DIABETES RESEARCH CENTERS

2016 Meeting of the Diabetes Centers' Directors

March 30, 2016

Hyatt Regency Bethesda

Bethesda, MD

DIABETES RESEARCH CENTERS

2016 Meeting of the Diabetes Research Centers' Directors

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Agenda
2016 Diabetes Center Directors' Meeting
Wednesday, March 30, 2016

Hyatt Regency Bethesda
1 Bethesda Metro Center
Bethesda, MD 20814

7:30 – 8:00 am	Registration
8:00 – 8:10 am	Welcome and opening remarks (Dr. Rodgers)
8:10 – 9:00 am	The view from NIDDK: • Updates (J. Hyde) • Perspectives & Opportunities (J. Fradkin)
9:00 – 9:20 am	NIDDK Summer Medical Student Program: report (A. Powers)
9:20 - 9:45 am	Center-Supported Research Publications: Resource Tool (A. Powers)
9:45 – 10:00 am	Common Fund: Stimulating Peripheral Activity to Relieve Conditions (K. Teff)
10:00 – 10:15 am	NIH Accelerating Medicines Partnership: Type 2 Diabetes (P. Smith)
10:15 – 10:30 am	Break
10:30 – 11:00 am	Helmsley Charitable Trust T1D Research: Priorities & Vision (G. Agiostratidou)
11:00 – 11:30 am	Common Fund: Molecular Transducers of Physical Activity in Humans (M. Laughlin)
11:30 – 11:45 am	General Discussion
11:45 – 1:00 pm	Lunch (on your own)
1:00 – 1:30 pm	Cystic Fibrosis Research and Translation Centers (T. Eggerman)
1:30 – 1:45 pm	Collaborative Interdisciplinary Team Science in NIDDK Research Areas (C. Silva)
1:45 – 2:00 pm	Updates: NIH Metabolomics Resources and Opportunities (A. Castle)
2:00 – 2:15 pm	Break
2:15 – 2:30 pm	Diabetes Research Centers: up-coming RFAs (J. Hyde)
2:30 – 2:45 pm	Submitting Complex Electronic Applications & Progress Reports (J. Hyde)
2:45 – 3:00 pm	Diabetes Research Centers website: updates/changes (J. Hyde)
3:00 – 3:15 pm	Wrap-up, final comments & adjourn

DIABETES RESEARCH CENTERS

2016 NIDDK Diabetes Research Center Directors' Annual Meeting

March 30, 2016

Hyatt Regency Bethesda Hotel
One Bethesda Metro Center
7400 Wisconsin Avenue
Bethesda, MD 20814

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DIABETES RESEARCH CENTERS

Current Funding Opportunity Announcements

PAR-16-121: Early-Stage Preclinical Validation of Therapeutic Leads for Diseases of Interest to the NIDDK (R01)

PAR-16-126: High Impact, Interdisciplinary Science in NIDDK Research Areas (RC2)

PAR-16-127: NIDDK Program Projects (P01)

PAR-16-064: Small Grants for New Investigators to Promote Diversity in Health-Related Research (R21)

RFA-DK-16-001: Improving Diabetes Management in Pre-teens, Adolescents and/or Young Adults with Type 1 Diabetes (DP3)

RFA-DK-16-002: Understanding Barriers and Facilitators to Type 1 Diabetes Management in Adults (DP3)

RFA-DK-16-003: Improving Diabetes Management in Children with Type 1 Diabetes (DP3)

RFA-DK-16-017: Psychosocial and Behavioral Mechanisms in Bariatric Surgery (R01)

RFA-DK-16-009: Clinical, Behavioral and Physiological Research Testing Current and Novel Closed Loop Systems (DP3)

RFA-DK-15-012: A Community Research Resource of Microbiome-Derived Factors Modulating Host Physiology in Obesity, Digestive and Liver Diseases, and Nutrition (R24)

RFA-DK-15-013: Exploratory Studies for Delineating Microbiome: Host Interactions in Obesity, Digestive and Liver Diseases and Nutrition (R21)

PAR-15-182: Interdisciplinary Training in Bioinformatics and Diabetes, Obesity and Metabolic Disease (T32)

PAR-15-176: Pilot and Feasibility Clinical Trials in Diabetes, and Endocrine and Metabolic Diseases (R21)

PA-15-169: Secondary Analyses in Obesity, Diabetes and Digestive and Kidney Diseases (R21)

PAR-15-157: Pragmatic Research in Healthcare Settings to Improve Diabetes and Obesity Prevention and Care (R18)

PAR-15-158: Planning Grants for Pragmatic Research in Healthcare Settings to Improve Diabetes and Obesity Prevention and Care (R34)

NIH Big Data to Knowledge (BD2K) Enhancing Training:

RFA-HG-14-005: Revisions to Add Biomedical Big Data Training to Active Institutional Training Grants (T32)

RFA-HG-14-004: Predoctoral Training in Biomedical Big Data Science (T32)

RFA-HG-14-008: Courses for Skills Development in Biomedical Big Data Science (R25)

National Institute of Diabetes and Digestive and Kidney Diseases

2016 Diabetes Center Directors' Meeting

Bethesda, MD

National Institute of Diabetes and Digestive and Kidney Diseases

2016 Diabetes Center Directors' Meeting

- JHU-UMD: Welcome to Dr. Mehboob Hussain
- Indiana University: Welcome to Dr. Raghu Mirmira

National Institute of Diabetes and Digestive and Kidney Diseases

2015-16 Diabetes Centers Executive Committee

- **Martin Myers, University of Michigan, Chair**
- Tim Garvey, University of Alabama at Birmingham
- George King, Joslin Diabetes Center
- Jerry Olefsky, University of California, San Diego
- Jerry Palmer, U Washington (Council liaison)
- Jeff Pessin, Albert Einstein College of Medicine

National Institute of Diabetes and Digestive and Kidney Diseases

2016 Diabetes Center Directors' Meeting

- Meeting Book: http://diabetescenters.org/niddk-directors-meeting
  - Agenda
  - Current Funding Opportunities (notification of additional opportunities will be sent via e-mail when published in NIH Guide)
  - Featured Publications from Centers (2014-2015)
  - Template for annual progress reports
    - RPPR: will be distributed in summer 2016
  - Additional materials for presentations at the meeting

National Institute of Diabetes and Digestive and Kidney Diseases

2016 Diabetes Center Directors' Meeting

- Brief Overview of Agenda:
  - 8:00 – 10:15: presentations
  - 10:15 – 10:30: break
  - 10:30 – 11:45: presentations/discussion
  - 11:45 – 1:00: Lunch (on your own; map in book)
  - 1:00 – 2:00: presentations
  - 2:00-2:15: break
  - 2:15-3:00 presentations
  - Transportation: if needed, see John at the registration desk to arrange for cab to airport

National Institute of Diabetes and Digestive and Kidney Diseases

2016 Diabetes Center Directors' Meeting

- Review conflicts due to external advisory committees

- Currently have three RFAs every 5 years for DRCs
  - **3**: Joslin, U Penn, Vanderbilt
  - **9**: Columbia, JHU-UMD, UAB, UCSD/UCLA, Chicago, Michigan, U Washington, Washington U, Yale
  - **4**: Boston Area, UCSF, Einstein-MSSM, Indiana

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P30 Grants: Human Research Subjects

- If there may ever be a P&F project (or core) that involves identifiable human subjects, please indicate “YES” for human subjects in your competing renewal application (Overall Component and Individual Component)
- NIH/NIDDK may use ‘delayed onset’ if the specifics are unknown for a project involving human research subjects
- P30 grants are now required to use the NIH electronic Inclusion Management System to report Inclusion Enrollment Data when human subjects are involved (cores, and/or P&F projects)
- Changing a P30 grant from “NO” to “YES” for human subjects is a LOT of work for everyone involved, especially you

Annual Progress Reports

- Overall, everyone (so far) did a very good job submitting electronic RPPRs.
- A new RPPR template will be circulated as a guide by summer 2016. Areas of clarification/change will be highlighted.
- Center-supported research publications continue to be difficult to track. Al Powers will make a brief presentation regarding a tool that Vanderbilt developed.

Annual Progress Reports: Publications

- It is critical for research publications that received Center support through cores or pilot projects cite the NIDDK Center grant number.
- When NIDDK analyzes Center-supported research publications, we use the grant number to search PubMed.
- Be sure that the list of publications (sorted by core, etc.) in the renewal application is accurate. If reviewers determine that there are some publications that were not clearly supported by a Center activity, this will likely decrease enthusiasm of the review panel.

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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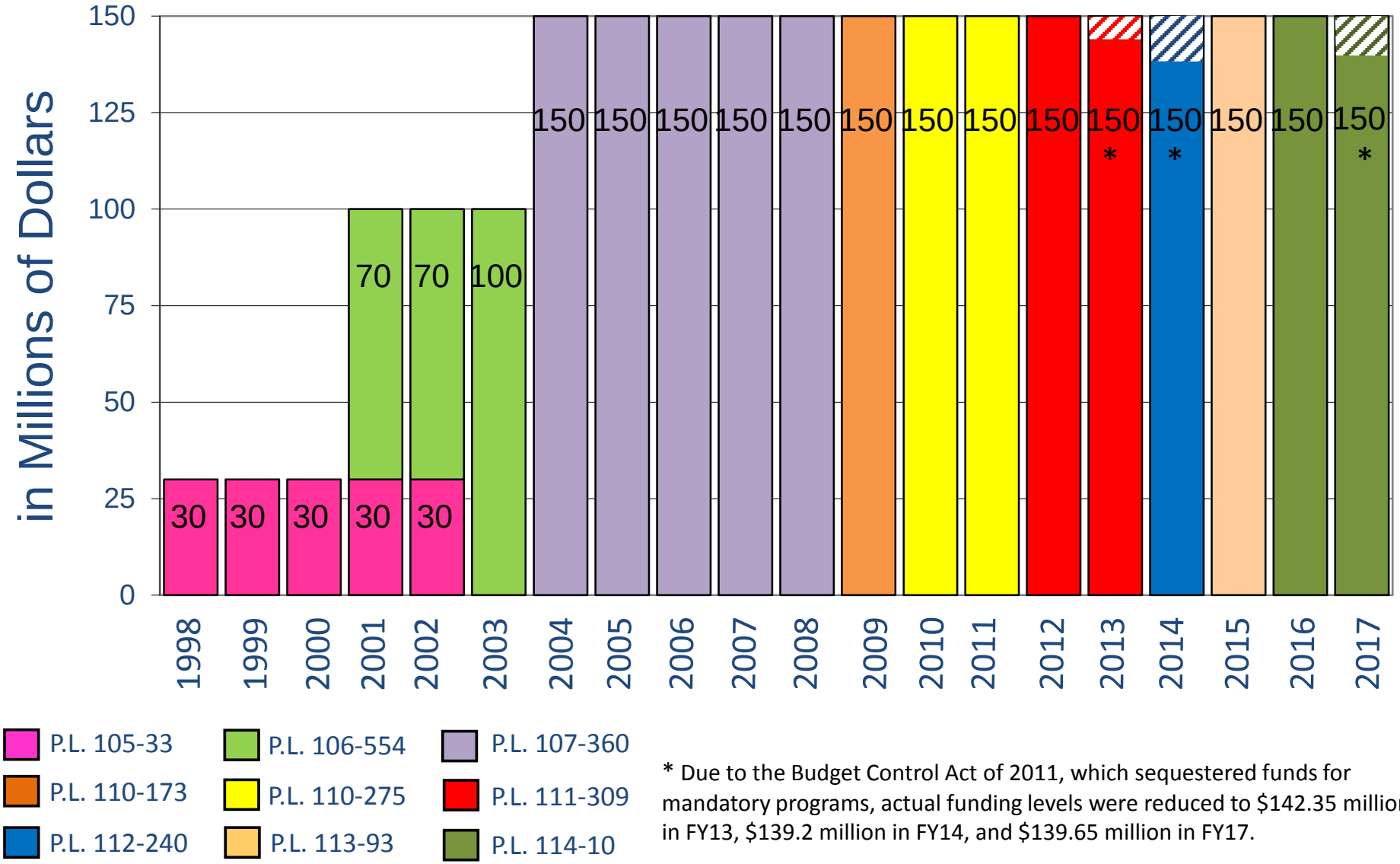
March 30, 2016

Topics

- Update on T1D Special Program
- Update on Initiatives with Regular NIDDK Funding
- Update on Major Clinical Research

National Institute of Diabetes and Digestive and Kidney Diseases

Laws Provide $2.46 Billion over 20 years*



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

Planning Meeting for Research Supported by the SDP
April *8-9, 2015*

- Coordinated by the statutory Diabetes Mellitus Interagency Coordinating Committee (DMICC)

- Panel of external scientific and lay experts will consider proposals and suggest opportunities for:

  - New research initiatives to be supported in FY16 and/or FY17 (should SDP be continued)

  - Continuations/expansions of ongoing programs

National Institute of Diabetes and Digestive and Kidney Diseases

Planning Meeting for Research Supported by the SDP
Panel Members

- *Autoimmunity*: Dr. Jeff Bluestone, Dr. Peter Gregersen, Dr. Jane Salmon
- *Transplantation*: Dr. Ronald Gill, Dr. Stanislaw Stepowski
- *Clinical*: Dr. Robert Sherwin, Dr. Betty Diamond
- *Behavior*: Dr. Georgeanna Klingensmith, Dr. Timothy Wysocki
- *Biostatistics*: Dr. Mark Espeland
- Epidemiology: Dr. James Meigs, Dr. Elizabeth Selvin
- *Beta cell biology*: Dr. Mike German, Dr. Maike Sander
- *Genetics*: Dr. Rudy Leibel
- *Complications*: Dr. Matthew Breyer, Dr. Robert Eckel, Dr. Nigel Calcutt, Dr. Thomas Gardner
- *Artificial pancreas*: Dr. Irl Hirsch, Dr. Richard Bergenstal
- *Patient perspective*: Ellen Leake

Note – Panel members have expertise in multiple areas.

NIH National Institute of Diabetes and Digestive and Kidney Diseases

High Enthusiasm for Continuation

- TEDDY, TrialNet, ITN
- SEARCH
- Diabetic Retinopathy Clinical Research, Diacomp
- Artificial Pancreas and Behavioral Research
- Living Biobank and Biosamples
- K12, T1D Pathfinder
- Islet distribution, CITR, assay standardization

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CC
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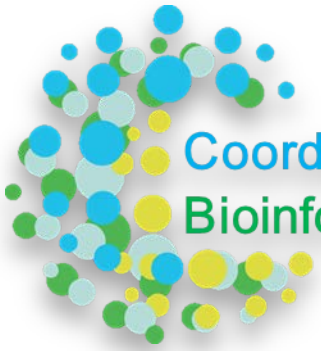
HIRN
HUMAN ISLET RESEARCH NETWORK

A Network Of Consortia

Consortium on Targeting And Regeneration (CTAR)

Consortium on Beta Cell Death and Survival (CBDS)



Coordinating Center
Bioinformatics Center

Consortium on Modeling Autoimmune Interactions (CMAI)

Consortium on Human Islet Biomimetics (CHIB)

Recent HIRN Related Initiatives

- **2015 HIRN-CBDS Initiative:** Single Cell-Resolution Technologies For Use On Human Pancreatic Tissues. (3 funded $11M)

- **2016 Initiative:** Human Pancreas Procurement and Analysis Program (HPPAP). ($15M)

- **2017 Initiative**: Small Business Innovation Research to Develop Cell Replacement for T1D (RFA-DK-16-004 $2.5M)

National Institute of Diabetes and Digestive and Kidney Diseases

Research Toward an Artificial Pancreas

- **Clinical Acceptance of the Artificial Pancreas: The International Diabetes Closed Loop Trial**
  - University of Virginia; PI: Boris Kovatchev
  - Cost: $12.7M
- **One Year Day-and-Night Home Closed Loop in Young People with T1D**
  - University of Cambridge; PI: Roman Hovorka
  - Cost: $6.4M
- **Home Use of MD-Logic Automated Insulin Delivery System**
  - HealthPartners Institute; PI: Richard Bergenstal
  - Cost: $2M
- **Final Clinical Studies for Submission of a Pre-market Approval Application to the FDA for a Bionic Pancreas that Automates T1D Management**
  - Boston University; PI: Ed Damiano
  - Cost: $1.5M
- **Comparison of Dual-hormone AP, Single-hormone AP, and Sensor-Augmented Pump Therapy in Outpatient Settings**
  - Clinical Research Institute of Montreal, Remi Rabasa-Lhorat
  - Cost: $2.5M

FY16 Funding Opportunity Announcements Artificial Pancreas

- **Advanced Clinical Trials to Test Artificial Pancreas Device Systems in Type 1 Diabetes** (RFA-DK-16-008)- **$20 million**

- **Clinical, Behavioral and Physiological Research Testing Current and Novel Closed Loop Systems** (RFA-DK-16-009)- **$5 million**

- **Small Business Innovation Research to Develop New or Improved Closed Loop Automated Technologies for Diabetes Therapy and Monitoring** (RFA-DK-15-022)- **$2 million**

National Institute of Diabetes and Digestive and Kidney Diseases

Funding Opportunity Announcements Improving Management of Type 1 Diabetes

- **Impact of the Use of Glucose Monitoring and Control Technologies on Health Outcomes and Quality of Life in Older Adults with Type 1 Diabetes** (RFA-DK-15-028)- **$5 million**
- **Improving Diabetes Management in Children with Type 1 Diabetes** (RFA-DK-16-003)- **$6 million- 6/22/2016**
- **Improving Diabetes Management in Pre-teens, Adolescents and/or Young Adults with Type 1 Diabetes** (RFA-DK-16-001)- **$6 million- 6/22/2016**
- **Understanding Barriers and Facilitators to Type 1 Diabetes Management in Adults** (RFA-DK-16-002)-
- **$5 million- 6/22/2016**

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2016 Funding Opportunity Announcements Diagnosis and Risk Assessment

- **Study to Assess the Incidence of Type 1 Diabetes in Young Adults** (RFA-DP-16-005)-CDC-**($2 million)**

- **Small Business Innovation Research to Develop New Methods and Technologies for Assessment of Risk and for Early Diagnosis and Prognosis of Type 1 Diabetes** (RFA-DK-15-024)-**($2 million)**

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Funding Opportunity Announcements Diabetes Complications

- **Research Using Biosamples and Subjects from Selected Type 1 Diabetes Clinical Studies—Complications (RFA-DK-15-019)-5 million**
- **Neurocognitive Effects of Glycemic Dysregulation in Type 1 Diabetes Planned for 2017**
- **Diabetes Complications Consortium**
  - Pilot and Feasibility Project Support
  - Applications due June 10, 2016

DiaComp

National Institute of Diabetes and Digestive and Kidney Diseases

2016 Funding Opportunity Announcements Pathogenesis of Type 1 Diabetes

- **Understanding the Pathogenesis and Etiology of Type 1 Diabetes Using Biosamples and Subjects from Clinical Studies (RFA-DK-15-018)- $4 million**

- **Mechanisms Underlying the Contribution of Type 1 Diabetes Risk-Associated Variants (RFA-DK-15-025)-$15 million**

National Institute of Diabetes and Digestive and Kidney Diseases

2016 Funding Opportunity Announcements Fostering the Next Generation of Researchers

- **Career Development Programs in Diabetes Research for Pediatric Endocrinologists** (RFA-DK-15-006)-**$2.5 million**

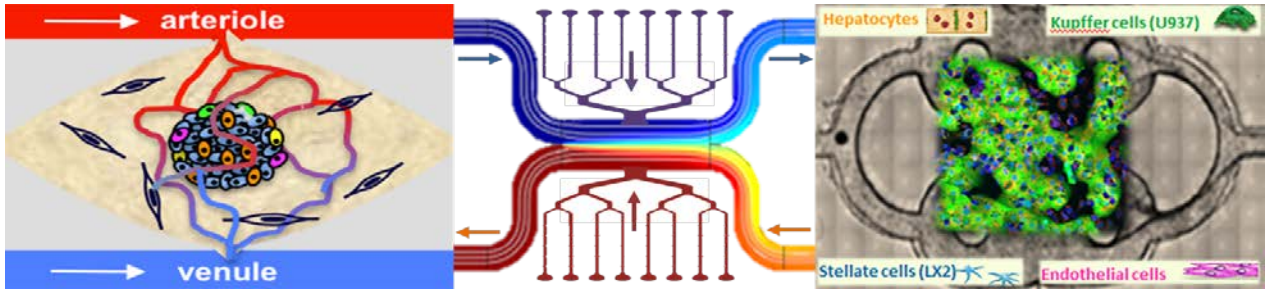
- **Type 1 Diabetes Pathfinder Award** (RFA-DK-15-030)-**$10 million**

National Institute of Diabetes and Digestive and Kidney Diseases

2017 Initiatives Concepts

National Institute of
Diabetes and Digestive
and Kidney Diseases

Engineering a Human Islet to Liver Axis

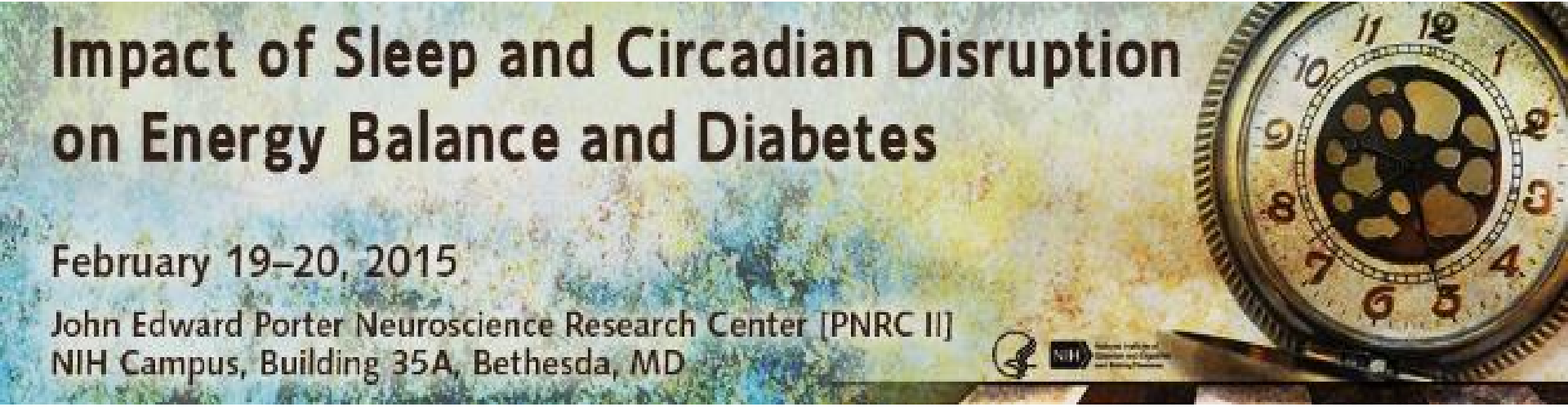


Take advantage of recent developments in the fields of human tissue chips and microfluidics to **build integrated in-vitro platforms** for disease modeling and drug discovery/drug testing;

Phase 1: Use microfluidics technology to develop a bimodal system relevant to diabetes using two advanced platforms: achieve functional integration of the existing islet and liver chips

Phase 2: Integrate other metabolically-relevant organ systems (muscle, fat depots, gut, etc.) to model normal and diseased human physiology.

DEPARTMENT OF HEALTH & HUMAN SERVICES · USA
NIH National Institute of Diabetes and Digestive and Kidney Diseases



- Invited basic and clinical investigators in diabetes, obesity, sleep dysregulation, and circadian biology
- 60 attendees including 10 junior investigators (supported by Sleep Research Society)
- Review current knowledge on the mechanisms mediating the effects of the circadian clock and sleep disruption on metabolism
- Discuss types of studies required to determine if modulation of sleep/circadian rhythms and timing of feeding can be used to attenuate and/or treat obesity and T2DM.

Mechanisms Mediating Effects of Sleep Disruption on Metabolism

Stimulate:

- In depth metabolic phenotyping approaches
- Innovation with regards to the population studied and/or perturbations investigated
- Multidisciplinary teams combining basic and clinical scientists with expertise in metabolism and sleep/circadian biology.

National Institute of Diabetes and Digestive and Kidney Diseases

Consortium for the Clinical Study of Diabetic Foot Ulcers

- Goal - Develop and test new outcome measures and wound and risk factor characterization through the implementation of standard protocols at clinical sites and the creation of a large, accessible database on the presentation, treatment and outcomes of diabetic foot ulcers.

National Institute of Diabetes and Digestive and Kidney Diseases

Neurocognition and Diabetes

- **The Opportunity**: BRAIN and National Plan to Address Alzheimer's Disease will provide financial support, tools, and research communities poised to make major advances within next 12 years

- **The Challenge:** Determine the mechanism(s) that explain the interactions between metabolic disease, brain biology, and cognitive dysfunction

- **2015 Strategic Planning**
  - July 2015 NIDDK workshop: "The Intersection of Metabolic and Neurocognitive Dysfunction"
  - September 2015 NIDDK webinar: "Neurocognition and diabetes"

National Institute of Diabetes and Digestive and Kidney Diseases

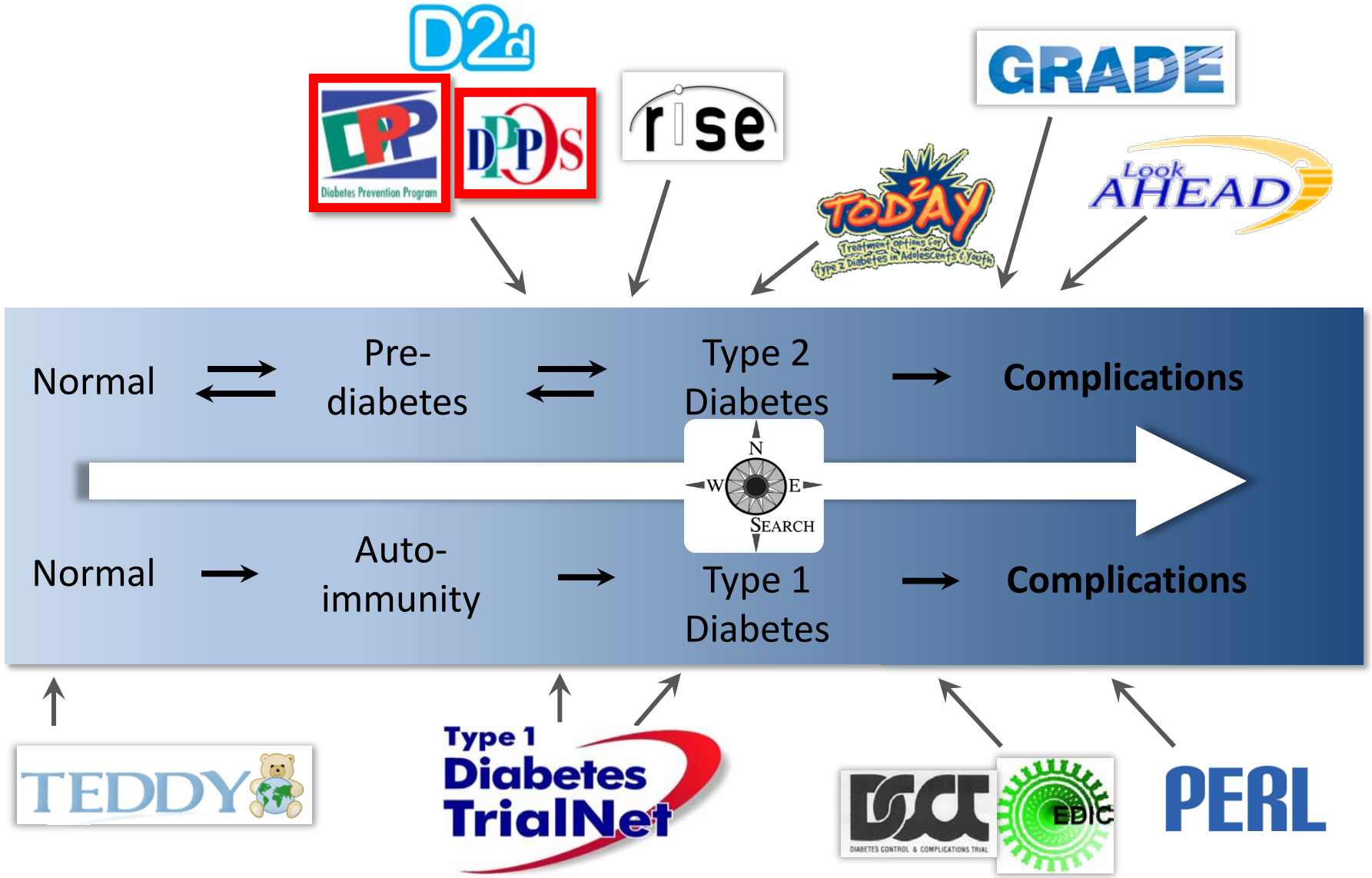
Neurocognition and Diabetes

- **Collaboration:**
  - Basic and clinical
  - Neuroscience and diabetes expertise

- **Ancillary studies:**
  - Addition of deep metabolic and clinical phenotyping to existing neurocognitive studies
  - Addition of cohorts with metabolic dysfunction/disease to existing neurocognitive studies
  - Addition of comprehensive neurocognitive measures to existing studies of cohorts with well-characterized metabolic function and course of disease

National Institute of Diabetes and Digestive and Kidney Diseases

NIDDK Clinical Studies: Diabetes



TEDDY Nested Case-Control Analysis

419 Cases of persistent confirmed islet antibodies
114 Cases of T1D

1:3 Matching cases : controls

- **Dietary biomarkers (n=23,594)**
  - Vit. C (n=4,048)
  - Vit. D (n=6,583)
  - Tocopherol, carotenoids (3,736)
  - RBC fatty acids (5,527)
- **Metabolomics (n=12,959)**

(number of samples to be tested)

1:1 Matching cases : controls

- **Gene Expression (n=5,200)**
- **Microbiome (n=13,403)**
- **Viral metagenomics (n=6,380)**
- **Proteomics (n~5,500)**

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Type 1 Diabetes TrialNet

Prevention Trials

- Screened **163,946** relatives of people with T1D
- **Three** ongoing trials

Prevention Trial	Status
Oral Insulin	Enrollment: complete Results: 2017
Anti-CD3 (Teplizumab)	Enrollment: 2016 Results: 2018
CTLA4-Ig (Abatacept)	Enrollment: 2017 Results: 2019

New-Onset Trials

- Completed **6** trials; partnered on **4** other trials with ITN
- **Two** new trials currently active

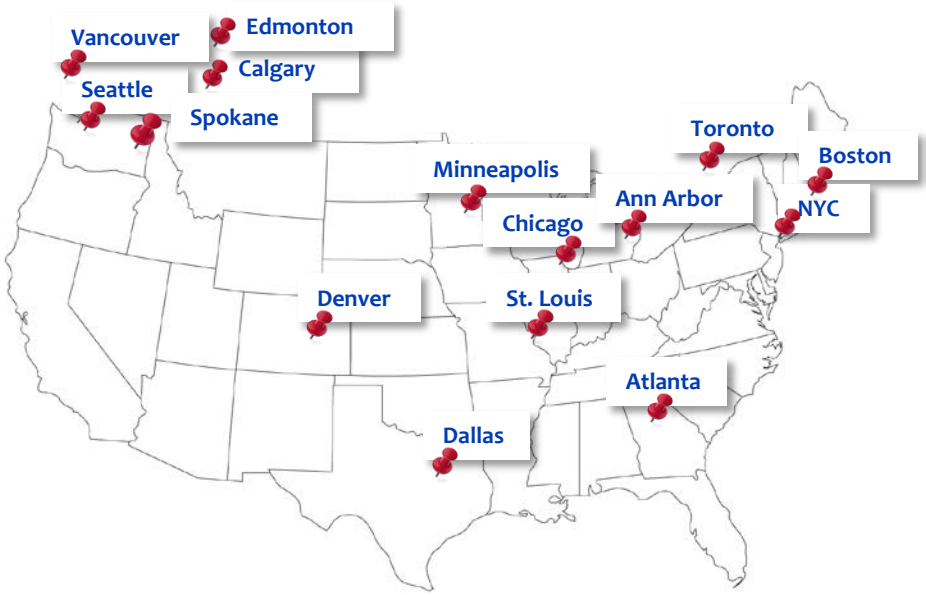
New-Onset Trial	Status
Antithymocyte globulin (ATG)/Granulocyte-colony stimulating factor (GCSF)	Enrollment: 2016 Results: 2017
Tocilizumab	Enrollment: 2016 Results: 2018

National Institute of Diabetes and Digestive and Kidney Diseases

PERL

PREVENTING EARLY RENAL LOSS IN DIABETES

- No new therapy for diabetic kidney disease since 1993
- Increased uric acid levels associated with loss of kidney function in T1D
- PERL is testing allopurinol in early renal loss, a stage most likely to be responsive to therapy
- Allopurinol is a safe, effective, inexpensive oral medication for lowering uric acid
- Timeline:
  - Enrollment complete: February 2016
  - Randomization: April 2016
  - End of intervention: April 2019
  - Data analysis: August 2019



National Institute of Diabetes and Digestive and Kidney Diseases

Facilitating Pragmatic Clinical Research

NIH Collaboratory
Distributed Research Network

- Infrastructure to facilitate multicenter studies using electronic clinical, administrative, and research data

- Facilitates multisite distributed querying of data resources, while allowing the data to remain in the control of the data owners

- Serves as a repository of tools to leverage electronic health records to support clinical research across multiple health systems

National Institute of Diabetes and Digestive and Kidney Diseases

New Policies on Ancillary Studies to Major Clinical Studies (PAR-16-034)

- **Goal: leveraging ongoing large, multi-center clinical research studies through ancillary studies**
  - **Includes clinical trials and prospective observational studies**
  - **Parent study need not be supported by NIDDK but ancillary must be in NIDDK mission**
- **Must propose to collect new information and/or biological samples directly from participants of the ongoing parent study**
- **Must address new research questions that are beyond those specified in the approved protocol of the parent study and are within the scientific mission of the NIDDK**
- **Cannot be used to extend the duration of the parent study.**

Major T2D Clinical Research Needs Webinar Themes

- Management of diabetes in the elderly
- Management of glycemia in hospitalized patients
- CVD prevention
- Treatment of GDM
- Improving diabetes care in primary care setting
- Defining heterogeneity for individualized care

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and Kidney Diseases

DIABETES RESEARCH CENTERS

Diabetes Centers EXECUTIVE COMMITTEE

2014-2015 Roster

Martin Myers, University of Michigan (Chair)

Tim Garvey, University of Alabama at Birmingham
George King, Joslin Diabetes Center
Jerry Olefsky, University of California, San Diego
Jeff Pessin, Albert Einstein College of Medicine

Jerry Palmer, University of Washington
NIDDK Advisory Council liaison

DIABETES RESEARCH CENTERS

The Medical Student Research Program in Diabetes is sponsored by the National Institutes of Health through the NIDDK and allows medical students to conduct research under the direction of an established scientist in the areas of diabetes, hormone action, physiology, islet cell biology or obesity at an institution with one of the NIDDK-funded Research Centers during the summer between the first and second year or second and third year of medical school.

