH CENTERS

AAMC, 2017

New DO Schools Starting Enrollment in Last 10 Years



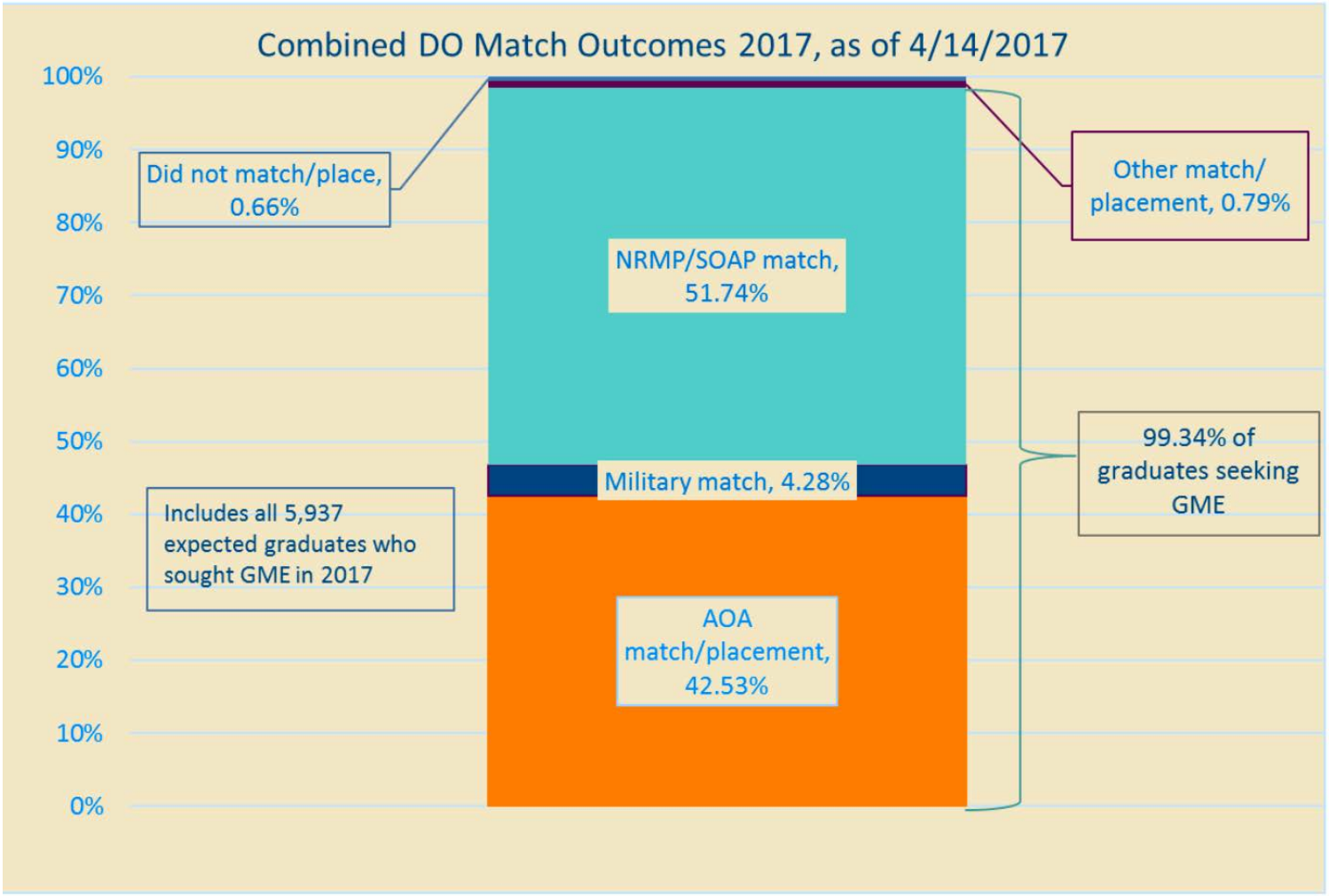
Endocrinology Certification Process

The American Osteopathic Board of Internal Medicine administers primary and subspecialty certification exams, as well as Osteopathic Continuous Certification requirements. The following stages represent key milestones in the process to obtain subspecialty board certification in Endocrinology.

Timeline

DIABETES RESEARCH CENTERS

New DO School Graduates - Match



DIABETES RESEARCH CENTERS

Challenges for Discussion

- Follow up of student participants
- Medical school curriculum revisions
- New US medical schools
- Increased applicants from osteopathic schools
- New Osteopathic schools

Application and Program Statistics
NIDDK Medical Student Summer Research Program
Summer 2016 and 2017

NIDDK Program Applications

Year 2017 - 501 applications from 113 medical schools for 88 positions
Year 2016 - 574 applications from 123 medical schools for 98 positions
Year 2015 - 685 applications from 138 medical schools for 99 positions
Year 2014 - 551 applications from 137 medicalschools for 87 positions
Year 2013 - 568 applications from 138 medical schools for 88 positions
Year 2012 - 395 applications from 114 medical schools for 78 positions
Year 2011 - 486 applications from 111 medical schools for 76 positions
Year 2010 - 431 applications from 104 medical schools for 68 positions
Year 2009 - 197 applications from 82 medical schools for 56 positions

2016 Program				2017 Program			
Center	# Participants	# Participants from medical schools associated with a DERC/DRTC	# applicants listing Center as #1 or 2 choice	Center	# Participants	# Participants from medical schools associated with a DERC/DRTC	# applicants listing Center as #1 or 2 choice
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UAB	6***	5	14	UAB	7*	6	20
UCLA/UCSD	6*	1	133	UCLA/UCSD	1	0	107
UCSF	4	0	83	Univ Chicago	4	0	100
Univ Chicago	4	0	90	Univ Colorado	6*	0	42
Univ Colorado	6	1	38	Univ Michigan	9*	2	31
Univ Michigan	9*	2	48	Univ Pennsylvania	4	0	50
Univ Pennsylvania	4	2	111	Univ Washington	8*	0	59
Univ Washington	8*	2	55	Vanderbilt - NIDDK	6	0	73
Vanderbilt - NIDDK	6	0	56	Washington Univ	4	0	22
Washington Univ	4	1	24	Yale	5*	1	54
Yale	5*	0	40	Sub-total	88	14	
Sub-total	95	20					

Medical Students Also Participating in the Summer Sympsoium		
Vanderbilt T35 grant	27	0
Vanderbilt O'Brien NIDDK Renal Ctr	14	0
SPORT	5**	0
TOTAL	**142**	**20**

Medical Students Also Participating in the Summer Sympsoium		
Vanderbilt T35 grant	32	1
Vanderbilt O'Brien NIDDK Renal Ctr	7	0
SPORT	9**	0
TOTAL	**136**	**15**

*indicates students added with support from the Diabetic Complications Consortium (DCC)

** University of Maryland Summer Program in Obesity, Diabetes and Nutrition Related Research Training (SPORT), supported by Maryland T35 DK095737

***Extra student with outside funding

Student Participant Demographics 2017		**US Medical School Enrollment 2016-17**		**US Census 2010**	
Race		Race		Race	
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Female	82 (60%)	Female	42089 (48%)	Female	156,964,000 (50.8%)
Male	54 (40%)	Male	46207 (52%)	Male	151,781,000 (49.2%)

^The category totals may not add to the total enrollees since a person could designate multiple categories. Source: AAMC

UAB Diabetes Research Center (DRC) and
Minority Health & Health Disparities Research Center (MHRC)

NIDDK Short-Term Education Program for Underrepresented Persons (STEP-UP)

UAB MEDICINE
Knowledge that will change your world

UAB Diabetes
Research Center

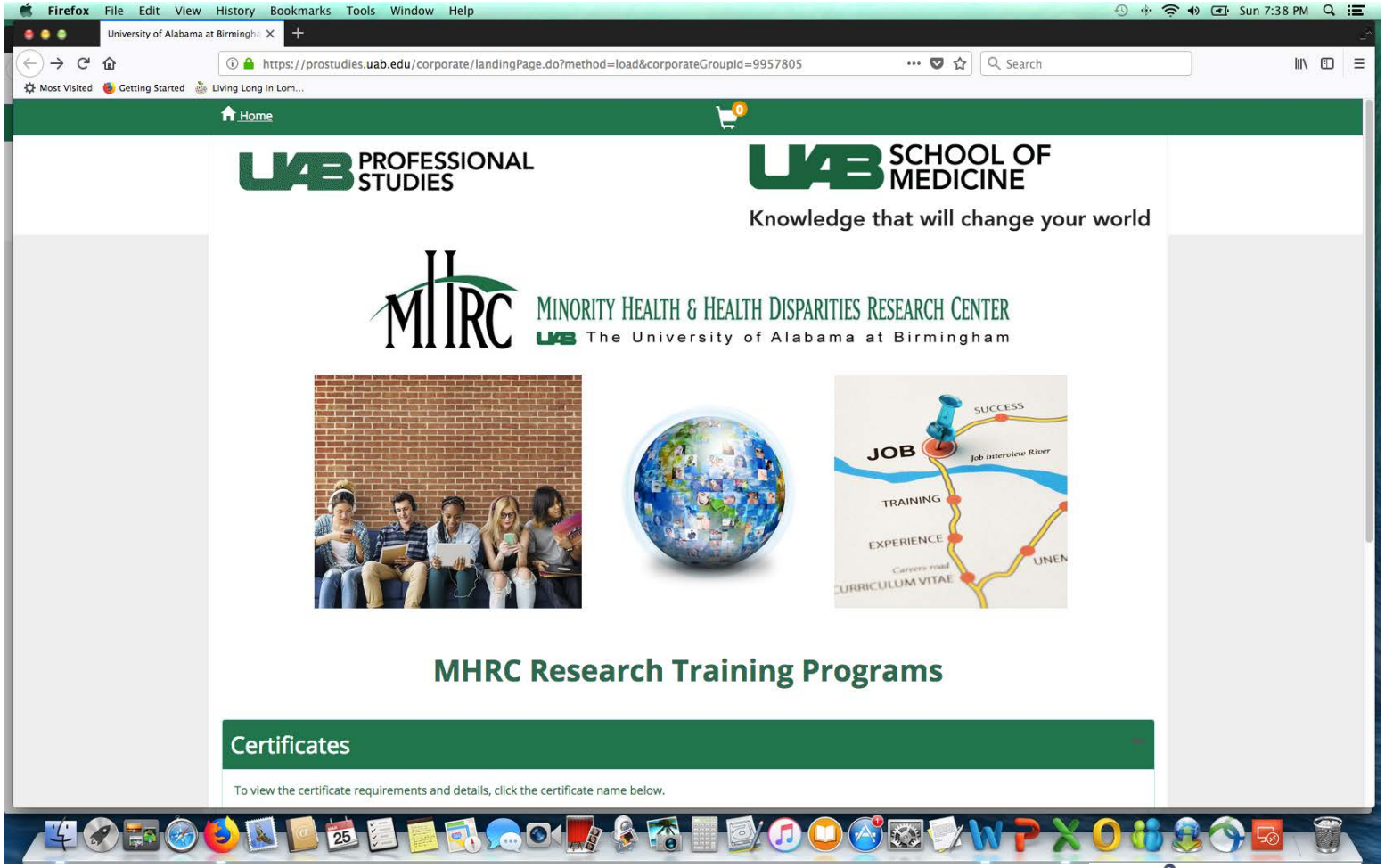
NIDDK STEP-UP

- Purpose: to expose underrepresented minority and disadvantaged undergraduate students to research in the NIDDK mission areas.
- Collaboration with NIDDK and OMHRC
- Long-term goal: to increase number of underrepresented minority students "in the pipeline," committed to careers in biomedical, behavioral, clinical, or social science research
- Component of NIDDK Strategic Plan on Minority Health Disparities: to reduce and eliminate health disparities, expand research education and training opportunities for underrepresented minority scientists.

NIDDK STEP-UP

- STEP-UP Summer Interns Receive:
- Biomedical research experience during 10-week summer program
- May 29 - August 2, 2018
- $5,000 Stipend
- All expense paid travel to present at the Annual STEP-UP Symposium at the NIH in Bethesda, MD (July 29-31, 2018)
- Year-round mentoring support and career development advice, and online curriculum

UAB DRC and UAB MHRC



NIDDK STEP-UP Student Demographics – Self-Identified - 2018 and 2017

Year	Total	AA	H/L	NH/ PI	AI/ AN	A	ME/ NA	W	F	M
2018	**20**	8	4 (6)	1	0	3	1	3	17	3
2017	**22**	8	5 (11)	0	1	1	1	6	18	4

- AA = African American
- H/L = Hispanic/Latino – Self Identified as Hispanic (as AA or W + Hispanic)
- NH/PI = Native Hawaiian/Pacific Islander
- AI/AN = American Indian/Alaska Native
- A = Asian
- ME/NA = Middle Eastern/North African
- W = White
- F = Female; M = Male

NIDDK STEP-UP 2018 Students

- 20 students from 17 institutions:

  - Albertus Magnus College
  - College of Micronesia
  - Emory U
  - Howard U
  - Hunter College
  - Johns Hopkins U
  - Lincoln U
  - Mississippi State U
  - Tuskegee U
  - UAB
  - UCLA
  - U of Illinois at Chicago
  - U of Puerto Rico, Río Piedras
  - U of Washington Tacoma
  - U of Wisconsin Madison
  - Wesleyan U
  - Williams College

- Students may or may not work at these sites

UAB DRC and UAB MHRC

- Students apply online: citizens or non-citizen nationals with GPA ≥ 3 in a STEM major, under-represented minority or disadvantaged background.
- Given guidance in identifying institution and mentor for project. Housing is not provided.
- Use network of DRCs, NORCs
  - Directors help identify mentors
  - DRC members refer students
  - Promote program on DRC/NORC websites and undergraduate student affairs website.
- www.uab.edu/medicine/mhrc/trainingprograms/undergraduate STEP-UP UAB annsmith@uab.edu

NIDDK STEP-UP Mentors

- Encourage so that students will appreciate the application of research to their future careers in science or medicine
- Encourage student's involvement in all steps of research from hypothesis generation through publication -
- Students may articulate a hypothesis, collect or extract data, analyze the data, reach an appropriate conclusion, and
- Create poster & oral presentation summarizing work.

NIDDK Information Network

Research
Resource
IDentification

Leveraging Research Resource Identifiers (RRIDs) for use by the NIDDK Diabetes Center Program

NIDDK Information Network

Research Resource IDentification

What are Research Resource Identifiers (RRIDs)?

RRIDs are designed to promote research transparency in the scientific literature by providing unique, persistent, machine readable identifiers for resources.

Catalog numbers can change, disappear or be reused for another resource, but RRIDs always resolve to the same research resource and endure beyond the existence of the research resource itself. It's an easy and practical method for improving reproducibility, transparency and tracking.

NIDDK Information Network

Research Resource IDentification

Why do we need Resource Identifiers?

Enhancing Rigor & Reproducibility

- It is difficult to query the biomedical literature to find out what research resources have been used to produce the results of a study
- Authors generally don't provide enough information to unambiguously identify key research resources
- Impossible to find all studies that used a resource

- Critical for reproducibility and data mining
- Critical for trouble-shooting

NIDDK Information Network

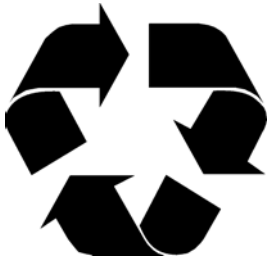
Research Resource IDentification

Why do we need Resource Identifiers?

Assuring proper Credit & Attribution for resource developers

ORCID
I am a researcher

RRID
I made this antibody



PubMed ID / DOI
I published this paper

RRIDs are now in use at many databases and catalogs

MMRRC
Mutant Mouse Resource & Research Centers supported by NIH

About MMRRC | Submissions | Catalog & Distribution

Strain Detail Sheet

AVAILABILITY

Strain Name: STOCK Tg(Sox9-EGFP)EB209Gsat/Mmucd
Stock Number: 011019-UCD
Citation ID: RRID:MMRRC_011019-UCD
Major Collection: GENSAT

COPY RRID CITATION TO CLIPBOARD

Gene Information

Purified anti-AKT1 Antibody

RRID AB_2566355 (BioLegend Cat. No. 680302)

Antigen Details

Structure 480 amino acids with a predicted molecular weight of ap

Distribution Cytoplasm, nucleus, cell membrane, phosphorylation on T
localization to the cell membrane where it is targeted for

ExPASy Bioinformatics Resource Portal

Cellosaurus cell lin

Search Clear

Cellosaurus 1-5c-4 (CVCL_2260)

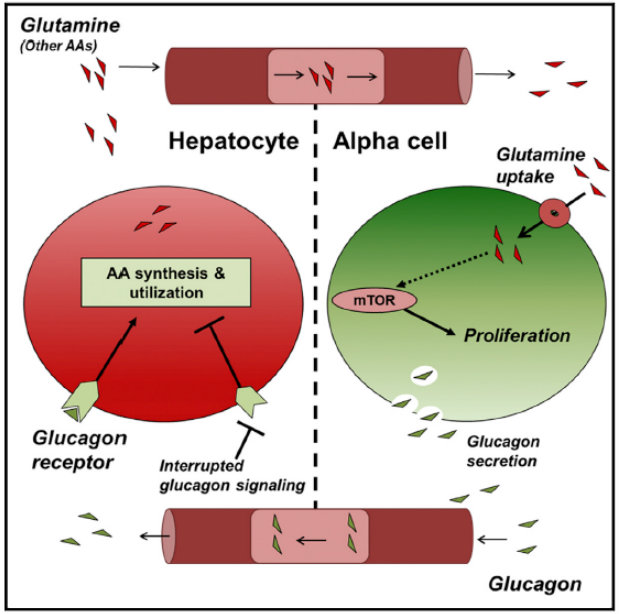
Cell line name	1-5c-4
Synonyms	Clone 1-5c-4; Clone 1-5c-4 WKD of Ch g Conjunctiva
Accession	CVCL_2260
Resource Identification Initiative	To cite this cell line use: 1-5c-4 (RRID:CVCL_2260)
Comments	Problematic cell line: Contaminated. Shown to be a He Transformant: NCBI_TaxID; 333761; Human papilloma Omics: Transcriptome analysis.
Disease	Human papillomavirus-related endocervical adenocarci
Species of origin	Homo sapiens (Human) (NCBI Taxonomy: 9606)
Hierarchy	Parent: CVCL_0030 (HeLa)
Sex of cell	Female
Category	Cancer cell line
	Source(s): ATCC; KCLB

Cell Metabolism

Interrupted Glucagon Signaling Reveals Hepatic α Cell Axis and Role for L-Glutamine in α Cell Proliferation

RRIDs in practice: an example

Graphical Abstract



Highlights

- Interrupted glucagon signaling (IGS) stimulates mouse and

Authors

E. Danielle Dean, Mingyu Li, Nripesh Prasad, ..., Roland Stein, Wenbiao Chen, Alvin C. Powers

Correspondence

al.powers@vanderbilt.edu

In Brief

Blocking glucagon action lowers blood

STAR★METHODS

KEY RESOURCES TABLE

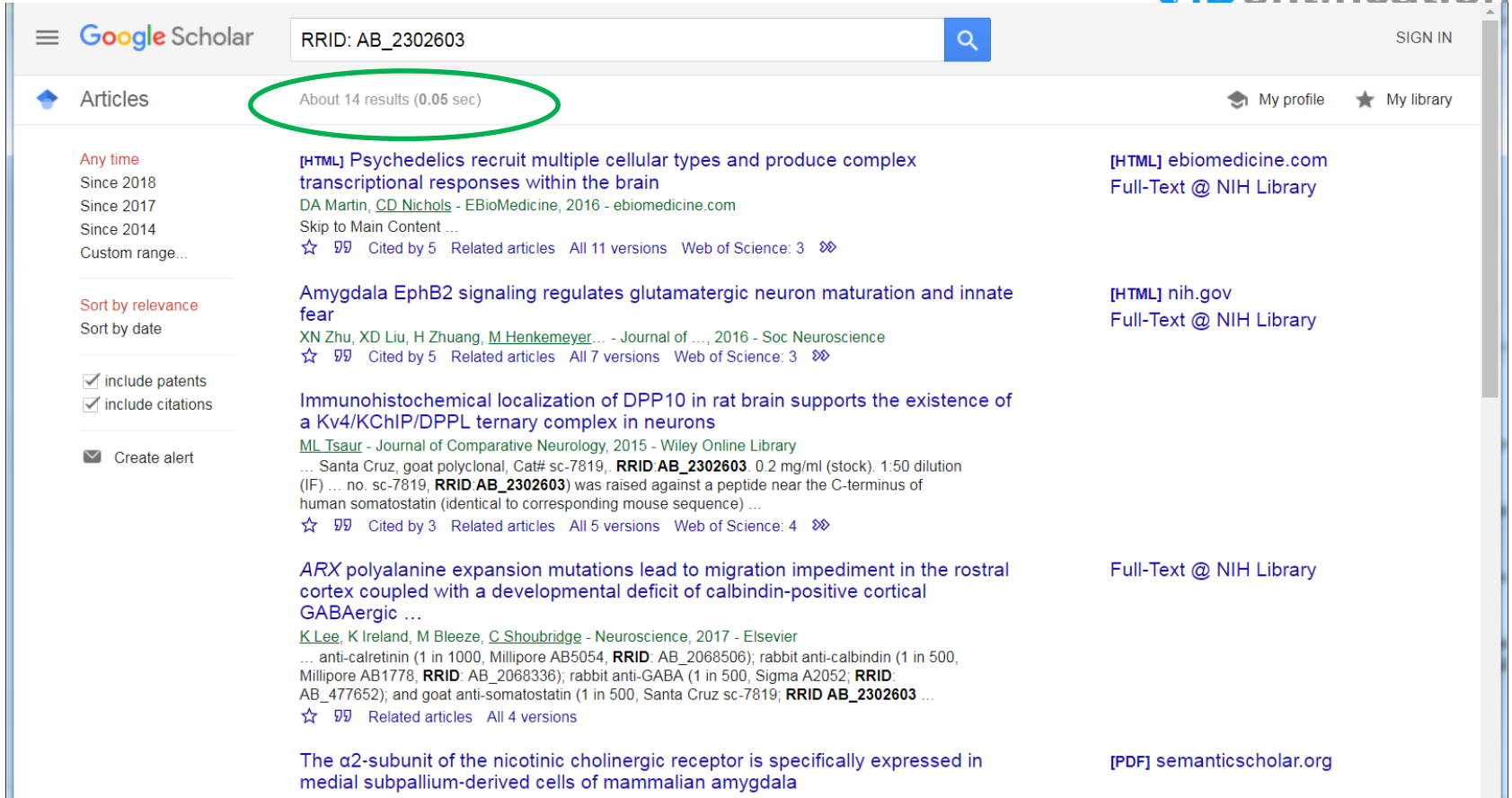
sc-7819; RRID: AB_2302603

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Anti-huGlucagon Receptor 2.59; huV/mdC IgG1 antibody (GCGR mAb)	Amgen; Gu et al., 2009	N/A
Mouse anti-glucagon	Abcam	ab10988; RRID: AB_297642
Rabbit anti-glucagon	Cell Signaling	#2760; RRID: AB_659831
Guinea pig anti-insulin	Dako	A0564; RRID: AB_10013624
Goat anti-SNAT5 (SLC38A5)	Santa Cruz (discontinued)	sc-50682; RRID: AB_785709
Rabbit anti-Ki67	Abcam	ab15580; RRID: AB_443209
Goat anti-somatostatin (D-20)	Santa Cruz (discontinued)	sc-7819; RRID: AB_2302603
Rabbit anti-amylase	Sigma-Aldrich	A-8273; RRID: AB_258380
Rabbit anti-phospho S6 (p235/p236)	Cell Signaling	#2211; RRID: AB_331679
Rabbit anti-Cre Recombinase	Novagen	#69050-3; RRID: AB_2314229
Biological Samples		
Isolated primary human islets	IIDP	https://iidp.coh.org/

NIDDK Information Network

RRID: AB_2302603

Research Resource IDentification



Google Scholar

RRID: AB_2302603

SIGN IN

Articles

About 14 results (**0.05** sec)

My profile My library

Any time
Since 2018
Since 2017
Since 2014
Custom range...

Sort by relevance
Sort by date

include patents
include citations

Create alert

[HTML] Psychedelics recruit multiple cellular types and produce complex transcriptional responses within the brain
DA Martin, CD Nichols - EBioMedicine, 2016 - ebiomedicine.com
Skip to Main Content ...
Cited by 5 Related articles All 11 versions Web of Science: 3

[HTML] ebiomedicine.com
Full-Text @ NIH Library

Amygdala EphB2 signaling regulates glutamatergic neuron maturation and innate fear
XN Zhu, XD Liu, H Zhuang, M Henkemeyer... - Journal of ..., 2016 - Soc Neuroscience
Cited by 5 Related articles All 7 versions Web of Science: 3

[HTML] nih.gov
Full-Text @ NIH Library

Immunohistochemical localization of DPP10 in rat brain supports the existence of a Kv4/KChIP/DPPL ternary complex in neurons
ML Tsaur - Journal of Comparative Neurology, 2015 - Wiley Online Library
... Santa Cruz, goat polyclonal, Cat# sc-7819,. **RRID:AB_2302603**. 0.2 mg/ml (stock). 1:50 dilution (IF) ... no. sc-7819, **RRID:AB_2302603**) was raised against a peptide near the C-terminus of human somatostatin (identical to corresponding mouse sequence) ...
Cited by 3 Related articles All 5 versions Web of Science: 4

ARX polyalanine expansion mutations lead to migration impediment in the rostral cortex coupled with a developmental deficit of calbindin-positive cortical GABAergic ...
K Lee, K Ireland, M Bleeze, C Shoubridge - Neuroscience, 2017 - Elsevier
... anti-calretinin (1 in 1000, Millipore AB5054, **RRID**: AB_2068506); rabbit anti-calbindin (1 in 500, Millipore AB1778, **RRID**: AB_2068336); rabbit anti-GABA (1 in 500, Sigma A2052; **RRID**: AB_477652); and goat anti-somatostatin (1 in 500, Santa Cruz sc-7819; **RRID AB_2302603** ...
Related articles All 4 versions

Full-Text @ NIH Library

The α2-subunit of the nicotinic cholinergic receptor is specifically expressed in medial subpallium-derived cells of mammalian amygdala

[PDF] semanticscholar.org

NIDDK Information Network

Research Resource IDentification

SciCrunch

INFORMATION RESOURCES COMMUNITIES DATA LITERATURE

Home / Search for RRIDs / View Resource

Search for RRIDs

Somatostatin (D-20) antibody

RRID:AB_2302603

DESCRIPTION USED IN LITERATURE

Antibody ID
AB_2302603

Target Antigen
Sst human, rat, mouse

Vendor
Santa Cruz Biotechnology

Cat Num
sc-7819

Proper Citation
(Santa Cruz Biotechnology Cat# sc-7819, RRID:AB_2302603)

Reference
References (18)

Clonality
polyclonal antibody

Host Organism
goat

Comments
discontinued 2016 due to animal welfare concerns see doi.org/10.1038/nature.2016.19958; validation status unknown check with seller; recommendations: western blot, ELISA, immunoprecipitation, immunocytochemistry [less]

Publications that use this research resource

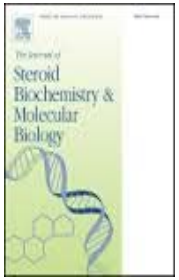
- PMID:27939219
- PMID:27649637
- PMID:28627419
- PMID:25355692
- PMID:25641263
- PMID:28591638
- PMID:27618674
- PMID:27683910
- PMID:28521139
- PMID:27719761
- PMID:26779909

ENDOCRINE SOCIETY

SfN SOCIETY for NEUROSCIENCE

PNAS

BioMed Central The Open Access Publisher



AJHG The American Journal of Human Genetics

frontiers

eLIFE

bioRxiv beta THE PREPRINT SERVER FOR BIOLOGY

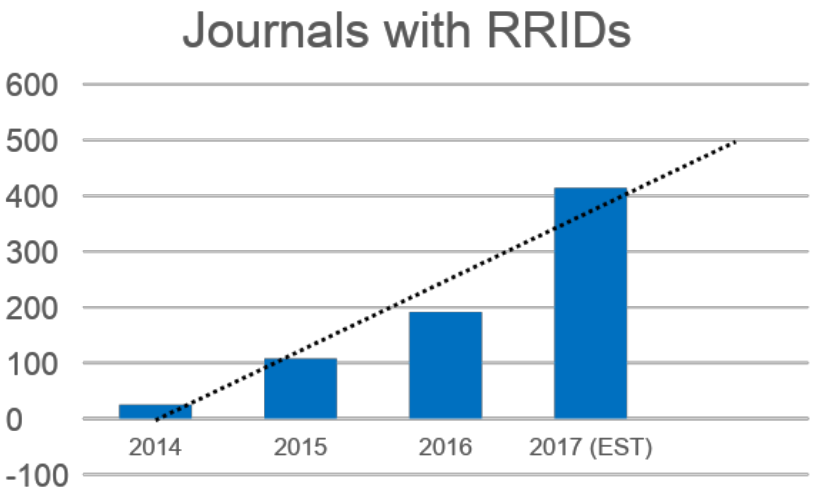
CellPress

PLOS ONE

the FEBS Journal

JOURNAL OF HEPATOLOGY The Home of Liver Research | EASL

RRID citations are growing in use by journals and publishers



NIDDK Information Network

Research Resource IDentification

Who mints RRIDs for resources?