The goal of the Program is to encourage medical students to consider research in diabetes and its complications as a career and to educate students about diabetes. Program Consultants assist students in selecting an appropriate research project and preceptor. **Prior research experience is not required**. In addition to working on his/her own research project, each student views web-cast seminars addressing clinical and research aspects of diabetes mellitus and its complications.

Students spend 2-3 months working on their research project at a Diabetes Center and receive a weekly stipend. Commencement dates and conclusion for the program are reasonably flexible; however, all students will present their results at a research symposium in Nashville, TN (travel funds provided).

You must be a U.S. citizen or a permanent resident to participate in this program.

Questions regarding the Program should be directed to:

NIDDK Medical Student Research Program in Diabetes
E-mail: niddk.diabetes.student.research@vanderbilt.edu

The NIDDK Medical Student Research Program in Diabetes is funded by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)

000000Application and Program Statistics
NIDDK Medical Student Symmer Research Program
Summers 2014 and 2015

NIDDK Program Applications:

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

	2014 Program			2015 Program		
Center	# participants	# participants from medical schools associated with a DERC/DRTC	# applicants listing Center as #1 or 2 choice	# participants	# participants from medical schools associated with a DERC/DRTC	# applicants listing Center as #1 or 2 choice
BADERC	7*	0	79	7*	0	79
Columbia	7*	1	148	7*	0	185
JHopkins/Univ MD	8*	0	95	8*	2	121
Joslin	9*	0	86	10*/***	0	102
UAB	6	4	15	7***	1	19
UCLA/UCSD	5*	0	153	7*	2	177
UCSF	4	1	94	4	1	115
Univ Chicago	4	0	102	5*	0	89
Univ Michigan	9*	3	44	9*	0	42
Univ Pennsylvania	4	2	86	4	2	103
Univ Washington	7*	1	48	8*	0	71
Vanderbilt - NIDDK	6	0	81	6	0	120
Washington Univ	6*	1	27	6*	0	46
Yale	5*	1	44	5*	0	56
Sub-total	87	14		93	9	
Vanderbilt - T35 grant	36			37***		
Univ Colorado				6*		
SPORT	3**			1**		
TOTAL	126			137		

*indicates students added with support from the Diabetic Complications Consortium (DCC)
** University of Maryland **S**ummer **P**rogram in **O**besity, Diabetes and Nutrition Related **R**esearch **T**raining (SPORT)
***Extra student with outside funding

Student Participant Demographics 2015^		**US Medical School Enrollment 2014-15^**		**US Census 2010^**	
Race		Race		Race	
African American	9 (7%)	African American	5263 (6%)	African American	38.9 million (13%)
American Indian	1 (<1%)	American Indian	181 (< 1%)	American Indian	2.9 million (0.9%)
Asian	49 (36%)	Asian	17330 (20%)	Asian	14.7 million (5%)
Caucasian	55 (40%)	Caucasian	47205 (55%)	Caucasian	173.1 million (56%)
Hispanic	10 (7%)	Hispanic	3944 (4%)	Hispanic	50.5 million (16%)
Native Hawaiian or Pacific Islander	0	Native Hawaiian or Pacific Islander	100 (<1%)	Native Hawaiian or Pacific Islander	0.5 million (0.2%)
Other/No answer	13 (9%)	Other/No answer	11134 (13%)	Other/two races	28.1 million (9%)
Gender		Gender		Gender	
Female	70 (51%)	Female	39837 (46.8%)	Female	156,964,000 (50.8%)
Male	67 (49%)	Male	45320 (53.2%)	Male	151,781,000 (49.2%)

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

NIDDK Medical Student Research Program in Diabetes

- Eighth summer (2009-2016)
- 4-9 students/Diabetes Center
- Funding
  - Supplement to T32s at Diabetes Centers
  - Diabetic Complications Consortium

DIABETES RESEARCH CENTERS

Additional Students Supported by the Diabetic Complications Consortium (DCC)

	2013	2014	2015
# Supported Students	10	19	22
Centers Accepting Students	3	10	10*

*plus the University of Colorado

NIDDK Medical Student Research Program - Summer 2015

November

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

March-April

- **Students apply and select 2 Diabetes Research Centers**
- **Each Center reviews and lists students**
- **“Matching” of students/centers**

DIABETES RESEARCH CENTERS

Some NIDDK Program Stats (See Handout)

	2009	2010	2011	2012	2013	2014	2015
# applicants	197	431	486	395	568	551	685
# medical schools	82	104	111	114	138	137	137*
# participants	83	92	101	102	114	119^	137^
# medical* schools	40	49	49	58	65	69^	80^**

* 116 MD-granting schools, 12 DO-granting schools, 9 foreign medical schools
** 75 MD-granting schools, 2 DO-granting schools, 3 foreign medical schools
^ Includes students supported by Diabetes Complications Consortium and students in Vanderbilt T35 program

Demographics

mographics 2015		US Medical School Enrollment 2014-15			
African American	9 (7%)	African American	5263 (6%)	African American	38.9 million (13%)

DIABETES RESEARCH CENTERS

NIDDK Medical Student Research Program - Summer 2015

May - June - July

- 8-12 weeks of research
- Webcasts

August

- Research symposium
- Poster presentations (some moderated)

DIABETES RESEARCH CENTERS

Summer Program in Obesity, Diabetes and Nutrition Related Research Training - T35 at Maryland

NIDDK Medical Student Research Symposium

- Visiting Professors
  - Carmella Evans-Molina, MD, PhD (Indiana)
  - W. Timothy Garvey, MD (UAB)
  - Nanette Steinle, MD, RDN (Maryland)
- Career pathways/advice
  - Residency Program Directors

DIABETES RESEARCH CENTERS

Program Oversight

- Advisory Committee
  - Art Castle (NIDDK)
  - James Hyde (NIDDK)
  - Steven Kahn (University of Washington)
  - Louis Philipson (University of Chicago)
  - Mike Rickels (Penn)

Evaluation, Challenges, Items for Discussion

- Students and centers mostly satisfied
  - Student interactions at some Centers
- NIDDK says… (Art Castle, Jim Hyde)
- Number of students applying to some Diabetes Centers
- How to select the most meritorious students

of Applicants/Research Center

Center	# participants	# participants from medical schools associated with a DERC/DRTC				
ale	5*	1	44	5*	0	56

DIABETES RESEARCH CENTERS

Evaluation, Challenges, Items for Discussion

- Students and centers mostly satisfied
  - Student interactions at some Centers
- NIDDK says… (Art Castle, Jim Hyde)
- Number of students applying to some Diabetes Centers
- How to select the most meritorious students
- Visiting professors - #
- Follow up
  - 1/3 do more research or present research

Residency Choices of NIDDK Participants

Specialty	2010 Participants (100% response rate)	2011 Participants (76% response rate)	2012 Participants (94% response rate)	National Average (2015 data)*
Internal Medicine	26%	25%	40%	25%
Pediatrics	13%	23%	15%	12%
Anesthesiology	3%	7%	9%	4%
Dermatology	3%	2%	2%	<1%
Emergency Medicine	8%	9%	7%	7%
Family Medicine	4%	5%	2%	12%
Neurology	2%	2%	4%	2%
Ob/Gyn	6%	0%	7%	5%
Ophthalmology	7%	2%	0%	
Otolaryngology	7%	2%	2%	1%
Pathology	4%	0%	2%	2%
Peds/Psych/Child Psych	2%	0%	0%	<1%
Physical Med & Rehabilitation	0%	4%	2%	<1%
Psychiatry	2%	4%	2%	5%
Radiation Oncology	3%	2%	0%	<1%
Radiology	5%	7%	2%	<1%
Surgery (General)	5%	4%	4%	10%

NIDDK Medical Student Research Program in Diabetes

Questions, Comments, or Suggestions?

DIABETES RESEARCH CENTERS

DIABETES RESEARCH CENTERS

Stimulating Peripheral Activity to Relieve Conditions (SPARC)

https://commonfund.nih.gov/sparc/index

Current Funding Opportunity Announcements

RFA-RM-16-009: Stimulating Peripheral Activity to Relieve Conditions (SPARC): Pre-clinical Development of Existing Market-approved Devices to Support New Market Indications (U18)

RFA-RM-16-002: Pre-application: Stimulating Peripheral Activity to Relieve Conditions (SPARC): Technologies to Understand the Control of Organ Function by the Peripheral Nervous System (OT1)

RFA-RM-16-003: Limited Competition - Stimulating Peripheral Activity to Relieve Conditions (SPARC): Technologies to Understand the Control of Organ Function by the Peripheral Nervous System (OT2)

RFA-RM-15-003: Pre-application: Stimulating Peripheral Activity to Relieve Conditions (SPARC): Comprehensive Functional Mapping of Neuroanatomy and Neurobiology of Organs (OT1)

RFA-RM-15-018: Limited Competition - Stimulating Peripheral Activity to Relieve Conditions (SPARC): Comprehensive Functional Mapping of Neuroanatomy and Neurobiology of Organs (OT2)

RFA-RM- 15-019: Limited Competition - Stimulating Peripheral Activity to Relieve Conditions (SPARC): Foundational Functional Mapping of Neuroanatomy and Neurobiology of Organs (OT2)

Stimulating Peripheral Activity to Relieve Conditions (SPARC): A New Common Fund Program

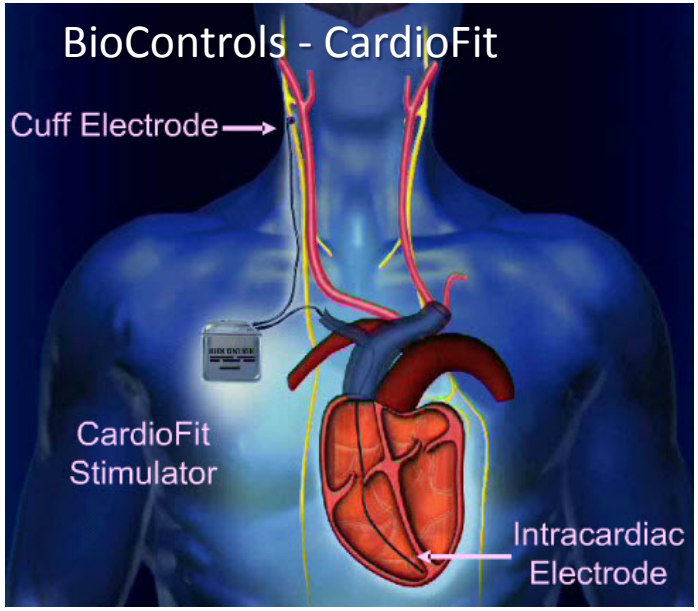
Karen Teff, Ph.D.

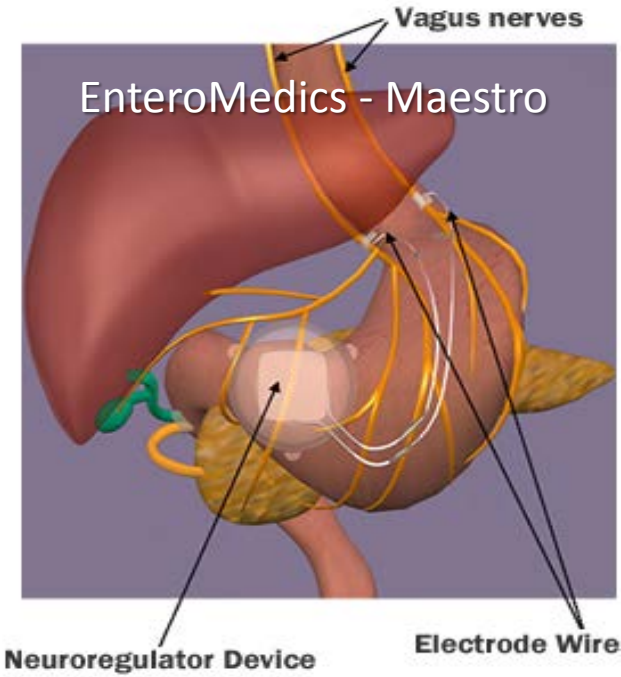
NIDDK,

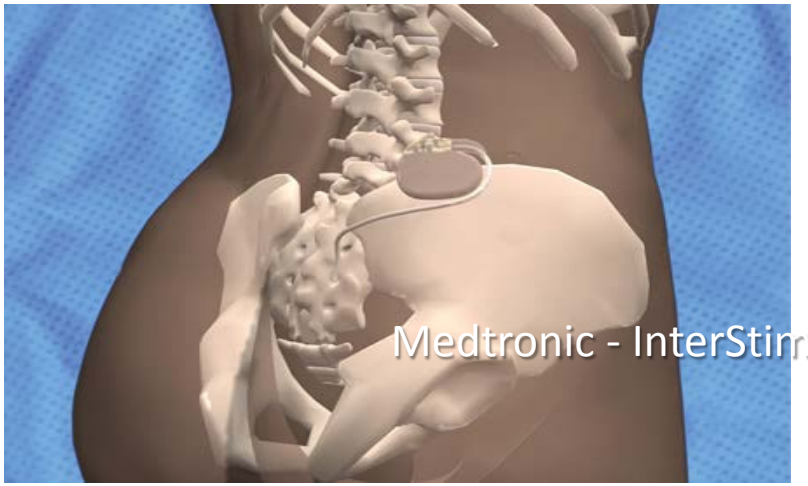
Division of Diabetes, Endocrinology and Metabolism

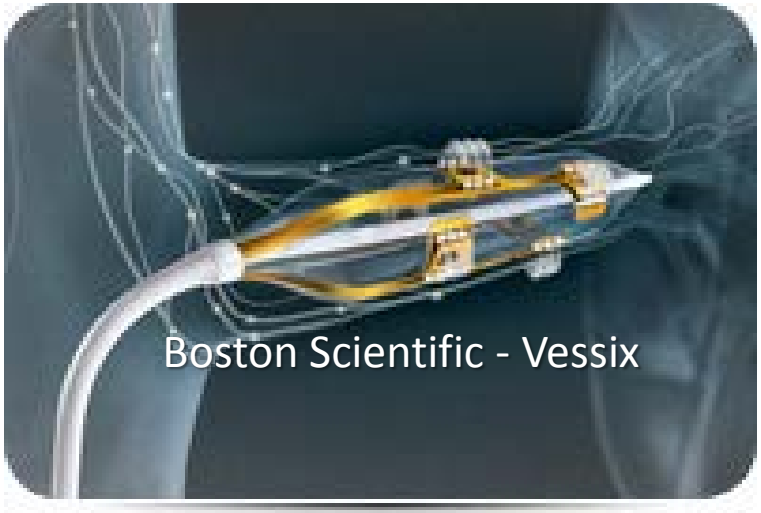
National Institutes of Health
Office of Strategic Coordination - The Common Fund

Neuromodulation: An Emerging Area of Research









Common Fund Programs

- Trans-NIH initiatives which address the missions of multiple institutes
- Projects are typically complex, unique and require a high level of coordination
- Projects must meet concrete milestones and achieve a defined set of high impact goals
- Rigorous planning process prior to commitment of funds with input from NIH, Scientific Community and Industry

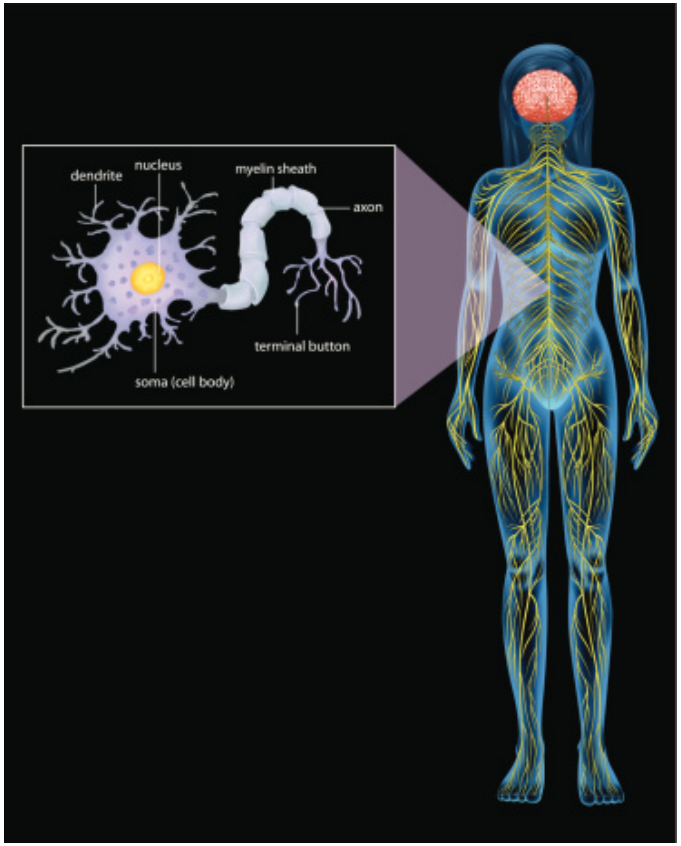
Neuromodulatory Devices: Current Limitations

Research Roadblocks

1. Knowledge gap in the area of the autonomic and sensory nervous systems on end organ function
2. Detailed neuroanatomical maps and neurophysiology are lacking for ANS innervation of end organ function
3. Lack of novel tools and technologies to understand the underlying biology
4. Need for ongoing partnerships among the NIH, FDA and Industry

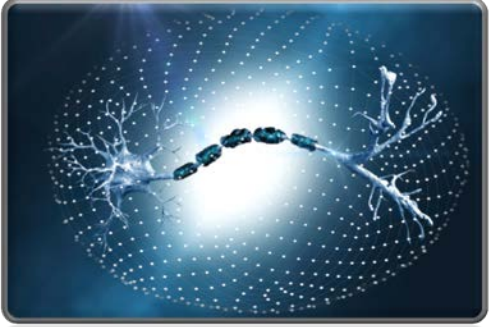
SPARC

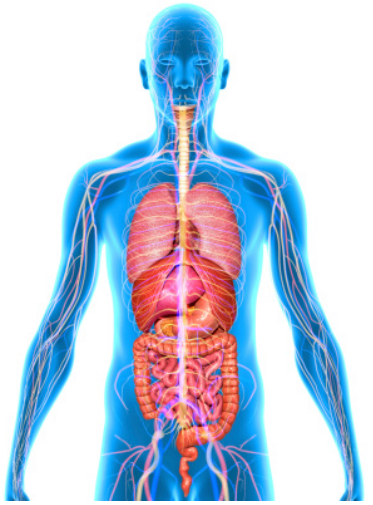
Opportunity: Neuromodulation of end-organ function holds promise in treating many diseases and conditions.



Challenge: The mechanisms of action for neuromodulation therapies remain poorly understood.

The **SPARC program** will advance neuromodulation therapies towards precise neural control of end-organ system function (~ 200 M over 7 years)





SPARC Strategic Planning Workshop
February 25-26, 2015

Experts from various disciplines, representing clinical expertise, basic biology, engineers, technology and data management discussed the neuromodulation field: overarching issues, knowledge gaps and roadblocks

SPARC – Stimulating Peripheral Activity to Relieve Conditions

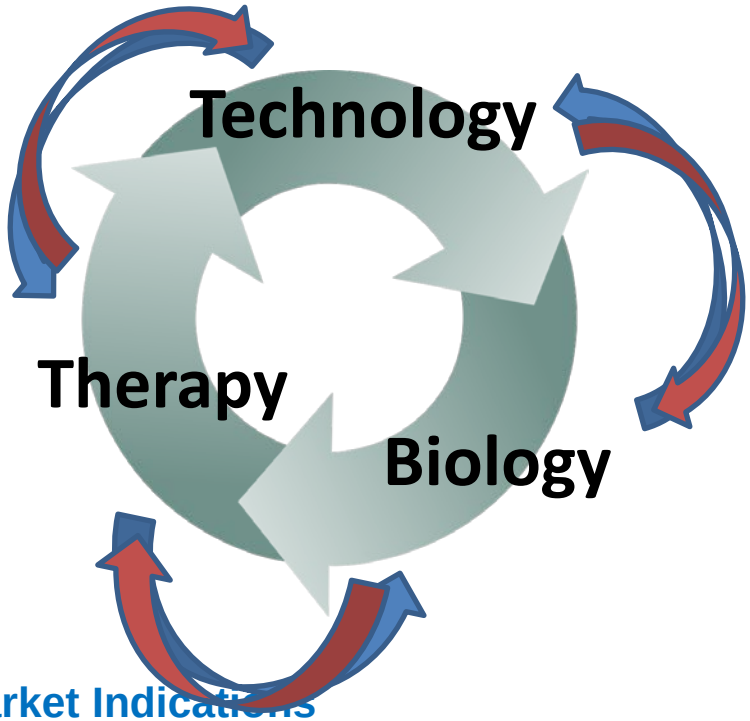
Research Components and Deliverables:

Anatomical and Functional Mapping of the Innervation of Major Internal Organs

- Anatomical and functional neural circuit maps for multiple major organs
- Novel surgical procedures, and stimulation protocols

Next Generation Tools and Technologies

- Novel and adapted technologies to define PNS control of organ function
- Next generation neuromodulation therapies



Use of Existing Market-Approved Technology for New Market Indications

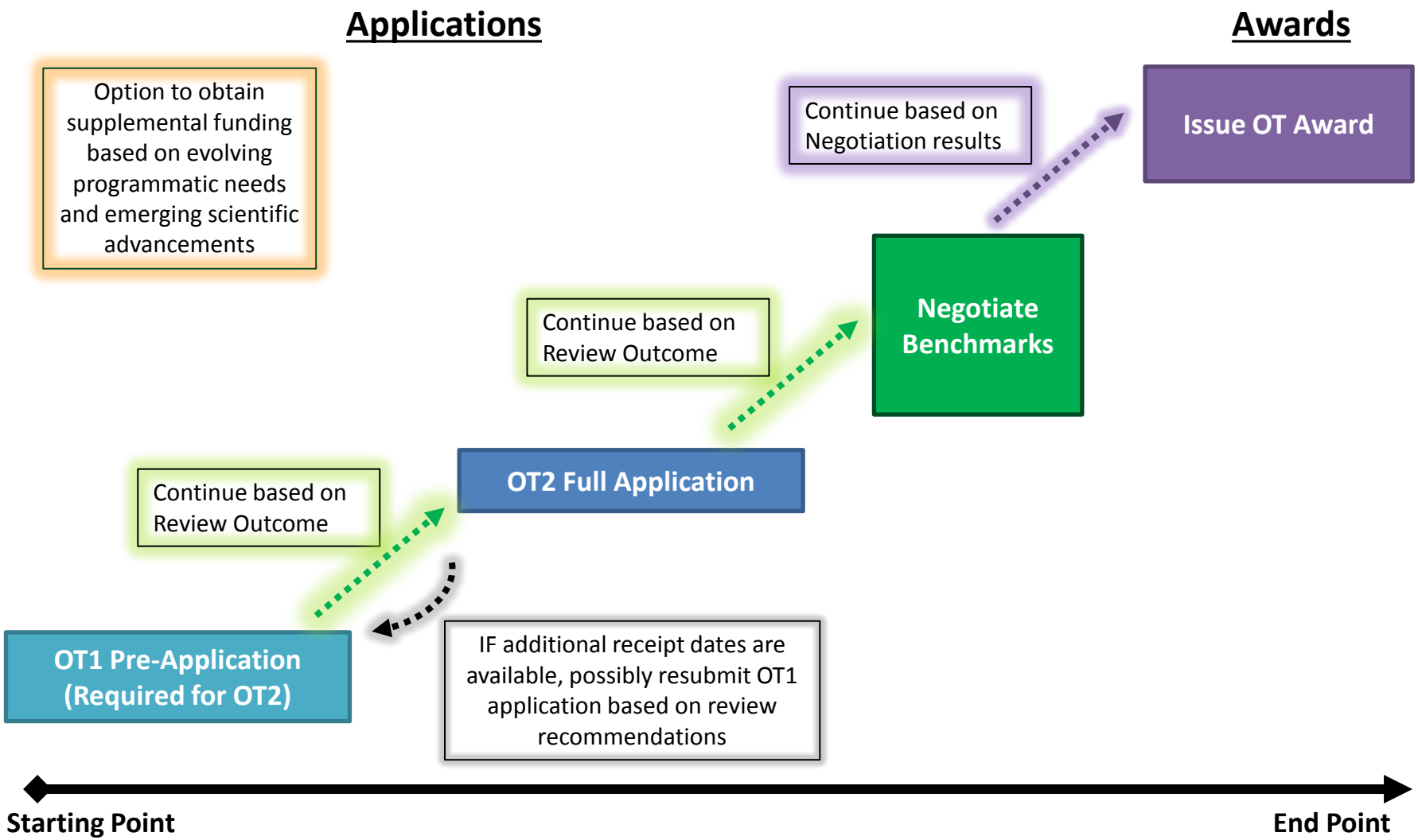
- New indications for existing, approved devices
- New therapeutic opportunities and methodologies

Data Coordination, Mapping, and Modeling Center

- Public data resource containing all SPARC data
- Integrated, Predictive, Anatomical and Functional Neural Circuit Maps

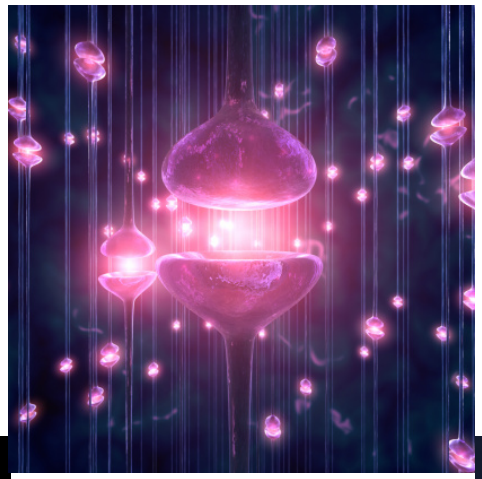
SPARC Other Transactions
A 2-Stage Process

https://commonfund.nih.gov/sparc/othertransactions



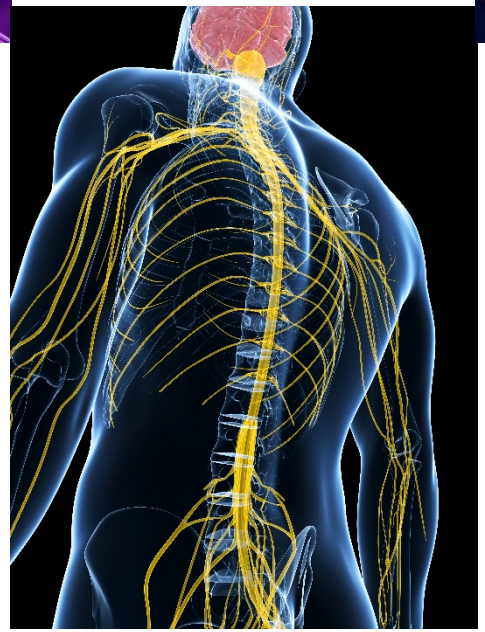
SPARC – Current Funding Opportunities

Foundational Functional Mapping of Neuroanatomy and Neurobiology of Organs (OT2)

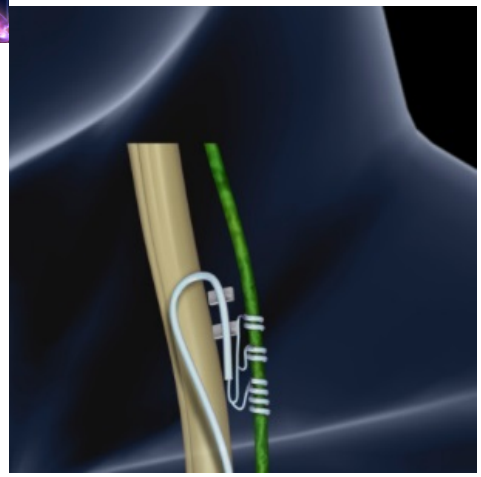


Pre-clinical Development of Existing Market-approved Devices to Support New Market Indications (U18)

Comprehensive Functional Mapping of Neuroanatomy a nd Neurobiology of Organs (OT1 and OT2)



Technologies to Understand the Control of Organ Function by the Peripheral Nervous System (OT1 and OT2)



SPARC

Comprehensive Functional Mapping of Neuroanatomy and Neurobiology of Organs (OT1 and OT2)

- Detailed, predictive functional and anatomical neural circuit map for neural control of medically relevant functions of a specific organ and its functionally-associated structures
- Large multidisciplinary team
- Identification of neuronal cell types, neurotransmitters, relationship to end-organ function
- Relevancy to humans and plans to validate in humans
- Detailed resource sharing plan
- OT1 Applications accepted on due dates every other month – Next **date is May 16, 2016.**

Contacts and Resources

- **Program Coordinator and Industry Liaison**

  Dr. Danilo Tagle: danilo.tagle@nih.gov, (301) 594-8064

- **Project Team Leaders**

  Biology – Dr. Jill Carrington: carringj@niddk.nih.gov, (301) 402-0671

  Technology – Dr. Grace Peng: penggr@mail.nih.gov, (301) 451-4778

  New Market Indications – Dr. Danilo Tagle: danilo.tagle@nih.gov, (301) 594-8064

  Data Coordination – Dr. Vinay Pai: paiv@mail.nih.gov, (301) 451-4781

- **Common Fund Program Leader**

  Dr. Mary Perry: perryma@mail.nih.gov, (301) 435-5082

- **Communications Point of Contact**

  Ms. Kristina Faulk: kfaulk@mail.nih.gov, (301) 402-9185

- **Program Website:** http://commonfund.nih.gov/sparc/index

The Accelerating Medicines Partnership Type 2 Diabetes (AMP T2D)

Diabetes Center Director's Meeting
March 30, 2016

The Accelerating Medicines Partnership in Type 2 Diabetes (AMP T2D)

The Accelerating Medicines Partnership in Type 2 Diabetes (AMP T2D) is a pre-competitive partnership among government, industry, and nonprofit organizations, whose goal is to understand T2D through identification of safe and effective novel therapeutic targets to accelerate the process of bringing new medicines to patients.

AMP T2D is part of the overall AMP Partnership, which also has projects in Alzheimer's Disease and Rheumatoid Arthritis/Lupus.

Collaborators of AMP T2D

The Accelerating Medicines Partnership Type 2 Diabetes Project (AMP T2D), is a multi-sector, pre-competitive partnership among government, industry, and nonprofit organizations, the goal of which is to harness collective capabilities, scale and resources toward improving current efforts to develop new therapies for complex, heterogeneous diseases.

Organizational Participation in AMP T2D: providing collaboration, funding and/or governance

How AMP/T2DM Can Make a Difference: Addressing the Gaps

- Problem statements
  - High attrition rate in diabetes drugs – large number of targets assessed in the past decade – few classes in late development – raises overall development costs
  - Higher "bar" in diabetes drug development – increasing timeline & cost, rising Regulatory requirements, need for more data in support of reimbursement, much more demanding required drug benefit profile
- Supporting / accelerating drug development in diabetes
  - Identifying promising targets *with needed profile of late 2020's diabetes drugs*
  - Providing human genetic validation of targets
  - Providing read on potential safety signals (CV, lipids, BP, general) to avoid clinical failures (including late stage)
  - Improve understanding of disease heterogeneity – responder population

AMP T2D Program Overview

AMP T2D, a 5-year, $40M + program:

Focused effort to identify human relevant targets

- **Problem Statement:** Many T2D therapies exist on the market, though a major unmet medical need remains: no therapies achieve long-term reversal of the progression of hyperglycemia or to prevent complications
- **Goal:** Enable access to broad harmonized genotype and phenotype data for T2D and its complications to support target identification
- **Strategy**: Aggregate human genetic data from 200K+ individuals on risk /protection for T2D & its complications with phenotypic data to identify novel drug targets and create knowledge portal with tools to allow easy, integrated interrogation across multiple datasets while maintaining individual level data privacy

Knowledge Portal will enable:

- Aggregation and harmonization of siloed data and creation of a user-friendly interface to enable querying of information
- Wealth of phenotypic data & scalability to add newly generated data
- Collection and generation of genotype/phenotype T2D & diabetic complications data
- Informatics approach to identify predictors of protection/risk for drug targets

Genetic association data are key for identifying new T2D drug targets

Proof of concept: genetic association studies identify known targets

- *PPARG* is the target for thiazolidinediones
  - P12A odds ratio ~1.20
  - HbA_{1C} reduction ~0.5%
- *KCNJ11* is the target for sulfonylureas
  - E23K odds ratio ~1.15
  - HbA_{1C} reduction ~1-1.5%
- *HMGCR* is the target for statins
  - SNP rs12654264 changes LDL by ~3 mg/dl
  - Statins lower LDL by 30-50% and save lives

More than 100 T2D-associated loci now known

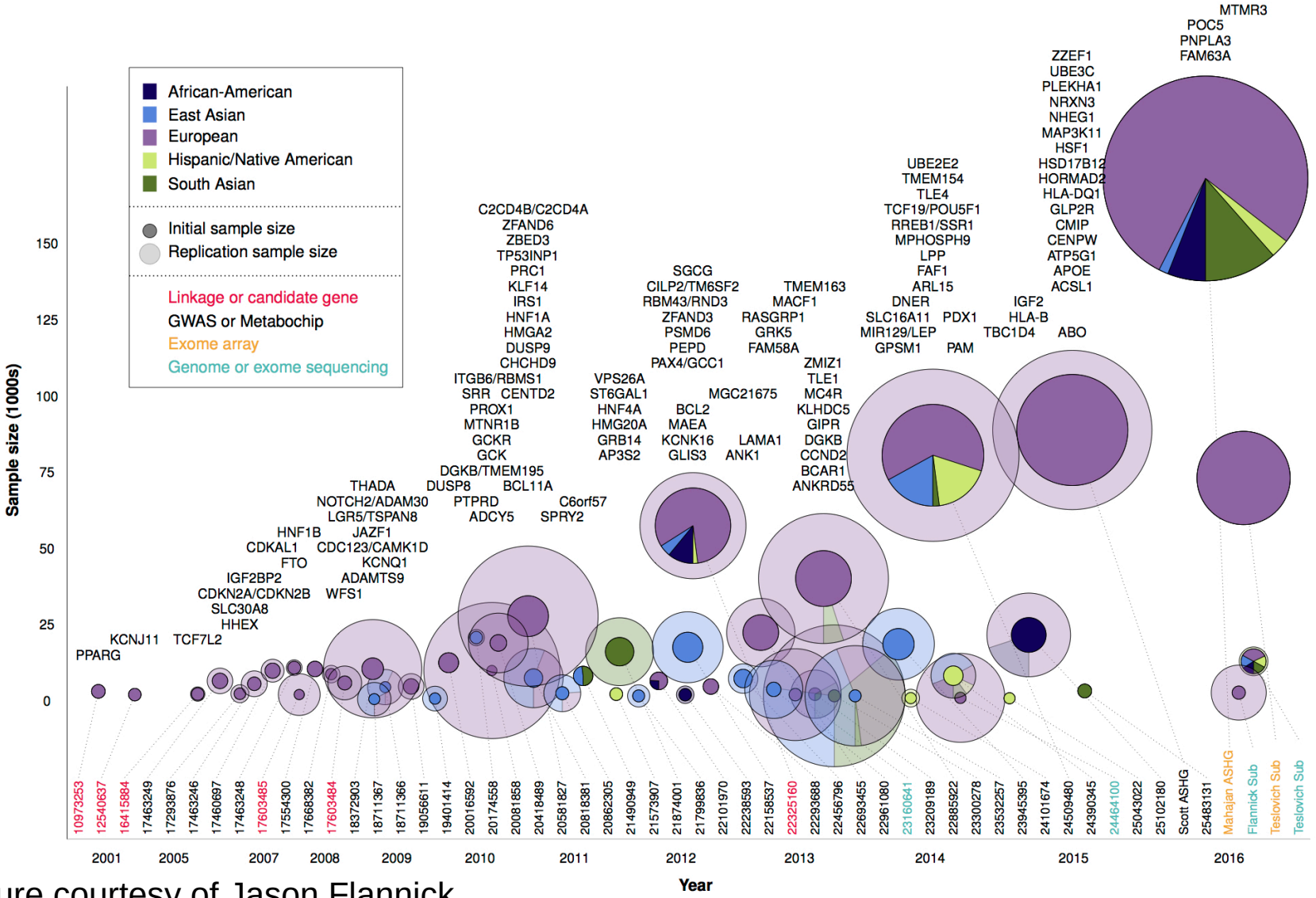


Figure courtesy of Jason Flannick

Genetic association data are not being mined to their full potential

- few investigators other than statistical geneticists in established consortia are leveraging human genetic findings
- no existing resource provides a knowledge base that can be queried, as opposed to a file cabinet of data
- data must be aggregated in order to promote discovery: nearly all known T2D loci have been identified by collaboration between groups and meta-analysis of multiple studies

Integrated and accessible genetic data could benefit:

The Scientific Community and Public

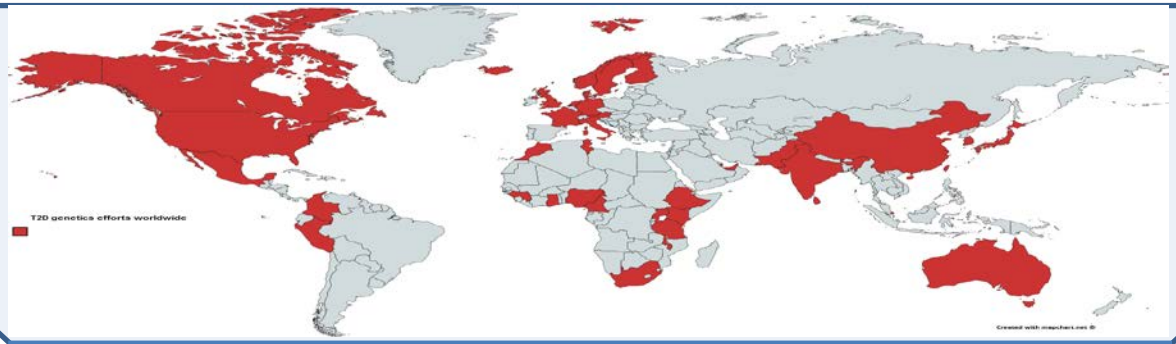
- the basic researcher testing a hypothetical association of a gene with T2D or related traits
- the biologist who seeks to translate data on T2D-associated loci into mechanistic insights about their effect on T2D
- the statistical geneticist who needs access to all available genetic association data in order to power meta-analyses
- the clinician who wishes to know the phenotypic effect of a sequence variant observed in a patient

The Pharmaceutical Industry

- the pharmaceutical researcher validating and developing novel targets for T2D and its complications

AMP T2D supports the T2D Knowledge Portal: a web resource that…

- **collects and organizes** comprehensive, harmonized human genetic and clinical data relevant to T2D and its complications
- **provides tools and software** for data intake, storage, analysis, and interpretation
- democratizes the study of T2D mechanism and targets by **disseminating genetic information** to researchers world-wide via a user-friendly website and community outreach
- provides access to summaries of pre-computed analyses and enables custom analyses, while protecting individual-level data



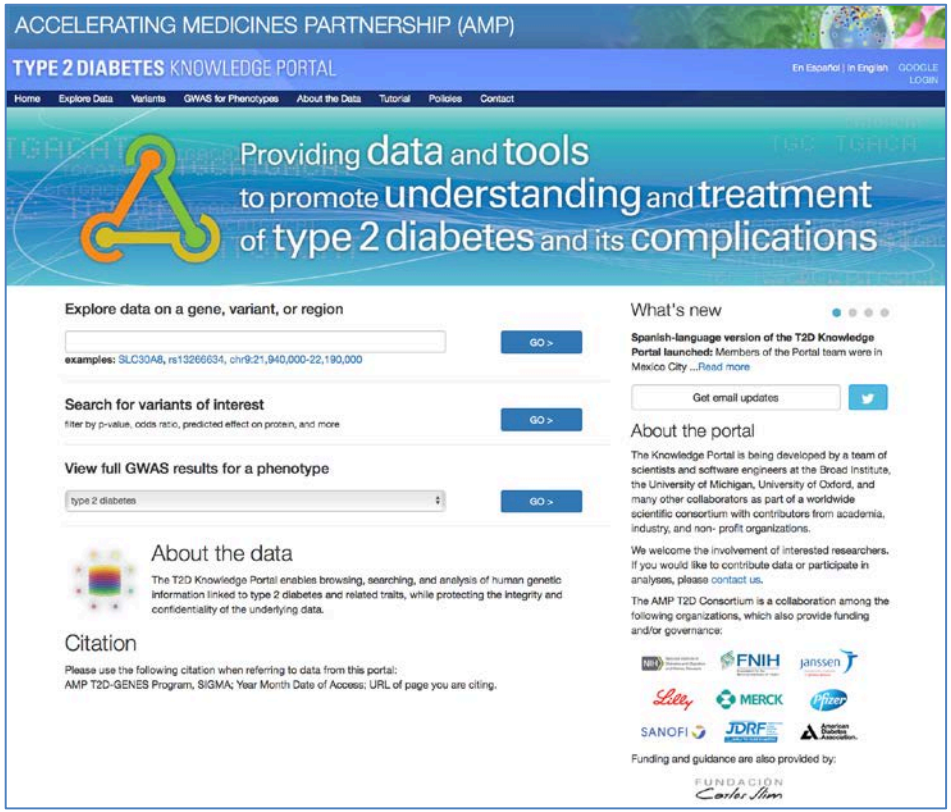
GWA Studies:
100,000/1,100,000
Exome variant genotyping projects:
80,000/820,000
Exome sequencing studies:
35,000/45,000
And more!

4K Childhood T2D
FNIH
RFP 4
DIAGRAM
Pfizer
1000G
25K T2D Exomes
MAGIC
T2D
COHORTS FOR HEART AND AGING RESEARCH IN GENOMIC EPIDEMIOLOGY
NIH
FNIH

AMP T2D Expansion
Additional Consortia
and
Expanded data sets

5 NIH sites
*4 FNIH funded sites
2 sites for DCC

The T2D Knowledge Portal today



- explore by gene or by variant
- compare strength of associations between variants and 25 phenotypes
- filter variants by location, significance, phenotype, and more

type2diabetesgenetics.org

The T2D Knowledge Portal project aims to engage the world-wide research community, academia, non-profits, and pharma

- Provide incentives for data providers and support workflows for data contribution
- Establish clear and consistent policies for contribution and use of data
- Manage collaboration among external groups that contribute data and methods; enable interaction across borders via federation
- Develop educational material and outreach to broaden the user base

Concluding comments

- the AMP T2D project and T2D Knowledge Portal will help advance T2D genetics and support discovery and translation
- development of a T2D portal will pave the way for portals for other diseases
- we welcome submission of additional data and community input on development and priorities

help@type2diabetesgenetics.org

AMP T2D Participants

NIH and FNIH funded investigators

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Andrew Hattersley
Frederick Karpe
Markku Laakso
Donna Lehman
Ruth Loos
Dan MacArthur
Mark McCarthy
Karen Mohlke
Ben Neale
Maggie Ng
Bing Ren
Maike Sander
Farook Thameem

AMP Type 2 Diabetes Steering Committee

Co-chairs
Peter Stein, Merck
Phil Smith, NIDDK
EC Liaison
Griffin Rodgers, NIDDK
Members
Hartmut Ruetten, Sanofi
Clarence Wang, Sanofi
Jim Lenhard, Janssen
Tony Parrado, Janssen
Keith Demarest, Janssen
Melissa Thomas, Lilly
Tao Wei, Lilly
Julia Brosnan, Pfizer
Eric Fauman, Pfizer
Jeffery Pfefferkorn, Pfizer
Martin Brenner, Merck
Dermot Reilly, Merck
Dan Rader, U Penn
Ellen Gadbois, NIH OD
Beena Alkokar, NIDDK
Olivier Blondel, NIDDK

Foundation for the National Institutes of Health

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Sanya Fanous Whitaker, FNIH
Nicole Spear, FNIH
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Michael Sanders
David Siedzek
Marcin von Grotthuss
Kaan Yuksel

A special thanks to all of the individuals whose participation in scientific studies makes discovery possible

The T2D-GENES, GoT2D, SIGMA, and DIAGRAM consortia contributed data to establish the AMP T2D Portal

TYPE 1 DIABETES (T1D) PROGRAM

NIDDK DIABETES CENTERS DIRECTORS MEETING

MARCH 30, 2016

THE LEONA M. AND HARRY B.
HELMSLEY
CHARITABLE TRUST

OUR MISSION

To improve lives by supporting exceptional nonprofits and other mission-aligned organizations in the U.S. and around the world in *health*, selected *place-based initiatives*, and *education* and *human services*.

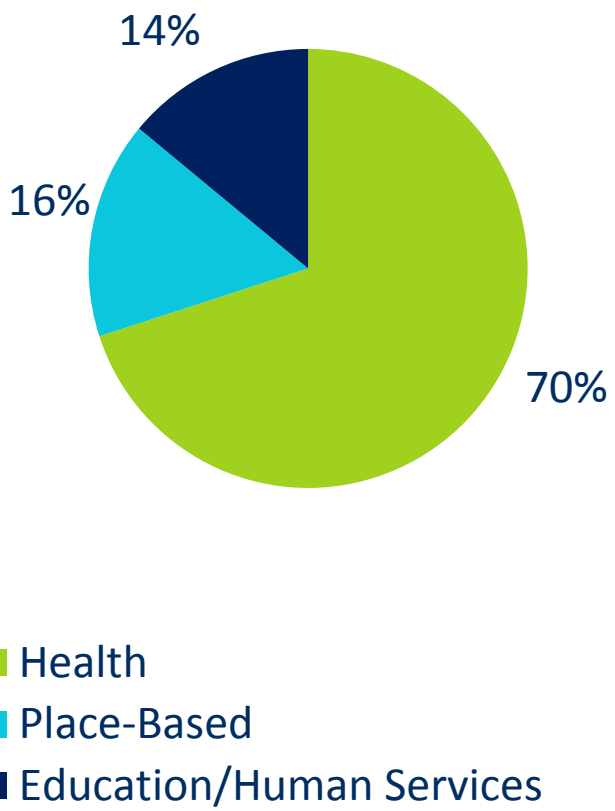
THE HELMSLEY CHARITABLE TRUST (HCT)

The Programs of the Trust

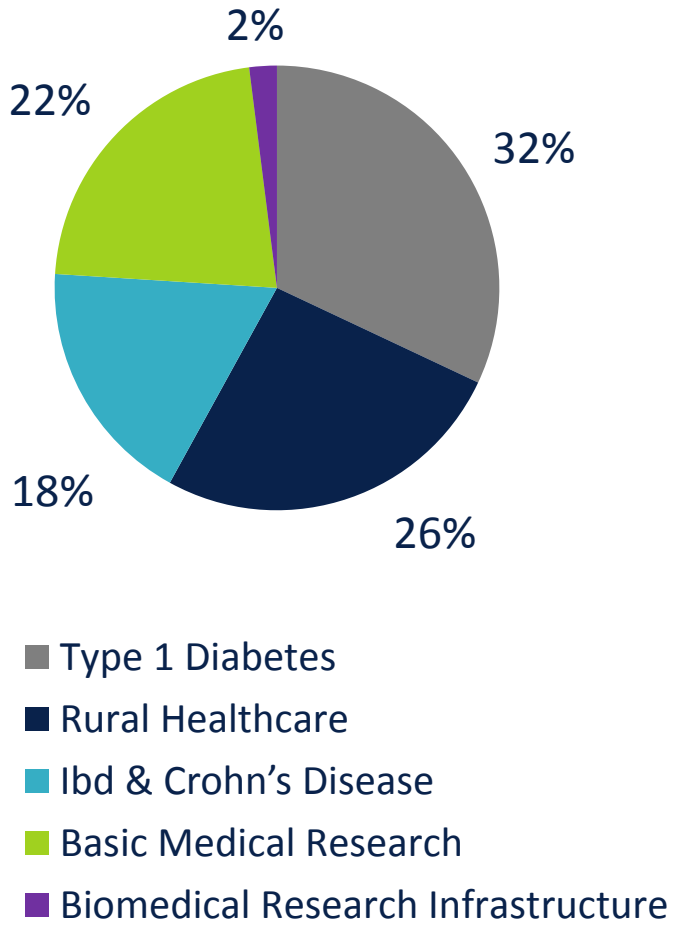
Health	Place-Based	Education & Human Service
• Basic Medical Research • Biomedical Research Infrastructure • IBD & Crohn's Disease • Rural Healthcare • Type 1 Diabetes	• Conservation • Israel • New York City	• U.S. K-12 & Higher Education • Vulnerable Children in Sub-Saharan Africa

GIVING BY FOCUS AREA

TOTAL FUNDING



TOTAL FUNDING IN HEALTH



T1D PROGRAM GOAL AND PHILOSOPHY

GOAL

To improve the lives of all people living with T1D

OUR PHILOSOPHY IS BASED ON THE FOLLOWING PRINCIPLES:

- We support projects that are high risk, high reward and have a long term vision
- We develop systems/tools to solve challenges and bridge gaps
- We want to create and support an environment of collaboration and sharing of data and knowledge

T1D PROGRAM

Information about the Program

- **The T1D Program accepts primarily proposals by invitation. Few RFAs to support novel ideas**
- **We have distributed $275 million over 7 years on more than 250 grants**
- **Our strategy is based on the following criteria:**
  - Impact on the life of people with type 1 diabetes
  - Novelty
  - Feasibility
  - Funding gap
- **Assess grants based on:**
  - Strategy
  - Reviewers/Advisors
  - Team Expertise

T1D PROGRAM PRIORITIES

The goal of the T1D Program is built on two pillars:

Improve Patient Outcomes

- Improve Glucose Control
- Improve T1D Clinical Care Models, Tools & Measures
- Improve Access to Care for All People with T1D
- Healthcare Professional's Training
- Community Education & Support

Drive Prevention & Treatments

- Build a Pipeline of Therapies
- Develop Tools & Resources for Impactful Research
- Build Systems to Prevent and Delay T1D

IMPROVE GLUCOSE CONTROL

The goal of this initiative is to improve outcomes while easing the burden of living with type 1 diabetes

PRIORITIES

- Begin to Automate Insulin Delivery
- Improve Sensing
- Adoption of Technology
- Alternative Insulin/Glucagon Delivery
- Decision Support

IMPROVE GLUCOSE CONTROL

The goal of this initiative is to improve clinical outcomes while easing the burden of living with type 1 diabetes

PRIORITIES

- Begin to Automate Insulin Delivery
- Improve Sensing
- Adoption of Technology
- Alternative Insulin/Glucagon Delivery
- Decision Support

HIGHLIGHTS:

Clinical studies to validate automated insulin delivery; E. Damiano, S. Russell

Develop stable glucagon, which will allow use in rescue and bi-hormonal systems

IMPROVE GLUCOSE CONTROL

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PRIORITIES

- Begin to Automate Insulin Delivery
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HIGHLIGHTS:

Medtronic

dexcom®

Support the development of the next generation sensors

IMPROVE GLUCOSE CONTROL

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HIGHLIGHTS:

HELMSLEY JDRF
Commitment • Collaboration • Results

&

unitio

Health Policy Initiative

IMPROVE GLUCOSE CONTROL

The goal of this initiative is to improve clinical outcomes while easing the burden of living with type 1 diabetes

PRIORITIES

- Begin to Automate Insulin Delivery
- Improve Sensing
- Adoption of Technology
- Alternative Insulin/Glucagon Delivery
- Decision Support

HIGHLIGHTS:

Massachusetts Institute of Technology

Smart Insulin and Glucagon; PIs: D. Anderson, M. Weiss

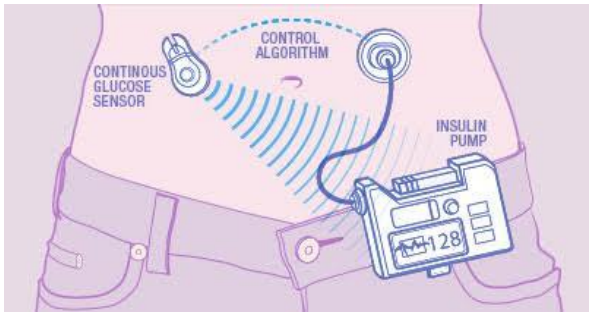
Case Western Reserve University EST. 1826

FUTURE EFFORTS

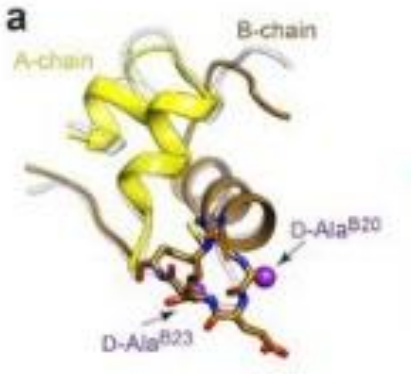
DECISION SUPPORT

EXERCISE AND GLUCOSE CONTROL

AUTOMATE INSULIN DELIVERY



SMART INSULIN AND GLUCAGON



INFUSION SET LONGEVITY

IMPROVE CLINICAL CARE AND ACCESS

The **goal** of this initiative is to identify new health models that will provide better clinical care and make it available to all people living with T1D

PRIORITIES

- Improve T1D Clinical Care Models, Tools & Measures
- Improve Access for All People Living with T1D
- Healthcare Professionals Training
- Community Education & Support

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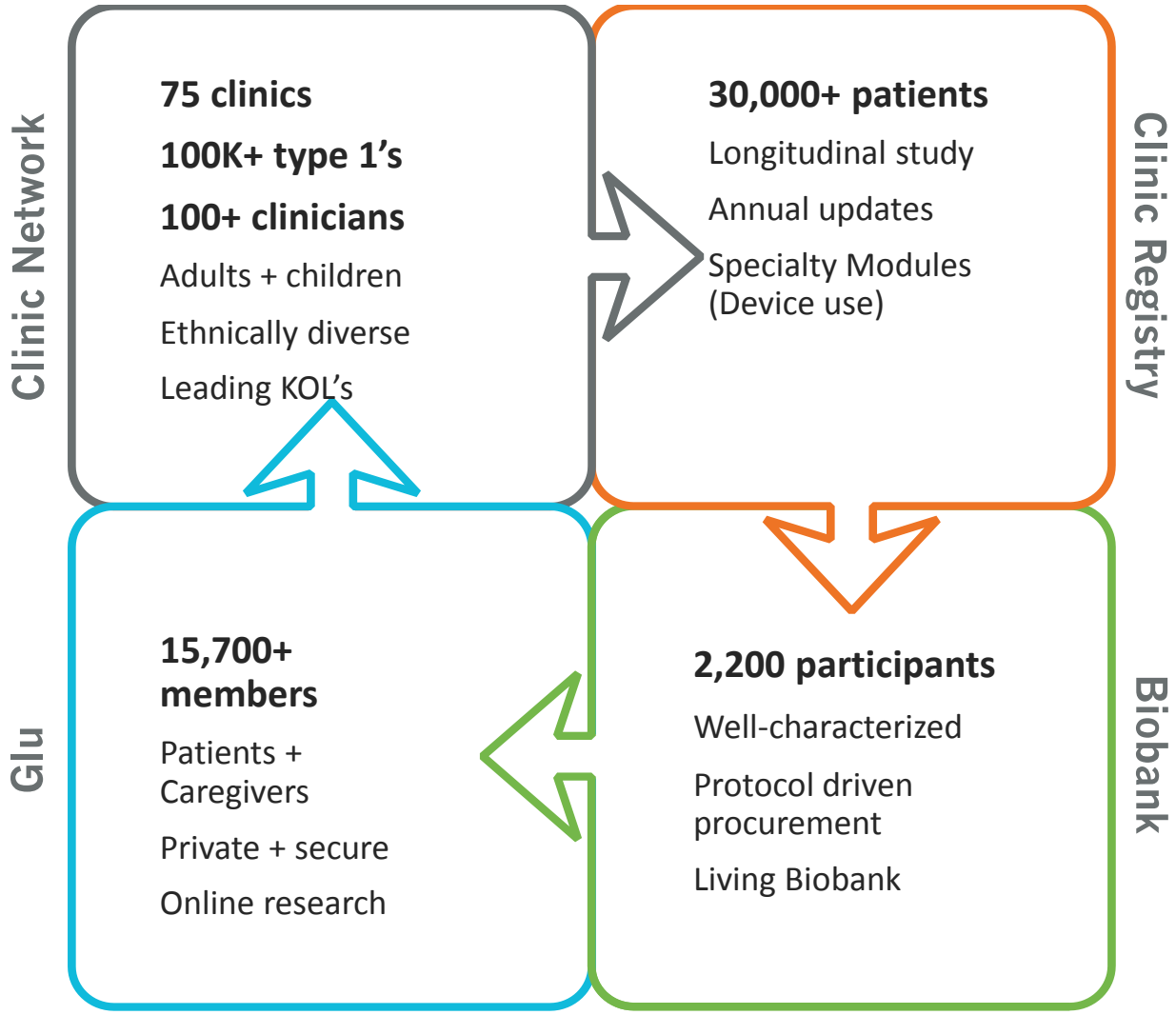
HIGHLIGHTS:

UNM SCHOOL of MEDICINE | Project ECHO®

UNITIO/T1D EXCHANGE

Accelerating Research ….Improving Outcomes



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Priorities

- Improve T1D Clinical Care Models, Tools & Measures
- Improve Access for All People Living with T1D
- Healthcare Professionals Training
- Community Education & Support

acciss
Addressing the Challenge and Constraints of Insulin Sources and Supply

IMPROVE CLINICAL CARE AND ACCESS

FUTURE EFFORTS

- **Health Models that engage the “hidden” patients**
- **Telemedicine**
- **Community Support: T1D Camps**
- **Healthcare Professionals Training (in collaboration with ADA and Endo Society)**

T1D THERAPY PIPELINE

The **goal** of this initiative is to identify and de-risk therapies that have a clear pathway to the clinic

PRIORITIES

- Beta Cell Therapies
  - Beta Cell Replacement
  - Regenerate or replace remaining beta cells
- Immunotherapies
  - Immunomodulation
  - Targeted Immunotherapies

BUILD A PIPELINE OF THERAPIES

The goal of this initiative is to identify and de-risk therapies that have a path to the clinic for those with T1D

PRIORITIES

Beta Cell Therapies:

- Beta cell replacement
- Regenerate or replace remaining beta cells

BETA CELL HIGHLIGHTS:

Beta cell replacement

Various Labs: Characterize stem cell-derived beta cells, reconstruct islets, confer immune protection

MIT (Anderson): Advanced biomaterials islet encapsulation. Co-funded with JDRF

DRI (Ricordi): New sites for islet transplant, new immunosuppression strategies. Co-funded with JDRF

Regenerate or protect remaining beta cells

UCSF (Papa): Type 2 kinase inhibitors of IRE1alpha to treat Type 1 Diabetes

UCSD (Sanders): Validation of a new pharmacological target for increasing beta cell regeneration

BUILD A PIPELINE OF THERAPIES

The goal of this initiative is to identify and de-risk therapies that have a path to the clinic for those with T1D

PRIORITIES

Immunotherapies:

- Immunomodulation
- Targeted immunotherapies

IMMUNOTHERAPY HIGHLIGHTS:

Immunomodulation

Florida (Haller): ATG/GCSF phase II clinical trial

Partnership with TrialNet, Sanofi, Amgen

Targeted cellular immunotherapies

Seattle Children's (Rawlings): Durable regulatory cell therapy of T1D using gene editing

Stanford (Kim): Targeting therapeutics to human islets with chimeric antigen receptor T cells

Upenn (Riley): Engineering T Regulatory Cells to put the Brakes on T1D

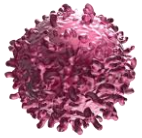
BUILD A PIPELINE OF THERAPIES

The goal of this initiative is to identify and de-risk therapies that have a path to the clinic for those with T1D

PRIORITIES

- Immunotherapies:
  - Immunomodulation
  - Targeted Immunotherapies
- Beta Cell Therapies:
  - Replacement
  - Protect or Regenerate

FUTURE EFFORTS



Targeted cell therapies
- Supporting additional approaches (antigen-specific dendritic cell therapies)
- Translational roadblocks

Targeted biologics (antigen-specific)
- Reduce effector response
- Enhance regulatory response

Beta cells
- Drug delivery
- Imaging

SUPPORT THE DEVELOPMENT OF TOOLS & RESOURCES FOR IMPACTFUL RESEARCH

The goal of this initiative is to develop resources and tools that can facilitate research and promote the understanding of T1D

PRIORITIES

- Develop Models to Recapitulate T1D
- Identify Targets & Validate New Interventions Using Human Samples

HIGHLIGHTS:

University of Massachusetts Medical School
UMASS

Umass (Greiner):Reconstruct human T1D

HELMSLEY
Cellular Research Hub

Columbia (Egli): Diabetes Cell Repository

nPOD
Network for Pancreatic Organ Donors with Diabetes

Miami (Pugliese): George Eisenbarth nPOD Award

T1D PREVENTION INITIATIVE

BUILD SYSTEMS & FACILITATE EXISTING ACTIVITIES TO PREVENT & DELAY T1D

BUILD SYSTEMS & FACILITATE EXISTING ACTIVITIES TO PREVENT & DELAY T1D

The goal of this initiative is to build a system to support international development efforts for prevention interventions by funding existing efforts and creating new resources

PRIORITIES

- Build a System to Perform Clinical Studies & Trials
- Identify/Validate Biomarkers and Diagnostic Technologies
- Provide Support for Data Sharing and Analysis

BUILD SYSTEMS & FACILITATE EXISTING ACTIVITIES TO PREVENT & DELAY T1D

The goal of this initiative is to build a system to support international development efforts for prevention interventions by funding existing efforts and creating new resources

PRIORITIES

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CLINICAL STUDIES TO UNDERSTAND THE DISEASE

PREGNANCY

Studies environmental factors in pregnancy & early life – Co-funded with JDRF Australia

BIRTH

Support for teams with innovative approaches to analysis of TEDDY data

CHILDHOOD

German state-wide general population screening for islet autoantibodies – Co-funded with JDRF

BUILD SYSTEMS & FACILITATE EXISTING ACTIVITIES TO PREVENT & DELAY T1D

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- Provide Support for Data Sharing and Analysis

SCREENING PLATFORMS FOR PREVENTION TRIALS

PRIMARY PREVENTION

HelmholtzZentrum münchen
German Research Center for Environmental Health

UNIVERSITY OF CAMBRIDGE

General population newborn screening for genetic risk and intervention clinical trials in Germany and the UK

SECONDARY PREVENTION

Frlda
Intervention

Oral insulin clinical trial in multiple islet autoantibody positive children

BUILD SYSTEMS & FACILITATE EXISTING ACTIVITIES TO PREVENT & DELAY T1D

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- Identify/Validate Biomarkers and Diagnostic Technologies
- Provide Support for Data Sharing and Analysis

TECHNOLOGIES FOR DIAGNOSIS

HELMSLEY JDRF
Commitment • Collaboration • Results

Improved clinical assays for diagnosis & screening

SCHOOL OF MEDICINE
Barbara Davis Center for Diabetes
UNIVERSITY OF COLORADO ANSCHUTZ MEDICAL CAMPUS

High-throughput multiplex islet autoantibody assay

FCSR
Fondazione
CENTRO SAN RAFFAELE

Target antigen variants for GADA and IAA detection

BUILD SYSTEMS & FACILITATE EXISTING ACTIVITIES TO PREVENT & DELAY T1D

The goal of this initiative is to build a system to support international development efforts for prevention interventions by funding existing efforts and creating new resources

PRIORITIES

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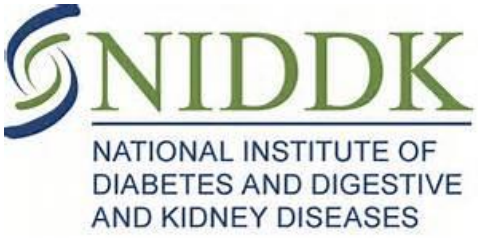
- Identify/Validate Biomarkers and Diagnostic Technologies
- Provide Support for Data Sharing and Analysis

FUTURE EFFORTS

- **Expansion of the clinical network**
  - Primary and secondary prevention
  - Different geographies
- **Piloting different models of screening**
  - In combination with other diseases
- **Biomarker landscape**

OUR PARTNERS

We can not solve type 1 diabetes alone…



OUR TEAM

- Gina Agiostratidou, PhD, MBA, Program Director
- Sean Sullivan, PhD, Senior Program Officer, Improve Patient Outcomes
- Deniz Dalton, Program Officer, Improve Patient Outcomes
- Lydia Guterman, MPH, Program Officer, Improve Patient Outcomes
- Anne Koralova, PhD, Program Officer, Drive Prevention & Treatments for T1D
- Ben Williams, PhD, Program Officer, Drive Prevention & Treatments for T1D
- Carly Maready, Executive Assistant
- Victoria Tralongo, Administrative Assistant

OUR INSPIRATION

When my daughter Morgan was diagnosed with T1D, I made a very simple promise to her: I will help you in any way possible. What started as heartbreak turned into an amazing opportunity. I have been drawn into this battle and given enormous resources to have positive impact on all those affected by this disease.