RRIDs are only as useful as the organization that stands behind them

- Points to a unique resource (de-duplication)
- Persistent metadata even if the resource is no longer available
- Resolvable

RRIDs are provided by *community-recognized databases* for each type of resource

- Open data
- Non-discriminatory
- Comprehensive

For example, authors publishing in participating journals during the pilot phases of the RRID project were asked to provide RRID's for their resources drawn from:

The Antibody Registry, **Model Organism Databases**, **NIF Resource Registry**, **Cellosaurus**

NIDDK Information Network

Research Resource IDentification

dkNET aggregates RRID source information for Researchers & Publishers

dkNET currently aggregates data from over 25 databases that develop/use RRIDs

ExPASy Bioinformatics Resource Portal

Cell Lines

MGI

Organisms

MMPC Mouse Metabolic Phenotyping Centers

Antibody Registry

Antibodies

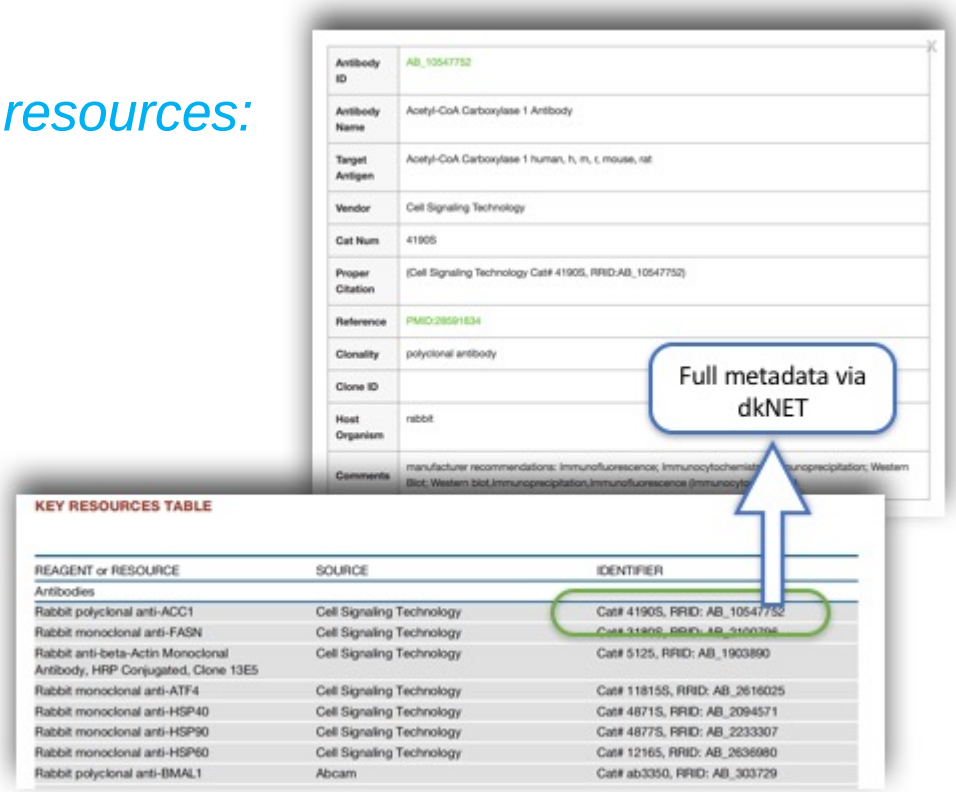
Resource Registry SciCrunch

Software

NIDDK Information Network

Research Resource IDentification

Use RRIDs to identify resources:



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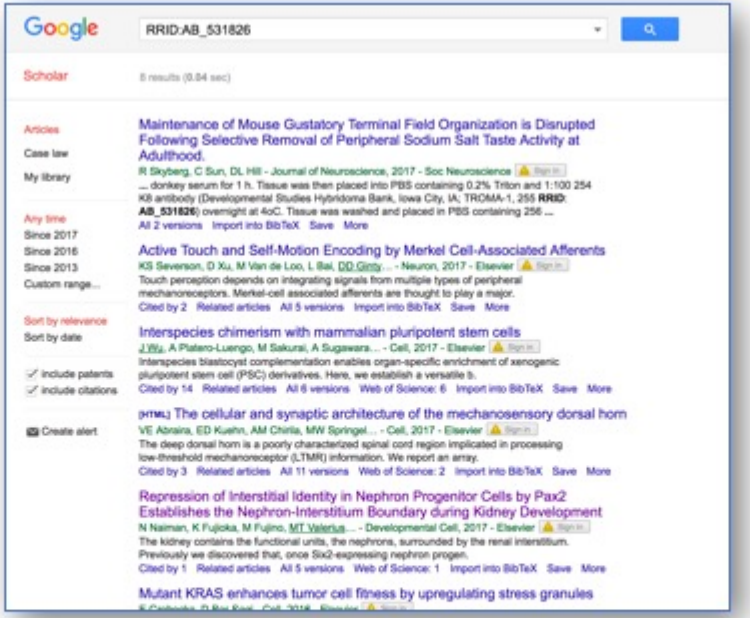
Research Resource IDentification

Use RRIDs to track resources:



Search for RRID in Google Scholar brings back a set of papers that used this resource

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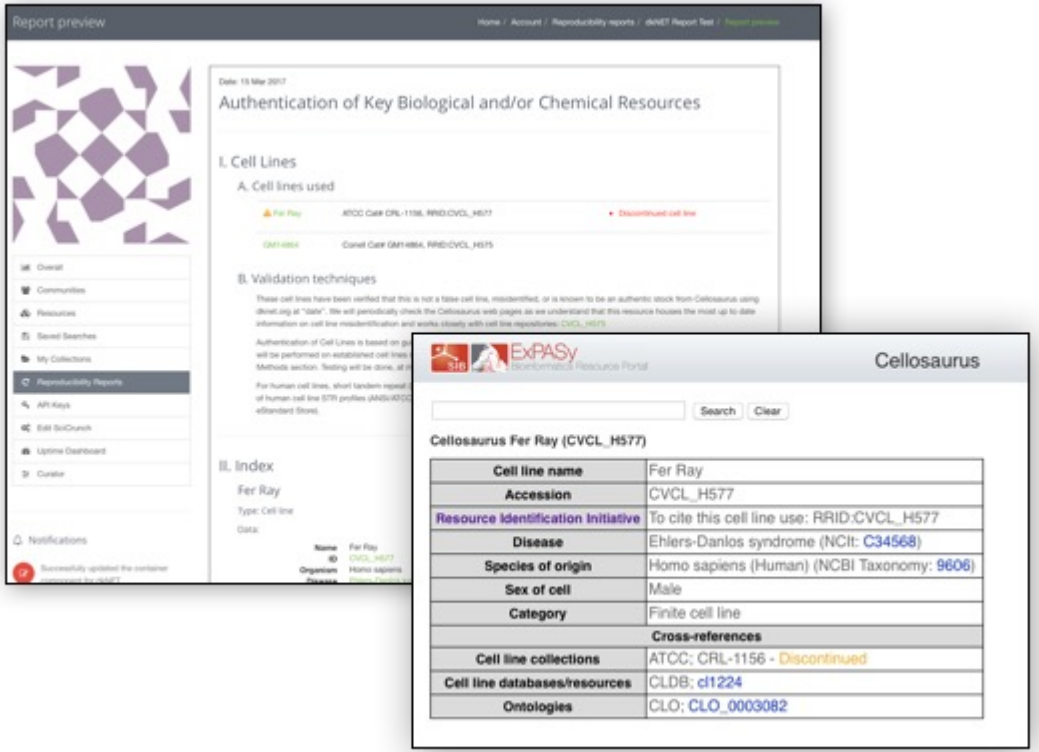
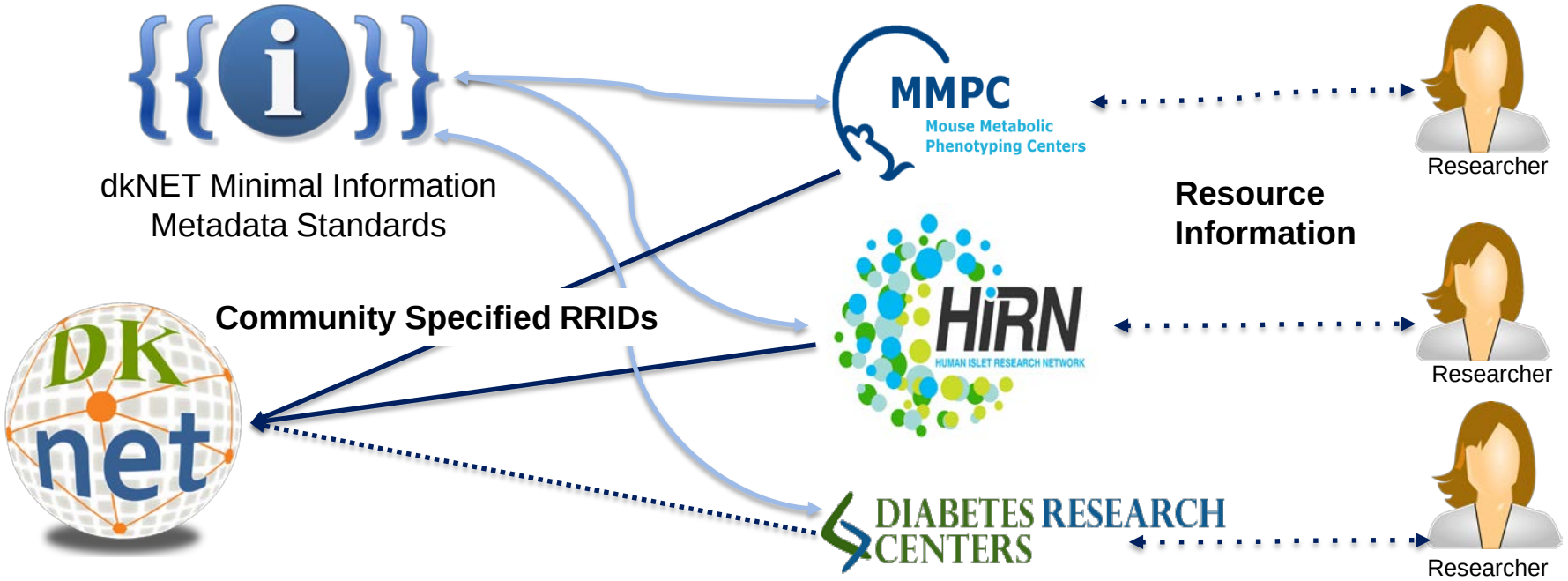
Research Resource IDentification

Use RRIDs to assist in generating authentication plans (in beta):



How might large, complex NIDDK programs benefit from this RRID "movement"?

Better tracking of activity and productivity in programs, centers and cores serves both NIH and PI needs: HIRN, MMPC & DCO

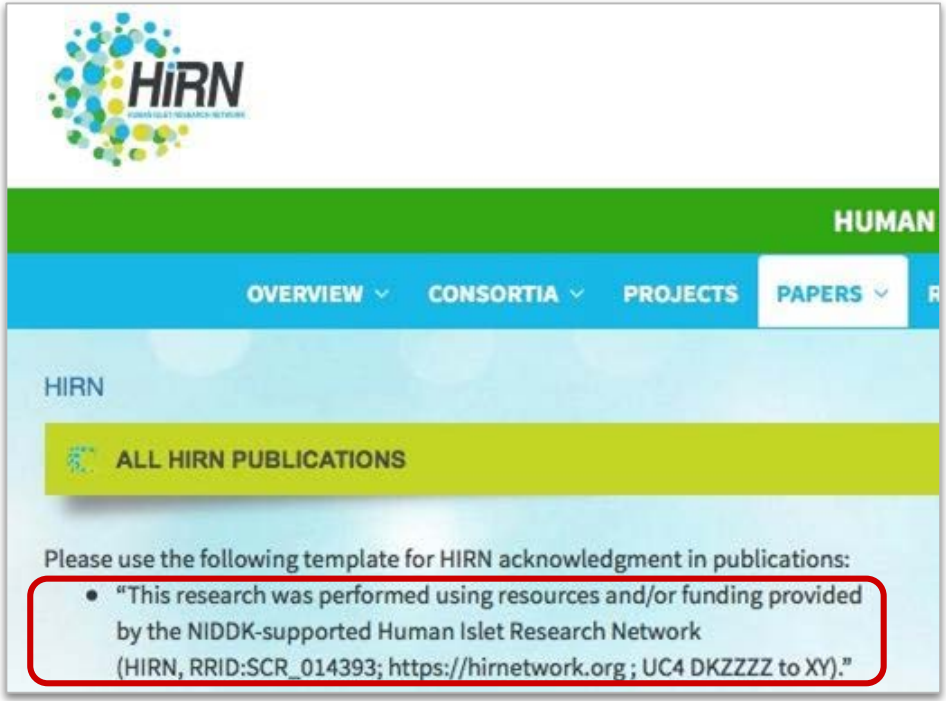


NIDDK Information Network

Research Resource IDentification

RRID Implementation example #1: identifying "programs"-HIRN

- Add RRID citation to language to website
- Ask researchers to include in publications:
  - Acknowledgments
  - Materials & Methods



Example citation policy- in the "Acknowledgments"

HIRN

HUMAN ISLET RESEARCH N

OVERVIEW | CONSORTIA | PROJECTS | PAPERS | RESOURCES | BIOIN

HIRN

ALL HIRN PUBLICATIONS

Please use the following

- "This research wa
by the NIDDK-sup
(HIRN, RRID:SCR_

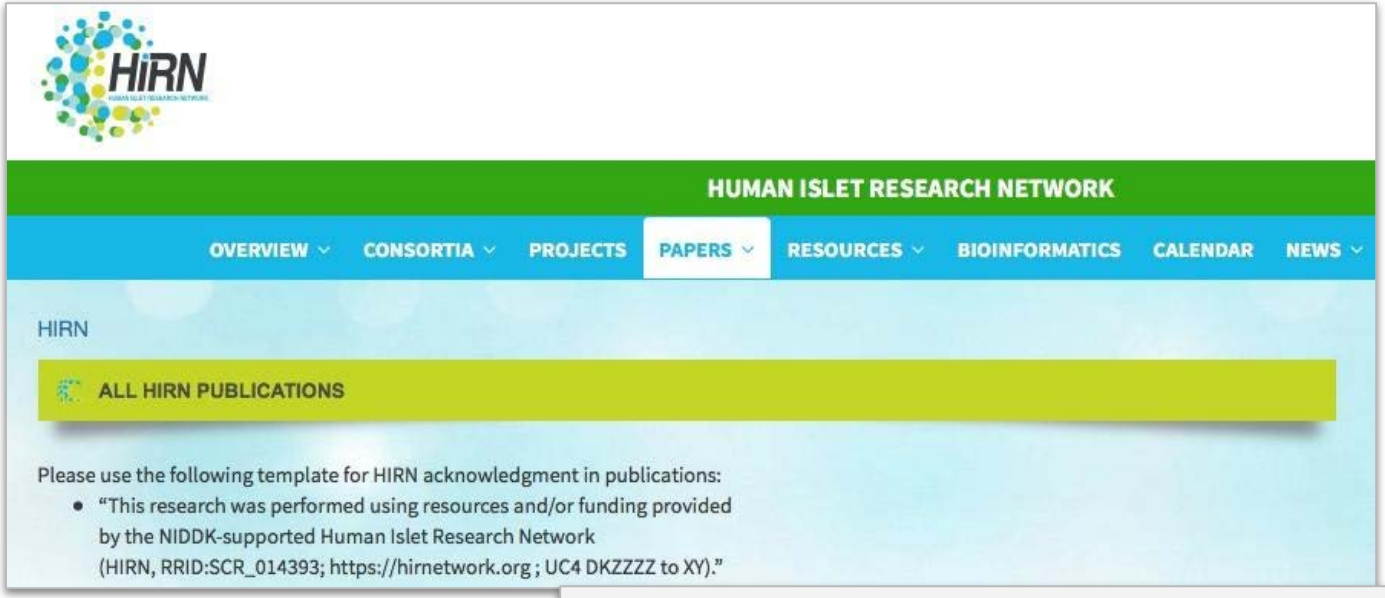
ACKNOWLEDGMENTS

We thank Dr. Seung Kim for providing critical comments on the manuscript. We are very grateful to Drs. Raphael Scharfmann, Michael German, and Gérard Gradwohl for helpful discussions and sharing reagents. Special thanks to Mr. David Scheel for experimental advice. This research was performed using resources and/or funding provided by the NIDDK-supported Human Islet Research Network (HIRN, RRID:SCR_014393; https://hirnetwork.org; UC4 DK104211, DK108120, and DK112232), by DK106755, DK72473, DK89572, DK97829, DK94199, the Vanderbilt Diabetes Research and Training Center (DK20593), and by grants from the JDRF (2-SRA-2015-68-Q-R, 2-SRA-2016-149-Q-R, 17-2013-321, 17-2013-324), the Leona M. and Harry B. Helmsley Charitable Trust, and the Department of Veterans Affairs (BX000666). Islet imaging and functional analysis were performed with

RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Rabbit anti-ARX (1:1000)	Beta Cell Biology Consortium (BCBC)/Patrick Collombat	N/A
Rabbit anti-Glucagon (1:200)	Cell Signaling	Cat# 2760; RRID: AB_659831
Mouse anti-Glucagon (1:500)	Abcam	Cat# ab10988; RRID: AB_297642
Mouse anti-Glucagon- Pacific Blue (1:600; flow cytometry)	Sigma-Aldrich	Cat# G2654; RRID: AB_259852

Example citation & Google-enabled search

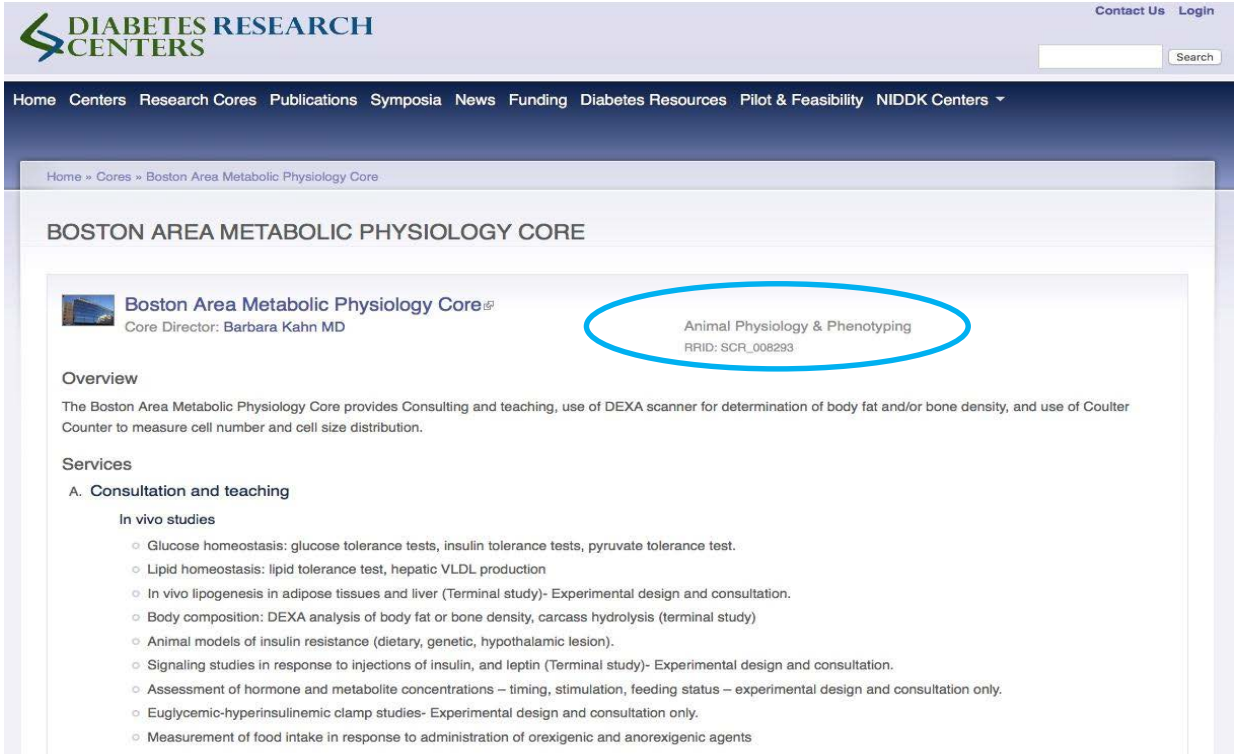


NIDDK Information Network

Research Resource IDentification

RRID Implementation example #2: identifying "centers & cores"- MMPC, DCO

- Centers & cores are registered & provided RRIDs via dkNET
- Core RRIDs can be included in the methods section when a Core is used or referenced; center RRIDs can be used in "Acknowledgments"
- dkNET can help with aggregating information on core use within the current dkNET RRID infrastructure

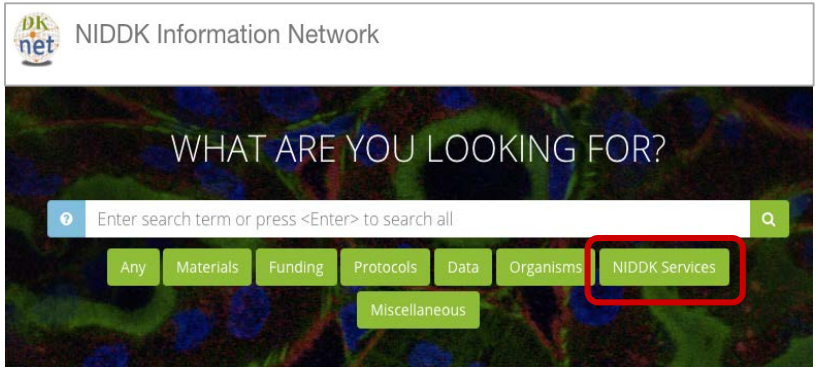


How to find my RRID?

STEP 1 - Find

Check if RRID exists:

https://dkNET.org > NIDDK Services



STEP 2 - Enter Center Name and Locate RRID:SCR_008997

Snippet View: Click ‘Cite This’

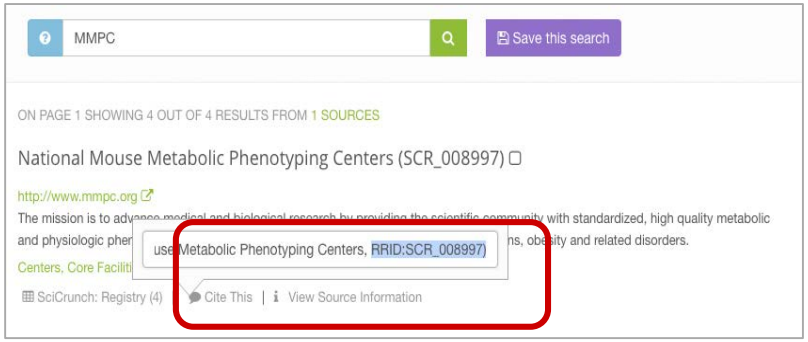
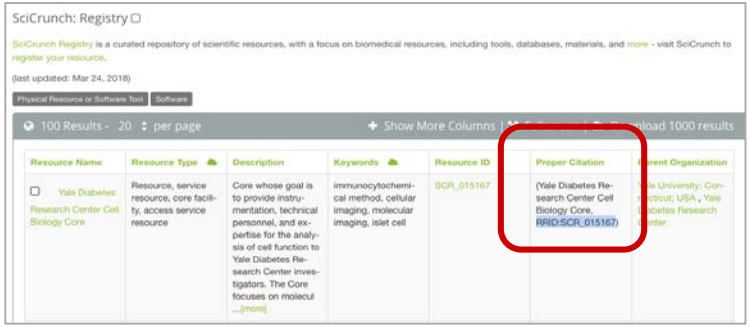


Table View: Proper Citation Column



CENTERS

Centers

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Diabetes Research

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EINSTEIN ANIMAL PHYSIOLOGY CORE

Einstein Animal Physiology Core

Core Director: Gary J Schwartz PhD

Animal Physiology & Phenotyping

RRID: SCR_015076

Overview

The Einstein Animal Physiology Core assists biomedical investigators in the in vivo assessment of glucose and fatty acid metabolism, insulin sensitivity and energy homeostasis in mice and rats. The Animal Physiology Core offers a wide range of specialized, high quality methodologies and tools relevant to understanding the behavior and physiology mediating the relationships among diabetes, nutrient sensing, obesity and diabetic cardiovascular complications in rodents.

Objectives

1. To offer advice and instruction to students, postdoctoral fellows, investigators and technical staff in the design and performance of physiological approaches and techniques necessary to evaluate the control of glucose homeostasis and insulin action in rodents.
2. To make available to investigators specialized measurements of whole body and tissue-specific glucose metabolism and insulin action in rodent models including

NIDDK Information Network

Research Resource IDentification

dkNET is developing a report routine based on Center RRIDs that will provide...

What our center is about? Which centers or cores are funded by me?

How my resources are used and who uses my resources?

a. Information on all resources from a parent resource
b. Proper citation
c. Usage: publication citations of the resource and child resources.
d. Listing of child resources for a particular entity (expand to research resources, i.e., antibodies in future)

If researcher uses my resources, how to properly cite it ?

What research resources are funded by me and their usage?

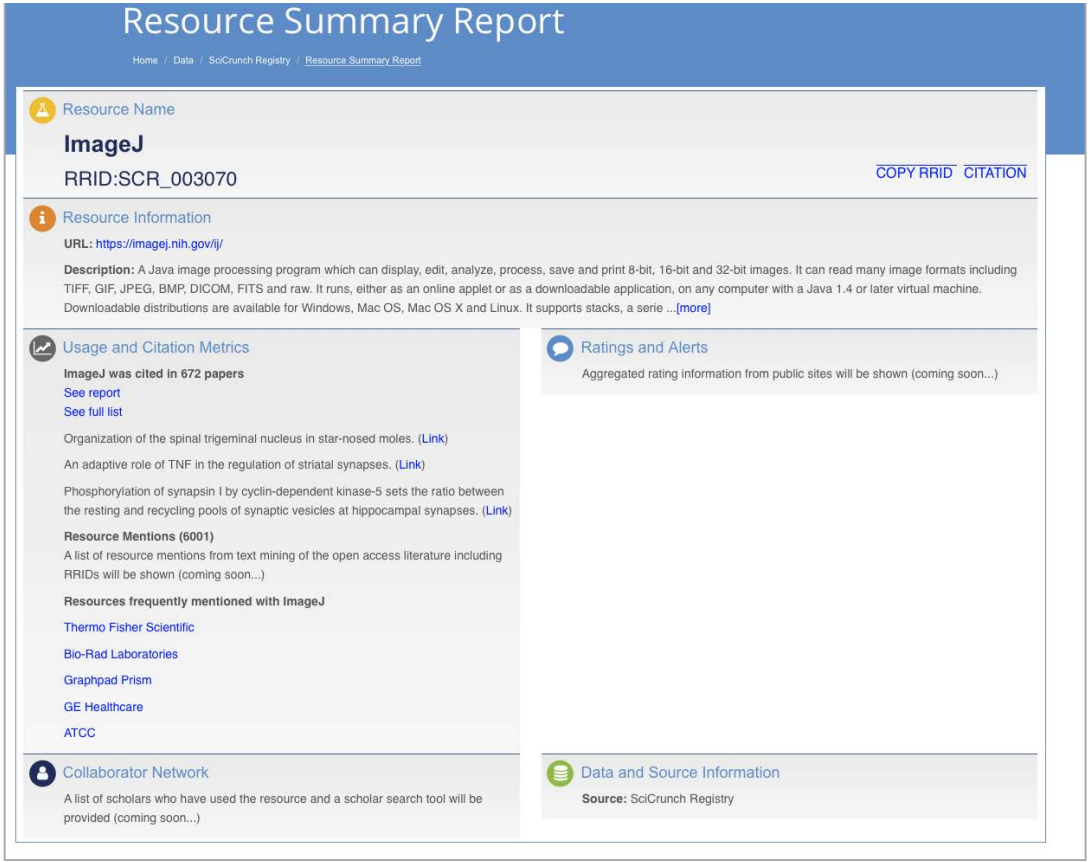
NIDDK Information Network

Research Resource IDentification

Resource Summary Report (under development)

Five major components:

1. Resource Information
2. Usage and Citation Metrics
3. Rating and Alerts (coming soon)
4. Collaborator Network (coming soon)
5. Data and Source Information



NIDDK Information Network

Research Resource IDentification

Resource Usage Report (under development)

Four components:

1. About the report
2. Usage
3. Additional filters
4. All mentions

Plan for Resource Reports for programs/centers/cores:

A user can download a resource report for each resource and its child resources

Resource	Child resource
Program center (i.e., Diabetes Research Centers)	University level center (i.e., Joslin DRC)
University level center (i.e., Joslin DRC)	Core facilities (i.e., Joslin DRC bioinformatic core)
Center of Core Facility (i.e., Joslin DRC)	Research resource (i.e., antibodies, software)

nb: The RRID does NOT take the place of citing the P30 in publications!

However:

Our hope is that by adopting the use of RRIDs, the centers & NIH will be able to track core & center activity through the literature using a multi-pronged approach…..

NIDDK Information Network

Research Resource IDentification

Adopting RRIDs for use by the DCO program Centers & Cores

- Find your center & core RRIDs (either through dkNET or as listed on DCO website)
- Provide author/citation instructions when services are provided

- Stay tuned for new search/report developments to be provided by dkNET!

NIH Clinical Trial Policies

Barbara Linder, M.D., Ph.D.
Division of Diabetes, Endocrinology and Metabolic Diseases
NIDDK
March 2018

Clinical Trial Definition

- Clinical Trial: A research study in which one or more human subjects are prospectively assigned to one or more interventions (which may include placebo or other control) to evaluate the effects of those interventions on health-related biomedical or behavioral outcomes.
- Therapeutic as well as mechanistic studies

Clinical Trial Definition

- Does the study involve human participants?
- Are the participants prospectively assigned to an intervention?
- Is the study designed to evaluate the effect of the intervention on the participants?
- Is the effect being evaluated a health-related biomedical or behavioral outcome?

Knowing Whether Your Study is a Clinical Trial is Crucial

- Clinical trial-specific FOAs
- Additional review criteria
- Register and report results in ClinicalTrials.gov

New FOA Policies

- Parent R01 FOA will no longer accept CTs (Jan. 2018)
- Each IC will develop their own clinical trial FOA(s)
- All FOAs will indicate in a new box on the template whether CTs are allowed (Required, Not Allowed, Optional)
- New application package -- includes Human Subjects and Clinical Trial Information form. Consolidates all human subjects information into one form and expands the information required for CTs

ClinicalTrials. gov

- All clinical trials that will be initiated on/after January 18, 2017 must register and report results in ClinicalTrials.gov
- ICMJE requires registration before the first participant is randomized
- Must be in consent form

ClinicalTrials.gov – Results Reporting

- Within 1 year of primary completion
- Participant flow
- Demographic/baseline characteristics
- Primary and secondary outcomes
- Adverse events
- Protocol and statistical analysis plan

https://nexus.od.nih.gov/all/2016/09/16/visibility-of-clinical-trial-activities-and-results/

Good Clinical Practice (GCP) Training

- All NIH-funded investigators involved in the conduct, oversight or management of clinical trials
- Effective January 2017
- Recertification every three years

Use of Single IRBs

- Domestic, multi-site, non-exempt human subjects research studies MUST use a single IRB of record
- Applications due on or after January 25, 2018
- Not applicable to Career Development (K), Research Training (T), or Fellowship (F) applications
- https://ncats.nih.gov/ctsa/projects/smartirb

Certificates of Confidentiality

- CoCs will now be issued automatically for any NIH-funded project using identifiable, sensitive information
- Policy takes effect October 1, 2017
- Covers all studies ongoing on/after December 13, 2016
- Part of Terms and Conditions of Award

NIH Policy Changes

:

All Research Involving Human Participants

- ✓ New forms to collect human subjects information
- ✓ Use of a single Institutional Review Board (IRB) for multi-site studies
- ✓ Certificates of confidentiality for all research that uses “identifiable, sensitive information”

Research that Meets the NIH Definition of a Clinical Trial

- ✓ Training in Good Clinical Practice (GCP)
- ✓ Clinical trial-specific Funding Opportunity Announcements (FOAs)
- ✓ New review criteria
- ✓ Expanded registration and results reporting in ClinicalTrials.gov

Learn more at https://grants.nih.gov/policy/clinical-trials.htm

https://grants.nih.gov/grants/guide/pa-files/PA-18-330.html

Investigator-Initiated Clinical Trials Targeting Diseases within the Mission of NIDDK (R01 Clinical Trial Required)

New Oversight Policies

- More structured programmatic review of:
  - --clinical trial design
  - --study progress
- Each Institute will develop its own processes

NIH Appendix Policy

- NO appendix materials allowed, except for the following in clinical trials:

  --Blank data collection forms, blank survey forms and blank questionnaire forms -- or screenshots thereof.

  --Simple lists of interview questions.

  --Blank informed consent/assent forms

  --Other items only if they are specified in the FOA as allowable Appendix materials

- Non-compliant applications being withdrawn

https://grants.nih.gov/grants/guide/notice-files/NOT-OD-18-126.html

National Institute of
Diabetes and Digestive
and Kidney Diseases

National Institute of Diabetes and Digestive and Kidney Diseases

NIDDK Diabetes Research Centers

Up-Coming RFAs

National Institute of Diabetes and Digestive and Kidney Diseases

Fiscal Year 2020

- **RFA:** Published by Fall 2018
- **RFA**: Application deadline: summer 2019
- Renewal Applications:
  - Boston Area DERC
  - Einstein-Mount Sinai DRC
  - Indiana DRC
  - UCSF

National Institute of Diabetes and Digestive and Kidney Diseases

National Institute of Diabetes and Digestive and Kidney Diseases

NIDDK Diabetes Research Centers

Interim-Final Progress Reports

National Institute of Diabetes and Digestive and Kidney Diseases

Interim Final Progress Report

NIH Guide Notice (OD-17-037): Effective February 9, 2017, if the recipient organization has submitted a renewal application on or before the date by which a Final Research Performance Progress Report (Final-RPPR) would be required for the current competitive segment, then submission of an "Interim-RPPR" via eRA Commons is now required. Based on this requirement, the NIH will discontinue the policy for renewal applications whereby, “whether funded or not,” the progress report contained in the renewal application may serve in lieu of a separate final progress report.

NIH National Institute of Diabetes and Digestive and Kidney Diseases

Interim Final Progress Report

Interim RPPR – Use when submitting a renewal (Type 2) application. If the Type 2 is not funded, the Interim RPPR will serve as the Final RPPR for the project. If the Type 2 is funded, the Interim RPPR will serve as the annual RPPR for the final year of the previous competitive segment. The data elements collected on the Interim RPPR are the same as for the Final RPPR, including project outcomes.

National Institute of Diabetes and Digestive and Kidney Diseases

Interim Final Progress Report

The format of the Interim-RPPR is very similar to that of the annual RPPR. The notable differences being the Interim RPPR does not have sections F (Changes), and H (Budget). Likewise, the following items do not require responses: B.1.a (changes to major goals), B.6 (plan for next reporting period), D.2 (personnel updates), G.10 (unobligated balance), G.11 (program income), and G.12 (change in F&A costs).

The Interim RPPR does have a new section: Outcomes

National Institute of Diabetes and Digestive and Kidney Diseases

Interim Final Progress Report

Section B: Overall Accomplishments.
B.1 should be prepopulated after the initial RPPR is submitted
B.2 combine ALL components of the P30 grant into this section (Admin Core, Biomedical Research Cores, P&F program and Enrichment Program); you may use the same format as listed in the annual RPPR instructions

Section C: Products.
C.1 Publications; should be populated via link to My Bibliography (the same as in the annual RPPR)

Section D: Participants. For Interim-RPPR only Section D.1 is required.

Section I: Outcomes. Information in this section will be made public in NIH RePORTER. The Outcomes section should be a concise summary of the outcomes or findings of the award, written for the general public in clear and comprehensible language, without including any proprietary, confidential information or trade secrets

National Institute of Diabetes and Digestive and Kidney Diseases

Interim Final Progress Report

Special Reporting Requirements:

G.4 Human Subjects
G.7 Vertebrate Animals

In most cases, the responses will be “Not Applicable”.

National Institute of Diabetes and Digestive and Kidney Diseases

National Institute of
Diabetes and Digestive
and Kidney Diseases

DIABETES RESEARCH CENTERS

2018 Institutional Diabetes Center Websites

Albert Einstein College of Medicine: http://www.einstein.yu.edu/centers/diabetes-research/

Baltimore Area (JHU/UMD): https://www.hopkinsmedicine.org/diabetes-research-center/index.html

Boston Area: http://www.baderc.org/

Columbia University: https://www.derc.cumc.columbia.edu/

Indiana University: https://medicine.iu.edu/research/centers-institutes/diabetes-metabolic-diseases/

Joslin Diabetes Center: https://joslinresearch.org/

Stanford University: https://sdrc.stanford.edu/

University of Alabama at Birmingham: http://www.uab.edu/shp/drc/

UCSD/UCLA: http://drc.ucsd.edu/index.shtml

UCSF: http://drc.ucsf.edu/

University of Chicago: http://drtc.bsd.uchicago.edu/

University of Michigan: http://diabetesresearch.med.umich.edu/

University of Pennsylvania: http://www.med.upenn.edu/idom/

University of Washington: http://depts.washington.edu/diabetes/

Vanderbilt University: https://labnodes.vanderbilt.edu/drtc

Washington University in St. Louis: https://diabetesresearchcenter.dom.wustl.edu/

Yale University: http://derc.yale.edu/

Opioid Receptor Activation Impairs Hypoglycemic Counterregulation in Humans

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Although intensive glycemic control improves outcomes in type 1 diabetes mellitus (T1DM), iatrogenic hypoglycemia limits its attainment. Recurrent and/or antecedent hypoglycemia causes blunting of protective counterregulatory responses, known as hypoglycemia-associated autonomic failure (HAAF). To determine whether and how opioid receptor activation induces HAAF in humans, 12 healthy subjects without diabetes (7 men, age 32.3 ± 2.2 years, BMI 25.1 ± 1.0 kg/m^2) participated in two study protocols in random order over two consecutive days. On day 1, subjects received two 120-min infusions of either saline or morphine (0.1 μg/kg/min), separated by a 120-min break (all euglycemic). On day 2, subjects underwent stepped hypoglycemic clamps (nadir 60 mg/dL) with evaluation of counterregulatory hormonal responses, endogenous glucose production (EGP, using 6,6-D2-glucose), and hypoglycemic symptoms. Morphine induced an ∼30% reduction in plasma epinephrine response together with reduced EGP and hypoglycemia-associated symptoms on day 2. Therefore, we report the first studies in humans demonstrating that pharmacologic opioid receptor activation induces some of the clinical and biochemical features of HAAF, thus elucidating the individual roles of various receptors involved in HAAF's development and suggesting novel pharmacologic approaches for safer intensive glycemic control in T1DM.

Intensive insulin therapy in type 1 diabetes mellitus (T1DM) has been clearly shown to reduce many diabetes-associated complications, and thus achievement of near-normal glycemia is an important management goal (1). However, despite clear clinical benefits, intensive therapy is associated with an increased risk of iatrogenic hypoglycemia, with a threefold increase in severe hypoglycemia reported in the intensively treated group in the Diabetes Control and Complications Trial (DCCT) (1) and even higher rates reported more recently among patients with T1DM by the U.K. Hypoglycemia Study Group (2). Despite medical advances in diabetes management, the problem of iatrogenic hypoglycemia has not been ameliorated (3) and remains both a clinical challenge and a costly public health problem. Indeed, there are an estimated nearly 100,000 emergency department visits and 30,000 hospital admissions for insulin-related hypoglycemia yearly in the U.S. alone (4). Furthermore, hypoglycemia per se causes morbidity and may even be fatal, with 6–10% of deaths in patients with T1DM attributed directly to hypoglycemic events (5).

Patients with T1DM are at particular risk of frequent hypoglycemia due to exogenous insulin treatment because they demonstrate blunted hormonal counterregulatory responses to hypoglycemia (6). In addition, it has been well established in both subjects without diabetes (7) and those with T1DM (6,8) that stressors such as recurrent hypoglycemia or exercise lead to blunting of protective glucagon and sympathoadrenomedullary counterregulatory responses as well as deterioration of hypoglycemia awareness and recovery, conditions known as hypoglycemia-associated autonomic failure (HAAF) and exercise-associated autonomic failure (EAAF), respectively (6).

Although the exact mechanisms underlying the development of HAAF and EAAF have not been fully elucidated, central nervous system (CNS) signals mediating the counterregulatory response have been implicated in its pathogenesis (9). Robust data point to a key role of the endogenous

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Cell Metabolism
Article

Astrocytic Process Plasticity and IKKβ/NF-κB in Central Control of Blood Glucose, Blood Pressure, and Body Weight

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SUMMARY

Central regulation of metabolic physiology is mediated critically through neuronal functions; however, whether astrocytes are also essential remains unclear. Here we show that the high-order processes of astrocytes in the mediobasal hypothalamus displayed shortening in fasting and elongation in fed status. Chronic overnutrition and astrocytic IKKβ/NF-κB upregulation similarly impaired astrocytic plasticity, leading to sustained shortening of high-order processes. In physiology, astrocytic IKKβ/NF-κB upregulation resulted in early-onset effects, including glucose intolerance and blood pressure rise, and late-onset effects, including body weight and fat gain. Appropriate inhibition in astrocytic IKKβ/NF-κB protected against chronic overnutrition impairing astrocytic plasticity and these physiological functions. Mechanistically, astrocytic regulation of hypothalamic extracellular GABA level and therefore BDNF expression were found partly accountable. Hence, astrocytic process plasticity and IKKβ/NF-κB play significant roles in central control of blood glucose, blood pressure, and body weight as well as the central induction of these physiological disorders leading to disease.

INTRODUCTION

Hypothalamic neurons are important for the regulation of feeding behavior and related metabolic physiology (Cone, 2005; Dietrich and Horvath, 2011; Flier and Maratos-Flier, 1998; Münzberg and Myers, 2005; Schwartz et al., 2000; Tschöp et al., 2006). Understanding the regulatory functions of hypothalamic neurons—for example, through secretion of various neuropeptides or formation of various neural circuits—has been a major focus of research, including many studies during recent years (Chiappini et al., 2014; Cohen et al., 2001; Coppari et al., 2005; Flak et al., 2014; Ghamari-Langroudi et al., 2015; Kievit et al., 2006; Kitamura et al., 2006; Kleinridders et al., 2013; Lam et al., 2005; Zeltser et al., 2012; Zhang et al., 2008). In contrast to neurons, astrocytes have not received adequate attention, despite the fact that they are not only the most abundant neural cells but also display remarkably high diversities in morphology and functions (Eroglu and Barres, 2010; Fields and Stevens-Graham, 2002; García-Cáceres et al., 2016; Ishibashi et al., 2006; Muroyama et al., 2005; Muthukumar et al., 2014; Nedergaard et al., 2003; Perea et al., 2014; Rangroo Thrane et al., 2013; Shigetomi et al., 2011; Theodosis et al., 2008; Wyss-Coray et al., 2003). Astrocytes and neurons are strongly interconnected and the astrocyte-to-neuron ratio surges with increased complexity of the brain (Nedergaard et al., 2003). Previous studies have shown that astrocytes secrete cytokines and neurotrophic factors to promote neuronal development and plasticity, modulate synaptic excitation through uptake and release of neurotransmitters, and maintain various types of homeostasis in brain tissues (Eroglu and Barres, 2010; Jo et al., 2014; Otsu et al., 2015; Rangroo Thrane et al., 2013; Schachtrup et al., 2015; Shigetomi et al., 2011). Also, astrocytes can influence the brain's metabolic condition by controlling glycogen storages and supplying fuel to neurons (Bélanger et al., 2011; Choi et al., 2012), and it has been reported that hypothalamic astrocytes can sense glucose via glucose transporter 2 and register leptin signaling via leptin receptor (Fuente-Martín et al., 2012; Marty et al., 2005). Recently, hypothalamic astrocyte processes were found to change in response to leptin (Kim et al., 2014), and lack of insulin signaling in astrocytes can alter the processes and brain glucose uptake (García-Cáceres et al., 2016). From the disease perspective, high-fat diet (HFD)-induced obesity in mice has been shown to be associated with hypothalamic astrogliosis (Thaler et al., 2012). Despite these observations, the role of astrocytes in hypothalamic control of metabolic physiology or disease still remains undetermined.

As is known, the majority of astrocytic regulatory functions rely on their astrocytic processes (Haydon and Carmignoto, 2006; Kimelberg and Nedergaard, 2010). Indeed, the processes of astrocytes are interspersed between neurons, shrouding neuronal somata, synapses, and dendrites (Theodosis et al., 2008). Astrocytes also express numerous transporters and receptors that are mainly localized in these processes (Chung et al., 2013; Minelli et al., 1996; Muthukumar et al., 2014; Shigetomi et al., 2011). The coating of neurons by astrocyte processes facilitates the containment of ions and neurotransmitters within neurons; such

System-wide Benefits of Intermeal Fasting by Autophagy

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SUMMARY

Autophagy failure is associated with metabolic insufficiency. Although caloric restriction (CR) extends healthspan, its adherence in humans is poor. We established an isocaloric twice-a-day (ITAD) feeding model wherein ITAD-fed mice consume the same food amount as *ad libitum* controls but at two short windows early and late in the diurnal cycle. We hypothesized that ITAD feeding will provide two intervals of intermeal fasting per circadian period and induce autophagy. We show that ITAD feeding modifies circadian autophagy and glucose/lipid metabolism that correlate with feeding-driven changes in circulating insulin. ITAD feeding decreases adiposity and, unlike CR, enhances muscle mass. ITAD feeding drives energy expenditure, lowers lipid levels, suppresses gluconeogenesis, and prevents age/obesity-associated metabolic defects. Using liver-, adipose-, myogenic-, and proopiomelanocortin neuron-specific autophagy-null mice, we mapped the contribution of tissue-specific autophagy to system-wide benefits of ITAD feeding. Our studies suggest that consuming two meals a day without CR could prevent the metabolic syndrome.

INTRODUCTION

Decreased quality control and accumulation of damaged organelles are factors contributing to chronic diseases including the metabolic syndrome. Autophagy, a lysosomal quality control pathway critical for cellular cleanliness, is compromised with age, setting the basis for chronic diseases (Rubinsztein et al., 2011). In fact, mice knocked out (KO) for the autophagy gene *Atg7* or lacking Beclin function display early lethality (Karsli-Uzunbas et al., 2014) and metabolic defects including fat accumulation (Singh et al., 2009a), muscle loss (Martinez-Lopez et al., 2013; Masiero et al., 2009), and glucose intolerance (He et al., 2012; Karsli-Uzunbas et al., 2014).

Caloric restriction (CR) extends healthspan and lifespan in multiple organisms (Colman et al., 2009; Mattison et al., 2012). Despite its remarkable benefits, humans adhere poorly to CR (Moreira et al., 2011), which has motivated the search for sustainable approaches to extend healthspan. Alternate feeding strategies, including intermittent fasting (Anson et al., 2003; Heilbronn et al., 2005; Varady et al., 2009), fasting-mimicking intervention (Brandhorst et al., 2015), and time-restricted feeding (Chaix et al., 2014) each mimic the effects of CR. Since fasting activates autophagy, it is conceivable that dietary interventions mediate their benefits, in part, through autophagy. The integrative physiology of autophagy and its ability to promote metabolic correction in a dietary intervention model remains unexplored.

Because fasting activates autophagy, we established an isocaloric twice-a-day (ITAD) feeding model wherein test mice eat the same amount of food as *ad libitum* (Ad-lib) controls (Con), albeit they eat their food at two 2 hr windows early and late in the diurnal cycle. We hypothesized that adopting the ITAD feeding strategy will eliminate scattered feeding, and provide two windows of intermeal fasting in each circadian period, which in principle will sustain autophagy without the need to restrict calories or alter the type of food consumed. Here we show that ITAD feeding promotes system-wide benefits including reduction of body fat and increased lean mass that accompany significant tissue remodeling. ITAD feeding sustains autophagy levels in aged mice, and prevents age-associated energy imbalance, dyslipidemia, and glucose intolerance. Using liver-, adipose-, myogenic-, and hypothalamic proopiomelanocortin (POMC) neuron-specific *Atg7* KO mice, we identified the contribution of cell-specific autophagy to system-wide benefits of ITAD feeding.

RESULTS

ITAD Feeding in Mice

To develop a feeding strategy that incorporates periods of fasting between feeding windows, we randomized 4-month-old C57BL/6J male mice into Ad-lib Con and ITAD groups. ITAD

Cell

An Ancient, Unified Mechanism for Metformin Growth Inhibition in *C. elegans* and Cancer

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SUMMARY

Metformin has utility in cancer prevention and treatment, though the mechanisms for these effects remain elusive. Through genetic screening in *C. elegans*, we uncover two metformin response elements: the nuclear pore complex (NPC) and acyl-CoA dehydrogenase family member-10 (ACAD10). We demonstrate that biguanides inhibit growth by inhibiting mitochondrial respiratory capacity, which restrains transit of the RagA-RagC GTPase heterodimer through the NPC. Nuclear exclusion renders RagC incapable of gaining the GDP-bound state necessary to stimulate mTORC1. Biguanide-induced inactivation of mTORC1 subsequently inhibits growth through transcriptional induction of ACAD10. This ancient metformin response pathway is conserved from worms to humans. Both restricted nuclear pore transit and upregulation of ACAD10 are required for biguanides to reduce viability in melanoma and pancreatic cancer cells, and to extend *C. elegans* lifespan. This pathway provides a unified mechanism by which metformin kills cancer cells and extends lifespan, and illuminates potential cancer targets.

INTRODUCTION

Metformin has been used to treat type 2 diabetes (T2D) for nearly 60 years. It also has potential benefit in cancer prevention and treatment (Evans et al., 2005; Yuan et al., 2013). The class of drugs to which metformin belongs, the biguanides, inhibit cellular growth in a variety of cancer cell lines, particularly in melanoma (Yuan et al., 2013) and pancreatic cancer cells (Kordes et al., 2015). While it is widely accepted that the mitochondrion is a primary target of metformin (Griss et al., 2015; Owen et al., 2000; Wheaton et al., 2014), exactly how mitochondrial inhibition by metformin is transduced to the drug's other health-promoting effects, including its anticancer properties, remains unclear.

Mitochondrial inhibition by metformin causes energetic stress, which results in activation of the energy sensor adenosine monophosphate-activated protein kinase (AMPK) (Zhou et al., 2001). However, multiple lines of evidence indicate that AMPK is dispensable for metformin's beneficial effects (Foretz et al., 2010; Griss et al., 2015; Kalender et al., 2010), invoking other major metformin effectors downstream of mitochondria.

The protein kinase mechanistic target of rapamycin complex 1 (mTORC1), which also serves as an energy and nutrient sensor, plays a central role in regulating cell growth, proliferation and survival (Schmelzle and Hall, 2000). Inhibition of mTORC1 activity has been reported in cells in culture treated with metformin, suggesting that reduced TOR activity may be important for the metabolic effects of biguanides (Kalender et al., 2010). In support of this idea, both metformin and canonical mTOR inhibitors have highly similar effects on the transcriptome, selectively decreasing mRNA levels of cell-cycle and growth regulators (Larsson et al., 2012). Several, distinct pathways are known to regulate mTORC1 signaling, including TSC-Rheb and Ras-related GTP-binding protein (Rag) GTPase-mediated signaling (Sancak et al., 2008). Metformin may inhibit mTORC1 via modulation of Rag GTPases (Kalender et al., 2010), but the mechanism by which this occurs is uncharacterized. It has been suggested that the pathway that leads to metformin-mediated inhibition of mTORC1 could represent a distinct mechanism of mTORC1 regulation, since no signaling pathway has been identified that connects the mitochondrion to mTORC1 without involvement of AMPK (Sengupta et al., 2010). Whether a

Cell

Article

Selective Chemical Inhibition of PGC-1α Gluconeogenic Activity Ameliorates Type 2 Diabetes

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SUMMARY

Type 2 diabetes (T2D) is a worldwide epidemic with a medical need for additional targeted therapies. Suppression of hepatic glucose production (HGP) effectively ameliorates diabetes and can be exploited for its treatment. We hypothesized that targeting PGC-1α acetylation in the liver, a chemical modification known to inhibit hepatic gluconeogenesis, could be potentially used for treatment of T2D. Thus, we designed a high-throughput chemical screen platform to quantify PGC-1α acetylation in cells and identified small molecules that increase PGC-1α acetylation, suppress gluconeogenic gene expression, and reduce glucose production in hepatocytes. On the basis of potency and bioavailability, we selected a small molecule, SR-18292, that reduces blood glucose, strongly increases hepatic insulin sensitivity, and improves glucose homeostasis in dietary and genetic mouse models of T2D. These studies have important implications for understanding the regulatory mechanisms of glucose metabolism and treatment of T2D.

INTRODUCTION

Type 2 diabetes (T2D) has become a worldwide epidemic that affected over 400 million people in 2014 according to the World Health Organization. Uncontrolled increased blood glucose concentration is a hallmark of T2D that can lead to long-term complications including micro- and macro-cardiovascular complications, neuropathy, kidney failure, and increased risk for developing cancer (Forbes and Cooper, 2013; Richardson and Pollack, 2005). Maintaining blood glucose within a normal range is the foremost objective in the treatment of T2D, as this dramatically reduces the risk of developing diabetes-associated complications (Holman et al., 2008; Kahn et al., 2014). Several drugs are currently being used for treatment of T2D in the clinic; these include primarily metformin, the first line drug used to treat T2D, but also sulfonylureas, thiazolidinediones (TZDs), incretin mimetics, and sodium-glucose cotransporter 2 (SGLT2) inhibitors (Bailey, 2013; Kahn et al., 2014). As most patients develop resistance to metformin treatment over time, a strategy of a combination therapy that includes treatment with an additional drug or insulin administration is commonly employed (Bailey, 2013). This highlights the need for developing additional drugs to reduce hyperglycemia that can be used either as a monotherapy or as part of a combination therapy.