David Panzirer

THANK YOU

THE LEONA M. AND HARRY B.
HELMSLEY
CHARITABLE TRUST

DIABETES RESEARCH CENTERS

Molecular Transducer of Physical Activity in Humans

https://commonfund.nih.gov/MolecularTransducers

Recent Funding Opportunity Announcements

Molecular Transducers of Physical Activity Genomics, Epigenomics and Transcriptomics Chemical Analysis Sites (U24)
(RFA-RM-15-010)
Due Date: March 18, 2016

Molecular Transducers of Physical Activity Metabolomics and Proteomics Chemical Analysis Sites (U24)
(RFA-RM-15-011)
Due Date: March 18, 2016

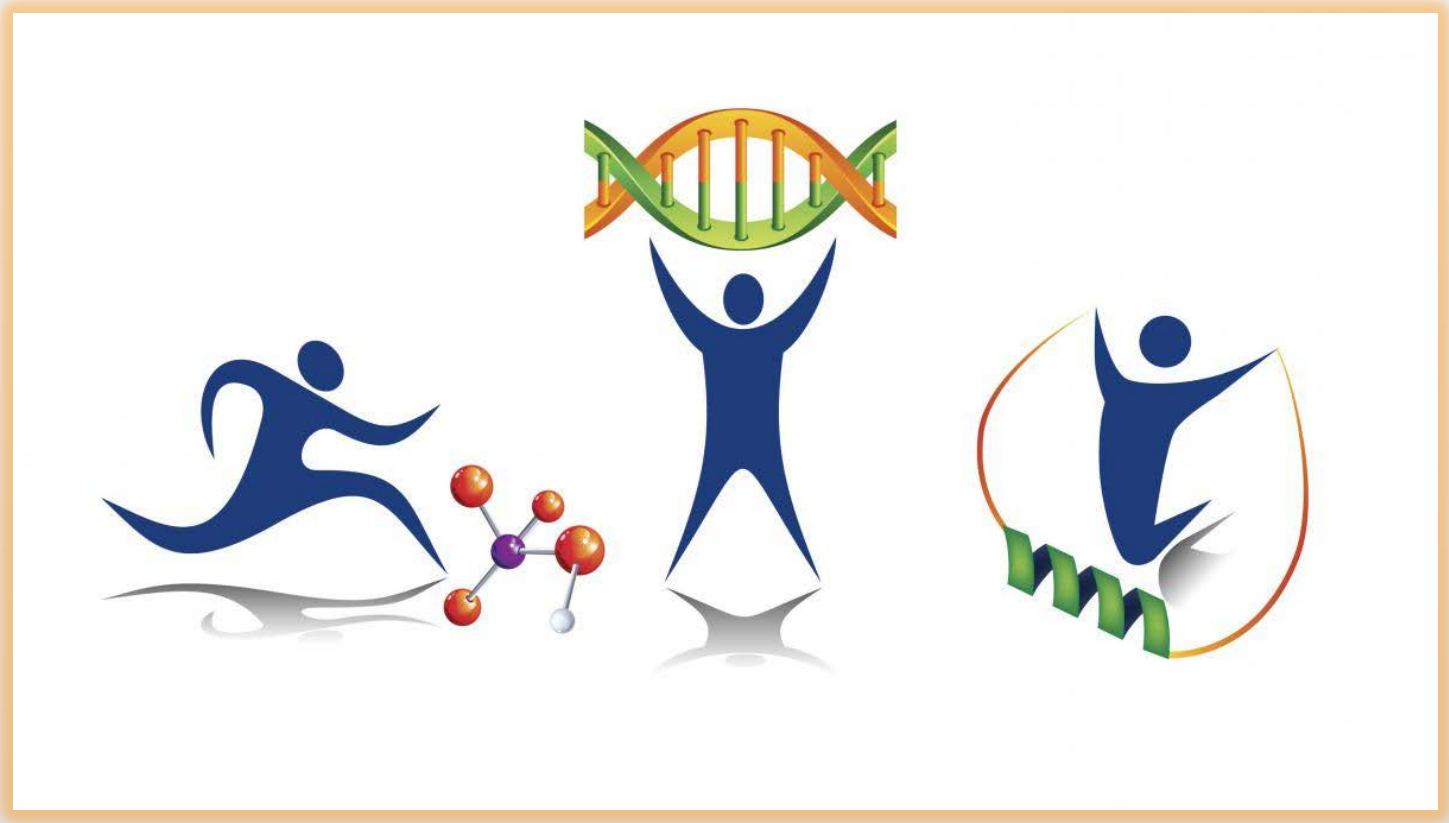
Molecular Transducers of Physical Activity Bioinformatics Center (U24)
(RFA-RM-15-012)
Due Date: March 18, 2016

Molecular Transducers of Physical Activity Preclinical Animal Study Sites (U01)
(RFA-RM-15-013)
Due Date: March 18, 2016

Molecular Transducers of Physical Activity Consortium Coordinating Center (CCC) (U24)
(RFA-RM-15-014)
Due Date: March 18, 2016

Molecular Transducers of Physical Activity Clinical Centers (U01)
(RFA-RM-15-015)
Due Date: March 18, 2016

Molecular Transducers of Physical Activity Consortium



Maren Laughlin, PhD, LaughlinM@extra.niddk.nih.gov

FY 2016 Common Fund Initiative

The Molecular Transducers of Physical Activity in Humans

http://commonfund.nih.gov/MolecularTransducers

6 RFAs due March 18, 2016: RFA-RM-15-010 - 015

Goal:

- Discover the molecules and pathways responsible for physical activity's benefits for **human health**.

Justification:

- Exploitation of the molecular mechanisms for the many well-documented health benefits of physical activity is hindered by a lack of knowledge about the **signaling molecules** that
  - are altered by physical activity and
  - communicate its effect among cells, tissues and organs.

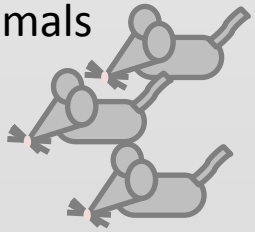
Project Overview

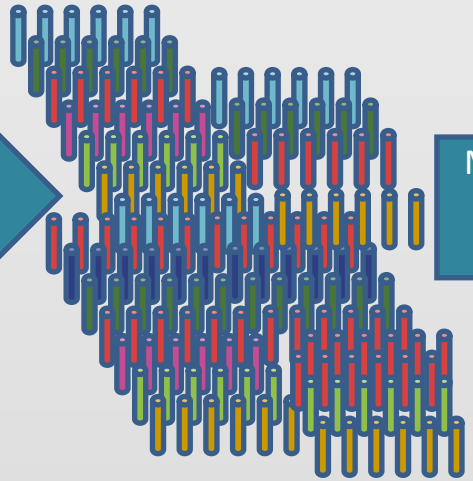
Human volunteers

Participant characteristics
Age, sex, fitness, response to training

Physical activity

Animals





Tissue Samples
Human blood, muscle, adipose
Animal heart, liver, lung, brain

Metabolomics,
transcriptomics,
genomics, etc.

Molecular Transducers of Physical Activity

Participant characteristics
Genes
Proteins
Peptides
Lipids
Sugars
Hormones
Epigenetics
mRNA
ncRNA

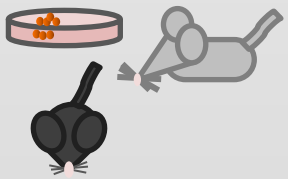
Project Overview

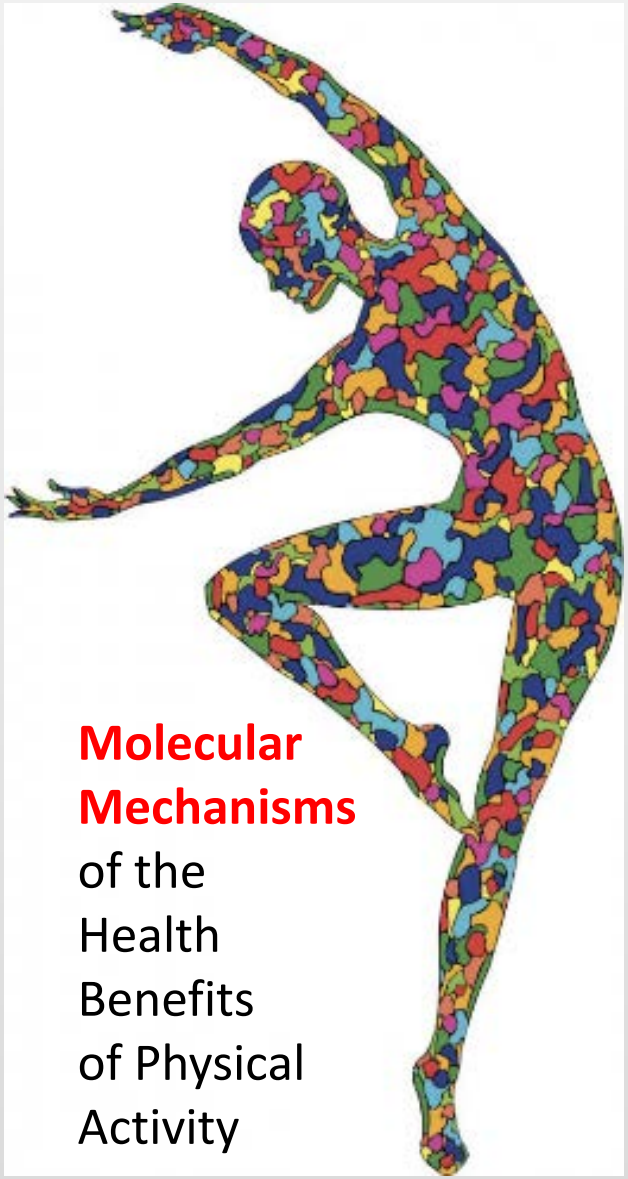
Molecular Transducers of Physical Activity

Participant characteristics
Genes
Proteins
Peptides
Lipids
Sugars
Hormones
Epigenetics
mRNA
ncRNA

Integrated Data Analysis

Mechanistic Studies (cells, animals)





Molecular Mechanisms of the Health Benefits of Physical Activity

Criteria for Common Fund Programs

Transformative: Programs are expected to have **exceptionally high and broadly applicable impact**. They should be relevant to many diseases and many ICs. They should create entirely new approaches to research or clinical care, or establish new biological paradigms.

Catalytic, Short Term and Goal-driven: Programs must achieve - not just work toward - a goal. They have **deliverables** - data sets, tools, technologies, approaches, or fundamental principles of biology, etc – that can be achieved within **5-10 years**. If the deliverable is expected to have ongoing maintenance costs, a vision for transition and sustainment must be articulated.

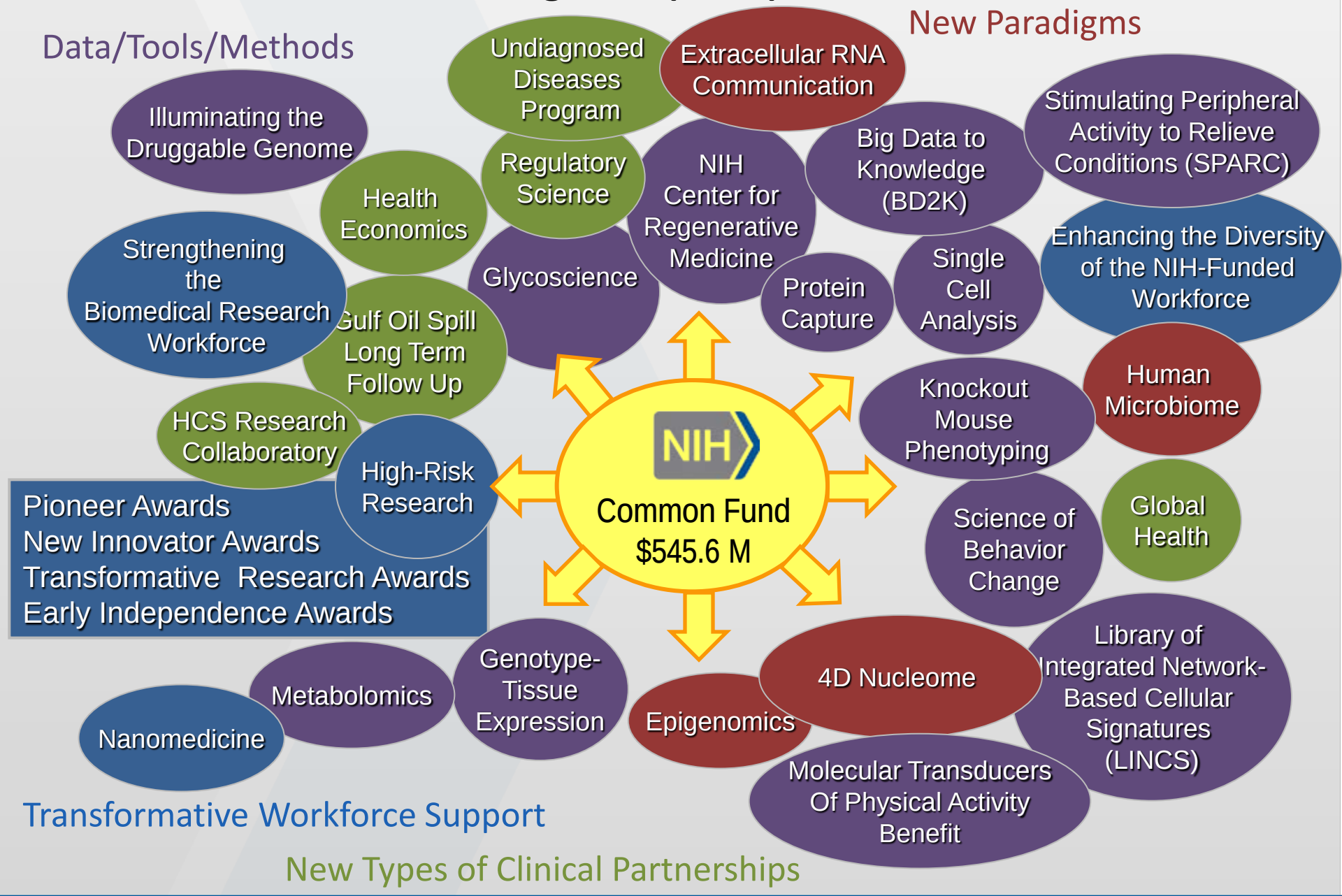
Synergistic /Enabling: Programs should be **value-added to the ICs**, with the output enabling the mission of many ICs.

Requires a High Level of Trans-NIH Coordination: CF programs should address complex issues that require trans-NIH teams, insights and perspectives to design and manage. There must be a **reason why strategic coordination is required**.

Novel: Programs should provide **new solutions to specific challenges**. If similar efforts exist, the CF program should be tightly coordinated to prevent duplication of effort. *Programs should not be something another entity would be likely to support.*

Designed to accomplish goals and deliverables within 5-10 years
Evaluation of program outputs/outcomes is essential

Current Common Fund Programs (FY15)

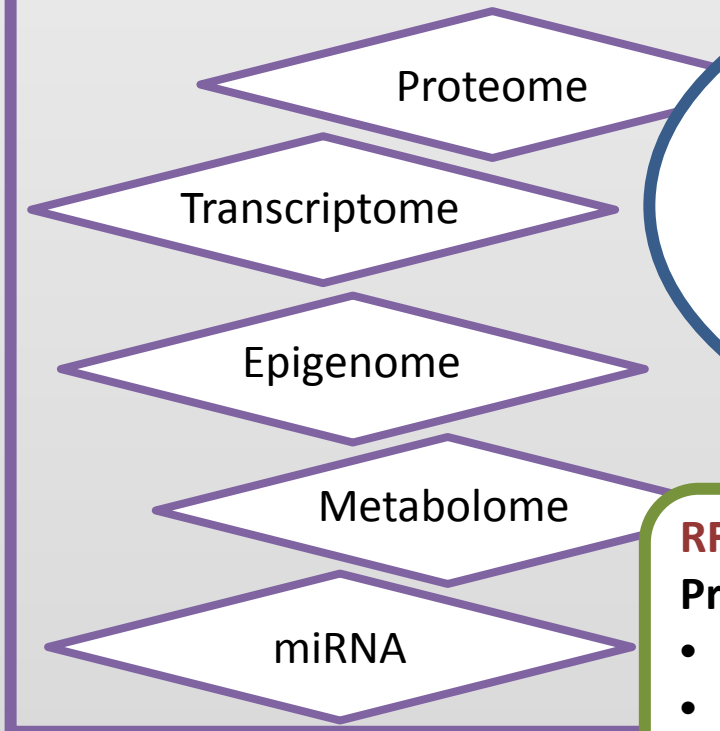


Molecular Transducers of Physical Activity Consortium (MoTrPAC)

6 Common Fund RFAs 2016-2021

RFA-RM-15-011 Metabolomics and Proteomics Chemical Analysis Sites

RFA-RM-15-010 Genomics, Epigenomics, and Transcriptomics Chemical Analysis Sites $86,000,000 TC



RFA-RM-15-015 Clinical Centers (U01)

5-7 Centers for PA study in healthy human subjects for discovery of molecular transducers of PA in blood, muscle, fat, etc. $43,000,000 TC

RFA-RM-15-014 Consortium Coordination Center (U24)

- Coordination
- Protocol Development
- Standardization
- Tissue Repository
- Pilot Funding

$10,000,000 TC

RFA-RM-15-012
Bioinformatics Center (U24)

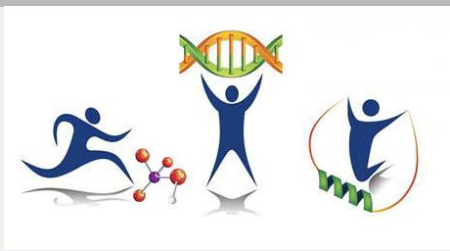
- Bioinformatics
- Data standards
- Data storage/retrieval
- Data analysis

$11,000,000 TC

RFA-RM-15-013 $7,000,000 TC
Preclinical Animal Study Sites (U01)

- Allow molecule discovery in all tissues
- Find target tissues and pathways
- Test hypotheses regarding mechanisms

Responsibilities

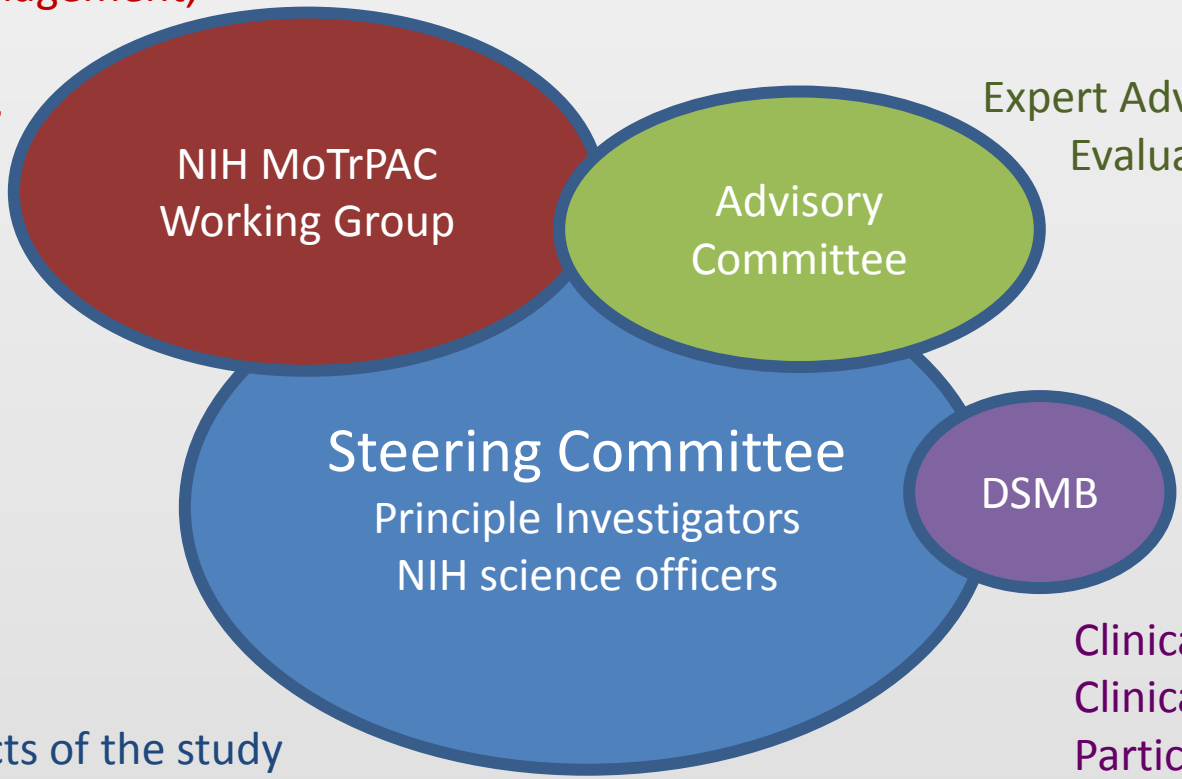


All MoTrPAC members will:

- Work together as a consortium
- Help design the overall study
- Use common protocols for clinical and animal physical activity studies
- Follow consortium plans for tissue analysis
- Submit tissues and data to the Consortium Coordinating Center and the Bioinformatics Center
- Participate in data analysis
- Inform mechanistic studies

MoTrPAC Consortium

Award management,
Oversight,
Evaluation,
Policy



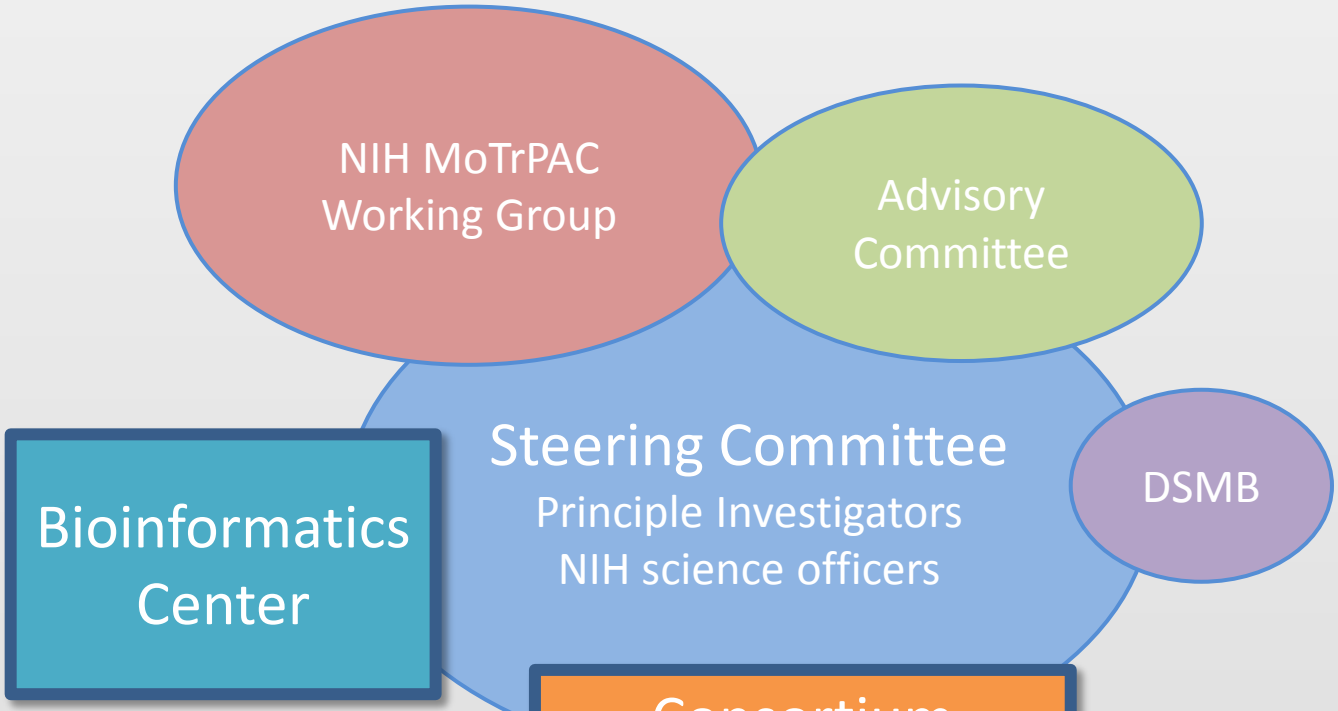
Expert Advice,
Evaluation

Clinical study oversight,
Clinical data quality,
Participant safety

All aspects of the study

- planning
- protocols
- execution
- data
- publication

MoTrPAC Consortium

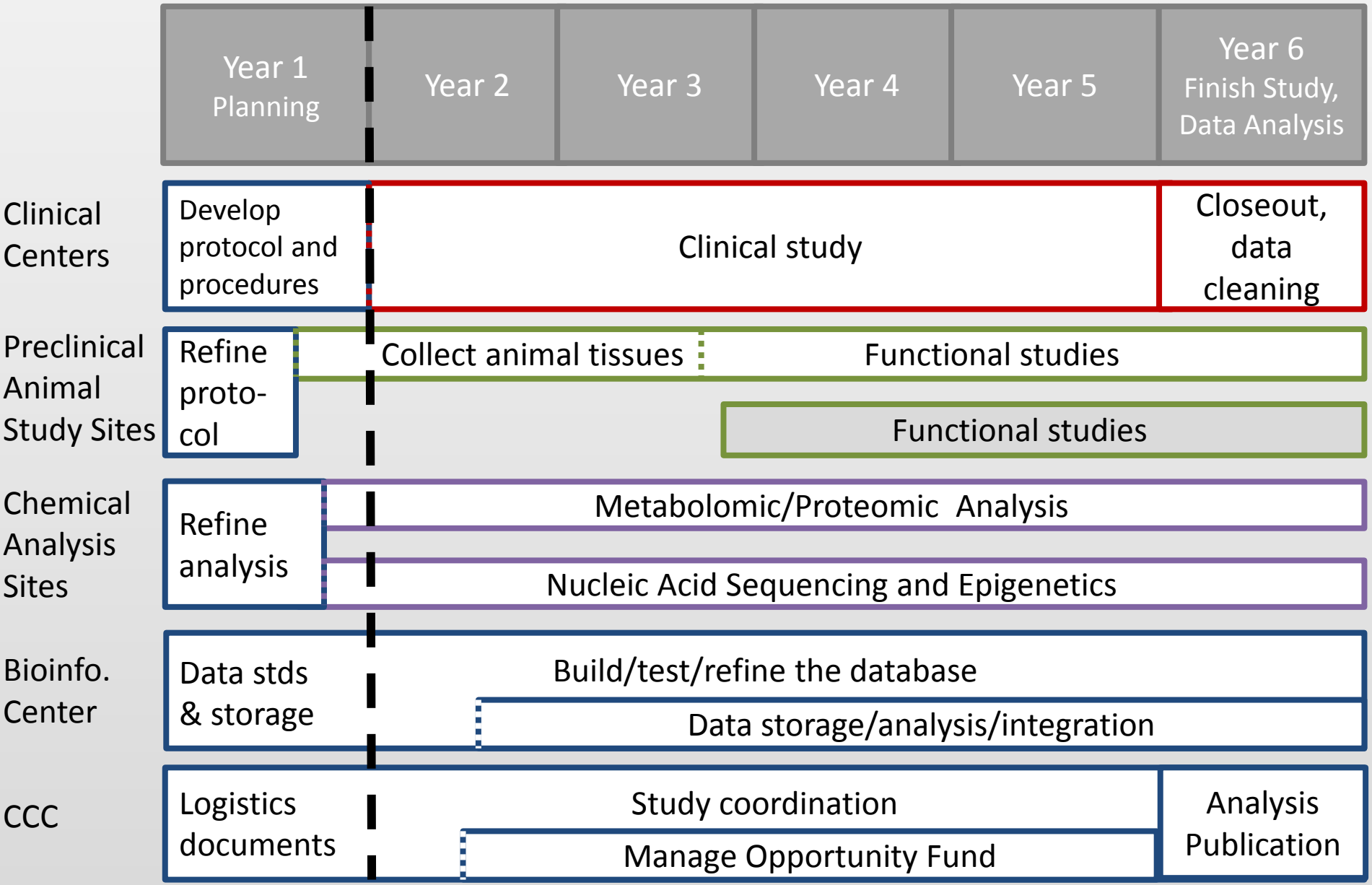


Assemble Data Resource:

- standards/syntax/metadata
- coordination/curation
- storage
- tools
- analysis

- Coordination
- Facilitation
- Training
- Document management
- Tissue storage/movement
- Opportunity Funds program

General Timeline



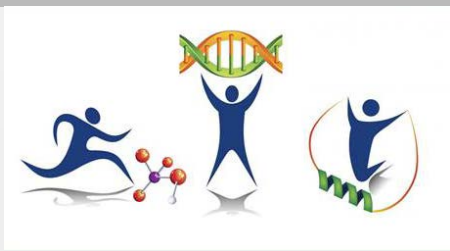
Clinical Centers Overview

- **Clinical Centers are central to overall success of project**
- **Clinical cohort: ~2700 to ~3000 adults and children**
  - Healthy and able to engage in physical activity programs
  - Participants will include: both sexes and wide range of ages, races/ethnicities, fitness, body type, etc.
  - Participant characterization: physiologic, morphometric, and metabolic fitness measures to be used in analyses, to relate the physiologic fitness measures to molecular transducers, and to relate these physiologic measures to existing literature

Study Design Overview

- Participants will be characterized and undergo single PA bout at start, and again after a ~12-wk exercise training program
- Biospecimens (blood, muscle, fat) will be collected before & at various appropriate time points after single bout of PA
- Endurance and Resistance exercise to be studied
- Control groups will include highly fit, athletic individuals and a non-exercised group

Preclinical Animal Study Sites (PASS) Overview



- Collect tissues from exercised animals that cannot be obtained from people, to complement the data and tissue collection in the human clinical protocol (Phase 1)

- Conduct mechanistic studies to explore the functions, sources, and target tissues of molecules that transduce the effects of physical activity (Phase 2)

– NIH anticipates releasing a second FOA for 5-7 more sites that will conduct additional mechanistic studies.

Human and Animal Tissue Samples for Analysis

Estimate:

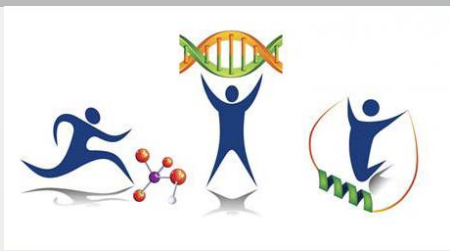
- **Human Participants: ~3000 healthy people**
- **Acute exercise bouts with tissue collection: ~5000**
- **Per acute exercise bout: ~5 blood and 2-3 other tissues**

  Total: ~25,000 human blood,
  ~7500 skeletal muscle, and
  ~7500 adipose tissue samples

- **From exercised animal models:**

  ~5,000 to 10,000 tissue samples
  (e.g., blood, muscle, heart, brain, kidney, liver, white and brown fat)

Chemical Analysis Sites for Tissue Characterization



Genomics, Epigenomics, and Transcriptomics Sites

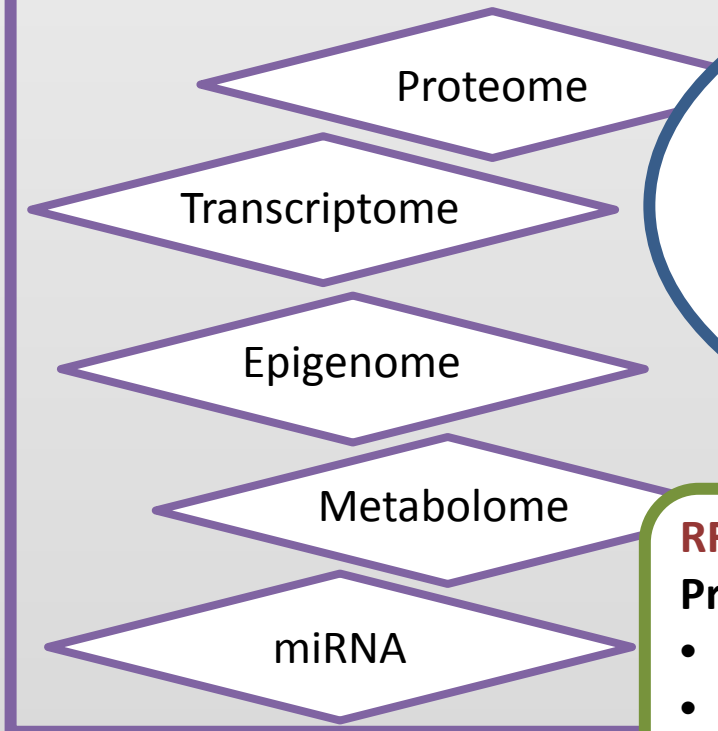
- Whole genome sequencing
- Transcriptional and epigenetic changes in relevant tissues
- Non-coding RNA
- RNA and DNA analysis of exosomes

Metabolomics and/or Proteomics Sites

- Targeted and untargeted metabolomics of plasma & tissues
- Global proteomics analysis
- Targeted plasma proteomics including PTMs
- Protein and metabolite analysis of exosomes

RFA-RM-15-011 **Metabolomics and Proteomics Chemical Analysis Sites**

RFA-RM-15-010 **Genomics, Epigenomics, and Transcriptomics Chemical Analysis Sites** $86,000,000 TC



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RFA-RM-15-012

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National Institute of Diabetes and Digestive and Kidney Diseases

NIDDK Cystic Fibrosis Research and Translation Centers: Overview

Thomas L. Eggerman, M.D., Ph.D.
Program Director
Division of Diabetes, Endocrinology & Metabolic Diseases
NIDDK/NIH

National Institute of Diabetes and Digestive and Kidney Diseases

Historical Highlights

1980s: Established Cystic Fibrosis Research and Training Centers

2014: Discontinued the NIDDK Gene Therapy Centers Program and allowed to compete as Cystic Fibrosis Centers.

2015: Established the NIDDK Cystic Fibrosis Centers website.

2016: First CFRTC PI meeting

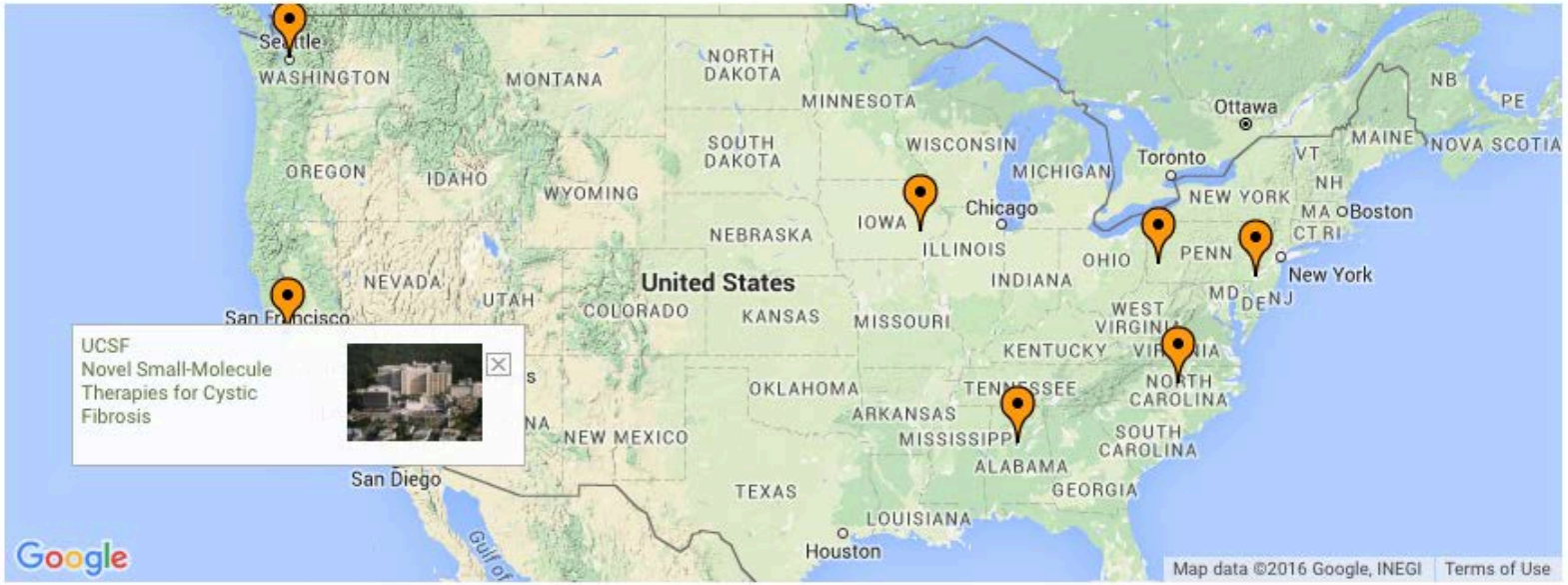
National Institute of Diabetes and Digestive and Kidney Diseases

Cystic Fibrosis Centers Structure

1. Efforts usually centered around a common theme
2. Administrative Cores
3. Basic Research support Cores
4. Clinical Research support Cores
5. Pilot and Feasibility studies

National Institute of Diabetes and Digestive and Kidney Diseases

CF CENTERS


Seattle
WASHINGTON
MONTANA
NORTH DAKOTA
MINNESOTA
SOUTH DAKOTA
WISCONSIN
MICHIGAN
Ottawa
Toronto
NB
PE
MAINE
NOVA SCOTIA
VT
NH
MA
Boston
CT
RI
NEW YORK
New York
OREGON
IDAHO
WYOMING
NEBRASKA
IOWA
Chicago
ILLINOIS
INDIANA
OHIO
PENN
MD
DE
NJ
NEVADA
UTAH
COLORADO
KANSAS
MISSOURI
United States
San Francisco
WEST VIRGINIA
KENTUCKY
NORTH CAROLINA
SOUTH CAROLINA
OKLAHOMA
TENNESSEE
ARKANSAS
MISSISSIPPI
ALABAMA
GEORGIA
NEW MEXICO
TEXAS
LOUISIANA
Houston
San Diego
UCSF
Novel Small-Molecule Therapies for Cystic Fibrosis
Google
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Common Sites for Diabetes and CF Centers

1. University of Washington
2. University of Alabama Birmingham
3. University of California San Francisco
4. University of North Carolina Chapel Hill

National Institute of Diabetes and Digestive and Kidney Diseases

University of Alabama at Birmingham

1. PI: Steven Rowe
2. Cystic Fibrosis Research and Translation Core Center
3. Cores:
    a. Cell Models and Assay
    b. Animal Models
    c. Clinical and Translational

National Institute of Diabetes and Digestive and Kidney Diseases

University of California San Francisco

1. PI: Alan Verkman
2. Novel Small Molecule Therapies for Cystic Fibrosis
3. Cores:
    a. High Throughput Screening
    b. Synthesis
    c. Cell and Tissue Model
    d. Cystic Fibrosis Cell and Tissue Bioassays

National Institute of Diabetes and Digestive and Kidney Diseases

University of Iowa

1. PI: John Engelhardt (Previous Gene Therapy Center)
2. Center for Gene Therapy for Cystic Fibrosis
3. Cores:
    a. Vector
    b. Animal models
    c. Comparative Pathology
    d. Cell and Tissue
    e. Clinical

National Institute of Diabetes and Digestive and Kidney Diseases

University of North Carolina

1. PI: Richard Boucher (Previous Gene Therapy Center)
2. UNC Cystic Fibrosis Research and Translation Core Center
3. Cores:
    a. Preclinical
    b. Cell Model
    c. Mucous Biochemistry and Biophysics
    d. Clinical Translation

National Institute of Diabetes and Digestive and Kidney Diseases

University of Pennsylvania

1. PI- James Wilson (Previous Gene Therapy Center)
2. Molecular Therapy for Cystic Fibrosis
3. Cores:
    a. Vector Core
    b. Immunology Core

National Institute of Diabetes and Digestive and Kidney Diseases

University of Pittsburgh

1. PI: Ray Frizzell, Joseph Pilewski, Jay Kolls and Simon Watkins
2. Basic and Translational Studies in Cystic Fibrosis
3. Cores:
    a. Human Airway Cells and Assays
    b. Translational Studies
    c. Imaging

National Institute of Diabetes and Digestive and Kidney Diseases

University of Washington

1. PIs- Bonnie Ramsey and E. Peter Greenberg
2. Translational Research Center to Expedite Novel Therapies in Cystic Fibrosis
3. Cores:
    a. Microbiology
    b. Cell Model and Assay
    c. Animal Models
    d. Clinical and Translational

National Institute of Diabetes and Digestive and Kidney Diseases

NIDDK CF Center-Related Websites

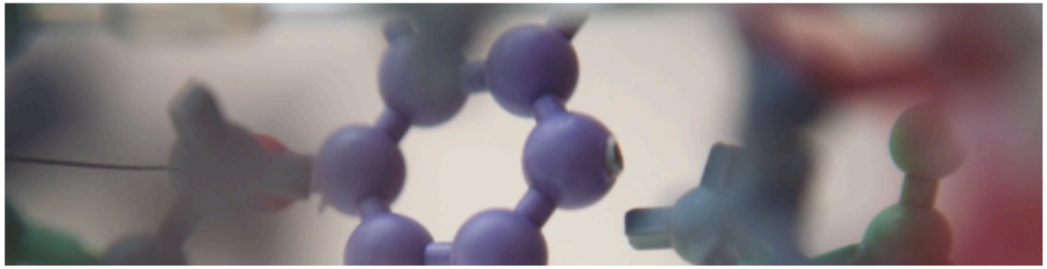
Cystic Fibrosis Research and Translation Centers:

http://www.cysticfibrosiscenters.org

CYSTIC FIBROSIS RESEARCH AND TRANSLATION CENTERS

CYSTIC FIBROSIS
RESEARCH AND TRANSLATION CENTERS

HOME CENTERS SERVICES PUBLICATIONS



Cystic Fibrosis Research and Translation Centers

The NIDDK-supported Cystic Fibrosis Research and Translation Centers are part of an integrated program of research support designed to enhance multidisciplinary research in pathogenesis and treatment of cystic fibrosis.

Cystic fibrosis-related diabetes (CFRD) is the most common complication of cystic fibrosis. Glucose abnormalities that precede diabetes are even more common, in fact, most adults living with CF have some degree of diabetes or glucose intolerance. Early recognition of the disease and therapeutic intervention may diminish the negative impact that diabetes has on lung health in CF.

The goal of the CF Research and Translation Centers is to support research to develop and test therapies for CF.

Centers provide increased, cost effective collaboration among multidisciplinary groups of investigators at institutions or a group of institutions with an established, comprehensive research base in cystic fibrosis and cystic fibrosis-related diabetes as well as promote communication and collaboration between basic and clinical researchers to study the multiple organ systems affect by Cystic Fibrosis.

The launch of the CF Research and Translation Centers will be COMING SOON.

Iowa
Center for Gene Therapy of Cystic Fibrosis

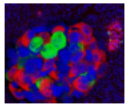
John F. Engelhardt
Director

NIH National Institute of Diabetes and Digestive and Kidney Diseases

SERVICES Overview

Animal Physiology and Phenotyping

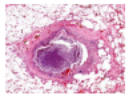
Cystic fibrosis is the first human genetic disease to benefit from the directed engineering of three different species of animal models (mice, pigs, and ferrets). Recent studies on the cystic fibrosis animal models are providing new information about the pathophysiology of cystic fibrosis in various organ systems.



Animal Physiology & Phenotyping

Cell Model and Assays

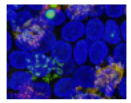
Various assays for measuring the cystic fibrosis transmembrane conductance regulator (CFTR) ion channel activity, as well as its stability in the membrane, can be used for basic research and drug discovery efforts.



Cell Model & Assays

Clinical and Translational Studies

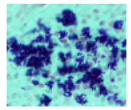
CF research often requires the use of specialized technologies and resources to support a cohesive research effort. One of the goals of the Cystic Fibrosis Research and Translation Centers (CF RTC) is to make state-of-the-art technologies and resources readily accessible to a broad spectrum of investigators working on CF.



Clinical & Translational Studies

Histology, Morphology, and Imaging

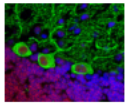
All states in the US now screen for CF at birth to enable presymptomatic support for nutrition and preventing infections that may further improve the quality of life for CF patients. Children identified at birth avoid the malnutrition that was the common presenting symptom for CF. In addition, these patients benefit from many new forms of therapy that are being developed to aggressively eradicate bacterial colonization of the lung, control inflammation and lung damage, treat diabetes and liver disease and provide nutritional support.



Histology, Morphology, Imaging

Immunology and Gene Therapy

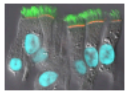
The accumulation of neutrophils and high concentrations of the chemokine interleukin-8 (IL-8) in the airways is a characteristic feature of chronic lung diseases, such as cystic fibrosis. Therapeutic interventions primarily emphasize the use of somatic gene transfer to correct inherited defects. Gene therapy approaches to deliver a normal CFTR gene are also being pursued. New vectors with less toxicity have been developed.



Immunology & Gene Therapy

Molecular Biology and Genomics

Screening of small molecules has identified lead molecules that increase either expression, processing, trafficking or function of the CFTR protein. This approach has led to the development of the drug, invacaftor (marketed as Kalydeco) which is approved for patients with G551D substitution in CFTR. In addition, other molecules that bind to CFTR are being studied for their ability to correct the most common mutation, ΔF508.



Molecular Biology & Genomics

Area of Collaboration

Cystic Fibrosis Associated Diabetes

Department of Health and Human Services
Part 1. Overview Information

Participating Organization(s)

National Institutes of Health (NIH)

Components of Participating Organizations

National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)

Funding Opportunity Title

NIDDK Program Projects (P01)

Activity Code

P01 Research Program Projects

Announcement Type

Reissue of PAR-13-266

Related Notices

- NOT-OD-16-004 - NIH & AHRQ Announce Upcoming Changes to Policies, Instructions and Forms for 2016 Grant Applications (November 18, 2015)

Funding Opportunity Announcement (FOA) Number

PAR-16-127

Companion Funding Opportunity

None

Number of Applications

See Section III. 3. Additional Information on Eligibility.

Catalog of Federal Domestic Assistance (CFDA) Number(s)

93.847

Funding Opportunity Purpose

This Funding Opportunity Announcement (FOA) invites submission of investigator-initiated program project applications. The proposed programs should address scientific areas relevant to the NIDDK mission including diabetes, selected endocrine and metabolic diseases, obesity, digestive diseases and nutrition, and kidney, urologic and hematologic diseases, as well as new approaches to prevent, treat and cure these diseases, including clinical research. A description of NIDDK scientific program areas can be found at http://www.niddk.nih.gov/about-niddk/research-areas/pages/research-areas.aspx .

Key Dates

Posted Date

March 9, 2016

Open Date (Earliest Submission Date)

April 25, 2016

Letter of Intent Due Date(s)

Six weeks before the application due date

Application Due Date(s)

Standard dates apply, by 5:00 PM local time of applicant organization. All types of non-AIDS applications allowed for this funding opportunity announcement are due on these dates.

Applicants are encouraged to apply early to allow adequate time to make any corrections to errors found in the application during the submission process by the due date.

AIDS Application Due Date(s)

Standard AIDS dates apply

Scientific Merit Review

Standard dates apply

Advisory Council Review

Standard dates apply

Earliest Start Date

Standard dates apply

Expiration Date

May 8, 2019

Due Dates for E.O. 12372

Not Applicable **** ELECTRONIC APPLICATION SUBMISSION REQUIRED****

NIH's new Application Submission System & Interface for Submission Tracking (ASSIST) is available for the electronic preparation and submission of multi-project applications through Grants.gov to NIH. Applications to this FOA must be submitted electronically; paper applications will not be accepted. ASSIST replaces the Grants.gov downloadable forms currently used with most NIH opportunities and provides many features to enable electronic multi-project application submission and improve data quality, including: pre-population of organization and PD/PI data, pre-submission validation of many agency business rules and the generation of data summaries in the application image used for review.

Required Application Instructions

It is critical that applicants follow the instructions in the SF424 (R&R) Application Guide, except where instructed to do otherwise (in this FOA or in a Notice from the *NIH Guide for Grants and Contracts*) and where instructions in the Application Guide are directly related to the Grants.gov downloadable forms currently used with most NIH opportunities. Conformance to all requirements (both in the Application Guide

and the FOA) is required and strictly enforced. Applicants must read and follow all application instructions in the Application Guide as well as any program-specific instructions noted in Section IV. When the program-specific instructions deviate from those in the Application Guide, follow the program-specific instructions. **Applications that do not comply with these instructions may be delayed or not accepted for review.**

There are several options to submit your application to the agency through Grants.gov. You can use the ASSIST system to prepare, submit and track your application online. You can download an application package from Grants.gov, complete the forms offline, submit the completed forms to Grants.gov and track your application in eRA Commons. Or, you can use other institutional system-to-system solutions to prepare and submit your application to Grants.gov and track your application in eRA Commons. Learn more.

Apply Online Using ASSIST Problems accessing or using ASSIST should be directed to the eRA Service Desk.

Table of Contents

Part 2. Full Text of Announcement
Section I. Funding Opportunity Description

New biologic knowledge will come from both sole investigators following their vision and from teams of scientists sharing their expertise. Some complex biomedical problems require a multidisciplinary vantage point to discover an innovative solution. This Funding Opportunity Announcement (FOA) invites grant applications for investigator-initiated Program Projects (P01) applications. The P01 program project award supports research that has multiple distinct but synergistic projects built around a unifying central theme within the scientific mission of the NIDDK.

The P01 is one of several grant mechanisms the NIDDK uses to foster multi-disciplinary, collaborative research projects. More information on and a comparison among these collaborative grant mechanisms supported by NIDDK can be found at: http://www.niddk.nih.gov/research-funding/process/apply/about-funding-mechanisms/niddk-collaborative-grants-comparison/Pages/default.aspx.

A central theme or well-defined major objective is the foundation of a P01 program project. Compared to the more narrow thrust of a traditional R01 research project, a program project is directed toward a range of scientific questions that elucidate various aspects of the central theme. The interrelationships of research projects and collaborations among investigators will yield synergy and results beyond those achievable if each research project were to be pursued independently. In addition, one or more shared research cores are required.

The project leaders and core leaders should be established investigators with strong records of innovative

research and independent funding support. The participation of experts in several disciplines or in several areas of one discipline will greatly enhance the goals of the program project. All investigators must contribute to, and share in, the responsibilities of fulfilling the program objectives.

The research of the program projects should advance the mission of the NIDDK to gain new knowledge relevant to understanding diabetes, selected endocrine and metabolic diseases, obesity, digestive diseases and nutrition, and kidney, urologic and hematologic diseases, and to develop new approaches to prevent, treat and cure these diseases.

A program project for this FOA requires: a) a minimum of three inter-related and synergistic research projects that complement and contribute to a unifying central theme; and b) at least one research core facility that provides services to at least two research projects. An administrative support core may also be proposed. The research projects and scientific and administrative cores are all under the leadership of an overall P01 leader responsible for program integration and management.

Investigators are allowed to submit each research project as an R01 application and as part of the program project for review in the same review cycle. If such a project were to receive impact scores that merit funding of both the R01 and P01 applications, funding of the project in the program project will take precedence, and the R01 application will be inactivated administratively.

Potential applicants are strongly encouraged to contact NIDDK while they are still in the process of developing conceptual plans for an application and at least 3 months and preferably 6 months before the due date to discuss a potential application. The discussion could include the choice of activity code, relevance of the topic to the NIDDK mission and the scope and approach of the project.

It is NIDDK policy that any P01 grant receiving a competing award in FY 2011 or later will be limited to one subsequent renewal.

See Section VIII. Other Information for award authorities and regulations.

Section II. Award Information

Funding Instrument

Grant: A support mechanism providing money, property, or both to an eligible entity to carry out an approved project or activity.

Application Types Allowed

New
Renewal - Only one subsequent renewal application is allowed for any application funded in FY 2011 or beyond.
Resubmission
Revision

The OER Glossary and the SF424 (R&R) Application Guide provide details on these application types.

Funds Available and Anticipated Number of Awards

The number of awards is contingent upon the NIDDK budget and the number of highly meritorious applications. A limited pool of funds for program projects and similar large grants makes these awards highly competitive.

Award Budget

Applications should not request more than $6.25 million in direct costs over 5 years. The indirect costs related to the subcontracts will be excluded from the requested direct cost levels prior to application of the cap.

Award Project Period

The maximum project period for these awards is 5 years.

NIH grants policies as described in the *NIH Grants Policy Statement* will apply to the applications submitted and awards made in response to this FOA.

Section III. Eligibility Information

1. Eligible Applicants

Eligible Organizations

Higher Education Institutions

- Public/State Controlled Institutions of Higher Education
- Private Institutions of Higher Education

The following types of Higher Education Institutions are always encouraged to apply for NIH support as Public or Private Institutions of Higher Education:

- Hispanic-serving Institutions
- Historically Black Colleges and Universities (HBCUs)
- Tribally Controlled Colleges and Universities (TCCUs)
- Alaska Native and Native Hawaiian Serving Institutions
- Asian American Native American Pacific Islander Serving Institutions (AANAPISIs)

Nonprofits Other Than Institutions of Higher Education

- Nonprofits with 501(c)(3) IRS Status (Other than Institutions of Higher Education)
- Nonprofits without 501(c)(3) IRS Status (Other than Institutions of Higher Education)

For-Profit Organizations

- Small Businesses
- For-Profit Organizations (Other than Small Businesses)

Governments

- State Governments
- County Governments
- City or Township Governments
- Special District Governments
- Indian/Native American Tribal Governments (Federally Recognized)
- Indian/Native American Tribal Governments (Other than Federally Recognized)
- Eligible Agencies of the Federal Government
- U.S. Territory or Possession

Other

- Independent School Districts
- Public Housing Authorities/Indian Housing Authorities
- Native American Tribal Organizations (other than Federally recognized tribal governments)
- Faith-based or Community-based Organizations
- Regional Organizations

Foreign Institutions

Non-domestic (non-U.S.) Entities (Foreign Institutions) **are not** eligible to apply.
Non-domestic (non-U.S.) components of U.S. Organizations **are not** eligible to apply.
Foreign components, as defined in the *NIH Grants Policy Statement*, **are** allowed.

Required Registrations

Applicant Organizations

Applicant organizations must complete and maintain the following registrations as described in the SF 424 (R&R) Application Guide to be eligible to apply for or receive an award. All registrations must be completed prior to the application being submitted. Registration can take 6 weeks or more, so applicants should begin the registration process as soon as possible. The NIH Policy on Late Submission of Grant Applications states that failure to complete registrations in advance of a due date is not a valid reason for a late submission.

- Dun and Bradstreet Universal Numbering System (DUNS) - All registrations require that applicants be issued a DUNS number. After obtaining a DUNS number, applicants can begin both SAM and eRA Commons registrations. The same DUNS number must be used for all registrations, as well as on the grant application.
- System for Award Management (SAM) (formerly CCR) – Applicants must complete and maintain an active registration, **which requires renewal at least annually**. The renewal process may require as much time as the initial registration. SAM registration includes the assignment of a Commercial and Government Entity (CAGE) Code for domestic organizations which have not already been assigned a CAGE Code.
    - NATO Commercial and Government Entity (NCAGE) Code – Foreign organizations must obtain an NCAGE code (in lieu of a CAGE code) in order to register in SAM.
- eRA Commons - Applicants must have an active DUNS number and SAM registration in order to complete the eRA Commons registration. Organizations can register with the eRA Commons as they are working through their SAM or Grants.gov registration. eRA Commons requires organizations to identify at least one Signing Official (SO) and at least one Program Director/Principal Investigator (PD/PI) account in order to submit an application.
- Grants.gov – Applicants must have an active DUNS number and SAM registration in order to complete the Grants.gov registration.

Program Directors/Principal Investigators (PD(s)/PI(s))

All PD(s)/PI(s) must have an eRA Commons account. PD(s)/PI(s) should work with their organizational officials to either create a new account or to affiliate their existing account with the applicant organization in eRA Commons.If the PD/PI is also the organizational Signing Official, they must have two distinct eRA Commons accounts, one for each role. Obtaining an eRA Commons account can take up to 2 weeks.

Eligible Individuals (Program Director/Principal Investigator)

Any individual(s) with the skills, knowledge, and resources necessary to carry out the proposed research as the Program Director(s)/Principal Investigator(s) (PD(s)/PI(s)) is invited to work with his/her organization to

develop an application for support. Individuals from underrepresented racial and ethnic groups as well as individuals with disabilities are always encouraged to apply for NIH support.

For institutions/organizations proposing multiple PDs/PIs, visit the Multiple Program Director/Principal Investigator Policy and submission details in the Senior/Key Person Profile (Expanded) Component of the SF424 (R&R) Application Guide.

The PD/PI should be an established research scientist who has the experience, ability, and time commitment to provide scientific leadership and to ensure quality control, effective administration and integration of all components of the program project. Each research project should be led by an experienced investigator with an established record of productivity and independent funding.

2. Cost Sharing

This FOA does not require cost sharing as defined in the *NIH Grants Policy Statement.*

3. Additional Information on Eligibility

Number of Applications

Applicant organizations may submit more than one application, provided that each application is scientifically distinct.

The NIH will not accept duplicate or highly overlapping applications under review at the same time. This means that the NIH will not accept:

- A new (A0) application that is submitted before issuance of the summary statement from the review of an overlapping new (A0) or resubmission (A1) application.
- A resubmission (A1) application that is submitted before issuance of the summary statement from the review of the previous new (A0) application.
- An application that has substantial overlap with another application pending appeal of initial peer review (see NOT-OD-11-101).

Section IV. Application and Submission Information

1. Requesting an Application Package

Applicants can access the SF424 (R&R) application package associated with this funding opportunity using the “Apply for Grant Electronically” button in this FOA or following the directions provided at Grants.gov.

Most applicants will use NIH’s ASSIST system to prepare and submit applications through Grants.gov to NIH. Applications prepared and submitted using applicant systems capable of submitting electronic multi-project applications to Grants.gov will also be accepted.

2. Content and Form of Application Submission

It is critical that applicants follow the instructions in the SF424 (R&R) Application Guide, including Supplemental Grant Application Instructions except where instructed in this funding opportunity announcement to do otherwise and where instructions in the Application Guide are directly related to the Grants.gov downloadable forms currently used with most NIH opportunities. Conformance to the requirements in the Application Guide is required and strictly enforced. Applications that are out of compliance with these instructions may be delayed or not accepted for review.

For information on Application Submission and Receipt, visit Frequently Asked Questions – Application

Guide, Electronic Submission of Grant Applications.

Letter of Intent

Although a letter of intent is not required, is not binding, and does not enter into the review of a subsequent application, the information that it contains allows IC staff to estimate the potential review workload and plan the review.