Whole body glucose homeostasis is achieved through an intricate balance between glucose production, mostly by the liver, and glucose uptake by peripheral tissues (DeFronzo, 2004; Moore et al., 2012). This is regulated primarily through the opposing pancreatic hormones insulin and glucagon. In diabetic states, the liver becomes resistant to the action of insulin and produces an elevated amount of glucose, which is a major contributor to the increased blood glucose levels observed in T2D (Rizza, 2010). Hence, reducing hepatic glucose production (HGP) is a feasible strategy to manage blood glucose levels in T2D. Importantly, increased HGP in T2D was shown to be primarily a result of dysregulated gluconeogenesis rather than glycogen breakdown (Magnusson et al., 1992), suggesting that targeting components within the gluconeogenic pathway could improve hyperglycemia. Indeed, metformin is believed to exert its effect mainly by reducing hepatic gluconeogenesis (Hundal et al., 2000; Madiraju et al., 2014).

The transcriptional coactivator PGC-1α plays a pivotal role in energy homeostasis by co-activating transcription factors that

Cell

Type 2 Diabetes Variants Disrupt Function of SLC16A11 through Two Distinct Mechanisms

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SUMMARY

Type 2 diabetes (T2D) affects Latinos at twice the rate seen in populations of European descent. We recently identified a risk haplotype spanning *SLC16A11* that explains ~20% of the increased T2D prevalence in Mexico. Here, through genetic fine-mapping, we define a set of tightly linked variants likely to contain the causal allele(s). We show that variants on the T2D-associated haplotype have two distinct effects: (1) decreasing *SLC16A11* expression in liver and (2) disrupting a key interaction with basigin, thereby reducing cell-surface localization. Both independent mechanisms reduce SLC16A11 function and suggest *SLC16A11* is the causal gene at this locus. To gain insight into how SLC16A11 disruption impacts T2D risk, we demonstrate that SLC16A11 is a proton-coupled monocarboxylate transporter and that genetic perturbation of SLC16A11 induces changes in fatty acid and lipid metabolism that are associated with increased T2D risk. Our findings suggest that increasing SLC16A11 function could be therapeutically beneficial for T2D.

INTRODUCTION

Type 2 diabetes (T2D) afflicts more than 415 million people and is a leading cause of morbidity and mortality worldwide. While T2D is influenced by environmental factors, it is also a highly heritable disorder (Prasad and Groop, 2015), with genetic variation contributing to a disparity in T2D prevalence both within and across populations (Diamond, 2003; Williams et al., 2014). For example, within American populations, the prevalence of T2D is nearly twice as high in individuals of Mexican or Latin American descent as compared to US non-Hispanic whites (Villalpando et al., 2010).

β-Cell Replacement in Mice Using Human Type 1 Diabetes Nuclear Transfer Embryonic Stem Cells

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β-Cells derived from stem cells hold great promise for cell replacement therapy for diabetes. Here we examine the ability of nuclear transfer embryonic stem cells (NT-ESs) derived from a patient with type 1 diabetes to differentiate into β-cells and provide a source of autologous islets for cell replacement. NT-ESs differentiate in vitro with an average efficiency of 55% into C-peptide–positive cells, expressing markers of mature β-cells, including MAFA and NKX6.1. Upon transplantation in immunodeficient mice, grafted cells form vascularized islet-like structures containing MAFA/C-peptide–positive cells. These β-cells adapt insulin secretion to ambient metabolite status and show normal insulin processing. Importantly, NT-ES-β-cells maintain normal blood glucose levels after ablation of the mouse endogenous β-cells. Cystic structures, but no teratomas, were observed in NT-ES-β-cell grafts. Isogenic induced pluripotent stem cell lines showed greater variability in β-cell differentiation. Even though different methods of somatic cell reprogramming result in stem cell lines that are molecularly indistinguishable, full differentiation competence is more common in ES cell lines than in induced pluripotent stem cell lines. These results demonstrate the suitability of NT-ES-β-cells for cell replacement for type 1 diabetes and provide proof of principle for therapeutic cloning combined with cell therapy.

Type 1 diabetes is a disorder characterized by the loss of β-cell mass and function. Because β-cells do not spontaneously regenerate sufficiently to correct diabetes, an exogenous source of β-cells could be useful (1). Transplantation of islets from a pancreatic organ donor can restore physiological regulation of blood glucose in human subjects (2) but require management of allo-immunity. Although autologous cells would not address the recurrence of autoimmunity against transplanted β-cells, it obviates the need to suppress allo-immunity. We have recently shown that pluripotent stem cells matched to a subject with type 1 diabetes can be derived from skin cells by somatic cell nuclear transfer (SCNT) (3). Stem cells can also be derived by induction of pluripotency (4), resulting in highly similar cell types with regard to gene expression and DNA methylation (5). However, the functionality of reprogrammed human stem cells has not been sufficiently tested. Notably, nuclear transfer (NT) from adult cells more consistently results in the production of viable mice (6) than in the production from induced pluripotent stem cells (iPSCs) (7), suggesting that reprogrammed cells derived by SCNT are more often fully differentiation competent (8). Reprogramming by NT recapitulates developmental events that occur upon normal fertilization and allows resetting of the epigenome of the

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Type 1 diabetes induction in humanized mice

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There is an urgent and unmet need for humanized in vivo models of type 1 diabetes to study immunopathogenesis and immunotherapy, and in particular antigen-specific therapy. Transfer of patient blood lymphocytes to immunodeficient mice is associated with xenogeneic graft-versus-host reactivity that complicates assessment of autoimmunity. Improved models could identify which human T cells initiate and participate in beta-cell destruction and help define critical target islet autoantigens. We used humanized mice (hu-mice) containing robust human immune repertoires lacking xenogeneic graft-versus-host reactivity to address this question. Hu-mice constructed by transplantation of HLA-DQ8$^+$ human fetal thymus and CD34$^+$ cells into HLA-DQ8–transgenic immunodeficient mice developed hyperglycemia and diabetes after transfer of autologous HLA-DQ8/insulin-B:9–23 (InsB:9–23)-specific T-cell receptor (TCR)-expressing human CD4$^+$ T cells and immunization with InsB:9–23. Survival of the infused human T cells depended on the preexisting autologous human immune system, and pancreatic infiltration by human CD3$^+$ T cells and insulitis were observed in the diabetic hu-mice, provided their islets were stressed by streptozotocin. This study fits Koch's postulate for pathogenicity, demonstrating a pathogenic role of islet autoreactive CD4$^+$ T-cell responses in type 1 diabetes induction in humans, underscores the role of the target beta-cells in their immunological fate, and demonstrates the capacity to initiate disease with T cells, recognizing the InsB:9–23 epitope in the presence of islet inflammation. This preclinical model has the potential to be used in studies of the pathogenesis of type 1 diabetes and for testing of clinically relevant therapeutic interventions.

type 1 diabetes | insulin | humanized mice

Type 1 diabetes mellitus (T1D) is an autoimmune disease resulting from immune destruction of insulin-producing β cells in the pancreatic islets. Autoreactive T cells recognizing β-cell antigens (e.g., insulin) are believed to play a critical role in disease onset and progression, but direct evidence for this is still lacking (1). The loss of β cells and the subsequent reduction or lack of insulin result in chronic elevation of blood glucose levels, leading to severe complications such as heart disease, stroke, and kidney failure. Patients with T1D rely on exogenous insulin to control the disease (2), but insulin therapy does not eliminate the risk for T1D complications and may be associated with life-threatening hypoglycemia (3). Thus, new therapies are urgently needed for T1D treatment. Recent insight into the human disease lesion in the pancreas indicated profound differences in immunopathology between patients with T1D and the nonobese diabetic mouse model that is regarded as the nearest preclinical model of autoimmune diabetes (4). These observations, combined with a range of other differences in the immune systems of humans versus mice, prompted us to design preclinical models in which the immune system is humanized to study both disease mechanisms and immunotherapeutic strategies (5).

In this study, we sought to overcome this issue, using humanized mice (hu-mice), by infusing autologous hu-mouse–derived human T cells engineered with a TCR that recognizes the HLA-DQ8–restricted insulin B chain peptide consisting of amino acids 9–23 (InsB:9–23). InsB:9–23 is a dominant MHC class II-restricted antigen recognized by islet-infiltrating insulin-specific T cells and serves as an essential target of the immune destruction of pancreatic β cells in nonobese diabetic (NOD) mice (6, 7). Previous observations suggested this epitope may also serve as a key autoantigenic target in humans, as it does in mice. HLA class II-restricted T-cell response to InsB:9–23 peptide is highly associated with T1D in humans (8). A recent study using HLA-DQ8/B:11–23^{R22E} tetramers further confirmed the presence of CD4 T cells recognizing the HLA-DQ8–restricted B:11–23 peptide in patients with T1D (9). More recently, 2.35% of CD4 T-cell clones isolated from inflamed islets of patients with T1D were found to recognize InsB:9–23 (10). However, there has been no direct evidence for human InsB:9–23-reactive T-cell–mediated in vivo destruction of pancreatic β cells in humans. Our data showed that adoptive transfer of HLA-DQ8–restricted InsB:9–23-specific human CD4 T cells is capable of inducing diabetes in HLA-DQ8–Tg hu-mice, consistent with the potential of T-cell responses to the InsB:9–23 epitope to initiate T1D in humans.

Results

The TCR α ($V_{\alpha 21}$) and β ($V_{\beta 11}$) chain cDNA was extracted from an InsB:9–23-specific human T-cell line (clone #5), which was established from blood from an 18-y-old Caucasoid HLA-DQ8 homozygous man diagnosed with T1D at the age of 8 y (11, 12), and linked by a P2A self-cleaving peptide gene. The TCRα-P2A-β gene fragment was then linked to a F2A-AcGFP gene fragment, and then cloned into a lentiviral vector (LV-insTCR; Fig. S1). To assess the

Significance

Type 1 diabetes (T1D) is known to be caused by immune destruction of insulin-producing β cells, but the disease pathogenesis remains poorly understood largely because of limitations in animal models to study the immunopathology. Here we established a humanized mouse T1D model, in which diabetes is driven by human T cells recognizing the HLA-DQ8–restricted insulin B chain peptide consisting of amino acids 9–23 (InsB:9–23). This study not only demonstrates the capacity of InsB:9–23-specific human CD4 T cells to initiate diabetes but also provides a preclinical humanized mouse model that has the potential to be used in studies of the immunopathogenesis and immunotherapy of T1D.

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Cell

Article

Selective Inhibition of FOXO1 Activator/Repressor Balance Modulates Hepatic Glucose Handling

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SUMMARY

Insulin resistance is a hallmark of diabetes and an unmet clinical need. Insulin inhibits hepatic glucose production and promotes lipogenesis by suppressing FOXO1-dependent activation of G6pase and inhibition of glucokinase, respectively. The tight coupling of these events poses a dual conundrum: mechanistically, as the FOXO1 corepressor of glucokinase is unknown, and clinically, as inhibition of glucose production is predicted to increase lipogenesis. Here, we report that SIN3A is the insulin-sensitive FOXO1 corepressor of glucokinase. Genetic ablation of SIN3A abolishes nutrient regulation of glucokinase without affecting other FOXO1 target genes and lowers glycemia without concurrent steatosis. To extend this work, we executed a small-molecule screen and discovered selective inhibitors of FOXO-dependent glucose production devoid of lipogenic activity in hepatocytes. In addition to identifying a novel mode of insulin action, these data raise the possibility of developing selective modulators of unliganded transcription factors to dial out adverse effects of insulin sensitizers.

INTRODUCTION

Insulin resistance predisposes to diabetes and metabolic diseases. Restoring insulin sensitivity is an effective approach to prevent and treat diabetes (Berkowitz et al., 1996) and to reduce its macrovascular complications (Kernan et al., 2016). However, currently available insulin sensitizers have significant adverse effects, such as weight gain due to triglyceride accumulation, fractures (possibly related to increased bone marrow adipogenesis), and hemodynamic changes (Cariou et al., 2012).

These "adverse" effects are part and parcel of increased insulin sensitivity and cannot be effectively separated from it. This conundrum is best illustrated by the role of insulin in the liver. Insulin has pleiotropic hepatic effects mediated by diverse signaling mechanisms, including the PI3K/AKT/FOXO pathway (Dong et al., 2008; Lu et al., 2012; Matsumoto et al., 2007). Others and we have shown that insulin inhibits FOXO (1, 3a, and 4), resulting in decreased glucose production and increased glucose utilization through glycolysis, glycogen synthesis, and de novo lipogenesis (Altomonte et al., 2003; Haeusler et al., 2010a, 2010b; Matsumoto et al., 2007; Samuel et al., 2006; Zhang et al., 2006). This is achieved through an elegant, if unexplained, mechanism whereby FOXOs inhibit expression of the rate-limiting enzyme of glucose utilization, glucokinase (*Gck*) (Dong et al., 2008; Haeusler et al., 2014; Zhang et al., 2006), while stimulating the rate-limiting enzyme of glucose production, glucose-6-phosphatase (*G6pc*) (Haeusler et al., 2014; Nakae et al., 2001a). When FOXOs are inhibited, glucose production decreases, potentially benefiting diabetes treatment, but hepatic lipid synthesis increases, predisposing to steatosis (Pajvani and Accili, 2015).

Whereas the activating functions of FOXO can be explained by binding to DNA via the forkhead domain (Cook et al., 2015a), the mechanism of its repressor functions in the liver is unknown. In this study, we sought to discover how FOXOs suppress hepatic *Gck* and then leveraged this knowledge to identify selective FOXO inhibitors with the ability to inhibit *G6pc* but bereft of *Gck*-stimulating activity.

RESULTS

Insulin Induction of Gck Requires Glucocorticoid-Induced Foxo1 Expression

Gck expression is induced within 1 hr of refeeding, suppressed after a 4-hr fast (Haeusler et al., 2014), and inversely correlated with *Foxo1* expression (Figure S1A). This regulation is abolished in liver-specific triple FOXO (1, 3a, and 4) knockout mice (*L-Foxo1,3,4*; Haeusler et al., 2014). To identify the corepressor(s) required for FOXO inhibition of *Gck*, we established an in vitro system that recapitulated hormonal control of *Gck* expression. We incubated primary murine hepatocytes in the

Glucagon-Like Peptide 1 Receptor Activation Augments Cardiac Output and Improves Cardiac Efficiency in Obese Swine After Myocardial Infarction

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This study tested the hypothesis that glucagon-like peptide 1 (GLP-1) therapies improve cardiac contractile function at rest and in response to adrenergic stimulation in obese swine after myocardial infarction. Obese Ossabaw swine were subjected to gradually developing regional coronary occlusion using an ameroid occluder placed around the left anterior descending coronary artery. Animals received subcutaneous injections of saline or liraglutide (0.005–0.015 mg/kg/day) for 30 days after ameroid placement. Cardiac performance was assessed at rest and in response to sympathomimetic challenge (dobutamine 0.3–10 μg/kg/min) using a left ventricular pressure/volume catheter. Liraglutide increased diastolic relaxation (dP/dt; Tau $^{1}/_{2}$; Tau $^{1}/_{e}$) during dobutamine stimulation ($P < 0.01$) despite having no influence on the magnitude of myocardial infarction. The slope of the end-systolic pressure volume relationship (i.e., contractility) increased with dobutamine after liraglutide ($P < 0.001$) but not saline administration ($P = 0.63$). Liraglutide enhanced the slope of the relationship between cardiac power and pressure volume area (i.e., cardiac efficiency) with dobutamine ($P = 0.017$). Hearts from animals treated with liraglutide demonstrated decreased β1-adrenoreceptor expression. These data support that GLP-1 agonism augments cardiac efficiency via attenuation of maladaptive sympathetic signaling in the setting of obesity and myocardial infarction.

PATHOPHYSIOLOGY

Use of glucagon like peptide 1 (GLP-1)–based therapies for the treatment of type 2 diabetes (T2DM) has increased significantly since their discovery in the 1980s (1). Although these agents demonstrate unequivocal efficacy in the control of blood glucose concentration, an emerging body of evidence indicates direct cardiovascular benefit, including improvements in cardiac contractile function (2), reductions in myocardial infarct size (3) in animal studies, and improved cardiovascular event rates in some but not all clinical trials (4–6). Thus, there is strong interest in these apparent cardioprotective effects of GLP-1–based therapies in clinical applications of obesity and T2DM (7,8).

Prior studies of the cardiovascular effects of GLP-1 have largely been performed in animal models lacking obesity/metabolic disease phenotypes, in contrast to the fact that most patient populations treated with agents from this therapeutic class are overweight or obese (9). Studies from our laboratory and others have demonstrated obesity-related impairment in cardiovascular effects of GLP-1 agonists, including attenuation of GLP-1–mediated increases in myocardial glucose uptake in obese swine (10) and humans with T2DM (10,11). Further evidence of different responses in obesity comes from our recent report that the GLP-1R agonist exendin-4 augmented end-diastolic volume (EDV) and systolic pressure generation during coronary reperfusion in lean swine while maintaining systolic pressure despite marked reduction in diastolic filling in obese swine (12).

The mechanisms responsible for these obesity-related differences are unknown. However, the capacity for GLP-1 to induce changes in cardiac inotropy (13) and lusitropy (12) implicate underlying differences in adrenergic responsiveness that are known to exist in obesity (14). Such metabolic state–dependent effects of GLP-1 combined with the inconsistent cardiovascular benefits of GLP-1–based therapeutics in clinical trials of patients with diabetes (4,5)

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Effectiveness of Computer Automation for the Diagnosis and Management of Childhood Type 2 Diabetes
A Randomized Clinical Trial

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IMPORTANCE Type 2 diabetes (T2D) is increasingly common in young individuals. Primary prevention and screening among children and adolescents who are at substantial risk for T2D are recommended, but implementation of T2D screening practices in the pediatric primary care setting is uncommon.

OBJECTIVE To determine the feasibility and effectiveness of a computerized clinical decision support system to identify pediatric patients at high risk for T2D and to coordinate screening for and diagnosis of prediabetes and T2D.

DESIGN, SETTING, AND PARTICIPANTS This cluster-randomized clinical trial included patients from 4 primary care pediatric clinics. Two clinics were randomized to the computerized clinical decision support intervention, aimed at physicians, and 2 were randomized to the control condition. Patients of interest included children, adolescents, and young adults 10 years or older. Data were collected from January 1, 2013, through December 1, 2016.

INTERVENTIONS Comparison of physician screening and follow-up practices after adding a T2D module to an existing computer decision support system.

MAIN OUTCOMES AND MEASURES Electronic medical record (EMR) data from patients 10 years or older were reviewed to determine the rates at which pediatric patients were identified as having a body mass index (BMI) at or above the 85th percentile and 2 or more risk factors for T2D and underwent screening for T2D.

RESULTS Medical records were reviewed for 1369 eligible children (712 boys [52.0%] and 657 girls [48.0%]; median [interquartile range] age, 12.9 [11.2-15.3]), of whom 684 were randomized to the control group and 685 to the intervention group. Of these, 663 (48.4%) had a BMI at or above the 85th percentile. Five hundred sixty-five patients (41.3%) met T2D screening criteria, with no difference between control and intervention sites. The T2D module led to a significant increase in the percentage of patients undergoing screening for T2D (89 of 283 [31.4%] vs 26 of 282 [9.2%]; adjusted odds ratio, 4.6; 95% CI, 1.5-14.7) and a greater proportion attending a scheduled follow-up appointment (45 of 153 [29.4%] vs 38 of 201 [18.9%]; adjusted odds ratio, 1.8; 95% CI, 1.5-2.2).

CONCLUSIONS AND RELEVANCE Use of a computerized clinical decision support system to automate the identification and screening of pediatric patients at high risk for T2D can help overcome barriers to the screening process. The support system significantly increased screening among patients who met the American Diabetes Association criteria and adherence to follow-up appointments with primary care clinicians.

TRIAL REGISTRATION clinicaltrials.gov Identifier: NCT01814787

Journal Club Slides and Supplemental content

CME Quiz at jamanetworkcme.com and CME Questions page 403

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Lack of liver glycogen causes hepatic insulin resistance and steatosis in mice

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Disruption of the *Gys2* gene encoding the liver isoform of glycogen synthase generates a mouse strain (LGSKO) that almost completely lacks hepatic glycogen, has impaired glucose disposal, and is pre-disposed to entering the fasted state. This study investigated how the lack of liver glycogen increases fat accumulation and the development of liver insulin resistance. Insulin signaling in LGSKO mice was reduced in liver, but not muscle, suggesting an organ-specific defect. Phosphorylation of components of the hepatic insulin-signaling pathway, namely IRS1, Akt, and GSK3, was decreased in LGSKO mice. Moreover, insulin stimulation of their phosphorylation was significantly suppressed, both temporally and in an insulin dose response. Phosphorylation of the insulin-regulated transcription factor FoxO1 was somewhat reduced and insulin treatment did not elicit normal translocation of FoxO1 out of the nucleus. Fat overaccumulated in LGSKO livers, showing an aberrant distribution in the acinus, an increase not explained by a reduction in hepatic triglyceride export. Rather, when administered orally to fasted mice, glucose was directed toward hepatic lipogenesis as judged by the activity, protein levels, and expression of several fatty acid synthesis genes, namely, acetyl-CoA carboxylase, fatty acid synthase, SREBP1c, chREBP, glucokinase, and pyruvate kinase. Furthermore, using cultured primary hepatocytes, we found that lipogenesis was increased by 40% in LGSKO cells compared with controls. Of note, the hepatic insulin resistance was not associated with increased levels of pro-inflammatory markers. Our results suggest that loss of liver glycogen synthesis diverts glucose toward fat synthesis, correlating with impaired hepatic insulin signaling and glucose disposal.

After ingestion of a meal, glucose is cleared from the bloodstream primarily by conversion to glycogen in skeletal muscle and liver, the liver disposing as much as one-third of the glucose load (1). Defects in insulin-mediated regulation of glycogen synthase (GS)[5] a key biosynthetic enzyme (2) are present in the muscle of most type 2 diabetes mellitus (T2DM) patients, who display post-prandial hyperglycemia and compromised glucose disposal (3, 4). Two GS genes exist in mammals, *GYS1*, encoding the isoform expressed in muscle and many other tissues, and *GYS2*, encoding an apparently liver-specific isoform (LGS). Mice lacking *GYS2* (LGSKO) have a severe decrease in their ability to store glycogen in hepatocytes (5), and display most of the symptoms of glycogen storage disease in patients who have loss-of-function mutations in the *GYS2* gene (6). LGSKO mice are glucose intolerant and exhibit impaired suppression of gluconeogenesis upon insulin stimulation (5). Mice with a disruption of the *GYS1* gene are unable to synthesize glycogen in several tissues, but surprisingly glucose tolerance was actually improved (7, 8). One conclusion from these studies, and studies using other genetically engineered murine models (9, 10), was that, in rodents, muscle glycogen represents a much smaller fraction of the total body glycogen stores than in humans, liver therefore having a greater role in overall glucose disposal and insulin sensitivity (11, 12).

Insulin resistance is often accompanied by increased hepatic steatosis (13, 14). However, it is still unclear whether insulin resistance is responsible for excessive fat deposition in the liver or whether increased fat content is a prerequisite for the development of insulin resistance (15). Compromised glucose disposal as glycogen may further contribute to hepatic steatosis by diverting excess carbohydrates into fatty acids by the *de novo* lipogenesis pathway (DNL) (16, 17). In patients with non-alcoholic fatty liver disease (NAFLD), it has been estimated that as much as 26% of the liver triglyceride derives from DNL (18). The first committed step in lipid synthesis is the conversion of acetyl-CoA to malonyl-CoA, catalyzed by acetyl-CoA carboxylase (ACC). Malonyl-CoA participates in two opposing pathways, as a precursor for fatty acid synthesis and as a negative regulator of fatty acid oxidation. There are two isoforms of ACC: ACC1, associated with fatty acid synthesis (19), and ACC2, linked to the regulation of fatty acid oxidation (20). For fatty acid biosynthesis, fatty acid synthase (FAS) uses malonyl-CoA as a two-carbon donor for chain elongation. The DNL pathway is activated at the transcriptional level by the synergis-

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[5] The abbreviations used are: GS, glycogen synthase; DNL, *de novo* lipogenesis; NAFLD, non-alcoholic fatty liver disease; SREBP, sterol regulatory element-binding protein; chREBP, carbohydrate response element-binding protein; GSK3, glycogen synthase kinase 3; ACC, acetyl-CoA carboxylase.

ARTICLE

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Adipose-derived circulating miRNAs regulate gene expression in other tissues

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Adipose tissue is a major site of energy storage and has a role in the regulation of metabolism through the release of adipokines. Here we show that mice with an adipose-tissue-specific knockout of the microRNA (miRNA)-processing enzyme Dicer (ADicerKO), as well as humans with lipodystrophy, exhibit a substantial decrease in levels of circulating exosomal miRNAs. Transplantation of both white and brown adipose tissue—brown especially—into ADicerKO mice restores the level of numerous circulating miRNAs that are associated with an improvement in glucose tolerance and a reduction in hepatic *Fgf21* mRNA and circulating FGF21. This gene regulation can be mimicked by the administration of normal, but not ADicerKO, serum exosomes. Expression of a human-specific miRNA in the brown adipose tissue of one mouse *in vivo* can also regulate its 3′ UTR reporter in the liver of another mouse through serum exosomal transfer. Thus, adipose tissue constitutes an important source of circulating exosomal miRNAs, which can regulate gene expression in distant tissues and thereby serve as a previously undescribed form of adipokine.

MicroRNAs are 19–22-nucleotide-long non-coding RNAs that function as negative regulators of translation and are involved in many cellular processes[1–5]. Many miRNAs exist in the circulation as well as in tissues[6], and a large fraction of these are found in exosomes[7] (50–200-nm vesicles that are released from multivesicular bodies[8]). Increased levels of specific miRNAs have been associated with a variety of diseases, including cancer[9], diabetes[3,10,11] obesity[12], and cardiovascular disease[13]. miRNAs have an important role in the differentiation and function of many cells, including those in adipose tissue[14]. The amount of white adipose tissue (WAT) miRNAs declines with age, owing to a decrease in the miRNA-processing enzyme Dicer[15], and similar decreases are also observed in humans with HIV-associated lipodystrophy[16] for the same reason. To understand more clearly the role of miRNAs in fat, we generated mice specifically lacking Dicer in adipose tissue using a Cre-*lox* gene-recombination strategy[15] (Fig. 1a). ADicerKO mice exhibited a defect in miRNA processing in adipose tissue, resulting in a decrease in WAT, the whitening of brown adipose tissue (BAT), insulin resistance and alterations to circulating lipids[16].

Adipose tissue is a major source of circulating miRNAs

To determine the extent to which adipose tissue contributes to circulating miRNAs, we isolated exosomes from the serum of 6-month-old male ADicerKO and wild-type control mice by differential ultracentrifugation[17]. These vesicles were 80–200 nm in diameter[18] (Extended Data Fig. 1a) and positively stained for the exosomal markers CD63 and CD9 (refs 19, 20) (Fig. 1b). The number of exosomes isolated from ADicerKO and control mice was comparable (Extended Data Fig. 1b, c). Quantitative PCR with reverse transcription (qRT–PCR) profiling of serum exosomes for 709 mouse miRNAs revealed 653 detectable miRNAs (defined as those with threshold cycle (C_t) below 34). ADicerKO mice exhibited significant ($P < 0.05$, R/Bioconductor limma package; see Methods) alterations in 422 exosomal miRNAs compared to control mice. Of these, three miRNAs were significantly increased, whereas 419 were significantly decreased (Fig. 1c, d, Extended Data Fig. 1d and Supplementary Table 1), with 88% reduced by more than fourfold, suggesting that adipose tissue is a major source of circulating exosomal miRNAs. Consistent with this finding, many of the miRNAs that were depleted in ADicerKO samples (Supplementary Table 1), including miR-221, miR-201, miR-222 and miR-16, have been shown to be highly expressed in fat[11,21–23].

miRNAs also exist in the circulation outside of exosomes. In a set of 80 miRNAs, there was a broad reduction in total miRNAs in the serum of ADicerKO mice when compared to the wild type (Extended Data Fig. 2a); however, this reduction was not as marked as the reduction in exosomal miRNAs, indicating that adipose tissue makes a particular contribution to the exosomal miRNA fraction. The loss of exosomal miRNA secretion in adipocytes lacking Dicer is cell-autonomous. Thus, in pre-adipocytes isolated from *Dicer*$^{fl/fl}$ mice and then transduced with Ad-Cre adenovirus *in vitro*, the levels of most of the detectable miRNAs (of the 380 miRNAs profiled) released in exosomes into the medium were decreased when compared to levels in Ad-GFP-transfected (control) cells (Extended Data Fig. 2b).