By the date listed in Part 1. Overview Information, prospective applicants are asked to submit a letter of intent that includes the following information:

- Descriptive title of proposed activity
- Name(s), address(es), and telephone number(s) of the PD(s)/PI(s)
- Names of other key personnel
- Participating institution(s)
- Number and title of this funding opportunity

The letter of intent, preferably electronically, should be sent to:

Michele Barnard, Ph.D.
Deputy Chief, Review Branch
National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)
6707 Democracy Boulevard, Room 7253
Bethesda, MD 20892-5452
(for express/courier service: Bethesda, MD 20817)
Telephone: 301-594-8898
Email: barnardm@mail.nih.gov

Page Limitations

Component Types Available in ASSIST	Research Strategy/Program Plan Page Limits
Overall	12
Admin Core	6
Core (Use for Research Cores)	6
Project	12

Additional page limits described in the SF424 Application Guide and the Table of Page Limits must be followed.

Instructions for the Submission of Multi-Component Applications

The following section supplements the instructions found in the SF424 (R&R) Application Guide, and should be used for preparing a multi-component application.

The application should consist of the following components:

- Overall: required
- Administrative Core: optional
- Research Core: at least 1 required

- Projects: at least 3 required

Overall Component

When preparing your application in ASSIST, use Component Type 'Overall'.

All instructions in the SF424 (R&R) Application Guide must be followed, with the following additional instructions, as noted.

SF424 (R&R) Cover (Overall)

Complete entire form.

PHS 398 Cover Page Supplement (Overall)

Note: Human Embryonic Stem Cell lines from other components should be repeated in cell line table in Overall component.

Research & Related Other Project Information (Overall)

Follow standard instructions.

Facilities and Other Resources: Describe the features of the institutional environment that would facilitate effective implementation of the program project. As appropriate, describe available resources, such as clinical and laboratory facilities, participating and affiliated units, patient populations, geographic distribution of space and personnel, and consultative resources. If the projects are not at the same location, describe the plans for communication and sharing of biologic material.

Project/Performance Site Location(s) (Overall)

Enter primary site only.

A summary of Project/Performance Sites in the Overall section of the assembled application image in eRA Commons compiled from data collected in the other components will be generated upon submission.

Research & Related Senior/Key Person Profile (Overall)

Include only the Project Director/Principal Investigator (PD/PI) and any multi-PDs/PIs (if applicable to this FOA) for the entire application. Include evidence that the PD/PI(s) has (have) a track record(s) of consistently producing highly significant research publications in one or more areas proposed in the program project and a track record of demonstrating the ability to effectively and productively manage a large, interdisciplinary project in the proposed research area. Describe PD/PI commitment and ability to develop a well-defined central research focus and demonstrated ability in mounting similar programs.

A summary of Senior/Key Persons followed by their Biographical Sketches in the Overall section of the assembled application image in eRA Commons will be generated upon submission.

Budget (Overall)

The only budget information included in the Overall component is the Estimated Project Funding

section of the SF424 (R&R) Cover.

A budget summary in the Overall section of the assembled application image in eRA Commons compiled from detailed budget data collected in the other components will be generated upon submission.

PHS 398 Research Plan (Overall)

Introduction to Application: For Resubmission and Revision applications, an Introduction to Application is required in the Overall component.

Specific Aims: Describe the specific aims of the overall program project. Outline how the individual cores and projects will contribute to these aims.

Research Strategy: The overall research strategy should include:

A. Program Background and Statement of Objectives: Present the background, rationale and hypotheses of the central scientific theme and the strategies to address specific questions and problems related to the central theme. Explain the strategy for achieving the objectives of the overall program.

B. Organization and Synergy of the Program Project: Describe the relationships among the projects and cores and their contribution to the overall program project theme and strategy and the cohesiveness and multidisciplinary scope of the program. Describe the unique advantages that would be gained by the proposed program project, potential impact on the field, the scientific synergy among the projects and cores, and the means by which the projects collectively will achieve the stated objectives of the proposed research. The overall scientific synergy (i.e., potential for scientific impact of the proposed program project as a whole to be greater than the sum of its component research projects and cores) should be addressed. Include in this description, the role of the research cores in promoting the collaboration among the projects and how proposed approaches in cores and projects are complementary. For new (Type 1) applications, this section should indicate any prior successful collaboration among investigators in the group and include peer-reviewed publications that resulted from the collaboration. For renewal (Type 2) applications, a description of the collaboration among the projects and cores during the previous funding period must be included. A justification for adding new projects or cores or for deleting components previously supported should also be included.

C. Relation of the Program Project to the Applicant Institution: Describe the relationships between the proposed program project and other existing research units at the applicant institution. List all NIDDK-supported Centers and Program Projects at the applicant institution and the relationship, if any, with the proposed Program Project. Indicate if any of the proposed cores will expand cores already existing at the institution.

Progress Report Publication List: For renewal (Type 2) applications, include a table of publications that directly resulted from the Program Project since it was last reviewed. The table should list the publications and note which Project(s) and Core(s) were directly involved in the research for each publication.

Protection of Human Subjects: Please specify which Projects and Cores will utilize human subjects.

Vertebrate Animals: Please specify which Projects and Cores will utilize vertebrate animals.

Letters of Support: Attach letters of support relevant to the program project as a whole e.g., letters of institutional support. Letters of support relevant to specific projects or cores should be attached in their

respective Research Plan forms.

Resource Sharing Plan: Individuals are required to comply with the instructions for the Resource Sharing Plans as provided in the SF424 (R&R) Application Guide, with the following modification:

All applications, regardless of the amount of direct costs requested for any one year, should address a Data Sharing Plan for the entire Program Project. The individual projects and cores can refer to this Data Sharing Plan.

Other Resource Sharing Plans (Sharing Model Organisms and Genomic Data Sharing (GDS) Plan) should be addressed in the Research Plan forms for the individual projects and cores, as needed.

Appendix: Do not use the Appendix to circumvent page limits. Follow all instructions for the Appendix as described in the SF424 (R&R) Application Guide.

Administrative Core

When preparing your application in ASSIST, use Component Type 'Admin Core.'

All instructions in the SF424 (R&R) Application Guide must be followed, with the following additional instructions, as noted.

SF424 (R&R) Cover (Administrative Core)

Complete only the following fields:

- Applicant Information
- Type of Applicant (optional)
- Descriptive Title of Applicant's Project
- Proposed Project Start/Ending Dates

PHS 398 Cover Page Supplement (Administrative Core)

Enter Human Embryonic Stem Cells in each relevant component.

Research & Related Other Project Information (Administrative Core)

Human Subjects: Answer only the 'Are Human Subjects Involved?' and 'Is the Project Exempt from Federal regulations?' questions. The Administrative Core should not involve Human Subjects Research.

Vertebrate Animals: Answer only the 'Are Vertebrate Animals Used?' question. The Administrative Core should not involve vertebrate animal Research.

Project Narrative: Do not complete. It is not required for the Administrative Core Note: ASSIST screens will show an asterisk for this attachment indicating it is required. However, eRA systems only enforce this requirement in the Overall component and applications will not receive an error if omitted in other components..

Project /Performance Site Location(s) (Administrative Core)

List all performance sites that apply to the specific component.

Research & Related Senior/Key Person Profile (Administrative Core)

- In the Project Director/Principal Investigator section of the form, use Project Role of 'Other' with Category of 'Core Lead' and provide a valid eRA Commons ID in the Credential field.
- In the additional Senior/Key Profiles section, list Senior/Key persons that are working in the component.
- Include a single Biographical Sketch for each Senior/Key person listed in the application regardless of the number of components in which they participate. When a Senior/Key person is listed in multiple components, the Biographical Sketch can be included in any one component.
- If more than 100 Senior/Key persons are included in a component, the Additional Senior Key Person attachments should be used.

Budget (Administrative Core)

Budget forms appropriate for the specific component will be included in the application package.

Minimum expected levels of effort for management of the overall P01 application are three person months, either for a single PD/PI or an aggregate of three months with multiple PDs/PIs. This includes level of effort requested for all components of the P01 for which the PDs/PIs participate.

The Administrative Core may include limited funds for program enrichment activities such as seminars and research workshops, if they are directly related to the goals of the program project. The funds cannot be used for general departmental activities.

Note: The R&R Budget form included in many of the component types allows for up to 100 Senior/Key Persons in section A and 100 Equipment Items in section C prior to using attachments for additional entries. All other SF424 (R&R) instructions apply.

PHS 398 Research Plan (Administrative Core)

Introduction to Application: For Resubmission and Revision applications, an Introduction to Application is allowed for each component.

Specific Aims: Describe the specific aims of the Administrative Core.

Research Strategy: The research strategy should provide a strong justification for the inclusion of an administrative core and its benefit to the program project. If an administrative core is included, it could provide a support structure for activities such as: coordinate the research mission, monitor timeline for achieving research milestones, coordinate and integrate program project activities, implement a plan for regular evaluation of scientific progress.

Letters of Support: Attach letters of support relevant to the Administrative Core..

Resource Sharing Plan: Individuals are required to comply with the instructions for the Resource Sharing Plans as provided in the SF424 (R&R) Application Guide, with the following modification:

The application can note that the Data Sharing Plan for the whole Program Project applies to the Administrative Core. Other resource sharing plans are not relevant to the Administrative Core.

Appendix: Do not use the Appendix to circumvent page limits. Follow all instructions for the Appendix as described in the SF424 (R&R) Application Guide.

Planned Enrollment Report

When conducting clinical research, follow all instructions for completing Planned Enrollment Reports as described in the SF424 (R&R) Application Guide.

PHS 398 Cumulative Inclusion Enrollment Report

When conducting clinical research, follow all instructions for completing Cumulative Inclusion Enrollment Report as described in the SF424 (R&R) Application Guide.

Research Core

When preparing your application in ASSIST, use Component Type ‘Core’.

All instructions in the SF424 (R&R) Application Guide must be followed, with the following additional instructions, as noted.

SF424 (R&R) Cover (Research Core)

Complete only the following fields:

- Applicant Information
- Type of Applicant (optional)
- Descriptive Title of Applicant’s Project
- Proposed Project Start/Ending Dates

PHS 398 Cover Page Supplement (Research Core)

Enter Human Embryonic Stem Cells in each relevant component.

Research & Related Other Project Information (Research Core)

Human Subjects: Answer only the ‘Are Human Subjects Involved?’ and 'Is the Project Exempt from Federal regulations?’ questions.

Vertebrate Animals: Answer only the ‘Are Vertebrate Animals Used?’ question.

Project /Performance Site Location(s) (Research Core)

List all performance sites that apply to the specific component.

Research & Related Senior/Key Person Profile (Research Core)

- In the Project Director/Principal Investigator section, use Project Role of ‘Other’ with Category of ‘Core Lead’ and provide a valid eRA Commons ID in the Credential field.
- In the additional Senior/Key Profiles section, list Senior/Key persons that are working in the component.
- Include a single Biographical Sketch for each Senior/Key person listed in the application regardless of the number of components in which they participate. When a Senior/Key person

is listed in multiple components, the Biographical Sketch can be included in any one component.
- If more than 100 Senior/Key persons are included in a component, the Additional Senior Key Person attachments should be used.

Budget (Research Core)

Budget forms appropriate for the specific component will be included in the application package.

Note: The R&R Budget form included in many of the component types allows for up to 100 Senior/Key Persons in section A and 100 Equipment Items in section C prior to using attachments for additional entries. All other SF424 (R&R) instructions apply.

PHS 398 Research Plan (Research Core)

Introduction to Application: For Resubmission and Revision applications, an Introduction to Application is allowed for each component.

Specific Aims: Describe the specific aims of the Research Core

Research Strategy: Describe the core and the various services it would provide, as well as core management and any special arrangements such as cooperation with other cores. The research strategy should also include a clear delineation of procedures, techniques, and quality control, and how core usage would be prioritized. If applicable, describe in detail statistical analyses and data management. Describe the role of the leaders of the Research Cores in developing the research directions of the Program Project. The research strategy could also include plans for the development of new methods and innovative procedures essential to advancing the research goals of the Program Project.

Research Core resources should not simply duplicate resources already available at the institution. The Research Core could be available to investigators outside the Program Project, but procedures for prioritization and funding for the outside users should be described.

For renewal (Type 2) applications, discuss progress in the Research Core during the prior funding period and the rationale for any changes.

Progress Report Publication List: Do not complete

Letters of Support: Attach letters of support relevant to the Research Core.

Resource Sharing Plan: Individuals are required to comply with the instructions for the Resource Sharing Plans as provided in the SF424 (R&R) Application Guide, with the following modifications:

The application can note that the Data Sharing Plan for the whole Program Project applies to the Research Core.

Other Resource Sharing Plans (Sharing Model Organisms and Genomic Data Sharing (GDS) Plan) should be addressed in this section, as needed.

Appendix: Do not use the Appendix to circumvent page limits. Follow all instructions for the Appendix as described in the SF424 (R&R) Application Guide.

Planned Enrollment Report (Research Core)

When conducting clinical research, follow all instructions for completing Planned Enrollment Reports as described in the SF424 (R&R) Application Guide.

PHS 398 Cumulative Inclusion Enrollment Report (Research Core)

When conducting clinical research, follow all instructions for completing Cumulative Inclusion Enrollment Report as described in the SF424 (R&R) Application Guide.

Project

When preparing your application in ASSIST, use Component Type 'Project'.

All instructions in the SF424 (R&R) Application Guide must be followed, with the following additional instructions, as noted.

SF424 (R&R) Cover (Project)

Complete only the following fields:

- Applicant Information
- Type of Applicant (optional)
- Descriptive Title of Applicant's Project
- Proposed Project Start/Ending Dates

PHS 398 Cover Page Supplement (Project)

Enter Human Embryonic Stem Cells in each relevant component.

Research & Related Other Project Information (Project)

Human Subjects: Answer only the 'Are Human Subjects Involved?' and 'Is the Project Exempt from Federal regulations?' questions.

Vertebrate Animals: Answer only the 'Are Vertebrate Animals Used?' question.

Project/Performance Site Location(s) (Project)

List all performance sites that apply to the specific component.

Note: The Project Performance Site form allows up to 300 sites, prior to using additional attachment for additional entries.

Research & Related Senior/Key Person Profile (Project)

- In the Project Director/Principal Investigator section, use Project Role of 'Other' with Category of 'Project Lead' and provide a valid eRA Commons ID in the Credential field.
- In the additional Senior/Key Profiles section, list Senior/Key persons that are working in the component.
- Include a single Biographical Sketch for each Senior/Key person listed in the application

regardless of the number of components in which they participate. When a Senior/Key person is listed in multiple components, the Biographical Sketch can be included in any one component.

- If more than 100 Senior/Key persons are included in a component, the Additional Senior Key Person attachments should be used.

Budget (Project)

Budget forms appropriate for the specific component will be included in the application package.

Minimum expected level of effort is 1.2 person months for the project leader.

PHS 398 Research Plan (Project)

Introduction to Application: For Resubmission and Revision applications, an Introduction to Application is allowed for each component.

Specific Aims: Describe the specific aims of the Project.

Research Strategy: Describe the research strategy of the Project in the same detail and format as required for an investigator-initiated R01 grant application. Even though all the Projects in the application need to be synergistic, this section should focus on the specific Project and not the potential synergy between this Project and the other Projects and Cores. The description of each Project should be explicit enough to enable peer reviewers to understand and evaluate the Project independently. For renewal (Type 2) applications, discuss progress in the Project during the prior funding period.

Progress Report Publication List: Do not complete

Letters of Support: Attach letters of support relevant to the Project.

Resource Sharing Plan: Individuals are required to comply with the instructions for the Resource Sharing Plans (as provided in the SF424 (R&R) Application Guide, with the following modifications:

The application can note that the Data Sharing Plan for the whole Program Project applies to the Project.

Other Resource Sharing Plans (Sharing Model Organisms and Genomic Data Sharing (GDS) Plan) should be addressed, as needed.

Appendix: Do not use the Appendix to circumvent page limits. Follow all instructions for the Appendix as described in the SF424 (R&R) Application Guide.

Planned Enrollment Report (Project)

When conducting clinical research, follow all instructions for completing Planned Enrollment Reports as described in the SF424 (R&R) Application Guide.

PHS 398 Cumulative Inclusion Enrollment Report (Project)

When conducting clinical research, follow all instructions for completing Cumulative Inclusion Enrollment Report as described in the SF424 (R&R) Application Guide.

3. Unique Entity Identifier and System for Award Management (SAM)

See Part 1. Section III.1 for information regarding the requirement for obtaining a unique entity identifier and for completing and maintaining active registrations in System for Award Management (SAM), NATO Commercial and Government Entity (NCAGE) Code (if applicable), eRA Commons, and Grants.gov.

4. Submission Dates and Times

Part I. Overview Information contains information about Key Dates and times. Applicants are encouraged to submit applications before the due date to ensure they have time to make any application corrections that might be necessary for successful submission. When a submission date falls on a weekend or Federal holiday, the application deadline is automatically extended to the next business day.

Organizations must submit applications to Grants.gov (the online portal to find and apply for grants across all Federal agencies) using ASSIST or other electronic submission systems. Applicants must then complete the submission process by tracking the status of the application in the eRA Commons, NIH's electronic system for grants administration. NIH and Grants.gov systems check the application against many of the application instructions upon submission. Errors must be corrected and a changed/corrected application must be submitted to Grants.gov on or before the application due date and time. If a Changed/Corrected application is submitted after the deadline, the application will be considered late. Applications that miss the due date and time are subjected to the NIH Policy on Late Application Submission.

Applicants are responsible for viewing their application before the due date in the eRA Commons to ensure accurate and successful submission.

Information on the submission process and a definition of on-time submission are provided in the SF424 (R&R) Application Guide.

5. Intergovernmental Review (E.O. 12372)

This initiative is not subject to intergovernmental review.

6. Funding Restrictions

All NIH awards are subject to the terms and conditions, cost principles, and other considerations described in the *NIH Grants Policy Statement*.

Pre-award costs are allowable only as described in the *NIH Grants Policy Statement*.

7. Other Submission Requirements and Information

Applications must be submitted electronically following the instructions described in the SF424 (R&R) Application Guide. Paper applications will not be accepted.

For information on how your application will be automatically assembled for review and funding consideration after submission go to: http://grants.nih.gov/grants/ElectronicReceipt/files/Electronic_Multi-project_Application_Image_Assembly.pdf.

Applicants must complete all required registrations before the application due date. Section III. Eligibility Information contains information about registration.

For assistance with your electronic application or for more information on the electronic submission process, visit Applying Electronically. If you encounter a system issue beyond your control that threatens your ability to complete the submission process on-time, you must follow the Guidelines for Applicants Experiencing System Issues. For assistance with application submission, contact the Application Submission Contacts in Section VII.

Important reminders:

All PD(s)/PI(s) and component Project Leads must include their eRA Commons ID in the Credential field of the Senior/Key Person Profile Component of the SF424(R&R) Application Package**.** Failure to register in the Commons and to include a valid PD/PI Commons ID in the credential field will prevent the successful submission of an electronic application to NIH.

The applicant organization must ensure that the DUNS number it provides on the application is the same number used in the organization's profile in the eRA Commons and for the System for Award Management (SAM). Additional information may be found in the SF424 (R&R) Application Guide.

See more tips for avoiding common errors.

Upon receipt, applications will be evaluated for completeness and compliance with application instructions by the Center for Scientific Review, NIH. Applications that are incomplete or non-compliant will not be reviewed.

Requests of $500,000 or more for direct costs in any year

Applicants requesting $500,000 or more in direct costs in any year (excluding consortium F&A) must submit a written request at least 6 weeks before submitting the application and follow the Policy on the Acceptance for Review of Unsolicited Applications that request $500,000 or More in Direct Costs as described in the SF 424 (R&R) Application Guide.

Although a written, pre-approval request is due at least 6 weeks prior to the application due date, NIDDK strongly encourages investigators to submit the pre-approval request much earlier (e.g., 6 months prior to the application due date). Early discussions with program staff and submission of the pre-approval request can significantly aid the investigators in the subsequent development of the application. NIDDK reviews pre-approval requests on a rolling basis and typically will inform investigators within 4 weeks of submission of the pre-approval request whether they will be allowed to submit an application.

The following criteria will be used in the administrative staff review of these requests:

A. Relevance to the NIDDK: Importance of the unifying central theme to the NIDDK mission.

B. Programmatic priority: Will the proposed research significantly advance the mission of NIDDK?

C. Programmatic balance: How does the proposed research relate to currently funded research in the NIDDK and by the investigative team?

D. Activity Code: Is the proposed work appropriate for the P01 activity code? Are there at least three discrete projects and a core that serves at least two projects?

If the NIDDK agrees to accept an application, a cover letter must be included with the application that identifies the NIDDK program staff who agreed to accept assignment of the application to the NIDDK. The NIDDK will also notify the NIH Division of Receipt and Referral of their willingness to accept the application.

Post Submission Materials

Applicants are required to follow the instructions for post-submission materials, as described in NOT-OD-13-030.

Section V. Application Review Information

1. Criteria

Only the review criteria described below will be considered in the review process. As part of the NIH mission, all applications submitted to the NIH in support of biomedical and behavioral research are evaluated for scientific and technical merit through the NIH peer review system.

Overall Impact - Overall

Reviewers will provide an overall impact score to reflect their assessment of the likelihood for the overall Program Project to exert a sustained, powerful influence on the research field(s) involved, in consideration of the following review criteria and additional review criteria (as applicable for the overall Program Project proposed).

Scored Review Criteria - Overall

Reviewers will consider each of the review criteria below in the determination of scientific merit, and give a separate score for each. An application does not need to be strong in all categories to be judged likely to have major scientific impact. For example, a overall Program Project that by its nature is not innovative may be essential to advance a field.

Significance

Does the overall Program Project address an important problem or a critical barrier to progress in the field? Is there a strong scientific premise for the project? If the aims of the overall Program Project are achieved, how will scientific knowledge, technical capability, and/or clinical practice be improved? How will successful completion of the aims change the concepts, methods, technologies, treatments, services, or preventative interventions that drive this field?

Investigator(s)

Are the PD(s)/PI(s), collaborators, and other researchers well suited to the overall Program Project? If Early Stage Investigators or New Investigators, or in the early stages of independent careers, do they have appropriate experience and training? If established, have they demonstrated an ongoing record of accomplishments that have advanced their field(s)? If the project is collaborative or multi-PD/PI, do the investigators have complementary and integrated expertise; are their leadership approach, governance and organizational structure appropriate for the project? Do(es) the overall Program Project PD/PI(s) have: (a) track record(s) of consistently producing highly significant research publications in one or more of the research areas proposed for the program project; (b) track record(s) demonstrating the ability to effectively and productively manage a large, interdisciplinary project in the proposed research area?

Innovation

Does the application challenge and seek to shift current research or clinical practice paradigms by utilizing novel theoretical concepts, approaches or methodologies, instrumentation, or interventions? Are the concepts, approaches or methodologies, instrumentation, or interventions novel to one field of

research or novel in a broad sense? Is a refinement, improvement, or new application of theoretical concepts, approaches or methodologies, instrumentation, or interventions proposed?

Approach

Are the overall strategy, methodology, and analyses well-reasoned and appropriate to accomplish the specific aims of the overall Program Project? Have the investigators presented strategies to ensure a robust and unbiased approach, as appropriate for the work proposed? Are potential problems, alternative strategies, and benchmarks for success presented? If the project is in the early stages of development, will the strategy establish feasibility and will particularly risky aspects be managed? Have the investigators presented adequate plans to address relevant biological variables, such as sex, for studies in vertebrate animals or human subjects?

If the overall Program Project involves human subjects and/or NIH-defined clinical research, are the plans to address 1) the protection of human subjects from research risks, and 2) inclusion (or exclusion) of individuals on the basis of sex/gender, race, and ethnicity, as well as the inclusion or exclusion of children, justified in terms of the scientific goals and research strategy proposed?

Are the approaches proposed in the individual projects and cores complementary?

Environment

Will the scientific environment in which the work will be done contribute to the probability of success? Are the institutional support, equipment and other physical resources available to the investigators adequate for the project proposed? Will the project benefit from unique features of the scientific environment, subject populations, or collaborative arrangements?

Additional Review Criteria - Overall

As applicable for the overall Program Project proposed, reviewers will evaluate the following additional items while determining scientific and technical merit, and in providing an overall impact score, but will not give separate scores for these items.

Synergy

Likelihood for the program project to exert a sustained, powerful influence on the research field(s) involved.

The relationship and contributions of each research component and core to the overall theme of the program project will be discussed and evaluated. In assigning the impact score for the application as a whole, the assessment of scientific synergy (i.e., the extent to which the potential for scientific impact of the proposed program project as a whole is deemed likely to be greater than the sum of its component research projects and cores) should contribute significantly to the overall score. This will include the following:

Scientific merit of the program as a whole, as well as that of individual projects, and its potential impact on the field;

Scientific gain of combining the component parts into a program project (beyond that achievable if each project were to be pursued separately);

Cohesiveness and multidisciplinary scope of the program and the coordination and interrelationship of

all individual research projects and cores to the common theme;

Leadership and scientific ability of the PD(s)/PI(s) and his or her commitment and ability to develop a well-defined central research focus (request of support for sufficient effort to provide effective oversight and administration of the program should be considered); and past accomplishments of the program or a demonstrated ability in mounting similar programs.

Protections for Human Subjects

For research that involves human subjects but does not involve one of the six categories of research that are exempt under 45 CFR Part 46, the committee will evaluate the justification for involvement of human subjects and the proposed protections from research risk relating to their participation according to the following five review criteria: 1) risk to subjects, 2) adequacy of protection against risks, 3) potential benefits to the subjects and others, 4) importance of the knowledge to be gained, and 5) data and safety monitoring for clinical trials.

For research that involves human subjects and meets the criteria for one or more of the six categories of research that are exempt under 45 CFR Part 46, the committee will evaluate: 1) the justification for the exemption, 2) human subjects involvement and characteristics, and 3) sources of materials. For additional information on review of the Human Subjects section, please refer to the Guidelines for the Review of Human Subjects.

Inclusion of Women, Minorities, and Children

When the proposed Program Project involves human subjects and/or NIH-defined clinical research, the committee will evaluate the proposed plans for the inclusion (or exclusion) of individuals on the basis of sex/gender, race, and ethnicity, as well as the inclusion (or exclusion) of children to determine if it is justified in terms of the scientific goals and research strategy proposed. For additional information on review of the Inclusion section, please refer to the Guidelines for the Review of Inclusion in Clinical Research.

Vertebrate Animals

The committee will evaluate the involvement of live vertebrate animals as part of the scientific assessment according to the following criteria: (1) description of proposed procedures involving animals, including species, strains, ages, sex, and total number to be used; (2) justifications for the use of animals versus alternative models and for the appropriateness of the species proposed; (3) interventions to minimize discomfort, distress, pain and injury; and (4) justification for euthanasia method if NOT consistent with the AVMA Guidelines for the Euthanasia of Animals. Reviewers will assess the use of chimpanzees as they would any other application proposing the use of vertebrate animals. For additional information on review of the Vertebrate Animals section, please refer to the Worksheet for Review of the Vertebrate Animal Section.

Biohazards

Reviewers will assess whether materials or procedures proposed are potentially hazardous to research personnel and/or the environment, and if needed, determine whether adequate protection is proposed.

Resubmissions

For Resubmissions, the committee will evaluate the application as now presented, taking into consideration the responses to comments from the previous scientific review group and changes made to the Program Project.

Renewals

For Renewals, the committee will consider the progress made in the last funding period. Applicants should include:

Progress and achievements specific to this program project during the past funding period and the evidence through publications, conferences, etc., that collaboration has occurred;

Evidence that the previous specific aims have been accomplished and that the new research goals are logical extensions of ongoing work;

Previous performance and estimated use of the core(s); and

Justification for adding new projects or cores or for deleting components previously supported.

Revisions

For Revisions, the committee will consider the appropriateness of the proposed expansion of the scope of the overall Program Project. If the Revision application relates to a specific line of investigation presented in the original application that was not recommended for approval by the committee, then the committee will consider whether the responses to comments from the previous scientific review group are adequate and whether substantial changes are clearly evident.

Additional Review Considerations - Overall

As applicable for the overall Program Project proposed, reviewers will consider each of the following items, but will not give scores for these items, and should not consider them in providing an overall impact score.

Applications from Foreign Organizations

Not Applicable

Select Agent Research

Reviewers will assess the information provided in this section of the application, including 1) the Select Agent(s) to be used in the proposed research, 2) the registration status of all entities where Select Agent(s) will be used, 3) the procedures that will be used to monitor possession use and transfer of Select Agent(s), and 4) plans for appropriate biosafety, biocontainment, and security of the Select Agent(s).

Resource Sharing Plans

Reviewers will comment on whether the following Resource Sharing Plans, or the rationale for not sharing the following types of resources, are reasonable: 1) Data Sharing Plan; 2) Sharing Model Organisms; and 3) Genomic Data Sharing Plan .

Authentication of Key Biological and/or Chemical Resources

For projects involving key biological and/or chemical resources, reviewers will comment on the brief plans proposed for identifying and ensuring the validity of those resources.

Budget and Period of Support

Reviewers will consider whether the budget and the requested period of support are fully justified and reasonable in relation to the proposed research.

Review Criteria for Individual Research Projects

It is important that each project fits and contributes to the theme of the overall program project, but this factor should be judged separately and have no bearing on a project's individual impact score. Instead, these considerations are addressed with respect to the merit of the overall program project.

Overall Impact

Reviewers will provide an overall impact score to reflect their assessment of the likelihood for the project to exert a sustained, powerful influence on the research field(s) involved, in consideration of the following five scored review criteria, and additional review criteria. An application does not need to be strong in all categories to be judged likely to have major scientific impact. The impact score for an individual project should assess its merit as a stand-alone project, and not take into consideration its relationship with other research projects and cores.

Scored Review Criteria

Reviewers will consider each of the review criteria below in the determination of scientific merit, and give a separate score for each. An application does not need to be strong in all categories to be judged likely to have major scientific impact. For example, a project that by its nature is not innovative may be essential to advance a field.

Significance

Does the project address an important problem or a critical barrier to progress in the field? Is there a strong scientific premise for the project? If the aims of the project are achieved, how will scientific knowledge, technical capability, and/or clinical practice be improved? How will successful completion of the aims change the concepts, methods, technologies, treatments, services, or preventative interventions that drive this field?

Investigator(s)

Are the PD/PIs, collaborators, and other researchers well suited to the project? If Early Stage Investigators or New Investigators, or in the early stages of independent careers, do they have appropriate experience and training? If established, have they demonstrated an ongoing record of accomplishments that have advanced their field(s)? If the project is collaborative or multi-PD/PI, do the investigators have complementary and integrated expertise; are their leadership approach, governance and organizational structure appropriate for the project?

Innovation

Does the application challenge and seek to shift current research or clinical practice paradigms by utilizing novel theoretical concepts, approaches or methodologies, instrumentation, or interventions? Are the concepts, approaches or methodologies, instrumentation, or interventions novel to one field of research or novel in a broad sense? Is a refinement, improvement, or new application of theoretical concepts, approaches or methodologies, instrumentation, or interventions proposed?

Approach

Are the overall strategy, methodology, and analyses well-reasoned and appropriate to accomplish the specific aims of the project? Have the investigators presented strategies to ensure a robust and unbiased approach, as appropriate for the work proposed? Are potential problems, alternative strategies, and benchmarks for success presented? If the project is in the early stages of development, will the strategy establish feasibility and will particularly risky aspects be managed? Have the investigators presented adequate plans to address relevant biological variables, such as sex, for studies in vertebrate animals or human subjects?

If the project involves human subjects and/or NIH-defined clinical research, are the plans to address 1) the protection of human subjects from research risks, and 2) inclusion (or exclusion) of individuals on the basis of sex/gender, race, and ethnicity, as well as the inclusion or exclusion of children, justified in terms of the scientific goals and research strategy proposed?

Environment

Will the scientific environment, in which the work will be done, contribute to the probability of success? Are the institutional support, equipment and other physical resources available to the investigators adequate for the project proposed? Will the project benefit from unique features of the scientific environment, subject populations, or collaborative arrangements?

Additional Review Criteria - Individual Research Projects

As applicable for the individual projects proposed, reviewers will evaluate the following additional items while determining scientific and technical merit, and in providing an impact score, but will not give separate scores for these items.

Protections for Human Subjects

For research that involves human subjects but does not involve one of the six categories of research that are exempt under 45 CFR Part 46, the committee will evaluate the justification for involvement of human subjects and the proposed protections from research risk relating to their participation according to the following five review criteria: 1) risk to subjects, 2) adequacy of protection against risks, 3) potential benefits to the subjects and others, 4) importance of the knowledge to be gained, and 5) data and safety monitoring for clinical trials.

For research that involves human subjects and meets the criteria for one or more of the six categories of research that are exempt under 45 CFR Part 46, the committee will evaluate: 1) the justification for the exemption, 2) human subjects involvement and characteristics, and 3) sources of materials. For additional information on review of the Human Subjects section, please refer to the Guidelines for the Review of Human Subjects.

Inclusion of Women, Minorities, and Children

When the proposed Individual Research Project involves human subjects and/or NIH-defined clinical research, the committee will evaluate the proposed plans for the inclusion (or exclusion) of individuals on the basis of sex/gender, race, and ethnicity, as well as the inclusion (or exclusion) of children to determine if it is justified in terms of the scientific goals and research strategy proposed. For additional information on review of the Inclusion section, please refer to the Guidelines for the Review of Inclusion in Clinical Research.

Vertebrate Animals

The committee will evaluate the involvement of live vertebrate animals as part of the scientific

assessment according to the following criteria: (1) description of proposed procedures involving animals, including species, strains, ages, sex, and total number to be used; (2) justifications for the use of animals versus alternative models and for the appropriateness of the species proposed; (3) interventions to minimize discomfort, distress, pain and injury; and (4) justification for euthanasia method if NOT consistent with the AVMA Guidelines for the Euthanasia of Animals. Reviewers will assess the use of chimpanzees as they would any other application proposing the use of vertebrate animals. For additional information on review of the Vertebrate Animals section, please refer to the Worksheet for Review of the Vertebrate Animal Section.

Review Criteria for Administrative Core

Reviewers will provide an overall impact score to reflect their assessment of the overall utility and quality of the proposed administrative core. The review criteria for the individual cores are given below (individual criterion scores are not provided):

Utility of the core to the program project;

Quality of the facilities or services provided by this core (administrative planning and leadership capability to provide for internal quality control of ongoing research, allocation of funds, enhancement of internal communication and cooperation among the investigators involved in the program, and replacement of the principal investigator/program director if required on an interim or permanent basis);

Qualifications, experience, and commitment of the personnel involved in the core; and

Appropriateness of the core in relation to the scope of the proposed administrative support.

Review Criteria for Individual Research Cores

Reviewers will provide an overall impact score to reflect their assessment of the utility and quality of proposed services the research cores will provide to the overall program project. The review criteria for the individual cores are given below (individual criterion scores are not provided):

Utility of the core to the program project; each core must provide essential facilities or service for two or more projects

Quality of the facilities or services provided by this core (including procedures, techniques, and quality control) and criteria for prioritization of usage;

Qualifications, experience, and commitment of the personnel involved in the core; and

Appropriateness of the core in relation to the scope of the proposed research support.

For projects involving key biological and/or chemical resources, reviewers will comment on the brief plans proposed for identifying and ensuring the validity of those resources

If human subjects, vertebrate animals, or biohazards are to be used in the core, the adequacy of these sections must be assessed and will be considered in determining the descriptor of the individual core.

Additional Review Criteria - Research Cores

As applicable for the Research Core(s) proposed, reviewers will evaluate the following additional items while determining scientific and technical merit, and in providing an impact score, but will not give separate

scores for these items.

Protections for Human Subjects

For research that involves human subjects but does not involve one of the six categories of research that are exempt under 45 CFR Part 46, the committee will evaluate the justification for involvement of human subjects and the proposed protections from research risk relating to their participation according to the following five review criteria: 1) risk to subjects, 2) adequacy of protection against risks, 3) potential benefits to the subjects and others, 4) importance of the knowledge to be gained, and 5) data and safety monitoring for clinical trials.

For research that involves human subjects and meets the criteria for one or more of the six categories of research that are exempt under 45 CFR Part 46, the committee will evaluate: 1) the justification for the exemption, 2) human subjects involvement and characteristics, and 3) sources of materials. For additional information on review of the Human Subjects section, please refer to the Guidelines for the Review of Human Subjects.

Inclusion of Women, Minorities, and Children

When the proposed Research Core involves human subjects and/or NIH-defined clinical research, the committee will evaluate the proposed plans for the inclusion (or exclusion) of individuals on the basis of sex/gender, race, and ethnicity, as well as the inclusion (or exclusion) of children to determine if it is justified in terms of the scientific goals and research strategy proposed. For additional information on review of the Inclusion section, please refer to the Guidelines for the Review of Inclusion in Clinical Research.

Vertebrate Animals

The committee will evaluate the involvement of live vertebrate animals as part of the scientific assessment according to the following criteria: (1) description of proposed procedures involving animals, including species, strains, ages, sex, and total number to be used; (2) justifications for the use of animals versus alternative models and for the appropriateness of the species proposed; (3) interventions to minimize discomfort, distress, pain and injury; and (4) justification for euthanasia method if NOT consistent with the AVMA Guidelines for the Euthanasia of Animals. Reviewers will assess the use of chimpanzees as they would any other application proposing the use of vertebrate animals. For additional information on review of the Vertebrate Animals section, please refer to the Worksheet for Review of the Vertebrate Animal Section.

2. Review and Selection Process

Applications will be evaluated for scientific and technical merit by (an) appropriate Scientific Review Group (s), convened by the NIDDK in accordance with NIH peer review policy and procedures, using the stated review criteria. Assignment to a Scientific Review Group will be shown in the eRA Commons.

As part of the scientific peer review, all applications:

- May undergo a selection process in which only those applications deemed to have the highest scientific and technical merit (generally the top half of applications under review) will be discussed and assigned an overall impact score.
- Will receive a written critique.

Applications will be assigned to the appropriate NIH Institute or Center. Applications will compete for available funds with all other recommended applications submitted in response to this FOA. Following initial

peer review, recommended applications will receive a second level of review by the National Diabetes and Digestive and Kidney Diseases Advisory Council. The following will be considered in making funding decisions:

- Scientific and technical merit of the proposed project as determined by scientific peer review.
- Availability of funds.
- Relevance of the proposed project to program priorities.

3. Anticipated Announcement and Award Dates

After the peer review of the application is completed, the PD/PI will be able to access his or her Summary Statement (written critique) via the eRA Commons. Refer to Part 1 for dates for peer review, advisory council review, and earliest start date.

Information regarding the disposition of applications is available in the *NIH Grants Policy Statement*.

Section VI. Award Administration Information

1. Award Notices

If the application is under consideration for funding, NIH will request "just-in-time" information from the applicant as described in the *NIH Grants Policy Statement*.

A formal notification in the form of a Notice of Award (NoA) will be provided to the applicant organization for successful applications. The NoA signed by the grants management officer is the authorizing document and will be sent via email to the grantee's business official.

Awardees must comply with any funding restrictions described in Section IV.5. Funding Restrictions. Selection of an application for award is not an authorization to begin performance. Any costs incurred before receipt of the NoA are at the recipient's risk. These costs may be reimbursed only to the extent considered allowable pre-award costs.

Any application awarded in response to this FOA will be subject to terms and conditions found on the Award Conditions and Information for NIH Grants website. This includes any recent legislation and policy applicable to awards that is highlighted on this website.

2. Administrative and National Policy Requirements

All NIH grant and cooperative agreement awards include the *NIH Grants Policy Statement* as part of the NoA. For these terms of award, see the *NIH Grants Policy Statement* Part II: Terms and Conditions of NIH Grant Awards, Subpart A: General and Part II: Terms and Conditions of NIH Grant Awards, Subpart B: Terms and Conditions for Specific Types of Grants, Grantees, and Activities. More information is provided at Award Conditions and Information for NIH Grants.

Recipients of federal financial assistance (FFA) from HHS must administer their programs in compliance with federal civil rights law. This means that recipients of HHS funds must ensure equal access to their programs without regard to a person's race, color, national origin, disability, age and, in some circumstances, sex and religion. This includes ensuring your programs are accessible to persons with limited English proficiency. HHS recognizes that research projects are often limited in scope for many reasons that are nondiscriminatory, such as the principal investigator's scientific interest, funding limitations, recruitment requirements, and other considerations. Thus, criteria in research protocols that target or exclude certain populations are warranted where nondiscriminatory justifications establish that such criteria are appropriate with respect to the health or safety of the subjects, the scientific study design, or the purpose of the research.

For additional guidance regarding how the provisions apply to NIH grant programs, please contact the Scientific/Research Contact that is identified in Section VII under Agency Contacts of this FOA. HHS provides general guidance to recipients of FFA on meeting their legal obligation to take reasonable steps to provide meaningful access to their programs by persons with limited English proficiency. Please see http://www.hhs.gov/ocr/civilrights/resources/laws/revisedlep.html. The HHS Office for Civil Rights also provides guidance on complying with civil rights laws enforced by HHS. Please see http://www.hhs.gov/ocr/civilrights/understanding/section1557/index.html; and http://www.hhs.gov/ocr/civilrights/understanding/index.html. Recipients of FFA also have specific legal obligations for serving qualified individuals with disabilities. Please see http://www.hhs.gov/ocr/civilrights/understanding/disability/index.html. Please contact the HHS Office for Civil Rights for more information about obligations and prohibitions under federal civil rights laws at http://www.hhs.gov/ocr/office/about/rgn-hqaddresses.html or call 1-800-368-1019 or TDD 1-800-537-7697. Also note it is an HHS Departmental goal to ensure access to quality, culturally competent care, including long-term services and supports, for vulnerable populations. For further guidance on providing culturally and linguistically appropriate services, recipients should review the National Standards for Culturally and Linguistically Appropriate Services in Health and Health Care at http://minorityhealth.hhs.gov/omh/browse.aspx?lvl=2&lvlid=53.

Cooperative Agreement Terms and Conditions of Award

Not Applicable

3. Reporting

When multiple years are involved, awardees will be required to submit the Research Performance Progress Report (RPPR) annually and financial statements as required in the *NIH Grants Policy Statement*..

A final progress report, invention statement, and the expenditure data portion of the Federal Financial Report are required for closeout of an award, as described in the *NIH Grants Policy Statement*.

The Federal Funding Accountability and Transparency Act of 2006 (Transparency Act), includes a requirement for awardees of Federal grants to report information about first-tier subawards and executive compensation under Federal assistance awards issued in FY2011 or later. All awardees of applicable NIH grants and cooperative agreements are required to report to the Federal Subaward Reporting System (FSRS) available at www.fsrs.gov on all subawards over $25,000. See the *NIH Grants Policy Statement* for additional information on this reporting requirement.

Section VII. Agency Contacts

We encourage inquiries concerning this funding opportunity and welcome the opportunity to answer questions from potential applicants.

Application Submission Contacts

eRA Service Desk (Questions regarding ASSIST, eRA Commons registration, submitting and tracking an application, documenting system problems that threaten submission by the due date, post submission issues)
Finding Help Online: http://grants.nih.gov/support/ (preferred method of contact)
Telephone: 301-402-7469 or 866-504-9552 (Toll Free)

Grants.gov Customer Support (Questions regarding Grants.gov registration and submission, downloading forms and application packages)
Contact Center Telephone: 800-518-4726

Web ticketing system: https://grants-portal.psc.gov/ContactUs.aspx
Email: support@grants.gov

GrantsInfo (Questions regarding application instructions and process, finding NIH grant resources)
Email: GrantsInfo@nih.gov (preferred method of contact)
Telephone: 301-435-0714

Scientific/Research Contact(s)

Chris Mullins, Ph.D.
Division of Kidney, Urologic and Hematologic Diseases (KUH)
National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)
Telephone: 301-451-4902
Email: mullinsC@mail.nih.gov

Bonnie Burgess-Beusse, Ph.D.
Division of Digestive Diseases and Nutrition (DDN)
National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)
Telephone: 301-594-4726
Email: Bonnie.Burgess-Beusse@nih.gov

Mr. Louis Martey
Division of Diabetes, Endocrinology, and Metabolic Diseases (DEM)
National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)
Telephone: 301-594-7733
Email: marteyl@mail.nih.gov

Peer Review Contact(s)

Michele Barnard, Ph.D.
National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)
Telephone: 301-594-8898
Email: barnardm@mail.nih.gov

Financial/Grants Management Contact(s)

Ms. Sharon T. Bourque
National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)
Telephone: 301-594-8846
Email: bourques@mail.nih.gov

Section VIII. Other Information

Recently issued trans-NIH policy notices may affect your application submission. A full list of policy notices published by NIH is provided in the *NIH Guide for Grants and Contracts*. All awards are subject to the terms and conditions, cost principles, and other considerations described in the NIH Grants Policy Statement.

Authority and Regulations

Awards are made under the authorization of Sections 301 and 405 of the Public Health Service Act as amended (42 USC 241 and 284) and under Federal Regulations 42 CFR Part 52 and 45 CFR Part 75.

Weekly TOC for this Announcement
NIH Funding Opportunities and Notices

National Institutes of Health
Office of Extramural Research

Department of Health
and Human Services (HHS)

USA.gov Government Made Easy

NIH... Turning Discovery Into Health®

Note: For help accessing PDF, RTF, MS Word, Excel, PowerPoint, Audio or Video files, see Help Downloading Files.

Department of Health and Human Services
Part 1. Overview Information

Participating Organization(s)

National Institutes of Health (NIH)

Components of Participating Organizations

National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)

Funding Opportunity Title

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

Activity Code

RC2 High Impact Research and Research Infrastructure Programs

Announcement Type

New

Related Notices

- NOT-OD-16-004 - NIH & AHRQ Announce Upcoming Changes to Policies, Instructions and Forms for 2016 Grant Applications (November 18, 2015)

Funding Opportunity Announcement (FOA) Number

PAR-16-126

Companion Funding Opportunity

None

Number of Applications

See Section III. 3. Additional Information on Eligibility.

Catalog of Federal Domestic Assistance (CFDA) Number(s)

93.847

Funding Opportunity Purpose

The purpose of the High Impact, Interdisciplinary Science grants program is to support high impact ideas that may lay the foundation for new fields of investigation within the mission of NIDDK. The interdisciplinary approach encouraged by this FOA is envisioned to generate a community research resource for the broader community, which may include discovery-based or hypothesis-generating science. The interdisciplinary research team should be able to provide an integrative plan of working together to effectively address the complex challenge at hand. This program will support research projects that accelerate critical breakthroughs, early and applied research on cutting-edge technologies, and new approaches to improve the synergy and interactions among multi- and interdisciplinary research teams, including sharing of data and other resources to further advance research in this area. This FOA seeks novel approaches in areas that address specific knowledge gaps, scientific opportunities, new technologies, data generation, or research methods that will advance the area in significant ways designed to accelerate scientific progress in

the understanding, treatment and prevention of diseases within the mission of NIDDK.

Key Dates

Posted Date

March 9, 2016

Open Date (Earliest Submission Date)

May 1, 2016

Letter of Intent Due Date(s)

Six weeks prior to the application due date

Application Due Date(s)

June 1, 2016; November 1, 2016; June 1, 2017; November 1, 2017; June 1, 2018; November 1, 2018, by 5:00 PM local time of applicant organization. All types of non-AIDS applications allowed for this funding opportunity announcement are due on these dates.

Applicants are encouraged to apply early to allow adequate time to make any corrections to errors found in the application during the submission process by the due date.

AIDS Application Due Date(s)

Not Applicable

Scientific Merit Review

October/November 2016; February/March 2017; October/November 2017; February/March 2018; October/November 2018; February/March 2019

Advisory Council Review

January 2017; May 2017; January 2018; May 2018; January 2019; May 2019

Earliest Start Date

April 2017; July 2017; April 2018; July 2018; April 2019; July 2019

Expiration Date

November 2, 2018

Due Dates for E.O. 12372

Not Applicable

Required Application Instructions

It is critical that applicants follow the instructions in the SF424 (R&R) Application Guide, except where instructed to do otherwise (in this FOA or in a Notice from the *NIH Guide for Grants and Contracts*). Conformance to all requirements (both in the Application Guide and the FOA) is required and strictly enforced. Applicants must read and follow all application instructions in the Application Guide as well as any program-specific instructions noted in Section IV. When the program-specific instructions deviate from those in the Application Guide, follow

the program-specific instructions. **Applications that do not comply with these instructions may be delayed or not accepted for review.**

There are several options to submit your application to the agency through Grants.gov. You can use the ASSIST system to prepare, submit and track your application online. You can download an application package from Grants.gov, complete the forms offline, submit the completed forms to Grants.gov and track your application in eRA Commons. Or, you can use other institutional system-to-system solutions to prepare and submit your application to Grants.gov and track your application in eRA Commons. Learn more.

Apply Online Using ASSIST | **Apply Using Downloadable Forms**

Problems accessing or using ASSIST should be directed to the eRA Service Desk.
Problems downloading forms should be directed to Grants.gov Customer Support.

Table of Contents

Part 2. Full Text of Announcement

Section I. Funding Opportunity Description

Research Objectives and Purpose

The mission of the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) is to conduct and support medical research and research training and to disseminate science-based information on diabetes and other endocrine and metabolic diseases; digestive diseases, nutritional disorders, and obesity; and kidney, urologic, and hematologic diseases, to improve people's health and quality of life. To that end, the NIDDK, through extramural grants programs of its Programmatic Divisions, supports a broad range of biomedical research. Previous research has enormously increased our understanding of the molecular, cellular and behavioral bases of disease and our approaches to health care. The most recent advances in technology and science create numerous opportunities for the public and private sectors to accelerate discoveries for the prevention, diagnosis and treatment of disease. The high complexity of the technologies and data systems required for this type of research, and the requirements for large interdisciplinary teams significantly limit progress and prevent private sector investments and expansions.

The purpose of the High Impact, Interdisciplinary Science grants program is to support high impact ideas that may lay the foundation for new fields of investigation within the mission of NIDDK. The interdisciplinary approach encouraged by this FOA could be used to generate a research resource for the broader community, which may include discovery-based or hypothesis-generating science. The interdisciplinary research team should be able to provide an integrative plan of working together to effectively address the complex challenge at hand. This program will support research projects that accelerate critical breakthroughs, early and applied research on cutting-edge technologies, and new approaches to improve the synergy and interactions among multi and interdisciplinary research teams. This FOA seeks novel approaches in areas that address specific knowledge gaps, scientific opportunities, new technologies, data generation, or research methods that will advance the area in significant ways designed to accelerate scientific progress in understanding, treatment and prevention of diseases within the mission of NIDDK.

Scope and Specific Requirements

The scope of this FOA includes, but is not limited to, the following:

- Groundbreaking, innovative, high impact and cross-cutting research projects that will improve and accelerate biomedical research.
- Basic, clinical and translational projects that could fundamentally enhance the research enterprise and that require the participation, interaction, coordination and integration of activities carried out in multiple research laboratories.
- Creation of large scale unique resources, accelerated application of high throughput, and other novel technologies.
- Deployment of critical infrastructure, resources, tools, and methodologies that substantially accelerate collaborative, multi and interdisciplinary basic, translational, and/or clinical research.
- Implementation of large scale research projects that are carried out using new and creative collaborative agreements and partnerships.
- Discovery-based and hypothesis-generating science.
- Creative approaches to overcome barriers to basic, translational, or clinical research using novel tools, technologies, and services.

RC2 projects are ***not intended to support:***

- Traditional investigator-initiated and highly focused studies (best supported by the R01 or P01 mechanisms).
- Research that is a logical extension of ongoing work.
- Core (or related) services to supplement the budgets of existing R01-type efforts.
- Groups of investigators at the same institution who would normally interact and collaborate in the absence of a collaborative grant.

Prior Consultation with NIDDK

Consultation with NIDDK staff at least **3 months (and preferably 6 months)** prior to the application due date (including resubmission applications) is strongly encouraged for submission of the High Impact, Interdisciplinary Science in NIDDK Research (RC2) application. If requested, NIDDK staff will consider whether the proposed RC2 meets the goals and mission of the Institute; whether it addresses one or more high priority research areas; and whether the application is best fit to the RC2 activity code. NIDDK staff will not evaluate the technical and scientific merit of the proposed project; technical and scientific merit will be determined during peer review using the review criteria indicated in this FOA. During the consultation phase, if the proposed project does not meet NIDDK's programmatic needs or is not appropriate for this FOA, applicants will be strongly encouraged to consider other Funding Opportunities.

See Section VIII. Other Information for award authorities and regulations.

Section II. Award Information

Funding Instrument

Grant: A support mechanism providing money, property, or both to an eligible entity to carry out an approved project or activity.

Application Types Allowed

New
Renewal: The number of renewals is not fixed; however, as it is anticipated that the underlying science will change over time, the maximum total funding period for any RC2 award is 10 years.
Resubmission

The OER Glossary and the SF424 (R&R) Application Guide provide details on these application types.

Funds Available and Anticipated Number of Awards

The number of awards is contingent upon NIH appropriations and the submission of a sufficient number of meritorious applications.

Award Budget

The total annual cost for individual awards is expected to vary, depending on the scope of the project and the number of participating institutions. Application budgets are not limited but need to reflect the actual needs of the proposed project.

Award Project Period

The scope of the proposed project should determine the project period. The maximum project period is 5 years.

NIH grants policies as described in the *NIH Grants Policy Statement* will apply to the applications submitted and awards made in response to this FOA.

Section III. Eligibility Information

1. Eligible Applicants

Eligible Organizations

Higher Education Institutions

- Public/State Controlled Institutions of Higher Education
- Private Institutions of Higher Education

The following types of Higher Education Institutions are always encouraged to apply for NIH support as Public or Private Institutions of Higher Education:

- Hispanic-serving Institutions
- Historically Black Colleges and Universities (HBCUs)
- Tribally Controlled Colleges and Universities (TCCUs)
- Alaska Native and Native Hawaiian Serving Institutions
- Asian American Native American Pacific Islander Serving Institutions (AANAPISIs)

Nonprofits Other Than Institutions of Higher Education

- Nonprofits with 501(c)(3) IRS Status (Other than Institutions of Higher Education)
- Nonprofits without 501(c)(3) IRS Status (Other than Institutions of Higher Education)

For-Profit Organizations

- Small Businesses
- For-Profit Organizations (Other than Small Businesses)

Governments

- State Governments
- County Governments

- City or Township Governments
- Special District Governments
- Indian/Native American Tribal Governments (Federally Recognized)
- Indian/Native American Tribal Governments (Other than Federally Recognized)
- Eligible Agencies of the Federal Government
- U.S. Territory or Possession

Other

- Independent School Districts
- Public Housing Authorities/Indian Housing Authorities
- Native American Tribal Organizations (other than Federally recognized tribal governments)
- Faith-based or Community-based Organizations
- Regional Organizations

Foreign Institutions

Non-domestic (non-U.S.) Entities (Foreign Institutions) **are not** eligible to apply.
Non-domestic (non-U.S.) components of U.S. Organizations **are** eligible to apply.
Foreign components, as defined in the *NIH Grants Policy Statement*, **are** allowed.

Required Registrations

Applicant Organizations

Applicant organizations must complete and maintain the following registrations as described in the SF 424 (R&R) Application Guide to be eligible to apply for or receive an award. All registrations must be completed prior to the application being submitted. Registration can take 6 weeks or more, so applicants should begin the registration process as soon as possible. The NIH Policy on Late Submission of Grant Applications states that failure to complete registrations in advance of a due date is not a valid reason for a late submission.

- Dun and Bradstreet Universal Numbering System (DUNS) - All registrations require that applicants be issued a DUNS number. After obtaining a DUNS number, applicants can begin both SAM and eRA Commons registrations. The same DUNS number must be used for all registrations, as well as on the grant application.
- System for Award Management (SAM) (formerly CCR) – Applicants must complete and maintain an active registration, **which requires renewal at least annually**. The renewal process may require as much time as the initial registration. SAM registration includes the assignment of a Commercial and Government Entity (CAGE) Code for domestic organizations which have not already been assigned a CAGE Code.
    - NATO Commercial and Government Entity (NCAGE) Code – Foreign organizations must obtain an NCAGE code (in lieu of a CAGE code) in order to register in SAM.
- eRA Commons - Applicants must have an active DUNS number and SAM registration in order to complete the eRA Commons registration. Organizations can register with the eRA Commons as they are working through their SAM or Grants.gov registration. eRA Commons requires organizations to identify at least one Signing Official (SO) and at least one Program Director/Principal Investigator (PD/PI) account in order to submit an application.
- Grants.gov – Applicants must have an active DUNS number and SAM registration in order to complete the Grants.gov registration.

Program Directors/Principal Investigators (PD(s)/PI(s))

All PD(s)/PI(s) must have an eRA Commons account. PD(s)/PI(s) should work with their organizational officials to either create a new account or to affiliate their existing account with the applicant organization in eRA

Commons. If the PD/PI is also the organizational Signing Official, they must have two distinct eRA Commons accounts, one for each role. Obtaining an eRA Commons account can take up to 2 weeks.

Eligible Individuals (Program Director/Principal Investigator)

Any individual(s) with the skills, knowledge, and resources necessary to carry out the proposed research as the Program Director(s)/Principal Investigator(s) (PD(s)/PI(s)) is invited to work with his/her organization to develop an application for support. Individuals from underrepresented racial and ethnic groups as well as individuals with disabilities are always encouraged to apply for NIH support.

For institutions/organizations proposing multiple PDs/PIs, visit the Multiple Program Director/Principal Investigator Policy and submission details in the Senior/Key Person Profile (Expanded) Component of the SF424 (R&R) Application Guide.

2. Cost Sharing

This FOA does not require cost sharing as defined in the *NIH Grants Policy Statement.*

3. Additional Information on Eligibility

Number of Applications

Applicant organizations may submit more than one application, provided that each application is scientifically distinct.

The NIH will not accept duplicate or highly overlapping applications under review at the same time. This means that the NIH will not accept:

- A new (A0) application that is submitted before issuance of the summary statement from the review of an overlapping new (A0) or resubmission (A1) application.
- A resubmission (A1) application that is submitted before issuance of the summary statement from the review of the previous new (A0) application.
- An application that has substantial overlap with another application pending appeal of initial peer review (see NOT-OD-11-101).

Section IV. Application and Submission Information

1. Requesting an Application Package

Applicants must obtain the SF424 (R&R) application package associated with this funding opportunity using the "Apply for Grant Electronically" button in this FOA or following the directions provided at Grants.gov.

2. Content and Form of Application Submission

It is critical that applicants follow the instructions in the SF424 (R&R) Application Guide, including Supplemental Grant Application Instructions except where instructed in this funding opportunity announcement to do otherwise. Conformance to the requirements in the Application Guide is required and strictly enforced. Applications that are out of compliance with these instructions may be delayed or not accepted for review.

For information on Application Submission and Receipt, visit Frequently Asked Questions – Application Guide, Electronic Submission of Grant Applications.

Letter of Intent

Although a letter of intent is not required, is not binding, and does not enter into the review of a subsequent application, the information that it contains allows IC staff to estimate the potential review workload and plan the

review.

By the date listed in Part 1. Overview Information, prospective applicants are asked to submit a letter of intent that includes the following information:

- Descriptive title of proposed activity
- Name(s), address(es), and telephone number(s) of the PD(s)/PI(s)
- Names of other key personnel
- Participating institution(s)
- Number and title of this funding opportunity

The letter of intent, preferably electronically, should be sent to:

Michele L. Barnard, Ph.D.
Deputy Chief, Review Branch
National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)
6707 Democracy Boulevard, Room 7353 MSC 5452
Bethesda, MD 20892-5452
(For UPS, FedEx use 20817)
Telephone: 301-594-8898
Fax: 301-480-3505
Email: barnardm@extra.niddk.nih.gov

Page Limitations

All page limitations described in the SF424 Application Guide and the Table of Page Limits must be followed.

Instructions for Application Submission

The following section supplements the instructions found in the SF424 (R&R) Application Guide and should be used for preparing an application to this FOA.

SF424(R&R) Cover

All instructions in the SF424 (R&R) Application Guide must be followed.

SF424(R&R) Project/Performance Site Locations

All instructions in the SF424 (R&R) Application Guide must be followed.

SF424(R&R) Other Project Information

All instructions in the SF424 (R&R) Application Guide must be followed.

Other Attachments: An attachment called "Team Science Organization and Resource Sharing" should be uploaded in Other Attachments. Although no specific page limitation applies to this section of the application, be succinct. Do not use this section to circumvent the page limits of the Research Strategy or your application will not proceed to review. The following points should be addressed in this attachment and are an important part of the RC2 application:

1) A description of the collaborative team aspect of the work proposed including the requirements and roles to be played by each member of the team.

Provide a plan to facilitate the interaction of PD/PIs and key personnel at different sites or institutions. The

justification for drawing investigators from varied disciplines should be well defined. A rationale must be provided explaining how this grant will enhance integration and collaboration amongst those participants, beyond what would normally be expected of a group of investigators with shared interests at the same institution. These activities should significantly enhance the investigators' existing capabilities and introduce new approaches to the research aims of the objective of the collaborative team. The role(s) for each member of the team and how this will provide the requisite synergies for answering the complex problem should be clearly articulated.

2) A clear plan of operation should be provided for the administrative structure and proposed interactions among the investigators. Lines of communication and exchange of data should be clearly established. The application is expected to address how data and resources will be easily shared among the collaborating investigators as appropriate and consistent with achieving the goals of the program. The plan for development and use of resources should help to promote the interdisciplinary and collaborative research aspect of the project. The PD/PI must provide a plan for the organization of collaborating investigators and institutions, including the need for electronic communication and/or travel. Depending on how the RC2 is structured, the PD/PI(s) might need to develop policies and procedures for the operations of project resources. The allocation of resources to the development of new technologies in comparison to provision of services with existing technologies should be addressed.

SF424(R&R) Senior/Key Person Profile

All instructions in the SF424 (R&R) Application Guide must be followed.

R&R Budget

All instructions in the SF424 (R&R) Application Guide must be followed.

With the following additional instructions:

- Key Personnel salaries derived from the grant will depend on the effort provided and institutional salary as well as existing NIH policies. It is recommended that the contact PD/PI devote at least three person months of his/her efforts to the project. The application should include salaries for individual PD/PIs only to the extent that they provide an essential function of the collaborative team. No overlap of time or effort between this award and separately-funded projects is permitted.
- It is anticipated that the research environment available to each team member will be sufficient to support the proposed work. However, requests for essential equipment must include a clear justification in terms of need and service to collaborative team investigators. General purpose equipment needs should be included only after surveying the availability of such items within the institution.
- Research patient care costs (both in-patient and out-patient expenses) will be considered in the context of other existing institutional clinical resources. Attempts should be made by the applicant institution to utilize existing clinical facilities, such as CTSAs. Costs relating to the clinical research efforts of investigators may be funded through this award, provided there is no overlap of funding. The RC2 is not intended to be a facility for health care delivery; thus, only those patient costs directly related to research activities may be charged to the grant.
- Domestic and foreign travel of project personnel directly related to the collaborative team activities of the award as well as travel of collaborative team members for attendance at annual meetings is allowable.
- Consultants and any associated costs (consultant fees, per diem, travel) may be included when their services are required within the award.

R&R Subaward Budget

All instructions in the SF424 (R&R) Application Guide must be followed.

PHS 398 Cover Page Supplement

All instructions in the SF424 (R&R) Application Guide must be followed.

PHS 398 Research Plan

All instructions in the SF424 (R&R) Application Guide must be followed, with the following additional instructions:

Research Strategy: In addition to describing the significance of the problem being addressed and the relevance to the mission of the NIDDK, the applicant should specifically address why the problem is best suited for this FOA, as described below. The application must provide an explanation of how the study proposed will fill a gap in the current knowledge in the field, or contribute a significant resource or technology that is currently lacking. Projects are expected to demonstrate the following:

- The work cannot be reasonably expected to be carried out successfully without support provided by this FOA.
- Specific outcomes of the proposed project promote and advance the mission of the NIDDK to improve health.
- Funding will accelerate current and future research across a broad range that comprehensively encompasses the particular scientific area of study.
- The proposed project is something that no other entity is likely or able to do, and there is a public health benefit to having the results of the research in the public domain.
- The project or generated results and resources can be expected to become integrated into the broader research community.
- There is a plan to sustain applicable research efforts and resources beyond funding.