To further dissociate altered metabolism from lipodystrophy as a cause of reduced exosomal miRNAs, we compared serum miRNAs from 4-week-old control and ADicerKO mice, since, at this age, the metabolic phenotypes of ADicerKO mice are similar to those of wild-type mice (Extended Data Fig. 2c). Of the 380 miRNAs profiled, 373 were detectable, with 202 downregulated and only 23 upregulated in ADicerKO mice, indicating that the reduction in circulating exosomal miRNAs primarily reflects differences in miRNA processing and production rather than the effects of chronic lipodystrophy.

To investigate whether circulating miRNAs in humans also originate from fat, we performed exosomal miRNA profiling on the serum of patients with congenital generalized lipodystrophy (CGL) and patients with HIV-associated lipodystrophy; the former have a generalized loss of adipose tissue and the latter have previously

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LETTERS

The cold-induced lipokine 12,13-diHOME promotes fatty acid transport into brown adipose tissue

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Brown adipose tissue (BAT) and beige adipose tissue combust fuels for heat production in adult humans, and so constitute an appealing target for the treatment of metabolic disorders such as obesity, diabetes and hyperlipidemia[1,2]. Cold exposure can enhance energy expenditure by activating BAT, and it has been shown to improve nutrient metabolism[3–5]. These therapies, however, are time consuming and uncomfortable, demonstrating the need for pharmacological interventions. Recently, lipids have been identified that are released from tissues and act locally or systemically to promote insulin sensitivity and glucose tolerance; as a class, these lipids are referred to as 'lipokines'[6–8]. Because BAT is a specialized metabolic tissue that takes up and burns lipids and is linked to systemic metabolic homeostasis, we hypothesized that there might be thermogenic lipokines that activate BAT in response to cold. Here we show that the lipid 12,13-dihydroxy-9Z-octadecenoic acid (12,13-diHOME) is a stimulator of BAT activity, and that its levels are negatively correlated with body-mass index and insulin resistance. Using a global lipidomic analysis, we found that 12,13-diHOME was increased in the circulation of humans and mice exposed to cold. Furthermore, we found that the enzymes that produce 12,13-diHOME were uniquely induced in BAT by cold stimulation. The injection of 12,13-diHOME acutely activated BAT fuel uptake and enhanced cold tolerance, which resulted in decreased levels of serum triglycerides. Mechanistically, 12,13-diHOME increased fatty acid (FA) uptake into brown adipocytes by promoting the translocation of the FA transporters FATP1 and CD36 to the cell membrane. These data suggest that 12,13-diHOME, or a functional analog, could be developed as a treatment for metabolic disorders.

Cold exposure activates the uptake and utilization of metabolic fuels in BAT in as little as 1 h in humans[9]. We hypothesized that thermogenic lipokines linked to BAT activation might increase in individuals exposed to an acute cold challenge, given that both lipokines and cold exposure can have beneficial effects on metabolism[3–5,10]. To test this hypothesis, we used liquid chromatography–tandem mass spectrometry (LC–MS/MS) to measure the concentrations of a panel of 88 lipids with annotated signaling properties in the plasma of human volunteers exposed to 1 h of cold at 14 °C[9] (**Supplementary Fig. 1a**,**b** and **Supplementary Table 1**). This approach to identify putative lipokines is highly sensitive and covers a broad range of oxidized fatty acid metabolites. Notably, three lipid species were significantly ($P < 0.05$) increased in human circulation by cold exposure after 1 h of cold challenge (**Fig. 1a** and **Supplementary Fig. 2**). The lipid 12,13-diHOME, however, was the only species that increased in all individuals measured (**Fig. 1b**), and 12,13-diHOME concentration was correlated with BAT activity, as measured by radiolabeled glucose uptake (**Fig. 1c**).

BAT activity and its mass are both decreased with obesity[1]; thus, to determine whether 12,13-diHOME is linked to human obesity and its related metabolic disorders, we measured 12,13-diHOME in a cohort of 55 individuals at room temperature. Importantly, we found significant, negative associations between the plasma concentration of 12,13-diHOME and body-mass index (BMI), insulin resistance (as measured by the homeostatic model of insulin resistance, or HOMA-IR), fasting plasma insulin and glucose concentrations (**Fig. 1d**,**e** and **Supplementary Fig. 3a–c**), although there was not a statistically significant correlation between 12,13-diHOME and either hemoglobin A1c or c-reactive peptide (**Supplementary Fig. 3d**,**e**). In agreement with the correlation between 12,13-diHOME and BMI, we found that circulating triglyceride and leptin levels were also negatively correlated with plasma concentrations of 12,13-diHOME (**Fig. 1f** and **Supplementary Fig. 3f**). Notably, circulating triglycerides remained significantly correlated with 12,13-diHOME concentration after we accounted for BMI as a covariate in a linear model

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Pyruvate kinase M2 activation may protect against the progression of diabetic glomerular pathology and mitochondrial dysfunction

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Diabetic nephropathy (DN) is a major cause of end-stage renal disease, and therapeutic options for preventing its progression are limited. To identify novel therapeutic strategies, we studied protective factors for DN using proteomics on glomeruli from individuals with extreme duration of diabetes (≥50 years) without DN and those with histologic signs of DN. Enzymes in the glycolytic, sorbitol, methylglyoxal and mitochondrial pathways were elevated in individuals without DN. In particular, pyruvate kinase M2 (PKM2) expression and activity were upregulated. Mechanistically, we showed that hyperglycemia and diabetes decreased PKM2 tetramer formation and activity by sulfenylation in mouse glomeruli and cultured podocytes. *Pkm*-knockdown immortalized mouse podocytes had higher levels of toxic glucose metabolites, mitochondrial dysfunction and apoptosis. Podocyte-specific *Pkm2*-knockout (KO) mice with diabetes developed worse albuminuria and glomerular pathology. Conversely, we found that pharmacological activation of PKM2 by a small-molecule PKM2 activator, TEPP-46, reversed hyperglycemia-induced elevation in toxic glucose metabolites and mitochondrial dysfunction, partially by increasing glycolytic flux and PGC-1α mRNA in cultured podocytes. In intervention studies using DBA2/J and *Nos3* (*eNos*) KO mouse models of diabetes, TEPP-46 treatment reversed metabolic abnormalities, mitochondrial dysfunction and kidney pathology. Thus, PKM2 activation may protect against DN by increasing glucose metabolic flux, inhibiting the production of toxic glucose metabolites and inducing mitochondrial biogenesis to restore mitochondrial function.

DN is the leading cause of end-stage renal disease[1–3]. Hyperglycemia is known to play a crucial part in the pathogenesis of DN. High glucose levels induce metabolic abnormalities in several glucose metabolic pathways and induce mitochondrial dysfunction, with subsequent overproduction of reactive oxygen species (ROS). These may in turn contribute to the development of the various microvascular pathologies observed in diabetes[4,5]. Elevated intracellular glucose leads to accumulation of the toxic glucose metabolites sorbitol, methylglyoxal (MG) and diacylglycerol (DAG), which have been proposed to contribute to DN development[6–9]. Furthermore, it has been proposed that mitochondrial dysfunction and ROS production induced by hyperglycemia may be irreversible owing to the persistence of epigenetic reprogramming (namely, metabolic memory)[10,11].

Hyperglycemia and its toxic end products can lead to marked glomerular abnormalities, a key component of DN. Podocytes, an important component of the glomerular filtration barrier, can become abnormal after chronic exposure to hyperglycemia. The loss of podocytes is one of the earliest glomerular morphologic changes and has important roles in DN progression[10,12,13].

In the hyperglycemic state, increased glycolytic flux has been postulated to induce mitochondrial dysfunction, increase flux through the polyol pathway and enhance synthesis of DAG and MG, which subsequently causes a variety of transcriptional and translational changes that lead to microvascular pathology, including DN. However, it is unclear whether increased flux through the tricarboxylic acid (TCA) cycle and the sorbitol, MG and DAG pathways are responsible for hyperglycemia-induced vascular pathology. Neither pharmacological agents that inhibit these pathways nor antioxidants have shown clinical success against DN or diabetic retinopathy (DR)[14–17].

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miR-204 Controls Glucagon-Like Peptide 1 Receptor Expression and Agonist Function

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Glucagon-like peptide 1 receptor (GLP1R) agonists are widely used to treat diabetes. However, their function is dependent on adequate GLP1R expression, which is downregulated in diabetes. GLP1R is highly expressed on pancreatic β-cells, and activation by endogenous incretin or GLP1R agonists increases cAMP generation, which stimulates glucose-induced β-cell insulin secretion and helps maintain glucose homeostasis. We now have discovered that the highly β-cell–enriched microRNA, miR-204, directly targets the 3′ UTR of GLP1R and thereby downregulates its expression in the β-cell–derived rat INS-1 cell line and primary mouse and human islets. Furthermore, in vivo deletion of miR-204 promoted islet GLP1R expression and enhanced responsiveness to GLP1R agonists, resulting in improved glucose tolerance, cAMP production, and insulin secretion as well as protection against diabetes. Since we recently identified thioredoxin-interacting protein (TXNIP) as an upstream regulator of miR-204, we also assessed whether in vivo deletion of TXNIP could mimic that of miR-204. Indeed, it also enhanced islet GLP1R expression and GLP1R agonist–induced insulin secretion and glucose tolerance. Thus, the present studies show for the first time that GLP1R is under the control of a microRNA, miR-204, and uncover a previously unappreciated link between TXNIP and incretin action.

Small, noncoding RNAs or microRNAs (miRNAs) have emerged as powerful regulators of gene expression, including that of pancreatic β-cells (1–3). Especially miR-204 has been shown to be highly enriched in β-cells as opposed to α-cells (2,4–6), suggesting that it may play a particularly important role in β-cell biology. Indeed, we recently found that miR-204 targets the insulin transcription factor MafA and thereby downregulates insulin production (4). Moreover, we found that miR-204 also targets PERK and thereby regulates the unfolded protein response and β-cell apoptosis (7). However, considering the fact that miRNAs can, by imperfect base pairing of their seed sequence, bind to the 3′ UTR of a multitude of mRNAs, leading to destabilization or translational inhibition of these target genes (8), we searched for additional putative genes that might be regulated by miR-204.

Intriguingly, using miRWalk as well as microrna.org target prediction software, we identified the glucagon-like peptide 1 receptor (GLP1R) as a potential target of miR-204. Conversely, when using these software programs to predict putative miRNAs that may target the GLP1R 3′ UTR, miR-204 again emerged as the most promising miRNA (all other predicted miRNAs were either not expressed in β-cells or had much shorter seed lengths, making actual targeting unlikely). The GLP1R is a G protein–coupled seven-transmembrane receptor highly expressed on pancreatic β-cells that plays a critical role in conferring the effects of GLP-1, the major incretin produced in intestinal L cells and pancreatic islet α-cells (9,10). GLP-1 is secreted in response to food intake, binds to the GLP1R, and leads to increased generation of cAMP, which stimulates glucose-induced β-cell insulin secretion and helps maintain glucose homeostasis (11). However, GLP1R not only mediates the effects of endogenously produced GLP-1, it is also responsible for the action of a large class of incretin mimetic diabetes drugs widely used in the treatment of type 2 diabetes (12), including injectable GLP-1 mimetics and analogs (e.g., exenatide and liraglutide) and oral dipeptidyl peptidase-4 (DPP-4) inhibitors that block GLP-1 degradation (e.g., sitagliptin and linagliptin).

β-Cell expression of GLP1R has been reported to be downregulated in type 2 diabetes (13), in response to glucose (14,15)

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Hepatic but Not Extrahepatic Insulin Clearance Is Lower in African American Than in European American Women

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African Americans (AAs) tend to have higher plasma insulin concentrations than European Americans (EAs); the increased insulin concentrations have been attributed to increased secretion and/or decreased insulin clearance by liver or other tissues. This work characterizes the contributions of hepatic versus extrahepatic insulin degradation related to ethnic differences between AAs and EAs. By using a recently developed mathematical model that uses insulin and C-peptide measurements from the insulin-modified, frequently sampled intravenous glucose tolerance test (FSIGT), we estimated hepatic versus extrahepatic insulin clearance in 29 EA and 18 AA healthy women. During the first 20 min of the FSIGT, plasma insulin was approximately twice as high in AAs as in EAs. In contrast, insulin was similar in AAs and EAs after the 20–25 min intravenous insulin infusion. Hepatic insulin first-pass extraction was two-thirds lower in AAs versus EAs in the overnight-fasted state. In contrast, extrahepatic insulin clearance was not lower in AAs than in EAs. The difference in insulin degradation between AAs and EAs can be attributed totally to liver clearance. The mechanism underlying reduced insulin degradation in AAs remains to be clarified, as does the relative importance of reduced liver clearance to increased risk for type 2 diabetes.

African Americans (AAs), particularly AA women, are at increased risk for type 2 diabetes compared with their white counterparts (1,2). Diabetes incidence is estimated to be 2.4-fold higher for AA women and 1.5-fold higher for AA men than for white women and men, respectively (3). The mechanisms underlying increased risk in AAs are unexplained. The elevated insulin in AA has been attributed to both increased insulin secretion (4–6) and decreased clearance (7,8), with these changes already apparent in AA adolescents (5,6,9). Whether hyperinsulinemia in AAs reflects a different metabolic pattern of insulin clearance relative to whites is of interest. Although substantial clearance of insulin occurs in the liver, with a single-pass degradation that can be >50% (10), insulin is also cleared by kidney and muscle tissue. Is it possible that the relative importance of insulin degradation by liver, with regard to other extrahepatic tissues, differs among ethnic groups, and do these divergences contribute to differences in peripheral insulin concentrations?

Methods for estimating hepatic insulin degradation from oral (10,11) and frequently sampled intravenous glucose tolerance (FSIGT) tests (12,13) have been developed on the basis of deconvolution of plasma C-peptide concentrations to calculate the insulin secretion rate (ISR) (14). A recently developed method (13) enables both hepatic and extrahepatic insulin degradation to be estimated from FSIGT data. This new model exploits the fact that during the insulin-modified FSIGT, all the insulin appearing during the first 20 min is from endogenous secretion (and hence is subjected to first-pass hepatic extraction before entering the systemic circulation), whereas the intravenous insulin infusion beginning at 20 min delivers insulin directly into the circulation. This approach was previously used to discriminate hepatic from extrahepatic clearance in a single population (13), but the method has not been used to differentiate patterns of insulin degradation in various ethnic groups. We used this method to compare degradation of insulin in AA versus European American (EA) participants.

RESEARCH DESIGN AND METHODS

Clinical Study

The experimental protocol and enrollment criteria were previously described (15–17). Participants were 23 AA and 30 EA premenopausal women. Exclusion criteria were type 1 or type 2 diabetes, polycystic ovary disease, disorders

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Clinical Science

Effects of acute hyperinsulinemia on skeletal muscle mitochondrial function, reactive oxygen species production, and metabolism in premenopausal women

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ABSTRACT

Background. Acute metabolic demands that promote excessive and/or prolonged reactive oxygen species production may stimulate changes in mitochondrial oxidative capacity.

Purpose. To assess changes in skeletal muscle H_2O_2 production, mitochondrial function, and expression of genes at the mRNA and protein levels regulating energy metabolism and mitochondrial dynamics following a hyperinsulinemic-euglycemic clamp in a cohort of 11 healthy premenopausal women.

Methods. Skeletal muscle biopsies of the vastus lateralis were taken at baseline and immediately following the conclusion of a hyperinsulinemic-euglycemic clamp. Mitochondrial production of H_2O_2 was quantified fluorometrically and mitochondrial oxidation supported by pyruvate, malate, and succinate (PMS) or palmitoyl carnitine and malate (PCM) was measured by high-resolution respirometry in permeabilized muscle fiber bundles. mRNA and protein levels were assessed by real time PCR and Western blotting.

Results. H_2O_2 emission increased following the clamp ($P < 0.05$). Coupled respiration (State 3) supported by PMS and the respiratory control ratio (index of mitochondrial coupling) for both PMS and PCM were lower following the clamp ($P < 0.05$). IRS1 mRNA decreased, whereas PGC1α and GLUT4 mRNA increased following the clamp ($P \leq 0.05$). PGC1α, IRS1, and phosphorylated AKT protein levels were higher after the clamp compared to baseline ($P < 0.05$).

Abbreviations: ACC, acetyl-CoA carboxylase; AKT, protein kinase B; AS160, AKT substrate of 160 kDa; BMI, body mass index; CD36, fatty acid translocase; CPT1B, carnitine palmitoyltransferase 1B; DNP, 2,4-dinitrophenol; DRP1, Dynamin-related protein 1; DXA, dual-energy X-ray absorptiometry; ETS, electron transport system; GDR, glucose disposal rate; GLUT4, glucose transporter type 4; GS1, glycogen synthase 1; HOMA-IR, homeostatic model assessment of insulin resistance; IAAT, intra-abdominal adipose tissue; IR, insulin resistance; IRS1, insulin receptor substrate 1; ISI, insulin sensitivity index; LPL, lipoprotein lipase; MFN1, mitofusin 1; MFN2, mitofusin 2; PCM, palmitoyl carnitine and malate substrate condition; PDK4, pyruvate dehydrogenase kinase 4; PGC1α, peroxisome proliferator-activated receptor gamma coactivator 1-alpha; PMS, pyruvate, malate, and succinate substrate condition; RCR, respiratory control ratio; ROS, reactive oxygen species; SLN, sarcolipin; SOD1, Cu-Zn superoxide dismutase; SOD2, Mn superoxide dismutase; T2D, type 2 diabetes; UCP3, uncoupling protein 3.

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RESEARCH ARTICLE

Impact of common genetic determinants of Hemoglobin A1c on type 2 diabetes risk and diagnosis in ancestrally diverse populations: A transethnic genome-wide meta-analysis

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Genetic Architecture of Insulin Resistance in the Mouse

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SUMMARY

Insulin resistance (IR) is a complex trait with multiple genetic and environmental components. Confounded by large differences between the sexes, environment, and disease pathology, the genetic basis of IR has been difficult to dissect. Here we examine IR and related traits in a diverse population of more than 100 unique male and female inbred mouse strains after feeding a diet rich in fat and refined carbohydrates. Our results show dramatic variation in IR among strains of mice and widespread differences between sexes that are dependent on genotype. We uncover more than 15 genome-wide significant loci and validate a gene, *Agpat5*, associated with IR. We also integrate plasma metabolite levels and global gene expression from liver and adipose tissue to identify metabolite quantitative trait loci (mQTL) and expression QTL (eQTL), respectively. Our results provide a resource for analysis of interactions between diet, sex, and genetic background in IR.

INTRODUCTION

Insulin resistance (IR) is characterized by the failure of tissues to respond appropriately to insulin. Driven by overconsumption of foods rich in fat and refined carbohydrates throughout the world and increasing rates of obesity, IR has become a serious global health problem. IR is an important contributing factor in type 2 diabetes (T2D) and other diseases, including coronary artery disease and fatty liver disease (Bugianesi et al., 2005; Howard et al., 1996). Decades of biochemical and physiological studies have revealed diverse biological mechanisms that contribute to IR ranging from inflammation and ER stress to aberrant lipid metabolism and gut microbiota dysbiosis (Johnson and Olefsky, 2013). While there have been significant advances in our understanding of the pathophysiology of IR, how genetic and environmental factors along with sex differences interact in IR remains poorly understood.

Genetic studies in humans have been successful at identifying genetic loci associated with T2D and other related traits, including obesity and plasma lipid levels (Grant et al., 2006; Scott et al., 2012; Sladek et al., 2007; Speliotes et al., 2010; Teslovich et al., 2010). Despite the successes of genome-wide association studies (GWASs) for T2D, there has been limited success in identifying genetic loci associated with IR in non-diabetic populations. One large GWAS including more than 40,000 non-diabetic individuals identified only two genome-wide significant loci associated with homeostatic model assessment of IR (HOMA-IR) (Dupuis et al., 2010). Recent meta-analyses of glycemic indices in more than 100,000 individuals have expanded this number to 19 loci; however, these loci explain only a few percent of the trait variation (Scott et al., 2012).

In humans, males and females differ dramatically in susceptibility to IR, whereby males are more prone to develop IR than females (Geer and Shen, 2009). Sex-specific distributions between visceral and subcutaneous adipose tissue depots as well as gonadal hormones are believed to be responsible in part for the dramatic sex differences in IR (Shi et al., 2009). Experimentally, females are less susceptible to fatty acid-induced peripheral IR and have increased insulin sensitivity in adipose tissue (Frias et al., 2001; Macotela et al., 2009). Targeted biochemical studies have even demonstrated that

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Adipose Tissue Macrophage-Derived Exosomal miRNAs Can Modulate *In Vivo* and *In Vitro* Insulin Sensitivity

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SUMMARY

MiRNAs are regulatory molecules that can be packaged into exosomes and secreted from cells. Here, we show that adipose tissue macrophages (ATMs) in obese mice secrete miRNA-containing exosomes (Exos), which cause glucose intolerance and insulin resistance when administered to lean mice. Conversely, ATM Exos obtained from lean mice improve glucose tolerance and insulin sensitivity when administered to obese recipients. miR-155 is one of the miRNAs overexpressed in obese ATM Exos, and earlier studies have shown that PPARγ is a miR-155 target. Our results show that miR-155KO animals are insulin sensitive and glucose tolerant compared to controls. Furthermore, transplantation of WT bone marrow into miR-155KO mice mitigated this phenotype. Taken together, these studies show that ATMs secrete exosomes containing miRNA cargo. These miRNAs can be transferred to insulin target cell types through mechanisms of paracrine or endocrine regulation with robust effects on cellular insulin action, *in vivo* insulin sensitivity, and overall glucose homeostasis.

INTRODUCTION

Insulin resistance is a key component in the etiology of type 2 diabetes mellitus, and obesity is clearly the most common cause of insulin resistance in humans (Johnson and Olefsky, 2013; Kahn et al., 2006; Romeo et al., 2012). As a result of the ongoing global obesity epidemic, there is a parallel rise in the prevalence of type 2 diabetes mellitus (Ogden et al., 2016). One of the hallmarks of obesity in both humans and rodents is a state of chronic, unresolved inflammation in adipose tissue, liver, and possibly skeletal muscle (Hirosumi et al., 2002; Holland et al., 2011; Hotamisligil et al., 1995, 1996; Shoelson et al., 2003; Xu et al., 2003). One of the striking components of this obesity-induced tissue inflammatory response is the accumulation of proinflammatory macrophages, particularly in adipose tissue and liver (Lumeng et al., 2007, 2008; Morinaga et al., 2015; Nguyen et al., 2007; Oh et al., 2012; Weisberg et al., 2003).

A number of earlier studies identified this chronic tissue inflammatory state and suggested that proinflammatory cytokines, such as tumor necrosis factor alpha (TNF-α) are secreted from tissue macrophages and directly inhibit insulin sensitivity, providing a potential cause for obesity-induced insulin resistance (De Taeye et al., 2007; Hotamisligil et al., 1996; Weisberg et al., 2003). However, anti-TNF-α antibody therapies have only led to limited benefits with respect to insulin resistance and glucose metabolism in humans (Dominguez et al., 2005; Lo et al., 2007; Ofei et al., 1996; Paquot et al., 2000), indicating that there must be other macrophage and immune cell factors contributing to decreased insulin sensitivity. Recently, the arachidonic acid-derived eicosanoid leukotriene B4, which works through its specific receptor BLT1 (Toda et al., 2002), has been proposed as one such factor that can directly decrease insulin signaling in hepatocytes and myocytes (Li et al., 2015; Spite et al., 2011). Galectin-3 is another macrophage-secreted factor that can both promote proinflammatory responses and directly block insulin action by inhibiting insulin receptor signaling (Li et al., 2016). In this current paper, we report a new mechanism whereby adipose tissue macrophages (ATMs) can modulate insulin action by secreting microRNA (miRNA) containing exosomes (Exos) into the circulation.

miRNAs are small non-coding RNAs that can serve as regulators of mRNA expression and translational efficiency in most cell types (Bartel, 2004). These miRNAs contain 6- to 8-nucleotide seed sequences, which correspond to complementary sequences in the 3′ UTR of target mRNAs (Brennecke et al., 2005; Lewis et al., 2003, 2005). Binding of the miRNAs to mRNAs leads to recruitment of the target mRNAs to the RNA-induced silencing complex (RISC) leading to translational arrest and mRNA degradation (Ameres et al., 2007; Mallory et al., 2004). Through these mechanisms, miRNAs can decrease protein expression of targeted mRNAs. In addition to their endogenous

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Repression of Adipose Tissue Fibrosis through a PRDM16-GTF2IRD1 Complex Improves Systemic Glucose Homeostasis

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SUMMARY

Adipose tissue fibrosis is a hallmark of malfunction that is linked to insulin resistance and type 2 diabetes; however, what regulates this process remains unclear. Here we show that the PRDM16 transcriptional complex, a dominant activator of brown/beige adipocyte development, potently represses adipose tissue fibrosis in an uncoupling protein 1 (UCP1)-independent manner. By purifying the PRDM16 complex, we identified GTF2IRD1, a member of the TFII-I family of DNA-binding proteins, as a cold-inducible transcription factor that mediates the repressive action of the PRDM16 complex on fibrosis. Adipocyte-selective expression of GTF2IRD1 represses adipose tissue fibrosis and improves systemic glucose homeostasis independent of body-weight loss, while deleting GTF2IRD1 promotes fibrosis in a cell-autonomous manner. GTF2IRD1 represses the transcription of transforming growth factor β-dependent pro-fibrosis genes by recruiting PRDM16 and EHMT1 onto their promoter/enhancer regions. These results suggest a mechanism by which repression of obesity-associated adipose tissue fibrosis through the PRDM16 complex leads to an improvement in systemic glucose homeostasis.

INTRODUCTION

Dysregulation of adipose tissue homeostasis is a primary cause of obesity-related metabolic disorders, including insulin resistance, hepatic steatosis, and diabetes mellitus (Crewe et al., 2017). Mounting evidence highlights the importance of the extracellular matrix (ECM) in maintaining adipose tissue homeostasis. The accumulation of ECM proteins (e.g., collagens) during the early stages of obesity is a part of the tissue remodeling process that accompanies healthy adipose expansion; however, pathologically excessive accumulation of the ECM in adipose tissues causes fibrosis, which is tightly associated with the increased infiltration of pro-inflammatory immune cells into the adipose tissues, and subsequently greater tissue inflammation (Sun et al., 2013b). Importantly, such changes to the ECM, and consequent fibrosis in human subcutaneous white adipose tissue (WAT) are strongly linked to insulin resistance and type 2 diabetes (Divoux et al., 2010; Henegar et al., 2008; Lackey et al., 2014; Muir et al., 2016; Reggio et al., 2016). For instance, collagen VI (Col6), a major ECM protein in adipose tissues, accumulates at much higher rates under obese and diabetic states (Dankel et al., 2014; Divoux et al., 2010; Khan et al., 2009; Pasarica et al., 2009; Spencer et al., 2010). In the absence of Col6, the WAT of obese mice is able to expand with less tissue fibrosis and inflammation; thus, the *Col6*-deficient mice display improved glucose tolerance and insulin sensitivity (Khan et al., 2009).

Two prominent pathways have been highlighted with regard to regulating adipose fibrosis: hypoxia-inducible factor 1α (HIF1α) and transforming growth factor β (TGF-β). In obesity, adipose tissue hypoxia results in the activation of HIF1α-dependent gene transcription (Hosogai et al., 2007; Rausch et al., 2008; Ye et al., 2007). Moreover, mouse models with ectopic activation of HIF1α in adipose tissues induce fibrosis and glucose intolerance (Halberg et al., 2009), whereas treatment with the HIF1α-selective inhibitor PX-478 ameliorates adipose fibrosis, inflammation, and glucose intolerance (Sun et al., 2013a). Similarly, levels of TGF-β, a primary factor promoting tissue fibrosis, are highly elevated in both the circulation and adipose tissues of obese mice and humans (Samad et al., 1997; Yadav et al., 2011).

It is notable that TGF-β signaling exerts effects on brown/beige adipocyte biogenesis that are reciprocal to those on

LETTERS
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Subcellular localization of MC4R with ADCY3 at neuronal primary cilia underlies a common pathway for genetic predisposition to obesity

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Most monogenic cases of obesity in humans have been linked to mutations in genes encoding members of the leptin–melanocortin pathway. Specifically, mutations in *MC4R*, the melanocortin-4 receptor gene, account for 3–5% of all severe obesity cases in humans[1–3]. Recently, *ADCY3* (adenylyl cyclase 3) gene mutations have been implicated in obesity[4,5]. ADCY3 localizes to the primary cilia of neurons[6], organelles that function as hubs for select signaling pathways. Mutations that disrupt the functions of primary cilia cause ciliopathies, rare recessive pleiotropic diseases in which obesity is a cardinal manifestation[7]. We demonstrate that MC4R colocalizes with ADCY3 at the primary cilia of a subset of hypothalamic neurons, that obesity-associated *MC4R* mutations impair ciliary localization and that inhibition of adenylyl cyclase signaling at the primary cilia of these neurons increases body weight. These data suggest that impaired signaling from the primary cilia of MC4R neurons is a common pathway underlying genetic causes of obesity in humans.

Most mammalian cells, including neurons, each possess a single, immotile primary cilium, an organelle that transduces select signals[7,8]. Defects in the genesis or function of primary cilia cause a range of overlapping human diseases, collectively termed ciliopathies[7,9,10]. Several ciliopathies, such as Bardet–Biedl syndrome and Alström syndrome, cause obesity[11], and mutations in genes encoding ciliary proteins, such as CEP19 and ANKRD26, cause nonsyndromic obesity in mice and humans[12,13]. Although the mechanisms underlying a number of ciliopathy-associated phenotypes, such as polycystic kidney disease or retinal degeneration, have been at least partly elucidated, how ciliary dysfunction leads to obesity remains poorly understood[7,11]. Ubiquitous ablation of the primary cilia of neurons in adult mice causes an increase in food intake and obesity, thus suggesting that ciliopathy-associated obesity involves the postdevelopmental disruption of anorexigenic neuronal signals[14]. Recently, genetic and epigenetic studies have suggested a role of *ADCY3* variations in human obesity[4,15], and loss-of-function mutations in *Adcy3* in mice have been found to lead to a severe obesity phenotype[5]. ADCY3, a member of the adenylyl cyclase family that mediates G_s signaling from G-protein-coupled receptors (GPCRs), is specifically expressed at the primary cilia of neurons[6].

The melanocortin 4 receptor (MC4R) is a G_s-coupled GPCR that transduces anorexigenic signals in the long-term regulation of energy homeostasis[16]. Heterozygous mutations in *MC4R* are the most common monogenic cause of severe obesity in humans, and individuals with homozygous null mutations display severe early-onset obesity[1–3]. Similarly, in mice, deletion of *Mc4r* causes severe obesity[17]. MC4R is a central component of the melanocortin system, a hypothalamic network of neurons that integrates information about peripheral energy stores and that regulates food intake and energy expenditure[18]. Despite being a major target for the pharmacotherapy of obesity, little is known about the subcellular localization of MC4R.

When expressed in unciliated heterologous cells, MC4R traffics to the cell membrane[2]. However, in ciliated cells such as mouse embryonic fibroblasts (MEFs), retinal pigment epithelium (RPE) or inner-medullary collecting duct (IMCD3) cells, we found that a previously well-characterized, functional, C-terminally GFP-tagged MC4R (MC4R-GFP)[19] localized to primary cilia (Fig. 1a). In a quantitative assay developed in IMCD3 cells, we found that the ciliary enrichment of MC4R was comparable to that of Smoothened (SMO), a known cilium-enriched protein[20,21], and was the strongest among members of the melanocortin-receptor family (Fig. 1b).

We set out to determine whether, and to what extent, MC4R localizes to primary cilia in mice. Most of the anorexigenic activity of MC4R is due to its function in a subset of single minded 1 (SIM1)-expressing neurons of the paraventricular nucleus of the hypothalamus (PVN)[22], and all MC4R-expressing neurons in the PVN express SIM1 (ref. [23]). Using a transgenic mouse line in which GFP was expressed in all SIM1-expressing neurons, we first investigated whether SIM1-expressing PVN neurons were ciliated. We found that adenylyl cyclase 3 (ADCY3)-positive primary cilia were present in most SIM1-expressing neurons of the PVN (Supplementary Fig. 1).

Previous attempts to determine the subcellular localization of MC4R in vivo in mice have been unsuccessful, owing to the small number of neurons in which it is expressed, its low abundance and the lack of tractable antibodies. To circumvent these limitations, we used Cas9-mediated recombination in mouse zygotes to insert a GFP tag in frame at the C terminus of the endogenous *Mc4r* locus (Fig. 2a). The MC4R-GFP/+ knock-in mice did not have an obvious energy-metabolism phenotype and were fertile, thus suggesting that the C-terminal GFP did not substantially impair the trafficking or function of MC4R in these mice. Confocal imaging of the PVN of these mice demonstrated that MC4R and ADCY3 colocalized to the primary cilia of a subset of PVN neurons (Fig. 2b–i).

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Targeting ABL-IRE1α Signaling Spares ER-Stressed Pancreatic β Cells to Reverse Autoimmune Diabetes

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SUMMARY

In cells experiencing unrelieved endoplasmic reticulum (ER) stress, the ER transmembrane kinase/endoribonuclease (RNase)—IRE1α—endonucleolytically degrades ER-localized mRNAs to promote apoptosis. Here we find that the ABL family of tyrosine kinases rheostatically enhances IRE1α's enzymatic activities, thereby potentiating ER stress-induced apoptosis. During ER stress, cytosolic ABL kinases localize to the ER membrane, where they bind, scaffold, and hyperactivate IRE1α's RNase. Imatinib—an anti-cancer tyrosine kinase inhibitor—antagonizes the ABL-IRE1α interaction, blunts IRE1α RNase hyperactivity, reduces pancreatic β cell apoptosis, and reverses type 1 diabetes (T1D) in the non-obese diabetic (NOD) mouse model. A mono-selective kinase inhibitor that allosterically attenuates IRE1α's RNase—KIRA8—also efficaciously reverses established diabetes in NOD mice by sparing β cells and preserving their physiological function. Our data support a model wherein ER-stressed β cells contribute to their own demise during T1D pathogenesis and implicate the ABL-IRE1α axis as a drug target for the treatment of an autoimmune disease.

INTRODUCTION

Diverse perturbations compromise folding and structural maturation of secretory proteins in the endoplasmic reticulum (ER). If uncorrected, such "ER stress" promotes cell degeneration and apoptosis. ER stress activates unfolded protein response (UPR) signaling pathways that determine cell fate. Remediable ER stress activates adaptive ("A")-UPR outputs that favor cell survival. But under irremediably high, chronic ER stress, these adaptive measures wane, as alternate terminal ("T")-UPR outputs trigger apoptosis.

High/chronic ER stress promotes numerous diseases of premature cell loss (Oakes and Papa, 2015). For example, pancreatic islet β cells, responsible for synthesizing and secreting sufficient quantities of insulin to maintain blood glucose homeostasis, commonly experience high ER stress and secretory exhaustion (Scheuner and Kaufman, 2008). Peripheral insulin resistance further elevates β cell insulin secretory demand during development of type 2 diabetes (T2D) (Back and Kaufman, 2012). Insulin gene mutations cause encoded proinsulin to become structurally arrested in the β cell ER, and various UPR gene deletions debilitate insulin production by β cells. Dysregulated UPR signaling promotes β cell autonomous apoptosis in these diverse diabetic syndromes (Ozcan et al., 2004; Papa, 2012).

Type 1 diabetes (T1D) is triggered by immune dysregulation and autoreactive T cell responses against β cells. However, the autoreactivity does not inevitably result in direct β cell destruction (fratricide), but will also induce β cells to autonomously undergo apoptosis (suicide) during disease progression (Atkinson et al., 2011; Bottazzo, 1986). Both human and mouse studies have suggested that, at the time of T1D diagnosis, as much as 30%–40% of β cells remain and are functionally unresponsive but can recover following removal of stress, suggesting that a window of opportunity may exist for therapies that prevent further β cell deterioration and restore β cell function (Alanentalo et al., 2010; Krogvold et al., 2015).

CellPress

Cell Metabolism
Short Article

Circadian Clock Interaction with HIF1α Mediates Oxygenic Metabolism and Anaerobic Glycolysis in Skeletal Muscle

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SUMMARY

Circadian clocks are encoded by a transcription-translation feedback loop that aligns energetic processes with the solar cycle. We show that genetic disruption of the clock activator BMAL1 in skeletal myotubes and fibroblasts increased levels of the hypoxia-inducible factor 1α (HIF1α) under hypoxic conditions. *Bmal1*$^{-/-}$ myotubes displayed reduced anaerobic glycolysis, mitochondrial respiration with glycolytic fuel, and transcription of HIF1α targets *Phd3*, *Vegfa*, *Mct4*, *Pk-m*, and *Ldha*, whereas abrogation of the clock repressors CRY1/2 stabilized HIF1α in response to hypoxia. HIF1α bound directly to core clock gene promoters, and, when co-expressed with BMAL1, led to transactivation of PER2-LUC and HRE-LUC reporters. Further, genetic stabilization of HIF1α in *Vhl*$^{-/-}$ cells altered circadian transcription. Finally, induction of clock and HIF1α target genes in response to strenuous exercise varied according to the time of day in wild-type mice. Collectively, our results reveal bidirectional interactions between circadian and HIF pathways that influence metabolic adaptation to hypoxia.

INTRODUCTION

The circadian system drives internal rhythms of physiology in synchrony with the rotation of the Earth. In mammals, the molecular clock is encoded by a transcription-translation feedback loop composed of activators (CLOCK/BMAL) that induce the transcription of repressors (PER/CRY) that feed back to inhibit the forward limb in a cycle that repeats itself every ~24 hr, including an additional stabilizing loop comprised of REV-ERB/ROR transcription factors (TFs) (Bass, 2012). Extensive investigation now has shown that peripheral tissue clocks influence metabolism in a tissue-specific manner (Mohawk et al., 2012). For example, abrogation of clock function within pancreas in adult life impairs glucose tolerance, whereas clock disruption within liver results in fasting-induced hypoglycemia and mitochondrial dysfunction (Marcheva et al., 2010; Peek et al., 2013).

While the suprachiasmatic nucleus (SCN) clock is entrained by light, peripheral clocks can be altered by serum factors, glucocorticoids, and core body temperature (Bass, 2012; Bass and Takahashi, 2010). Environmental signals that impact ATP/AMP levels, redox state, NAD^+-dependent class III deacetylases (sirtuins), and nuclear receptor ligands also directly impact clock TFs (Bass and Takahashi, 2010; Peek et al., 2012), raising the possibility that the clock system functions not only to anticipate the light cycle but also to alter timing in response to metabolic flux, although a gap remains in understanding how the circadian clock regulates adaptation to hypoxia.

Clues as to how hypoxia may impact the clock derive from the discovery that both clock and hypoxia-inducible factors belong to the basic helix-loop-helix PER-ARNT-SIM (bHLH-PAS) TF superfamily. HIF proteins are present as heterodimers, consisting of HIF1α and HIF1β (ARNT), that bind to E-box-like hypoxia-response elements (HREs) within gene promoters that contain the sequence 5′-[A/G]CGTG-3′ (Kaelin and Ratcliffe, 2008). In normoxic conditions, HIF1α is post-translationally modified by prolyl-hydroxylases and marked for proteasomal degradation by the Von Hippel-Lindau (VHL) E3 ubiquitin ligase. In contrast, during hypoxia when oxygen levels are limiting or during mitochondrial stress, HIF1α subunits are protected from degradation due to the inactivation of the oxygen-dependent prolyl-hydoxylases (Semenza, 2007). The stabilized HIF1α/β heterodimer activates gene transcription pathways involved in angiogenesis, erythropoiesis, and anaerobic glycolysis (Semenza, 2012). Although in silico and in vitro biochemical models suggest that the core clock TF BMAL1 dimerizes with the related bHLH-PAS proteins HIF1α and HIF2α, whether these interactions occur in a physiological setting is unknown (Bersten et al., 2013; Hogenesch et al., 1998), particularly during exercise when oxygen depletion in skeletal muscle increases energy production through HIF-mediated anaerobic glycolysis.

Here we applied a combination of genetic, biochemical, and cell-type-specific physiological approaches to dissect the interplay between molecular clock and HIF proteins in oxygenic metabolism. Our findings indicate that circadian clock-HIF interactions regulate skeletal muscle anaerobic glycolysis during exercise and that hypoxia reciprocally regulates muscle circadian function. Our

Preservation of Reduced Numbers of Insulin-Positive Cells in Sulfonylurea-Unresponsive *KCNJ11*-Related Diabetes

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Context: The most common genetic cause of permanent neonatal diabetes mellitus is activating mutations in *KCNJ11*, which can usually be treated using oral sulfonylureas (SUs) instead of insulin injections, although some mutations are SU unresponsive. In this work, we provide a report of the pancreatic islet endocrine cell composition and area in a patient with an SU-unresponsive *KCNJ11* mutation (p.G334D), in comparison with age-matched controls.

Case Description: Pancreatic autopsy tissue sections from a 2-year-old female child diagnosed with *KCNJ11*-related diabetes at 4 days of age and 13 age-matched controls were stained with insulin, glucagon, somatostatin, pancreatic polypeptide, and Ki67 antibodies to determine islet endocrine cell composition and area. β-cell ultrastructure was assessed by electron microscopic (EM) analysis. The patient's pancreas (sampling from head to tail) revealed insulin-positive cells in all regions. The pancreatic β-cell (insulin) area was significantly reduced compared with controls: 0.50% $\pm$ 0.04% *versus* 1.67% $\pm$ 0.20%, respectively ($P < 0.00001$). There were no significant differences in α-cell (glucagon) or δ-cell (somatostatin) area. EM analysis revealed secretory granules with a dense core typical of mature β-cells as well as granules with a lighter core characteristic of immature granules.

Conclusions: Our results suggest that mechanisms exist that allow preservation of β-cells in the absence of insulin secretion. It remains to be determined to what extent this reduction in β-cells may be reversible. **(*J Clin Endocrinol Metab* 102: 1–5, 2017)**

Activating mutations in the genes (*KCNJ11* and *ABCC8*) encoding the adenosine triphosphate–sensitive potassium (K_{ATP}) channel are the most common causes of permanent neonatal diabetes (1). Most of these patients can be successfully treated with high doses of oral sulfonylureas (SUs) that close K_{ATP} channels and restore insulin secretion. However, rare mutations are SU unresponsive, and these patients require lifelong exogenous insulin injections.

It has not previously been possible to study the structure of the pancreatic islets and β-cells in patients with *KCNJ11*-related diabetes. It has thus been unclear whether β-cells that are incapable of insulin secretion continue to synthesize insulin and what might be the fate and distribution of such cells. In this study, we report islet cell composition in the first autopsy case of a patient with SU-unresponsive *KCNJ11*-related diabetes in comparison with 13 age-matched control specimens.

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†Deceased.

Abbreviations: EM, electron microscopic; K_{ATP}, adenosine triphosphate–sensitive potassium; PP, pancreatic polypeptide; SU, sulfonylurea.

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ARTICLE

In vivo measurement and biological characterisation of the diabetes-associated mutant insulin p.R46Q (GlnB22-insulin)

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Abstract
Aims/hypothesis Heterozygous mutations in the insulin gene that affect proinsulin biosynthesis and folding are associated with a spectrum of diabetes phenotypes, from permanent neonatal diabetes to MODY. In vivo studies of these mutations may lead to a better understanding of insulin mutation-associated diabetes and point to the best treatment strategy. We studied an 18-year-old woman with MODY heterozygous for the insulin mutation p.R46Q (GlnB22-insulin), measuring the secretion of mutant and wild-type insulin by LC-MS. The clinical study was combined with in vitro studies of the synthesis and secretion of p.R46Q-insulin in rat INS-1 insulinoma cells.
Methods We performed a standard 75 g OGTT in the 18-year-old woman and measured plasma glucose and serum insulin (wild-type insulin and GlnB22-insulin), C-peptide, proinsulin, glucagon and amylin. The affinity of GlnB22-insulin was tested on human insulin receptors expressed in baby hamster kidney (BHK) cells. We also examined the subcellular localisation, secretion and impact on cellular stress markers of p.R46Q-insulin in INS-1 cells.
Results Plasma GlnB22-insulin concentrations were 1.5 times higher than wild-type insulin at all time points during the OGTT. The insulin-receptor affinity of GlnB22-insulin was 57% of that of wild-type insulin. Expression of p.R46Q-insulin in INS-1 cells was associated with decreased insulin secretion, but not induction of endoplasmic reticulum stress.
Conclusions/interpretation The results show that beta cells can process and secrete GlnB22-insulin both in vivo and in vitro. Our combined approach of immunoprecipitation and LC-MS to measure mutant and wild-type insulin may be useful for the study of other mutant insulin proteins. The ability to process and secrete a mutant protein may predict a more benign course of insulin mutation-related diabetes. Diabetes develops when the beta cell is stressed because of increased demand for insulin, as observed in individuals with other insulin mutations that affect the processing of proinsulin to insulin or mutations that reduce the affinity for the insulin receptor.

Keywords Insulin gene · Maturity-onset diabetes of the young · Mutant insulin

Julie Støy, Jørgen Olsen and Soo-Young Park contributed equally to this study.

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Abbreviations

BHK	Baby hamster kidney
eIF2α	Eukaryotic Initiation Factor 2α
ER	Endoplasmic reticulum
NDM	Neonatal diabetes mellitus

A leptin-regulated circuit controls glucose mobilization during noxious stimuli

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Adipocytes secrete the hormone leptin to signal the sufficiency of energy stores. Reductions in circulating leptin concentrations reflect a negative energy balance, which augments sympathetic nervous system (SNS) activation in response to metabolically demanding emergencies. This process ensures adequate glucose mobilization despite low energy stores. We report that leptin receptor–expressing neurons (LepRb neurons) in the periaqueductal gray (PAG), the largest population of LepRb neurons in the brain stem, mediate this process. Application of noxious stimuli, which often signal the need to mobilize glucose to support an appropriate response, activated PAG LepRb neurons, which project to and activate parabrachial nucleus (PBN) neurons that control SNS activation and glucose mobilization. Furthermore, activating PAG LepRb neurons increased SNS activity and blood glucose concentrations, while ablating LepRb in PAG neurons augmented glucose mobilization in response to noxious stimuli. Thus, decreased leptin action on PAG LepRb neurons augments the autonomic response to noxious stimuli, ensuring sufficient glucose mobilization during periods of acute demand in the face of diminished energy stores.

Introduction

The hormone leptin, which is produced by adipocytes to signal the repletion of body energy (fat) stores, acts via the leptin receptor (LepRb) on specialized populations of brain neurons to control food intake, energy expenditure, and other metabolic parameters (1–4). The fall in circulating leptin concentrations during negative energy balance increases hunger and initiates an energy-sparing neuroendocrine program characterized by decreased sympathetic nervous system (SNS) tone and diminished activity of the thyroid, reproductive, and growth axes, among others. This response not only tends to replenish body energy stores by increasing feeding relative to energy utilization when food is available, but also preserves remaining energy stores in the face of continued famine, increasing the chances of survival until food becomes available.

The withdrawal of leptin action from LepRb neurons in the hypothalamus mediates most of these orexigenic and energy-sparing responses to negative energy balance (5). While brain stem sites contain many LepRb neurons, roles for most populations of brain stem LepRb neurons, including the largest population (located in the periaqueductal gray [PAG]), remain undefined (6, 7).

In isolation, the energy-sparing neuroendocrine response to negative energy balance carries a potential risk, however. The reduction of energy stores coupled with decreased SNS tone impairs the ability to respond to acute, potentially life-threatening emergencies that require the robust mobilization of metabolic fuels, such as glucose. Thus, during negative energy balance, meeting acutely elevated metabolic needs requires a system that increases the drive to mobilize glucose beyond that which is required when energy reserves are replete. Furthermore, this system must activate specifically in response to metabolic emergencies without otherwise altering SNS tone and glucose mobilization (otherwise, this would blunt the energy-sparing response to negative energy balance). Indeed, we previously defined a system that augments the counterregulatory response (CRR) to hypoglycemia when energy stores are diminished: hypoglycemia activates cholecystokinin-containing (CCK-containing) neurons in the hindbrain parabrachial nucleus (PBN), which project to the ventromedial hypothalamic nucleus (VMN) (which plays a crucial role in the CRR) (8, 9). Some PBN CCK neurons contain LepRb; during negative energy balance, the withdrawal of leptin from these PBN LepRb cells augments their response to low glucose, enhancing glucose mobilization during hypoglycemia without altering baseline endocrine function, energy balance, or glucose homeostasis (8).

We reasoned that other stimuli that signal the need for robust SNS activation and glucose mobilization might similarly engage the PBN→VMN circuit to meet acutely increased metabolic demands when energy stores are low. Because noxious stimuli (such as pain) increase blood glucose and often indicate the need for SNS activation and nutrient mobilization, we sought to understand the potential role for hindbrain LepRb neurons in modulating the response to noxious stimuli in line with the repletion state of body energy stores. We find that LepRb neurons in the PAG (which plays crucial roles in responding to noxious stimuli; refs. 10–13) lie

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JCI insight

RESEARCH ARTICLE

Tissue-specific metabolic reprogramming drives nutrient flux in diabetic complications

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Diabetes is associated with altered cellular metabolism, but how altered metabolism contributes to the development of diabetic complications is unknown. We used the BKS *db/db* diabetic mouse model to investigate changes in carbohydrate and lipid metabolism in kidney cortex, peripheral nerve, and retina. A systems approach using transcriptomics, metabolomics, and metabolic flux analysis identified tissue-specific differences, with increased glucose and fatty acid metabolism in the kidney, a moderate increase in the retina, and a decrease in the nerve. In the kidney, increased metabolism was associated with enhanced protein acetylation and mitochondrial dysfunction. To confirm these findings in human disease, we analyzed diabetic kidney transcriptomic data and urinary metabolites from a cohort of Southwestern American Indians. The urinary findings were replicated in 2 independent patient cohorts, the Finnish Diabetic Nephropathy and the Family Investigation of Nephropathy and Diabetes studies. Increased concentrations of TCA cycle metabolites in urine, but not in plasma, predicted progression of diabetic kidney disease, and there was an enrichment of pathways involved in glycolysis and fatty acid and amino acid metabolism. Our findings highlight tissue-specific changes in metabolism in complication-prone tissues in diabetes and suggest that urinary TCA cycle intermediates are potential prognostic biomarkers of diabetic kidney disease progression.

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Introduction

Diabetes is characterized by altered carbohydrate and lipid metabolism. Despite medications that reduce hyperglycemia, hypertension, and hyperlipidemia, diabetes is often associated with highly morbid complications such as diabetic kidney disease (DKD), diabetic neuropathy (DN), and diabetic retinopathy (DR). Diabetes is the most common cause of end-stage renal disease, lower extremity amputation, and blindness in the United States (1). Despite the fact that most diabetic patients develop one or more of these complications, the pathophysiology leading to development and progression of these complications remains poorly understood.

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Hypothalamic Vitamin D Improves Glucose Homeostasis and Reduces Weight

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Despite clear associations between vitamin D deficiency and obesity and/or type 2 diabetes, a causal relationship is not established. Vitamin D receptors (VDRs) are found within multiple tissues, including the brain. Given the importance of the brain in controlling both glucose levels and body weight, we hypothesized that activation of central VDR links vitamin D to the regulation of glucose and energy homeostasis. Indeed, we found that small doses of active vitamin D, 1α,25-dihydroxyvitamin D_3 ($1,25D_3$) (calcitriol), into the third ventricle of the brain improved glucose tolerance and markedly increased hepatic insulin sensitivity, an effect that is dependent upon VDR within the paraventricular nucleus of the hypothalamus. In addition, chronic central administration of $1,25D_3$ dramatically decreased body weight by lowering food intake in obese rodents. Our data indicate that $1,25D_3$-mediated changes in food intake occur through action within the arcuate nucleus. We found that VDR colocalized with and activated key appetite-regulating neurons in the arcuate, namely proopiomelanocortin neurons. Together, these findings define a novel pathway for vitamin D regulation of metabolism with unique and divergent roles for central nervous system VDR signaling. Specifically, our data suggest that vitamin D regulates glucose homeostasis via the paraventricular nuclei and energy homeostasis via the arcuate nuclei.

Vitamin D is a fat-soluble vitamin available in some foods but also produced from sunlight. Whatever the source, vitamin D requires hydroxylation in the liver and kidney to produce the active form, 1α,25-dihydroxyvitamin D_3 ($1,25D_3$), also called calcitriol. $1,25D_3$ binds to the vitamin D receptor (VDR), which forms a heterodimer with the retinoid X receptor and modulates gene expression. Although vitamin D has important roles in calcium/phosphorus regulation and bone health, it also has important actions in immunity, inflammation, and differentiation (1).

Interestingly, low vitamin D status is associated with obesity and impaired glucose tolerance (2,3). Whether this is a causal relationship is unclear. When combined with a low-energy diet, vitamin D supplementation results in greater decreases in body weight and fat mass in humans (4–6) and prevents dietary-induced weight gain in rodents (7). Additionally, vitamin D intake at breakfast can increase thermogenesis and fat oxidation rates in subsequent meals, suggesting direct metabolic action (8). Preclinical studies also demonstrate a clear pathway by which vitamin D affects glucose homeostasis. VDR is present in pancreatic β-cells (9), the VDR-null mouse has impaired glucose tolerance with decreased insulin levels (10), and supplementation with $1,25D_3$ improves fasting glucose levels in mice (11). Given the strong association between vitamin D status and the metabolic syndrome, the inconsistency of vitamin D supplementation in improving weight or abnormal glucose tolerance is perplexing (4,12–17).

Understanding how vitamin D might regulate glucose and body weight is paramount to creating effective

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LIM domain–binding 1 maintains the terminally differentiated state of pancreatic β cells

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The recognition of β cell dedifferentiation in type 2 diabetes raises the translational relevance of mechanisms that direct and maintain β cell identity. LIM domain–binding protein 1 (LDB1) nucleates multimeric transcriptional complexes and establishes promoter-enhancer looping, thereby directing fate assignment and maturation of progenitor populations. Many terminally differentiated endocrine cell types, however, remain enriched for LDB1, but its role is unknown. Here, we have demonstrated a requirement for LDB1 in maintaining the terminally differentiated status of pancreatic β cells. Inducible ablation of LDB1 in mature β cells impaired insulin secretion and glucose homeostasis. Transcriptomic analysis of LDB1-depleted β cells revealed the collapse of the terminally differentiated gene program, indicated by a loss of β cell identity genes and induction of the endocrine progenitor factor neurogenin 3 (NEUROG3). Lineage tracing confirmed that LDB1-depleted, insulin-negative β cells express NEUROG3 but do not adopt alternate endocrine cell fates. In primary mouse islets, LDB1 and its LIM homeodomain–binding partner islet 1 (ISL1) were coenriched at chromatin sites occupied by pancreatic and duodenal homeobox 1 (PDX1), NK6 homeobox 1 (NKX6.1), forkhead box A2 (FOXA2), and NK2 homeobox 2 (NKX2.2) — factors that co-occupy active enhancers in 3D chromatin domains in human islets. Indeed, LDB1 was enriched at active enhancers in human islets. Thus, LDB1 maintains the terminally differentiated state of β cells and is a component of active enhancers in both murine and human islets.

Introduction

All forms of diabetes are characterized by reduced numbers and/or dysfunction of insulin-producing pancreatic islet β cells (1, 2). The β cell is unique in its ability to synthesize and secrete insulin and is 1 of 5 endocrine lineages that arise from neurogenin 3–expressing (NEUROG3-expressing) progenitors (3). As these lineages differentiate and mature, orchestrated mechanisms establish the epigenetic landscape and transcriptional networks that reinforce the mature, functional identity of each lineage (4, 5). The transcription factors pancreatic and duodenal homeobox 1 (PDX1) and paired box 4 (PAX4) specify fate, and MAF bZIP transcription factor A (MAFA) and teashirt zinc finger family member 1 (TSHZ1) drive the functional maturation of β cells (6–9). Ablation of *Pdx1* in the mature β cell is sufficient to activate ectopic α cell features, illustrating the critical role PDX1 plays in maintaining β cell identity (10). Likewise, maintenance of the pancreatic endocrine cell epigenetic landscape is critical; inhibition of histone methyltransferases in human and murine islets leads α cells to ectopically express insulin and PDX1 (11).

LIM domain–binding protein 1 (LDB1), a nuclear protein lacking DNA-binding capacity and enzymatic activity (12), is also required for the maturation of pancreatic endocrine precursors (13). LDB1 homodimers dictate cell fate during fetal development in various progenitor populations (14). Two distinct LDB1 functional paradigms have been described and are distinguished by the high-affinity protein-protein interactions between LDB1 and LIM homeodomain (LIM-HD) transcription factors or LIM-only (LMO) scaffolding proteins (15). The LIM-HD–based paradigm involves LDB1-nucleating tetrameric and hexameric LIM-HD complexes and is exemplified by V2 interneuron and somatic motor neuron fate determination (16, 17). The LMO-based paradigm is implemented during erythropoiesis and hematopoiesis and involves the formation of a pentameric complex, in which LDB1 binds an LMO factor that in turn bridges a GATA factor and an E-box heterodimer (18–20). These LDB1-mediated complexes recruit chromatin-remodeling complexes and enlist transcriptional machinery (21–24).

▶ Related Commentary: p. 94

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Tissue-specific exosome biomarkers for noninvasively monitoring immunologic rejection of transplanted tissue

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In transplantation, there is a critical need for noninvasive biomarker platforms for monitoring immunologic rejection. We hypothesized that transplanted tissues release donor-specific exosomes into recipient circulation and that the quantitation and profiling of donor intra-exosomal cargoes may constitute a biomarker platform for monitoring rejection. Here, we have tested this hypothesis in a human-into-mouse xenogeneic islet transplant model and validated the concept in clinical settings of islet and renal transplantation. In the xenogeneic model, we quantified islet transplant exosomes in recipient blood over long-term follow-up using anti-HLA antibody, which was detectable only in xenoislet recipients of human islets. Transplant islet exosomes were purified using anti-HLA antibody–conjugated beads, and their cargoes contained the islet endocrine hormone markers insulin, glucagon, and somatostatin. Rejection led to a marked decrease in transplant islet exosome signal along with distinct changes in exosomal microRNA and proteomic profiles prior to appearance of hyperglycemia. In the clinical settings of islet and renal transplantation, donor exosomes with respective tissue specificity for islet β cells and renal epithelial cells were reliably characterized in recipient plasma over follow-up periods of up to 5 years. Collectively, these findings demonstrate the biomarker potential of transplant exosome characterization for providing a noninvasive window into the conditional state of transplant tissue.

Introduction

Immunologic rejection and immunosuppressive regimen–related complications remain the major causes of morbidity and mortality in transplant recipients. This is most evident in the fields of heart and lung transplantation, where the highest rates of immunologic rejection and patient mortality are seen despite routine surveillance biopsies to monitor organ status (1–4). In kidney transplantation, monitoring allograft rejection by rise in serum creatinine does not specifically portray immunologic rejection (5). In islet transplantation, where blood glucose monitoring remains the clinical standard, hyperglycemia typically heralds an advanced stage of rejection. Collectively, the current standards for monitoring transplant rejection reveal the critical need for more accurate, time-sensitive, and noninvasive biomarker platforms.

Several groups have reported on whole plasma/bodily fluid profiling of free nucleic acids and proteins as biomarker platforms for monitoring rejection, especially in the context of renal transplantation (6–17). But diagnostic accuracy remains a critical problem, as free nucleic acids and proteins are typically nonspecific and unstable in circulation, requiring a high steady state for reliable quantitation. Exosomes are extracellular vesicles released by many tissue types into bodily fluids, including blood, urine, and bronchoalveolar secretions. Exosomes represent stable and tissue-specific proteomic and RNA signature profiles that reflect the conditional state of their tissue of origin (8–10, 12, 17). But similar to quantitative assays based on circulating free proteins and nucleic acids, whole plasma exosome analysis also introduces a high noise-to-signal ratio, as many tissue types contribute to the total plasma exosome pool. Therefore, quantitation and characterization of tissue-specific exosome profiles from bodily fluids would overcome this problem associated with whole plasma analysis, and would serve as a more accurate biomarker platform. In the context of transplantation, we hypothesized that transplanted tissue releases distinct, donor-specific exosomes into recipient plasma/bodily fluids, and its characterization would constitute a more accurate noninvasive biomarker platform for monitoring the conditional status of the transplanted organ. To quantify, purify, and characterize transplant exosomes and their intra-exosomal proteomic and RNA cargoes, we took advantage of 2 concepts: (a) exosomes express surface MHC antigens identical to their tissue counterparts, and (b) donor-recipient MHC mismatch introduced by transplantation enables characterization of transplant tissue–specific exosomes from recipient bodily fluids.

In this report, we detail our investigation of transplant tissue–specific exosome purification and characterization in an animal model of islet xenotransplantation (human into mouse), and validate the biomarker potential of this platform in the clinical settings of islet and renal transplantation.

Results

Transplanted human islets release donor MHC–specific exosomes into recipient plasma. First, we confirmed that exosomes released by

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LETTER

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Histone deacetylase 3 prepares brown adipose tissue for acute thermogenic challenge

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Brown adipose tissue is a thermogenic organ that dissipates chemical energy as heat to protect animals against hypothermia and to counteract metabolic disease[1]. However, the transcriptional mechanisms that determine the thermogenic capacity of brown adipose tissue before environmental cold are unknown. Here we show that histone deacetylase 3 (HDAC3) is required to activate brown adipose tissue enhancers to ensure thermogenic aptitude. Mice with brown adipose tissue-specific genetic ablation of HDAC3 become severely hypothermic and succumb to acute cold exposure. Uncoupling protein 1 (UCP1) is nearly absent in brown adipose tissue lacking HDAC3, and there is also marked downregulation of mitochondrial oxidative phosphorylation genes resulting in diminished mitochondrial respiration. Remarkably, although HDAC3 acts canonically as a transcriptional corepressor[2], it functions as a coactivator of oestrogen-related receptor α (ERRα) in brown adipose tissue. HDAC3 coactivation of ERRα is mediated by deacetylation of PGC-1α and is required for the transcription of *Ucp1*, *Ppargc1a* (encoding PGC-1α), and oxidative phosphorylation genes. Importantly, HDAC3 promotes the basal transcription of these genes independently of adrenergic stimulation. Thus, HDAC3 uniquely primes *Ucp1* and the thermogenic transcriptional program to maintain a critical capacity for thermogenesis in brown adipose tissue that can be rapidly engaged upon exposure to dangerously cold temperature.

Brown adipose tissue (BAT) is a major site of mammalian non-shivering thermogenesis mediated through UCP1-dependent respiration[1]. Cold temperature triggers fuel oxidation and UCP1-mediated dissipation of the mitochondrial proton gradient to rapidly generate heat in BAT[1,3], and C57BL/6J (B6) mice acclimated to room temperature (22 °C) survive acute exposure to 4 °C through this mechanism[1,4]. While UCP1-deficient mice can utilize other thermogenic mechanisms upon gradual acclimation to cold[5–8], UCP1 is required to prevent lethal hypothermia upon rapid decreases in ambient temperature, such as from 22 °C to 4 °C (ref. 4). Although much is known about brown adipose commitment and differentiation[9], the transcriptional mechanisms that ensure readiness of mature BAT for immediate heat production remain unclear[10].

The ubiquitously expressed class I histone deacetylase HDAC3 is an epigenomic modulator of nuclear receptor controlled gene expression, functioning as a stoichiometric component of the nuclear receptor co-repressor (NCoR) complex[2] to modulate deacetylation of histones as well as non-histone targets[11]. Global HDAC3 deletion is embryonic lethal[12], but studies of its tissue-specific functions link HDAC3 to hepatic steatosis[13], macrophage function[14], atherosclerosis[15], bone density[16], intestinal homeostasis[17], and cardiac energy metabolism[18,19]. However, its physiological role in BAT is not known.

We bred B6 mice with a floxed HDAC3 allele to B6 mice harbouring the pan-adipose *adiponectin-cre* (*Adipoq-cre*) and the BAT-specific *Ucp1-cre* for conditional pan-adipose and BAT-specific knockout (KO) (Extended Data Fig. 1a), and challenged adults with a drop in ambient temperature from 22 °C to 4 °C. As expected, control littermates maintained their core body temperature in the face of the acute environmental change (Fig. 1a). Strikingly, both the *Adipoq-cre* and *Ucp1-cre* HDAC3 KO mice exhibited a rapid loss of core body temperature, becoming severely hypothermic within just a few hours of moving to 4 °C (Fig. 1a). The inability to maintain core body temperature was lethal for every mouse lacking HDAC3 in BAT, whereas all the control littermates survived (Fig. 1b). Notably, the severe cold susceptibility of the HDAC3 KO mice was similar to that observed in congenic *Ucp1* KO mice (Fig. 1a, b).

The requirement for HDAC3 in regulating BAT-thermogenic capacity was examined by measuring noradrenaline-induced whole-body oxygen consumption in anaesthetized mice. Control littermates exhibited a rapid and robust increase in oxygen consumption following noradrenaline treatment whereas HDAC3 KO mice had a blunted response comparable to that observed in *Ucp1* KO mice[1] (Fig. 1c). Despite severely impaired BAT metabolic respiration, loss of HDAC3 had little effect on interscapular BAT mass, BAT mitochondrial content, or total body mass (Extended Data Fig. 1b–d).

BAT mitochondrial function was tested by high-resolution respirometry. As expected, palmitoylcarnitine and pyruvate induced respiration in control BAT mitochondria and, consistent with UCP1-dependence, this was inhibited by guanosine diphosphate[1] (Fig. 1d). Remarkably, mitochondria from HDAC3 KO BAT exhibited impaired substrate-induced respiration, with reduced function of complexes I, II, and IV of the electron transport chain (Fig. 1d). Consistent with this mitochondrial dysfunction, histological analysis revealed the presence of larger lipid droplets in HDAC3 KO BAT (Fig. 1e). By contrast, the histology of inguinal white adipose tissue (iWAT) lacking HDAC3 was similar to that of wild-type (WT) mice (Extended Data Fig. 1e),

To elucidate the molecular basis through which HDAC3 controls the thermogenic capacity of BAT, we next performed RNA sequencing (RNA-seq) on BAT from control and KO mice housed at thermoneutrality (29 °C) to avoid the potentially confounding influence of cold-stress. Consistent with the well-known function of HDAC3 as a corepressor, many genes were induced by loss of HDAC3, including

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Neprilysin Is Required for Angiotensin-(1–7)'s Ability to Enhance Insulin Secretion via Its Proteolytic Activity to Generate Angiotensin-(1–2)

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Recent work has renewed interest in therapies targeting the renin-angiotensin system (RAS) to improve β-cell function in type 2 diabetes. Studies show that generation of angiotensin-(1–7) by ACE2 and its binding to the Mas receptor (MasR) improves glucose homeostasis, partly by enhancing glucose-stimulated insulin secretion (GSIS). Thus, islet ACE2 upregulation is viewed as a desirable therapeutic goal. Here, we show that, although endogenous islet ACE2 expression is sparse, its inhibition abrogates angiotensin-(1–7)–mediated GSIS. However, a more widely expressed islet peptidase, neprilysin, degrades angiotensin-(1–7) into several peptides. In neprilysin-deficient mouse islets, angiotensin-(1–7) and neprilysin-derived degradation products angiotensin-(1–4), angiotensin-(5–7), and angiotensin-(3–4) failed to enhance GSIS. Conversely, angiotensin-(1–2) enhanced GSIS in both neprilysin-deficient and wild-type islets. Rather than mediating this effect via activation of the G-protein–coupled receptor (GPCR) MasR, angiotensin-(1–2) was found to signal via another GPCR, namely GPCR family C group 6 member A (GPRC6A). In conclusion, in islets, intact angiotensin-(1–7) is not the primary mediator of beneficial effects ascribed to the ACE2/angiotensin-(1–7)/MasR axis. Our findings warrant caution for the concurrent use of angiotensin-(1–7) compounds and neprilysin inhibitors as therapies for diabetes.

The renin-angiotensin system (RAS) is a major regulator of blood pressure and fluid balance, with its role in mediating other physiological effects recently being the subject of intense investigation. The classic view is that angiotensinogen is hydrolyzed by renin to produce angiotensin I, which is subsequently hydrolyzed by ACE to generate angiotensin II. Angiotensin II is biologically active, primarily binding angiotensin II receptor type I (AT_1) to mediate its effects. This cascade is known as the ACE/angiotensin II/AT_1 axis.

Discovery of more components of the RAS has seen the classic model evolve, wherein the ACE2/angiotensin-(1–7)/Mas receptor (MasR) axis was proposed. This involves the generation of angiotensin-(1–7) directly from angiotensin I or angiotensin II by ACE2 or indirectly from angiotensin-(1–9) (1–3). Angiotensin-(1–7) binds the G-protein–coupled receptor (GPCR) MasR (4) to elicit responses that can counteract those of the ACE/angiotensin II/AT_1 axis.

Another peptidase capable of generating angiotensin-(1–7) is neprilysin (5–7). Kinetic studies of peptide cleavage showed that neprilysin more efficiently hydrolyzed angiotensin I to angiotensin-(1–7) compared with ACE2 (1). Also, angiotensin-(1–9) was cleaved preferentially by neprilysin to generate angiotensin-(1–7), which was further cleaved by neprilysin to several smaller peptides (1,6,8). Whether these small peptides bind the MasR remains unknown.

The existence of a local RAS has been reported in many tissues, including the pancreatic islet (9). The islet RAS plays important roles in regulating local blood flow, insulin biosynthesis and secretion, and β-cell survival (10,11). Previously, we demonstrated that neprilysin is expressed in islets (12). Despite this, the effects of angiotensin-(1–7) on islet

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Cell Metabolism
Article

Microglial Inflammatory Signaling Orchestrates the Hypothalamic Immune Response to Dietary Excess and Mediates Obesity Susceptibility

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SUMMARY

Dietary excess triggers accumulation of pro-inflammatory microglia in the mediobasal hypothalamus (MBH), but the components of this microgliosis and its metabolic consequences remain uncertain. Here, we show that microglial inflammatory signaling determines the immunologic response of the MBH to dietary excess and regulates hypothalamic control of energy homeostasis in mice. Either pharmacologically depleting microglia or selectively restraining microglial NF-κB-dependent signaling sharply reduced microgliosis, an effect that includes prevention of MBH entry by bone-marrow-derived myeloid cells, and greatly limited diet-induced hyperphagia and weight gain. Conversely, forcing microglial activation through cell-specific deletion of the negative NF-κB regulator A20 induced spontaneous MBH microgliosis and cellular infiltration, reduced energy expenditure, and increased both food intake and weight gain even in absence of a dietary challenge. Thus, microglial inflammatory activation, stimulated by dietary excess, orchestrates a multicellular hypothalamic response that mediates obesity susceptibility, providing a mechanistic rationale for non-neuronal approaches to treat metabolic diseases.

INTRODUCTION

Energy homeostasis depends on the integrated function of hypothalamic neurons that detect changes in nutrient availability through adiposity hormones, such as leptin, and coordinately control feeding behavior and metabolic rate (Schwartz et al., 2000). However, the high prevalence of obesity indicates that environmental influences, such as dietary excess, can override this control system to promote weight gain.

Clear public health concerns have spurred efforts to determine how to maintain CNS control over energy balance in the face of dietary excess. While most studies have focused on hypothalamic neurons (Waterson and Horvath, 2015), comparatively few have investigated non-neuronal cells, which outnumber neurons in the brain. Here, we provide the first mechanistic evidence that microglia, the self-renewing population of CNS macrophages, orchestrate both the immunologic and physiologic responses of the hypothalamus to dietary excess and instruct the hypothalamic control of food intake, energy expenditure, and body weight.

Diet-induced obesity (DIO) is associated with a form of low-grade inflammation involving macrophages and other immune cells in white adipose and other metabolic tissues and is implicated in the development of insulin resistance (Gregor and Hotamisligil, 2011). This process is paralleled by a more rapid response involving glial cell accumulation (gliosis) in the mediobasal hypothalamus (MBH), both in mice and humans (Buckman et al., 2013; Gao et al., 2014; Schur et al., 2015; Thaler et al., 2012; Valdearcos et al., 2014), and the inflammatory activation of MBH microglia is prominent in the gliosis induced by high-fat diet (HFD) or saturated fat consumption (Thaler et al., 2012; Valdearcos et al., 2014). The porous blood-brain barrier (BBB) in the MBH may also allow infiltrating myeloid cells from the circulation to augment gliosis, as is seen in other CNS inflammatory conditions that alter BBB integrity (Ginhoux et al., 2010; Sheng et al., 2015). However, because prior analyses of "microgliosis" in mice with DIO (e.g., Thaler et al., 2012; Valdearcos et al., 2014; Morari et al., 2014) used either common myeloid markers (e.g., Iba1, CD11b, Emr1) or methods that might damage the

Apoptosis Repressor With Caspase Recruitment Domain Ameliorates Amyloid-Induced β-Cell Apoptosis and JNK Pathway Activation

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Islet amyloid is present in more than 90% of individuals with type 2 diabetes, where it contributes to β-cell apoptosis and insufficient insulin secretion. Apoptosis repressor with caspase recruitment domain (ARC) binds and inactivates components of the intrinsic and extrinsic apoptosis pathways and was recently found to be expressed in islet β-cells. Using a human islet amyloid polypeptide transgenic mouse model of islet amyloidosis, we show ARC knockdown increases amyloid-induced β-cell apoptosis and loss, while ARC overexpression decreases amyloid-induced apoptosis, thus preserving β-cells. These effects occurred in the absence of changes in islet amyloid deposition, indicating ARC acts downstream of amyloid formation. Because islet amyloid increases c-Jun N-terminal kinase (JNK) pathway activation, we investigated whether ARC affects JNK signaling in amyloid-forming islets. We found ARC knockdown enhances JNK pathway activation, whereas ARC overexpression reduces JNK, c-Jun phosphorylation, and c-Jun target gene expression (*Jun* and *Tnf*). Immunoprecipitation of ARC from mouse islet lysates showed ARC binds JNK, suggesting interaction between JNK and ARC decreases amyloid-induced JNK phosphorylation and downstream signaling. These data indicate that ARC overexpression diminishes amyloid-induced JNK pathway activation and apoptosis in the β-cell, a strategy that may reduce β-cell loss in type 2 diabetes.

In type 2 diabetes, a number of factors, including hyperglycemia, elevated free fatty acids, and islet amyloid, contribute to the dysfunction and death of islet β-cells, and this in turn contributes to insufficient insulin secretion (1–3). Islet amyloid is found in the vast majority of individuals with type 2 diabetes and contains as its unique peptide component human islet amyloid polypeptide (hIAPP) (4,5), which is cosecreted with insulin by the pancreatic β-cell (6). Islet amyloid formation is linked to β-cell apoptosis and loss in humans (7), and this process has also been observed in transgenic animal models of islet amyloidosis (8–10).

To gain insight into the mechanisms of hIAPP toxicity, in vitro studies have used two general approaches: *1*) application of synthetic hIAPP to β-cell lines or islets, and *2*) transgenic expression of hIAPP in the β-cell. Both of these approaches have been used to show hIAPP aggregation activates c-Jun N-terminal kinase (JNK) pathway signaling in the β-cell (11–13). JNK pathway activation is commonly characterized by increased phosphorylation of JNK and its downstream target c-Jun as well as increased c-Jun target gene expression (14–16). Notably, inhibition of JNK signaling almost completely abrogates β-cell apoptosis both when hIAPP is applied exogenously (11) or when hIAPP is expressed endogenously (13), indicating the central importance of this pathway in amyloid-induced β-cell toxicity.

Apoptosis repressor with caspase recruitment domain (ARC) is an inhibitor of apoptosis that has been shown to reduce cell death in cardiac and skeletal muscle (17,18), neurons (19,20), hepatocytes (21,22), and more recently, in islet β-cells (23). In these tissues, ARC antagonizes several proapoptotic signaling pathways implicated in amyloid-induced cell death (24,25), including the JNK pathway (21,22). Physical interaction between ARC's caspase recruitment domain (CARD) and proapoptotic molecules has been shown to govern its antiapoptotic effects (24,26).

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Combined Deletion of *Slc30a7* and *Slc30a8* Unmasks a Critical Role for ZnT8 in Glucose-Stimulated Insulin Secretion

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Polymorphisms in the *SLC30A8* gene, which encodes the ZnT8 zinc transporter, are associated with altered susceptibility to type 2 diabetes (T2D), and *SLC30A8* haploinsufficiency is protective against the development of T2D in obese humans. *SLC30A8* is predominantly expressed in pancreatic islet β-cells, but surprisingly, multiple knockout mouse studies have shown little effect of *Slc30a8* deletion on glucose tolerance or glucose-stimulated insulin secretion (GSIS). Multiple other *Slc30a* isoforms are expressed at low levels in pancreatic islets. We hypothesized that functional compensation by the *Slc30a7* isoform, which encodes ZnT7, limits the impact of *Slc30a8* deletion on islet function. We therefore analyzed the effect of *Slc30a7* deletion alone or in combination with *Slc30a8* on in vivo glucose metabolism and GSIS in isolated islets. Deletion of *Slc30a7* alone had complex effects in vivo, impairing glucose tolerance and reducing the glucose-stimulated increase in plasma insulin levels, hepatic glycogen levels, and pancreatic insulin content. *Slc30a7* deletion also affected islet morphology and increased the ratio of islet α- to β-cells. However, deletion of *Slc30a7* alone had no effect on GSIS in isolated islets, whereas combined deletion of *Slc30a7* and *Slc30a8* abolished GSIS. These data demonstrate that the function of ZnT8 in islets can be unmasked by removal of ZnT7 and imply that ZnT8 may affect T2D susceptibility through actions in other tissues where it is expressed at low levels rather than through effects on pancreatic islet function.
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SLC30A8 encodes the zinc transporter ZnT8 and is highly expressed in pancreatic islets (1, 2). ZnT8 transports zinc into insulin secretory granules, which is thought to promote the proper maturation, storage, and secretion of insulin (1, 2). In humans a nonsynonymous *SLC30A8* single nucleotide polymorphism (SNP) rs13266634 is associated with modest changes in proinsulin to insulin conversion (3), glucose tolerance (4), first phase insulin secretion (5), and susceptibility for the development of type 2 diabetes (T2D) (6). Similarly, studies in *Slc30a8* knockout (KO) mice have generally shown modest effects of global *Slc30a8* deletion on glucose tolerance and glucose-stimulated insulin secretion (GSIS) (7–10). Although *Slc30a8* is the most highly expressed *Slc30a* isoform in human and mouse islets (8, 10), various studies have implicated a role for other *Slc30a* isoforms in islet function, including *Slc30a3*, which encodes ZnT3 (11), and *Slc30a7*, which encodes ZnT7 (12). We therefore hypothesized that functional compensation by one of these isoforms may be limiting the impact of *Slc30a8* deletion on

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Abbreviations: DKO, Double ZnT7 and ZnT8 Knockout; EM, electron microscopy; GSIS, glucose-stimulated insulin secretion; HET, heterozygous; IPGTT, intraperitoneal glucose tolerance test; ITT, insulin tolerance test; KO, knockout; OGTT, oral glucose tolerance test; SNP, single nucleotide polymorphism; T2D, type 2 diabetes; WT, wild type.

CellPress

Cell Metabolism
Article

Interrupted Glucagon Signaling Reveals Hepatic α Cell Axis and Role for L-Glutamine in α Cell Proliferation

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SUMMARY

Decreasing glucagon action lowers the blood glucose and may be useful therapeutically for diabetes. However, interrupted glucagon signaling leads to α cell proliferation. To identify postulated hepatic-derived circulating factor(s) responsible for α cell proliferation, we used transcriptomics/proteomics/metabolomics in three models of interrupted glucagon signaling and found that proliferation of mouse, zebrafish, and human α cells was mTOR and FoxP transcription factor dependent. Changes in hepatic amino acid (AA) catabolism gene expression predicted the observed increase in circulating AAs. Mimicking these AA levels stimulated α cell proliferation in a newly developed in vitro assay with L-glutamine being a critical AA. α cell expression of the AA transporter *Slc38a5* was markedly increased in mice with interrupted glucagon signaling and played a role in α cell proliferation. These results indicate a hepatic α islet cell axis where glucagon regulates serum AA availability and AAs, especially L-glutamine, regulate α cell proliferation and mass via mTOR-dependent nutrient sensing.

INTRODUCTION

Blood glucose homeostasis is primarily the result of coordinated action of two pancreatic islet-derived hormones, insulin and glucagon. Nutrient ingestion stimulates insulin secretion from islet β cells, which promotes glucose uptake and suppresses liver glucose production, while hypoglycemia stimulates glucagon secretion from islet α cells to promote liver glucose production via gluconeogenesis and glycogenolysis. While traditionally thought to result from absolute or relative deficiency of insulin action, more recently the contribution of hyperglucagonemia to diabetes has been emphasized (Unger and Cherrington, 2012). Consequently, efforts to reduce glucagon action using small molecule antagonists, small interfering RNA (siRNA), aptamers, or antibodies that target the glucagon receptor (GCGR) have successfully improved glycemic control, especially in models of diabetes and in humans; however, interrupting glucagon signaling by multiple approaches (proglucagon gene knockout, interruption of *Gcgr* expression or downstream signaling, GCGR small molecule inhibitors, GCGR antibodies, or *Gcgr* antisense oligonucleotides) results in hyperglucagonemia and α cell hyperplasia (Campbell and Drucker, 2015). In fact, efforts over 35 years ago to develop antibodies against glucagon offered the first clue that neutralizing circulating glucagon leads to α cell hyperplasia (von Dorsche and Ziegler, 1981).

α cells from $Gcgr^{-/-}$ and liver-specific $Gcgr^{-/-}$ ($Gcgr^{Hep-/-}$) mice proliferate at rates 3- to 7-fold higher than control mice independently of the pancreatic environment or islet innervation (Longuet et al., 2013). To test the hypothesis that interrupted glucagon signaling in the liver leads to the production of a hepatic factor that promotes α cell proliferation, we used an integrated strategy of proteomics/metabolomics/transcriptomics in three models with altered glucagon signaling, and a new in vitro islet culture α cell proliferation assay to discover that high concentrations of the gluconeogenic amino acid (AA) L-glutamine (Q) in

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STEM CELLS AND REGENERATION

RESEARCH ARTICLE

The mammal-specific *Pdx1* Area II enhancer has multiple essential functions in early endocrine cell specification and postnatal β-cell maturation

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ABSTRACT

The transcription factor Pdx1 is required for multiple aspects of pancreatic organogenesis. It remains unclear to what extent *Pdx1* expression and function depend upon *trans*-activation through 5′ conserved *cis*-regulatory regions and, in particular, whether the mammal-specific Area II (−2139 to −1958 bp) affects minor or major aspects of organogenesis. We show that Area II is a primary effector of endocrine-selective transcription in epithelial multipotent cells, nascent endocrine progenitors, and differentiating and mature β cells *in vivo*. *Pdx1*$^{\Delta AREAII/-}$ mice exhibit a massive reduction in endocrine progenitor cells and progeny hormone-producing cells, indicating that Area II activity is fundamental to mounting an effective endocrine lineage-specification program within the multipotent cell population. Creating an Area II-deleted state within already specified *Neurog3*-expressing endocrine progenitor cells increased the proportion of glucagon^{+} α relative to insulin^{+} β cells, associated with the transcriptional and epigenetic derepression of the α-cell-determining *Arx* gene in endocrine progenitors. There were also glucagon and insulin co-expressing cells, and β cells that were incapable of maturation. Creating the *Pdx1*$^{\Delta AREAII}$ state after cells entered an insulin-expressing stage led to immature and dysfunctional islet β cells carrying abnormal chromatin marking in vital β-cell-associated genes. Therefore, *trans*-regulatory integration through Area II mediates a surprisingly extensive range of progenitor and β-cell-specific *Pdx1* functions.

KEY WORDS: *Cis*-regulatory function, Lineage diversification, Pancreatic endocrine progenitors, *Pdx1* enhancer Area II, Mouse

INTRODUCTION

Various pancreas-enriched transcription factors have been linked to the programs that direct the differentiation of early and later pancreas progenitors into functional islet β cells (e.g. Sox9, Nkx6.1, Neurog3, Pdx1) (reviewed by Pan and Wright, 2011). Although the integrated, likely highly cross-regulatory gene-regulatory networks are not well defined, the dynamic expression pattern of *Pdx1*, and profound defects incurred with global or cell type-specific inactivation (e.g. Fujitani et al., 2006; Gannon et al., 2001; Hale et al., 2005; Kodama et al., 2016; Offield et al., 1996; Swift et al., 1998), clearly point to its pervasive and orchestrating role during organogenesis. Thus, a *Pdx1* null mutation in human (Stoffers et al., 1997a,b) or mouse (Jonsson et al., 1994; Offield et al., 1996) results in pancreatic agenesis, and a heterozygous mutation leads to human early-onset diabetes (Stoffers et al., 1997a,b). Moreover, conditional deletion of *Pdx1* has revealed the requirement for this transcription factor in several of the later stages of pancreatic endocrine cell development and in adult islet β-cell function (reviewed by Pan and Wright, 2011).

Much of *Pdx1* transcriptional regulation appears to be exerted by *trans*-acting factors acting within four conserved upstream *cis*-regulatory regions (termed Areas I-IV), located within 6.5 kb of the transcriptional start site (Gannon et al., 2001; Gerrish et al., 2000; Van Velkinburgh et al., 2005). Whereas Areas I, III and IV are present in widely differing vertebrate species, Area II is restricted, somewhat surprisingly, to mammals (Gerrish et al., 2000). In mouse, combined deletion of Areas I-II-III (*Pdx1*$^{\Delta I\text{-}II\text{-}III}$) *in vivo* produces severely deficient *Pdx1* expression and impairs formation of the early pancreatic buds (Fujitani et al., 2006), an effect similar to the pancreatic agenesis in *Pdx1* germline nulls (Offield et al., 1996). Complementary experiments showed that *Pdx1* expression driven by Areas I-II-III, with only a small portion of Area IV, restored full pancreatic development to *Pdx1* null mice (Boyer et al., 2006; Gannon et al., 2001). These results imply that the embryonic *Pdx1* expression required for complete production of a differentiated pancreatic organ is principally, if not exclusively, regulated by Areas I-II-III.

Enhancer-like activities for Areas I, II and III have been documented in reporter assays in β-cell lines and a limited number of transgenic mouse assays. Such studies assigned β-cell-specific enhancer-like activities to Area II. For example, while Area I or Area II imparted β-cell-specific activation in cell lines (Gerrish et al., 2000), only Area II independently directed expression to islet β cells *in vivo*, although expression was variegated. When placed together, Areas I and II seemed to show functional interactions that were now able to induce high *Pdx1* expression throughout the entire β-cell population from around embryonic day (E) 13.5, which represents the start of the major phase of insulin^{+} cell production (Van Velkinburgh et al., 2005). Whereas the region representing Areas I-II-III is bivalently marked in early endodermal progenitors, it is subsequently derepressed in nascent pancreatic progenitors leading to a relative deficit of repressive chromatin markings (van Arensbergen et al., 2010; Xie et al., 2013; Xu et al., 2011). Together with Area I-II-III transgene analysis (Wiebe et al., 2007), these findings supported the idea that Areas I-II-III are involved in driving *Pdx1* expression in pancreatic endocrine as well as exocrine progenitors.

Although these combined findings support a central role for Area II in driving *Pdx1* transcription, the effect of removing just Area II

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RESEARCH PAPER - BASIC SCIENCE

Intermittent fasting preserves beta-cell mass in obesity-induced diabetes via the autophagy-lysosome pathway

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ABSTRACT

Obesity-induced diabetes is characterized by hyperglycemia, insulin resistance, and progressive beta cell failure. In islets of mice with obesity-induced diabetes, we observe increased beta cell death and impaired autophagic flux. We hypothesized that intermittent fasting, a clinically sustainable therapeutic strategy, stimulates autophagic flux to ameliorate obesity-induced diabetes. Our data show that despite continued high-fat intake, intermittent fasting restores autophagic flux in islets and improves glucose tolerance by enhancing glucose-stimulated insulin secretion, beta cell survival, and nuclear expression of NEUROG3, a marker of pancreatic regeneration. In contrast, intermittent fasting does not rescue beta-cell death or induce NEUROG3 expression in obese mice with lysosomal dysfunction secondary to deficiency of the lysosomal membrane protein, LAMP2 or haplo-insufficiency of BECN1/Beclin 1, a protein critical for autophagosome formation. Moreover, intermittent fasting is sufficient to provoke beta cell death in nonobese *lamp2* null mice, attesting to a critical role for lysosome function in beta cell homeostasis under fasting conditions. Beta cells in intermittently-fasted LAMP2- or BECN1-deficient mice exhibit markers of autophagic failure with accumulation of damaged mitochondria and upregulation of oxidative stress. Thus, intermittent fasting preserves organelle quality via the autophagy-lysosome pathway to enhance beta cell survival and stimulates markers of regeneration in obesity-induced diabetes.

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KEYWORDS
autophagy; beta cells; diabetes; intermittent fasting; lysosomes

Introduction

Diabetes is a major contributor to morbidity and mortality from multiple causes, including cardiovascular disease and chronic kidney disease. With an increasingly obese population, type 2 diabetes (T2D) is becoming a worldwide pandemic, with nearly one in 10 adults affected and more than 1.5 million deaths per year. By 2050, nearly one in 3 adults may be diagnosed with T2D.[1] The pathogenesis of T2D involves a downward spiral of hyperglycemia, insulin resistance, impairments in insulin secretion and beta cell death culminating in overt insulin-dependence.[2] Given the increasing burden of T2D, developing strategies to reverse or prevent these metabolic derangements and beta cell loss is of utmost importance.

The prevention of T2D by dietary modifications, including caloric restriction and intermittent fasting (IF), has been described in human clinical trials.[3] Caloric restriction can extend life span in rodents, an effect that is associated with improvement in insulin sensitivity [4] and markers of metabolic health in humans;[5] however, caloric restriction is often difficult to sustain. An alternative dietary regimen to caloric restriction is intermittent fasting (IF), which may have beneficial effects on longevity and metabolism in humans.[6-8] IF prevents the development of diabetes and promotes fat loss and lean body mass retention in rodent models.[9-12] However, the mechanisms by which IF leads to these benefits are unknown.

The autophagy-lysosome pathway is both induced by IF and implicated in the development of T2D.[13-18] Our group has recently demonstrated that IF preconditions the myocardium towards ischemia-reperfusion injury, via stimulation of the autophagy-lysosome pathway.[19] Here we have tested the hypothesis that IF improves glucose handling in obesity-induced diabetes via the autophagy-lysosome pathway. Our results demonstrate that IF improves glucose tolerance in high-fat diet (HFD) fed mice compared to ad libitum controls via preservation of beta cell mass and function. In LAMP2-deficient mice, a model of lysosomal insufficiency, the benefits of IF on glucose metabolism are lost, and IF paradoxically

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HEPATOLOGY

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Targeting the Mitochondrial Pyruvate Carrier Attenuates Fibrosis in a Mouse Model of Nonalcoholic Steatohepatitis

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Diseases of the liver related to metabolic syndrome have emerged as the most common and undertreated hepatic ailments. The cause of nonalcoholic fatty liver disease is the aberrant accumulation of lipid in hepatocytes, though the mechanisms whereby this leads to hepatocyte dysfunction, death, and hepatic fibrosis are still unclear. Insulin-sensitizing thiazolidinediones have shown efficacy in treating nonalcoholic steatohepatitis (NASH), but their widespread use is constrained by dose-limiting side effects thought to be due to activation of the peroxisome proliferator–activated receptor γ. We sought to determine whether a next-generation thiazolidinedione with markedly diminished ability to activate peroxisome proliferator–activated receptor γ (MSDC-0602) would retain its efficacy for treating NASH in a rodent model. We also determined whether some or all of these beneficial effects would be mediated through an inhibitory interaction with the mitochondrial pyruvate carrier 2 (MPC2), which was recently identified as a mitochondrial binding site for thiazolidinediones, including MSDC-0602. We found that MSDC-0602 prevented and reversed liver fibrosis and suppressed expression of markers of stellate cell activation in livers of mice fed a diet rich in trans-fatty acids, fructose, and cholesterol. Moreover, mice with liver-specific deletion of MPC2 were protected from development of NASH on this diet. Finally, MSDC-0602 directly reduced hepatic stellate cell activation *in vitro*, and MSDC-0602 treatment or hepatocyte MPC2 deletion also limited stellate cell activation indirectly by affecting secretion of exosomes from hepatocytes. *Conclusion:* Collectively, these data demonstrate the effectiveness of MSDC-0602 for attenuating NASH in a rodent model and suggest that targeting hepatic MPC2 may be an effective strategy for pharmacologic development. (HEPATOLOGY 2017;65:1543-1556).

The epidemic of obesity has dramatically increased the incidence of a variety of related metabolic diseases, including nonalcoholic fatty liver disease (NAFLD).[1] NAFLD encompasses a spectrum of diseases from isolated steatosis to steatohepatitis (NASH), which includes steatosis with inflammation, hepatocyte ballooning injury, and fibrosis.[2] NASH is a leading cause of cirrhosis and liver failure,[2] increases risk for hepatocellular carcinoma,[3] and is currently the second leading indication for liver transplantation in the United States.[4,5] Fibrosis, and thus parenchymal remodeling, is the best predictor of progression, need for liver transplant, and mortality.[6-8] Despite the prevalence of NAFLD and

Abbreviations: ALT, alanine transaminase; AST, aspartate transaminase; BRET, bioluminescence energy transfer; HTF-C, high trans-fat, fructose, cholesterol; LF, low-fat; MPC, mitochondrial pyruvate carrier; NAFLD, nonalcoholic fatty liver disease; NAS, NAFLD activity score; NASH, nonalcoholic steatohepatitis; PPARγ, peroxisome proliferator–activated receptor γ; RESPYR, reporter sensitive to pyruvate; TZD, thiazolidinedione.

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Rpl13a small nucleolar RNAs regulate systemic glucose metabolism

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Small nucleolar RNAs (snoRNAs) are non-coding RNAs that form ribonucleoproteins to guide covalent modifications of ribosomal and small nuclear RNAs in the nucleus. Recent studies have also uncovered additional non-canonical roles for snoRNAs. However, the physiological contributions of these small RNAs are largely unknown. Here, we selectively deleted four snoRNAs encoded within the introns of the ribosomal protein L13a (*Rpl13a*) locus in a mouse model. Loss of *Rpl13a* snoRNAs altered mitochondrial metabolism and lowered reactive oxygen species tone, leading to increased glucose-stimulated insulin secretion from pancreatic islets and enhanced systemic glucose tolerance. Islets from mice lacking *Rpl13a* snoRNAs demonstrated blunted oxidative stress responses. Furthermore, these mice were protected against diabetogenic stimuli that cause oxidative stress damage to islets. Our study illuminates a previously unrecognized role for snoRNAs in metabolic regulation.

Introduction

Box C/D snoRNAs are short noncoding RNAs containing conserved C and D box consensus motifs that form ribonucleoproteins with NOP56, NOP58, 15.5 kDa, and the methyltransferase fibrillarin (1). These ribonucleoproteins localize to nucleoli, where their canonical function is to serve as guides to target specific sites on ribosomal RNAs (rRNAs) or small nuclear RNAs (snRNAs) for 2′-*O*-methylation through a short stretch of antisense complementarity (10–21 nucleotides). In vertebrates, this function has been demonstrated experimentally for some box C/D snoRNAs and is predicted for others. The observation that animals homozygous for knockout of fibrillarin are inviable indicates that functions of box C/D snoRNAs as a class are essential for normal development (2).

Genetic studies have suggested an expanding functional repertoire for snoRNAs beyond their well-established roles in modification and processing of rRNAs and snRNAs (3). Dysregulated snoRNA expression has been associated with altered splicing in the developmental syndromes, Prader-Willi and tetralogy of Fallot (4, 5), and snoRNAs U50, ACA11, and ACA42 have been shown to modulate tumor proliferation in vivo (6–9). Nonetheless, the physiological consequences of perturbation of expression of most mammalian snoRNAs remain unexplored, and no animal models exist with selective and complete loss of function for any snoRNAs.

Our laboratory's studies have demonstrated that box C/D snoRNAs encoded by introns of the *Rpl13a* locus function as critical mediators of cell death in response to metabolic and oxidative stress in cultured cells (10–12). The observations that this role can be dissociated from changes in the 2′-*O*-methylation status of predicted ribosomal RNA targets and that the *Rpl13a* snoRNAs accumulate in the cytosol during oxidative stress suggest that the *Rpl13a* snoRNAs may function through noncanonical mechanisms. The goal of this study was to determine the physiological role of these noncoding RNAs.

Results

Generation of Rpl13a-snoless mice. Our previous work demonstrated a critical role for box C/D snoRNAs U32a, U33, U34, and U35a, embedded within 4 introns of the *Rpl13a* locus (Figure 1A), in the cellular response to lipotoxic and oxidative stress (10). The observation that loss of function of the individual snoRNAs in cultured cells is not sufficient to confer resistance to metabolic stress suggests that these 4 snoRNAs function in concert in stress response pathways. Therefore, to probe the long-term physiological consequences of loss of function of *Rpl13a* snoRNAs, we generated a model with simultaneous loss of all 4 box C/D snoRNAs encoded in this locus. Since deletion of the RPL13a protein in *Drosophila* is embryonic lethal (13), our goal was to selectively modify 4 snoRNA-hosting introns without perturbing expression of the exon-encoded ribosomal protein. This was accomplished using a single recombination event to replace the locus with an allele from which the snoRNAs were selectively deleted (Figure 1, A–C, and Supplemental Figure 1; supplemental material available online with this article; doi:10.1172/JCI88069DS1). No known regulatory regions overlap with these 4 snoRNAs, and these intronic sequences are relatively depleted of H3K27 histone marks that are often found near regulatory regions.

Homozygous *Rpl13a* snoRNA loss-of-function mice (referred to hereafter in text as *Rpl13a*-snoless and in figures as -/-) were live-born at normal Mendelian ratios, were fertile, and displayed no overt pathophysiological phenotype under standard housing,

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Frequency of Evidence-Based Screening for Retinopathy in Type 1 Diabetes

The DCCT/EDIC Research Group*

ABSTRACT

BACKGROUND

In patients who have had type 1 diabetes for 5 years, current recommendations regarding screening for diabetic retinopathy include annual dilated retinal examinations to detect proliferative retinopathy or clinically significant macular edema, both of which require timely intervention to preserve vision. During 30 years of the Diabetes Control and Complications Trial (DCCT) and its longitudinal follow-up Epidemiology of Diabetes Interventions and Complications (EDIC) study, retinal photography was performed at intervals of 6 months to 4 years.

METHODS

We used retinal photographs from the DCCT/EDIC study to develop a rational screening frequency for retinopathy. Markov modeling was used to determine the likelihood of progression to proliferative diabetic retinopathy or clinically significant macular edema in patients with various initial retinopathy levels (no retinopathy or mild, moderate, or severe nonproliferative diabetic retinopathy). The models included recognized risk factors for progression of retinopathy.

RESULTS

Overall, the probability of progression to proliferative diabetic retinopathy or clinically significant macular edema was limited to approximately 5% between retinal screening examinations at 4 years among patients who had no retinopathy, 3 years among those with mild retinopathy, 6 months among those with moderate retinopathy, and 3 months among those with severe nonproliferative diabetic retinopathy. The risk of progression was also closely related to mean glycated hemoglobin levels. The risk of progression from no retinopathy to proliferative diabetic retinopathy or clinically significant macular edema was 1.0% over 5 years among patients with a glycated hemoglobin level of 6%, as compared with 4.3% over 3 years among patients with a glycated hemoglobin level of 10%. Over a 20-year period, the frequency of eye examinations was 58% lower with our practical, evidence-based schedule than with routine annual examinations, which resulted in substantial cost savings.

CONCLUSIONS

Our model for establishing an individualized schedule for retinopathy screening on the basis of the patient's current state of retinopathy and glycated hemoglobin level reduced the frequency of eye examinations without delaying the diagnosis of clinically significant disease. (Funded by the National Institute of Diabetes and Digestive and Kidney Diseases and others; DCCT/EDIC ClinicalTrials.gov numbers, NCT00360893 and NCT00360815.)

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Cell Metabolism
Article

β Cells that Resist Immunological Attack Develop during Progression of Autoimmune Diabetes in NOD Mice

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SUMMARY

Type 1 diabetes (T1D) is a chronic autoimmune disease that involves immune-mediated destruction of β cells. How β cells respond to immune attack is unknown. We identified a population of β cells during the progression of T1D in non-obese diabetic (NOD) mice that survives immune attack. This population develops from normal β cells confronted with islet infiltrates. Pathways involving cell movement, growth and proliferation, immune responses, and cell death and survival are activated in these cells. There is reduced expression of β cell identity genes and diabetes antigens and increased immune inhibitory markers and stemness genes. This new subpopulation is resistant to killing when diabetes is precipitated with cyclophosphamide. Human β cells show similar changes when cultured with immune cells. These changes may account for the chronicity of the disease and the long-term survival of β cells in some patients.

INTRODUCTION

Type 1 diabetes (T1D) is a chronic autoimmune disease involving the destruction of insulin-producing β cells by diabetes-antigen-specific T cells. Despite widespread acceptance of this paradigm, several key questions about the disease pathogenesis remain. For example, it is not clear why β cell killing takes such an extended period of time. Some have proposed that there is waxing and waning of the immune response, possibly due to environmental factors, and our previous studies support the periodic nature of β cell killing during the period prior to dysglycemia in individuals at risk (Herold et al., 2015; von Herrath et al., 2007).

In addition, β cell destruction is not always complete, and some β cells survive immune attack. Recent observational studies have shown that there are detectable levels of insulin production in individuals who have had diabetes for many years (Keenan et al., 2010; Liu et al., 2009). Consistent with this is the finding that the immunologic destruction is not uniform throughout the islets, and regions that are heavily infiltrated with immune cells may be located next to regions that are free of inflammation (Atkinson et al., 2011). These observations point to heterogeneity of β cells, but the nature and distribution of subpopulations, their ontogeny, and immunologic properties have not been identified.

Tissues can adapt to chronic inflammation. Adaptation may involve expression of inflammatory mediators, such as cytokines, or factors that may improve the viability of the cells, such as VEGF (Akirav et al., 2011; Eizirik et al., 2009). Little is known about how immune responses change β cells and how β cells may respond to immune mediators. The question is of clinical importance because despite the success of some immune mediators in reducing decline in β cell function short term, permanent remission of T1D has not been achieved. It is possible that intrinsic β cell factors lead to destruction.

Our lab and others have shown increased rates of β cell proliferation during the prediabetes period in non-obese diabetic (NOD) mice (Sherry et al., 2006; Sreenan et al., 1999). At diabetes onset, there is a population of degranulated β cells that may recover function. These β cells were assumed to have released all of their insulin granules, but the characteristics and durability of these cells are not known. In other settings of metabolic stressors, Talchai et al. described adaptation of β cells to metabolic stressors involving dedifferentiation with expression of transcripts associated with β cell precursors such as Neurog3, Oct4, and Nanog (Talchai et al., 2012).

Studies of β cells in humans, particularly those at risk of T1D, are difficult because of the inaccessibility of tissue for study. Herein, we describe how β cells adapt to chronic autoimmunity in NOD mice by development of a novel subpopulation that can resist immune attack. This subpopulation has reduced expression of genes associated with their parent cells and acquires stem-like features, including increased rates of proliferation. These cells avoid immune attack by reducing the expression of diabetogenic antigens and increasing expression of immunomodulatory molecules. We postulate that adaptation in these cells may account for the

RESEARCH ARTICLE

CELL BIOLOGY

Lipid transport by TMEM24 at ER–plasma membrane contacts regulates pulsatile insulin secretion

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Insulin is released by β cells in pulses regulated by calcium and phosphoinositide signaling. Here, we describe how transmembrane protein 24 (TMEM24) helps coordinate these signaling events. We showed that TMEM24 is an endoplasmic reticulum (ER)–anchored membrane protein whose reversible localization to ER-plasma membrane (PM) contacts is governed by phosphorylation and dephosphorylation in response to oscillations in cytosolic calcium. A lipid-binding module in TMEM24 transports the phosphatidylinositol 4,5-bisphosphate [PI(4,5)P_2] precursor phosphatidylinositol between bilayers, allowing replenishment of PI(4,5)P_2 hydrolyzed during signaling. In the absence of TMEM24, calcium oscillations are abolished, leading to a defect in triggered insulin release. Our findings implicate direct lipid transport between the ER and the PM in the control of insulin secretion, a process impaired in patients with type II diabetes.

Eukaryotic cells harbor close appositions (10 to 30 nm) between the plasma membrane (PM) and the endoplasmic reticulum (ER), the organelle where the synthesis of most membrane lipids begins. These ER-PM contact sites are important in cellular calcium dynamics and also play critical roles in lipid homeostasis and signaling (*1*–*4*). Identification and functional characterization of the protein machinery at ER-PM appositions, including lipid-transfer proteins that carry lipids between membranes, have provided key insights into the molecular mechanisms underlying processes that occur at these sites (*5*–*10*).

Here, we investigated the properties of TMEM24, one of two similar proteins, TMEM24/C2CD2L and C2CD2, which bioinformatics studies predict to contain a module typically found in proteins that act at membrane contact sites (*11*, *12*). TMEM24, which is highly expressed in professional neurosecretory cells, is a regulator of insulin secretion (*13*). Insulin is produced by pancreatic β cells, where it is generated in the Golgi complex via the cleavage of proinsulin, then packaged into granules and stored (*14*). A rise in blood glucose activates tightly coupled calcium and phosphoinositide signaling pathways in these cells and leads to granule exocytosis, which occurs in two phases (*15*, *16*). Immediately after glucose stimulation, a rise in intracellular calcium levels triggers secretion of the readily releasable granule pool (~5%), consisting of granules already docked and primed at the PM (*17*). Next, a reserve granule pool undergoes docking and priming steps and is therefore released more gradually in a series of small bursts dependent on pulsatile rises in calcium levels (*15*, *18*). Insulin secretion is impaired in patients with type II diabetes, highlighting the importance of understanding the mechanisms that underlie this process (*14*).

Here, we probed the molecular mechanism by which TMEM24 modulates insulin exocytosis and identified TMEM24 as a lipid transport protein resident at ER-PM contact sites implicated in the cross-talk between calcium and PM phosphoinositide dynamics that regulates insulin release. We found that TMEM24 plays a role in transporting glycerolipids with a preference for phosphatidylinositol (PI) from its site of synthesis in the ER to the PM, where it is a precursor for phosphatidylinositol (4,5)-bisphosphate [PI(4,5)P_2]. At the PM, PI(4,5)P_2 has multiple roles in the cell, including in signal transduction (*19*, *20*) and in the direct control of insulin granule exocytosis (*14*, *21*–*23*) and PM ion channel function (*14*, *22*, *23*). Whereas TMEM24 localization to ER-PM contact sites and its activity in transporting PI to the PM are regulated by calcium dynamics, the lipid transfer function of TMEM24 is required for calcium pulsatility.

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Results and discussion

TMEM24 is an ER-anchored protein that localizes at ER-PM contacts

Bioinformatics studies predict that the transmembrane protein TMEM24 comprises a lipid-binding module of the tubular lipid-binding (TULIP) superfamily (*12*) (Fig. 1A), which we confirmed by crystallography (see below). All previously characterized members of the intracellular subfamily of TULIP proteins containing this module—known as synaptotagmin-like, mitochondrial, and lipid-binding protein (SMP) domain—are concentrated at membrane contact sites (*10*, *11*, *24*), suggesting that TMEM24 might also localize to sites of membrane apposition. To address this possibility, we assessed localization of versions of the human protein [TMEM24-enhanced green fluorescent protein (EGFP)] in INS-1 cells, a rat insulinoma cell line that, like pancreatic islets, exhibits high levels of endogenous TMEM24 (*13*) (fig. S1, A and B), or in HeLa cells (TMEM24-mCherry) that are not specialized for triggered secretion and have undetectable endogenous levels of TMEM24 (*13*) (fig. S1, A and B). Confocal microscopy of the transfected cells showed that moderate levels of fluorescent TMEM24 colocalized with cotransfected ER markers, mRFP-Sec61β or ER-oxGFP, indicating a localization throughout the ER (Fig. 1, B and C, left insets in C). In addition, hot spots of fluorescence were observed at the cell periphery, in a pattern consistent with a concentration at ER-PM contacts (*24*) (Fig. 1, B and C, right insets in C).

To rule out a possible subcellular mistargeting of exogenous TMEM24 due to the fluorescent tags, we assessed the localization of the overexpressed untagged protein and of the endogenous protein using newly generated antibodies directed against TMEM24 (Fig. 1D and fig. S1D), followed by confocal microscopy. An equatorial focal acquisition plane showed that overexpressed untagged TMEM24 immunofluorescence accumulated at the cell periphery in spots with the characteristic pattern for ER-PM contact sites (fig. S1D, upper panel) and partially colocalized with the ER marker calreticulin (Fig. 1D and fig. S1D). This peripheral localization was further confirmed by image acquisition of the ventral plane of cells, which highlighted the portions of the ER in close proximity with the plasma membrane (fig. S1D, lower panel). The same localization was observed for endogenous TMEM24 (Fig. 1D), which also partially colocalized with the ER marker protein disulfide isomerase (PDI). Absence of TMEM24 immunoreactivity in INS-1 clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9–edited cells lacking TMEM24 validated the specificity of the signal (Figs. 1D and 6B and fig. S1C).

The localization of TMEM24 in the ER with a concentration in the cortical ER differs from the reported localization of endogenous TMEM24 immunoreactivity on insulin granules based on a commercially available TMEM24 antibody [Aviva #ARP47080_P050 (*13*)]. We confirmed this finding using the same commercial antibodies. However, we found that such immunoreactivity was still