Letters of Support: Any resources or expertise outside of the team of investigators, including institutional support through core facilities or resources, should be evidenced by appropriate letters of support from the relevant individual.

Resource Sharing Plan: Individuals are required to comply with the instructions for the Resource Sharing Plans as provided in the SF424 (R&R) Application Guide, with the following modification:

> *A description of the development, sharing and sustainability of resources generated.* If the application proposes the generation of a research resource, the application must provide a description of the resource to be generated. A resource could be something tangible such as biosamples, reagents, antibodies, reporters, cell lines, etc.; or could be the types of datasets generated by discovery research (e.g. RNAseq, omic profiles, epigenetic maps, etc). The application is expected to address how the successful completion of this project will provide a research resource useful for the broader research community. In this section, the applicant should also adequately address how the resource will be shared and sustained beyond the funding period of the RC2 as appropriate and consistent with achieving the goals of the program.

All applications, regardless of the amount of direct costs requested for any one year, should address a Data Sharing Plan.

Appendix: Do not use the Appendix to circumvent page limits. Follow all instructions for the Appendix as described in the SF424 (R&R) Application Guide.

PHS Inclusion Enrollment Report

When conducting clinical research, follow all instructions for completing PHS Inclusion Enrollment Report as described in the SF424 (R&R) Application Guide.

PHS Assignment Request Form

All instructions in the SF424 (R&R) Application Guide must be followed.

3. Unique Entity Identifier and System for Award Management (SAM)

See Part 1. Section III.1 for information regarding the requirement for obtaining a unique entity identifier and for completing and maintaining active registrations in System for Award Management (SAM), NATO Commercial and Government Entity (NCAGE) Code (if applicable), eRA Commons, and Grants.gov

4. Submission Dates and Times

Part I. Overview Information contains information about Key Dates and times. Applicants are encouraged to submit applications before the due date to ensure they have time to make any application corrections that might be necessary for successful submission. When a submission date falls on a weekend or Federal holiday, the application deadline is automatically extended to the next business day.

Organizations must submit applications to Grants.gov (the online portal to find and apply for grants across all Federal agencies). Applicants must then complete the submission process by tracking the status of the application in the eRA Commons, NIH's electronic system for grants administration. NIH and Grants.gov systems check the application against many of the application instructions upon submission. Errors must be corrected and a changed/corrected application must be submitted to Grants.gov on or before the application due date and time. If a Changed/Corrected application is submitted after the deadline, the application will be considered late. Applications that miss the due date and time are subjected to the NIH Policy on Late Application Submission.

Applicants are responsible for viewing their application before the due date in the eRA Commons to ensure accurate and successful submission.

Information on the submission process and a definition of on-time submission are provided in the SF424 (R&R) Application Guide.

5. Intergovernmental Review (E.O. 12372)

This initiative is not subject to intergovernmental review.

6. Funding Restrictions

All NIH awards are subject to the terms and conditions, cost principles, and other considerations described in the *NIH Grants Policy Statement*.

Pre-award costs are allowable only as described in the *NIH Grants Policy Statement*.

7. Other Submission Requirements and Information

Applications must be submitted electronically following the instructions described in the SF424 (R&R) Application Guide. Paper applications will not be accepted.

Applicants must complete all required registrations before the application due date. Section III. Eligibility Information contains information about registration.

For assistance with your electronic application or for more information on the electronic submission process, visit Applying Electronically. If you encounter a system issue beyond your control that threatens your ability to complete the submission process on-time, you must follow the Guidelines for Applicants Experiencing System Issues. For assistance with application submission, contact the Application Submission Contacts in Section VII.

Important reminders:

All PD(s)/PI(s) must include their eRA Commons ID in the Credential field of the Senior/Key Person Profile Component of the SF424(R&R) Application Package**.** Failure to register in the Commons and to include a valid PD/PI Commons ID in the credential field will prevent the successful submission of an electronic application to NIH. See Section III of this FOA for information on registration requirements.

The applicant organization must ensure that the DUNS number it provides on the application is the same number used in the organization's profile in the eRA Commons and for the System for Award Management. Additional information may be found in the SF424 (R&R) Application Guide.

See more tips for avoiding common errors.

Upon receipt, applications will be evaluated for completeness and compliance with application instructions by the Center for Scientific Review, NIH. Applications that are incomplete or non-compliant will not be reviewed.

Requests of $500,000 or more for Direct Costs in any year

Applicants requesting $500,000 or more in direct costs in any year (excluding consortium F&A) must submit a written request at least 6 weeks before submitting the application and follow the Policy on the Acceptance for Review of Unsolicited Applications that request $500,000 or More in Direct Costs as described in the SF 424 (R&R) Application Guide.

Although a written, pre-approval request is due at least 6 weeks prior to the application due date, NIDDK strongly encourages investigators to submit the pre-approval request much earlier (at least 3 months, and preferably up to 6 months, before the submission date). Early discussions with program staff and submission of the pre-approval request can significantly aid the investigators in the subsequent development of the application. NIDDK reviews pre-approval requests on a rolling basis and typically will inform investigators within 4 weeks of submission of the pre-approval request whether they will be allowed to submit an application.

The following criteria will be used in the administrative staff review of these requests:

A. Relevance to the NIDDK: Importance of the complex problem or resource to the NIDDK mission.

B. Programmatic priority: Will the proposed research significantly advance the mission of NIDDK?

C. Programmatic balance: How does the proposed research relate to currently funded research in the NIDDK and by the investigative team?

D. Activity Code: Is the proposed work appropriate for the RC2 activity code?

If the NIDDK agrees to accept an application, a cover letter must be included with the application that identifies the NIDDK program staff who agreed to accept assignment of the application to the NIDDK. The NIDDK will also notify the NIH Division of Receipt and Referral of their willingness to accept the application.

Post Submission Materials

Applicants are required to follow the instructions for post-submission materials, as described in NOT-OD-13-030.

Section V. Application Review Information

1. Criteria

Only the review criteria described below will be considered in the review process. As part of the NIH mission, all

applications submitted to the NIH in support of biomedical and behavioral research are evaluated for scientific and technical merit through the NIH peer review system.

Overall Impact

Reviewers will provide an overall impact score to reflect their assessment of the likelihood for the project to exert a sustained, powerful influence on the research field(s) involved, in consideration of the following review criteria and additional review criteria (as applicable for the project proposed).

Scored Review Criteria

Reviewers will consider each of the review criteria below in the determination of scientific merit, and give a separate score for each. An application does not need to be strong in all categories to be judged likely to have major scientific impact. For example, a project that by its nature is not innovative may be essential to advance a field.

Significance

Does the project address an important problem or a critical barrier to progress in the field? Is there a strong scientific premise for the project? If the aims of the project are achieved, how will scientific knowledge, technical capability, and/or clinical practice be improved? How will successful completion of the aims change the concepts, methods, technologies, treatments, services, or preventative interventions that drive this field? Does the application address a significant research topic that fills a gap in the current knowledge?

Investigator(s)

Are the PD(s)/PI(s), collaborators, and other researchers well suited to the project? If Early Stage Investigators or New Investigators, or in the early stages of independent careers, do they have appropriate experience and training? If established, have they demonstrated an ongoing record of accomplishments that have advanced their field(s)? If the project is collaborative or multi-PD/PI, do the investigators have complementary and integrated expertise; are their leadership approach, governance and organizational structure appropriate for the project?

Innovation

Does the application challenge and seek to shift current research or clinical practice paradigms by utilizing novel theoretical concepts, approaches or methodologies, instrumentation, or interventions? Are the concepts, approaches or methodologies, instrumentation, or interventions novel to one field of research or novel in a broad sense? Is a refinement, improvement, or new application of theoretical concepts, approaches or methodologies, instrumentation, or interventions proposed?

Approach

Are the overall strategy, methodology, and analyses well-reasoned and appropriate to accomplish the specific aims of the project? Have the investigators presented strategies to ensure a robust and unbiased approach, as appropriate for the work proposed? Are potential problems, alternative strategies, and benchmarks for success presented? If the project is in the early stages of development, will the strategy establish feasibility and will particularly risky aspects be managed? Have the investigators presented adequate plans to address relevant biological variables, such as sex, for studies in vertebrate animals or human subjects?

If the project involves human subjects and/or NIH-defined clinical research, are the plans to address 1) the protection of human subjects from research risks, and 2) inclusion (or exclusion) of individuals on the basis

of sex/gender, race, and ethnicity, as well as the inclusion or exclusion of children, justified in terms of the scientific goals and research strategy proposed?

Environment

Will the scientific environment in which the work will be done contribute to the probability of success? Are the institutional support, equipment and other physical resources available to the investigators adequate for the project proposed? Will the project benefit from unique features of the scientific environment, subject populations, or collaborative arrangements?

Additional Review Criteria

As applicable for the project proposed, reviewers will evaluate the following additional items while determining scientific and technical merit, and in providing an overall impact score, but will not give separate scores for these items.

Interdisciplinary Team

Has a plan been developed to facilitate the interaction of PD/PIs and key personnel at different sites or institutions? Will data and resources be easily shared with the interdisciplinary team within the collaboration in order to address the application in an integrated, interdisciplinary way as appropriate and consistent with achieving the goals of the program? Does the administrative organization reflect a coordination of ongoing research and establish and maintain internal communication and cooperation among investigators? Are mechanisms to prioritize the usage of shared resources provided?

Research Resource

If the application proposes the generation of a research resource, will successful completion of this project generate a research resource for the broader community? If so, how will it be sustained beyond the funding period?

Protections for Human Subjects

For research that involves human subjects but does not involve one of the six categories of research that are exempt under 45 CFR Part 46, the committee will evaluate the justification for involvement of human subjects and the proposed protections from research risk relating to their participation according to the following five review criteria: 1) risk to subjects, 2) adequacy of protection against risks, 3) potential benefits to the subjects and others, 4) importance of the knowledge to be gained, and 5) data and safety monitoring for clinical trials.

For research that involves human subjects and meets the criteria for one or more of the six categories of research that are exempt under 45 CFR Part 46, the committee will evaluate: 1) the justification for the exemption, 2) human subjects involvement and characteristics, and 3) sources of materials. For additional information on review of the Human Subjects section, please refer to the Guidelines for the Review of Human Subjects.

Inclusion of Women, Minorities, and Children

When the proposed project involves human subjects and/or NIH-defined clinical research, the committee will evaluate the proposed plans for the inclusion (or exclusion) of individuals on the basis of sex/gender, race, and ethnicity, as well as the inclusion (or exclusion) of children to determine if it is justified in terms of the scientific goals and research strategy proposed. For additional information on review of the Inclusion section, please refer to the Guidelines for the Review of Inclusion in Clinical Research.

Vertebrate Animals

The committee will evaluate the involvement of live vertebrate animals as part of the scientific assessment according to the following criteria: (1) description of proposed procedures involving animals, including species, strains, ages, sex, and total number to be used; (2) justifications for the use of animals versus alternative models and for the appropriateness of the species proposed; (3) interventions to minimize discomfort, distress, pain and injury; and (4) justification for euthanasia method if NOT consistent with the AVMA Guidelines for the Euthanasia of Animals. Reviewers will assess the use of chimpanzees as they would any other application proposing the use of vertebrate animals. For additional information on review of the Vertebrate Animals section, please refer to the Worksheet for Review of the Vertebrate Animal Section.

Biohazards

Reviewers will assess whether materials or procedures proposed are potentially hazardous to research personnel and/or the environment, and if needed, determine whether adequate protection is proposed.

Resubmissions

For Resubmissions, the committee will evaluate the application as now presented, taking into consideration the responses to comments from the previous scientific review group and changes made to the project.

Renewals

For Renewals, the committee will consider the progress made in the last funding period.

Revisions

Not Applicable

Additional Review Considerations

As applicable for the project proposed, reviewers will consider each of the following items, but will not give scores for these items, and should not consider them in providing an overall impact score.

Applications from Foreign Organizations

Not Applicable

Select Agent Research

Reviewers will assess the information provided in this section of the application, including 1) the Select Agent(s) to be used in the proposed research, 2) the registration status of all entities where Select Agent(s) will be used, 3) the procedures that will be used to monitor possession use and transfer of Select Agent(s), and 4) plans for appropriate biosafety, biocontainment, and security of the Select Agent(s).

Resource Sharing Plans

Reviewers will comment on whether the following Resource Sharing Plans, or the rationale for not sharing the following types of resources, are reasonable: (1) Data Sharing Plan; (2) Sharing Model Organisms; and (3) Genomic Data Sharing Plan (GDS).

Authentication of Key Biological and/or Chemical Resources:

For projects involving key biological and/or chemical resources, reviewers will comment on the brief plans proposed for identifying and ensuring the validity of those resources.

Budget and Period of Support

Reviewers will consider whether the budget and the requested period of support are fully justified and reasonable in relation to the proposed research.

2. Review and Selection Process

Applications will be evaluated for scientific and technical merit by (an) appropriate Scientific Review Group(s) convened by NIDDK, in accordance with NIH peer review policy and procedures, using the stated review criteria. Assignment to a Scientific Review Group will be shown in the eRA Commons.

As part of the scientific peer review, all applications:

- May undergo a selection process in which only those applications deemed to have the highest scientific and technical merit (generally the top half of applications under review) will be discussed and assigned an overall impact score.
- Will receive a written critique.

Applications will be assigned to the appropriate NIH Institute or Center. Applications will compete for available funds with all other recommended applications . Following initial peer review, recommended applications will receive a second level of review by the National Diabetes and Digestive and Kidney Diseases Advisory Council (NDDKAC). The following will be considered in making funding decisions:

- Scientific and technical merit of the proposed project as determined by scientific peer review.
- Availability of funds.
- Relevance of the proposed project to program priorities.

3. Anticipated Announcement and Award Dates

After the peer review of the application is completed, the PD/PI will be able to access his or her Summary Statement (written critique) via the eRA Commons. Refer to Part 1 for dates for peer review, advisory council review, and earliest start date.

Information regarding the disposition of applications is available in the *NIH Grants Policy Statement*.

Section VI. Award Administration Information

1. Award Notices

If the application is under consideration for funding, NIH will request "just-in-time" information from the applicant as described in the *NIH Grants Policy Statement*.

A formal notification in the form of a Notice of Award (NoA) will be provided to the applicant organization for successful applications. The NoA signed by the grants management officer is the authorizing document and will be sent via email to the grantee's business official.

Awardees must comply with any funding restrictions described in Section IV.5. Funding Restrictions. Selection of an application for award is not an authorization to begin performance. Any costs incurred before receipt of the NoA are at the recipient's risk. These costs may be reimbursed only to the extent considered allowable pre-award costs.

Any application awarded in response to this FOA will be subject to terms and conditions found on the Award

Conditions and Information for NIH Grants website. This includes any recent legislation and policy applicable to awards that is highlighted on this website.

2. Administrative and National Policy Requirements

All NIH grant and cooperative agreement awards include the *NIH Grants Policy Statement* as part of the NoA. For these terms of award, see the *NIH Grants Policy Statement* Part II: Terms and Conditions of NIH Grant Awards, Subpart A: General and Part II: Terms and Conditions of NIH Grant Awards, Subpart B: Terms and Conditions for Specific Types of Grants, Grantees, and Activities. More information is provided at Award Conditions and Information for NIH Grants.

Recipients of federal financial assistance (FFA) from HHS must administer their programs in compliance with federal civil rights law. This means that recipients of HHS funds must ensure equal access to their programs without regard to a person's race, color, national origin, disability, age and, in some circumstances, sex and religion. This includes ensuring your programs are accessible to persons with limited English proficiency. HHS recognizes that research projects are often limited in scope for many reasons that are nondiscriminatory, such as the principal investigator's scientific interest, funding limitations, recruitment requirements, and other considerations. Thus, criteria in research protocols that target or exclude certain populations are warranted where nondiscriminatory justifications establish that such criteria are appropriate with respect to the health or safety of the subjects, the scientific study design, or the purpose of the research.

For additional guidance regarding how the provisions apply to NIH grant programs, please contact the Scientific/Research Contact that is identified in Section VII under Agency Contacts of this FOA. HHS provides general guidance to recipients of FFA on meeting their legal obligation to take reasonable steps to provide meaningful access to their programs by persons with limited English proficiency. Please see http://www.hhs.gov/ocr/civilrights/resources/laws/revisedlep.html. The HHS Office for Civil Rights also provides guidance on complying with civil rights laws enforced by HHS. Please see http://www.hhs.gov/ocr/civilrights/understanding/section1557/index.html; and http://www.hhs.gov/ocr/civilrights/understanding/index.html. Recipients of FFA also have specific legal obligations for serving qualified individuals with disabilities. Please see http://www.hhs.gov/ocr/civilrights/understanding/disability/index.html. Please contact the HHS Office for Civil Rights for more information about obligations and prohibitions under federal civil rights laws at http://www.hhs.gov/ocr/office/about/rgn-hqaddresses.html or call 1-800-368-1019 or TDD 1-800-537-7697. Also note it is an HHS Departmental goal to ensure access to quality, culturally competent care, including long-term services and supports, for vulnerable populations. For further guidance on providing culturally and linguistically appropriate services, recipients should review the National Standards for Culturally and Linguistically Appropriate Services in Health and Health Care at http://minorityhealth.hhs.gov/omh/browse.aspx?lvl=2&lvlid=53.

Cooperative Agreement Terms and Conditions of Award

Not Applicable

3. Reporting

When multiple years are involved, awardees will be required to submit the Research Performance Progress Report (RPPR) annually and financial statements as required in the NIH Grants Policy Statement.

A final progress report, invention statement, and the expenditure data portion of the Federal Financial Report are required for closeout of an award, as described in the *NIH Grants Policy Statement*.

The Federal Funding Accountability and Transparency Act of 2006 (Transparency Act), includes a requirement for awardees of Federal grants to report information about first-tier subawards and executive compensation under Federal assistance awards issued in FY2011 or later. All awardees of applicable NIH grants and

cooperative agreements are required to report to the Federal Subaward Reporting System (FSRS) available at www.fsrs.gov on all subawards over $25,000. See the *NIH Grants Policy Statement* for additional information on this reporting requirement.

Section VII. Agency Contacts

We encourage inquiries concerning this funding opportunity and welcome the opportunity to answer questions from potential applicants.

Application Submission Contacts

eRA Service Desk (Questions regarding ASSIST, eRA Commons registration, submitting and tracking an application, documenting system problems that threaten submission by the due date, post submission issues)
Finding Help Online: http://grants.nih.gov/support/ (preferred method of contact)
Telephone: 301-402-7469 or 866-504-9552 (Toll Free)

Grants.gov Customer Support (Questions regarding Grants.gov registration and submission, downloading forms and application packages)
Contact CenterTelephone: 800-518-4726
Web ticketing system: https://grants-portal.psc.gov/ContactUs.aspx
Email: support@grants.gov

GrantsInfo (Questions regarding application instructions and process, finding NIH grant resources)
Email: GrantsInfo@nih.gov (preferred method of contact)
Telephone: 301-435-0714

Scientific/Research Contact(s)
Corinne M. Silva, Ph.D.
Division of Diabetes, Endocrinology and Metabolic Diseases
National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)
Telephone: 301-451-7335
Email: silvacm@mail.nih.gov

Peter J. Perrin, Ph.D.
Division of Digestive Diseases and Nutrition
National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)
Telephone: 301-451-3759
Email: Peter.Perrin@nih.gov

Chris Mullins, Ph.D.
Division of Kidney, Urologic and Hematologic Diseases
National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)
Telephone: 301-451-4902
Email: mullinsC@extra.niddk.nih.gov

Peer Review Contact(s)

Michele L. Barnard, Ph.D.
National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)
Telephone: 301-594-8898
Email: barnardm@extra.niddk.nih.gov

Financial/Grants Management Contact(s)

Sharon Bourque
National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)
Telephone: 301-594-8846
Email: bourques@extra.niddk.nih.gov

Section VIII. Other Information

Recently issued trans-NIH policy notices may affect your application submission. A full list of policy notices published by NIH is provided in the *NIH Guide for Grants and Contracts*. All awards are subject to the terms and conditions, cost principles, and other considerations described in the NIH Grants Policy Statement.

Authority and Regulations

Awards are made under the authorization of Sections 301 and 405 of the Public Health Service Act as amended (42 USC 241 and 284) and under Federal Regulations 42 CFR Part 52 and 45 CFR Part 75.

Weekly TOC for this Announcement
NIH Funding Opportunities and Notices

Department of Health
and Human Services (HHS)

NIH... Turning Discovery Into Health®

Note: For help accessing PDF, RTF, MS Word, Excel, PowerPoint, Audio or Video files, see Help Downloading Files.

National Institute of Diabetes and Digestive and Kidney Diseases

Collaborative Interdisciplinary Team Science in NIDDK Research Areas

Corinne M. Silva, Ph.D

Program Director

DEM/NIDDK

National Institute of Diabetes and Digestive and Kidney Diseases

Collaborative Grant Mechanisms

Program Project Grant (P01)

PAR-16-127

High Impact, Interdisciplinary Science (RC2)

PAR-16-126

Multiple-Principal Investigator Initiated Research Project (Multi-PI R01)

PAR-13-302

Scientific Scope

- P01: multiple projects (at least 3) and cores (at least 1) broadly and *synergistically* address a central theme

- Multi-PI R01: focused project to address an overall hypothesis

(*supporting preliminary data is essential*)

RC2 Scientific Scope (1)

- Single project using an interdisciplinary team approach
- generate research resource for community; discovery-based; hypothesis-generating
- accelerate critical breakthroughs in biomedical research relevant to NIDDK

(*preliminary data is not required*)

RC2 Scientific Scope (2)

- novel approaches in specific knowledge gaps, scientific opportunities, new technologies, data generation, or research methods

- sharing of data and other resources to further advance research in this area

Scientific Scope Comparison

P01 or Multi-PI R01

New studies or extensions of current or previous research efforts; hypothesis-addressing

RC2

High impact ideas that may lay the foundation for new fields of research; groundbreaking, high impact, cross-cutting; hypothesis-generating

Principal Investigators (1)

Multi-PI R01

- Prior collaboration not required
- But prior successful collaboration would strengthen the study
- Complementary and integrated expertise
- Multi-PI plan required-leadership approach, governance and organizational structure

Principal Investigators (2)

- P01: Prior collaboration not required but prior successful collaboration would strengthen the study; ability to manage large, interdisciplinary project

- RC2: Prior collaboration not required but the interdisciplinary team must provide an plan for working together that will enhance integration and collaboration

Administrative Considerations

	Program Project Grant (P01)*	Multiple-Principal Investigator Initiated Research Project (Multi-PI R01)	High Impact, Interdisciplinary* Science (RC2)
Typical Budget (Direct Costs per Year)	$1 million. Capped at $6.25 million over five years	Up to $499,999. Higher budgets may be requested with NIDDK approval	Usually greater than $500K. Budgets vary and have no cap
Required Pre-Approval	Applicants requesting $500K or more in Direct Costs must contact NIDDK Program Staff at least 6 weeks prior to submission	Applicants requesting $500K or more in Direct Costs must contact NIDDK Program Staff at least 6 weeks prior to submission	Applicants requesting $500K or more in Direct Costs must contact NIDDK Program Staff at least 6 weeks prior to submission
Recommended Pre-Approval	Applicants are strongly encouraged to discuss proposed studies with NIDDK staff and generate the Pre-Approval letter at least 3 months, and preferably 6 months, before submission	Applicants are always encouraged to develop studies in consultation with the NIDDK. However, there is no formal recommended timeline for these discuss	Applicants are strongly encouraged to discuss proposed studies with NIDDK staff and generate the Pre-Approval letter at least 3 months, and preferably 6 months, before submission
Average Number of Investigators	4 to 5	2 to 3	3 to 4
Application Review	NIDDK Special Emphasis Panel	Center for Scientific Review	NIDDK Special Emphasis Panel

* 10 year funding limit

* 10 year funding limit

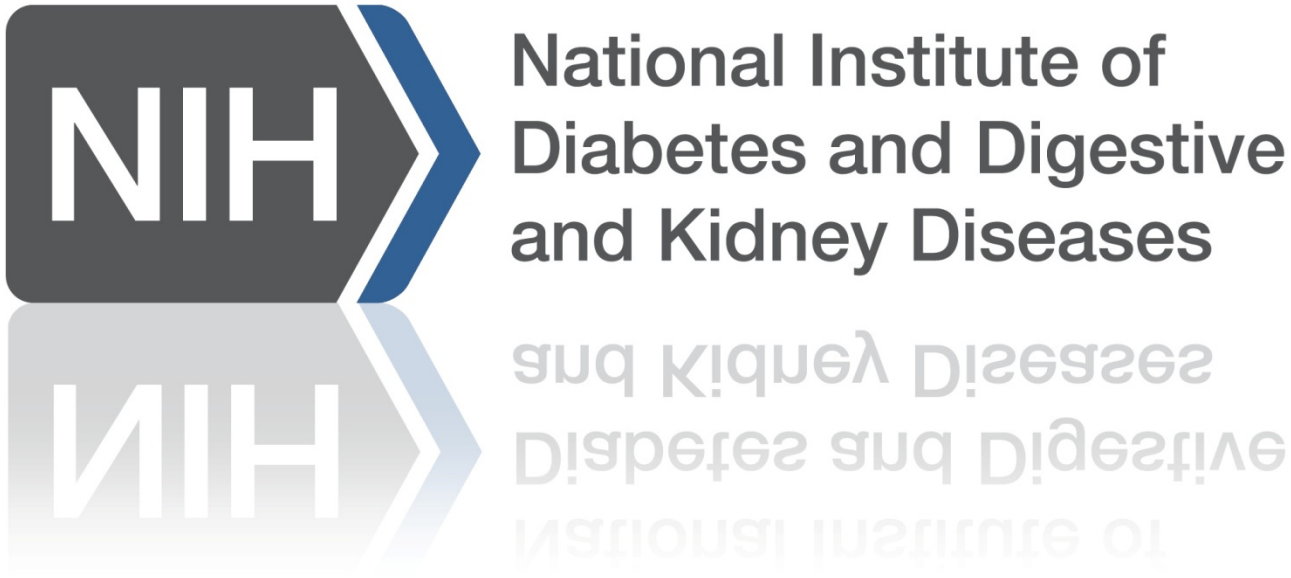
Preapproval Process is Key

- Relevance to the NIDDK: Importance of the complex problem or resource to the NIDDK mission.
- Programmatic priority: Will the proposed research significantly advance the mission of NIDDK?
- Programmatic balance: How does the proposed research relate to currently funded research in NIDDK and by the investigative team?
- Activity Code: Is the proposed work appropriate for the RC2 or P01 activity code?

www.NIDDK.NIH.GOV

NIDDK
> Research & Funding for Scientists
> Funding Process
> Apply
> About Funding Mechanisms
> NIDDK Collaborative Grants Comparison

QUESTIONS?


NIH
National Institute of
Diabetes and Digestive
and Kidney Diseases

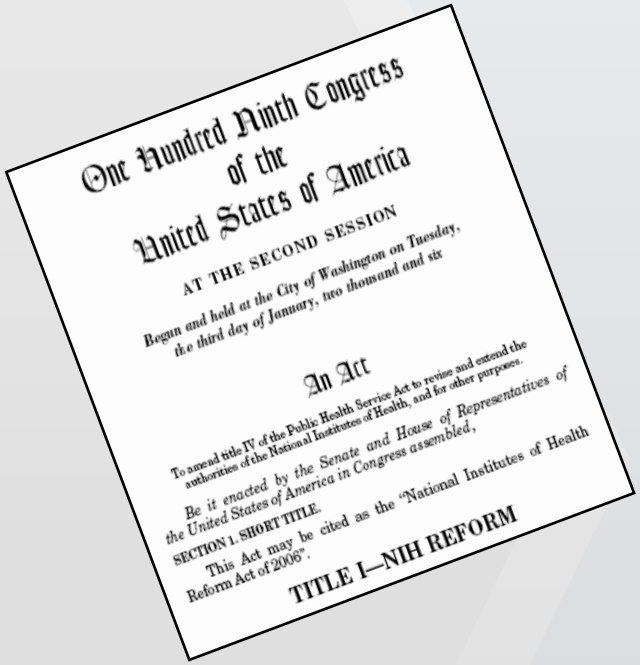
NIH Common Fund Metabolomics Program

Art Castle, PhD
DEM, NIDDK

March 30, 2016

National Institutes of Health
Office of Strategic Coordination - The Common Fund

The NIH Common Fund: A Different Approach to Science Management



One Hundred Ninth Congress
of the
United States of America

AT THE SECOND SESSION

Begun and held at the City of Washington on Tuesday, the third day of January, two thousand and six

An Act

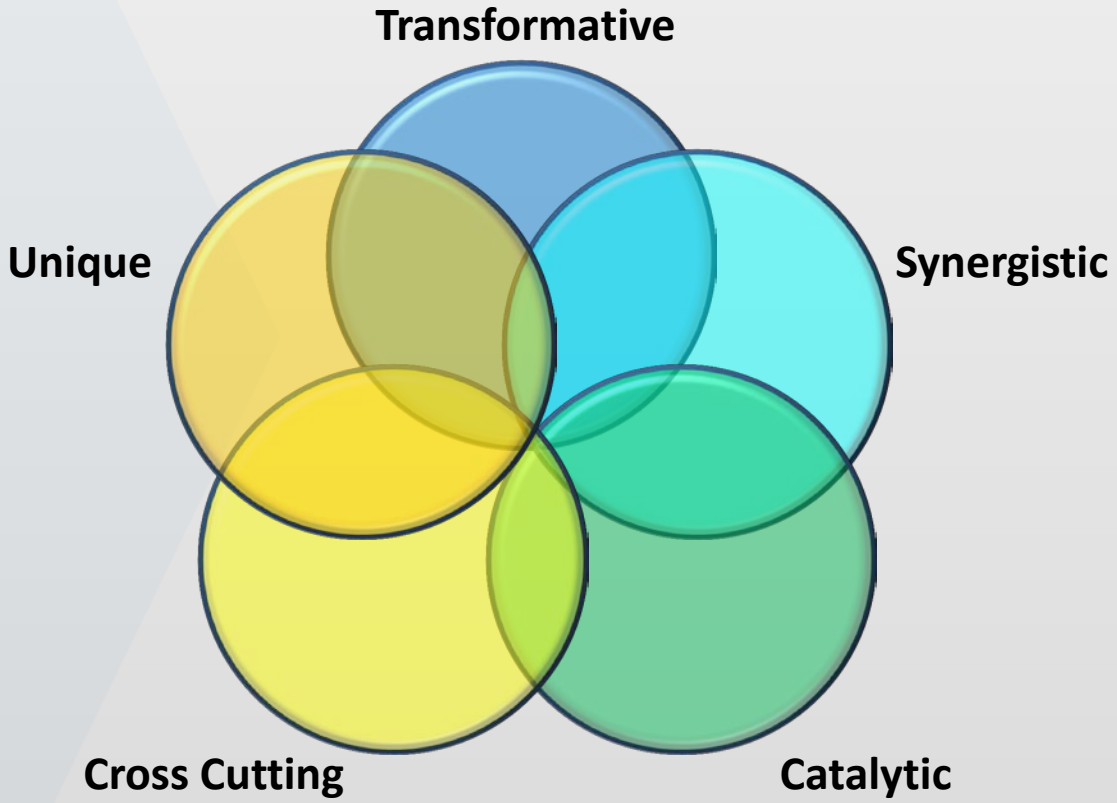
To amend title IV of the Public Health Service Act to revise and extend the authorities of the National Institutes of Health, and for other purposes.

Be it enacted by the Senate and House of Representatives of the United States of America in Congress assembled,

SECTION 1. SHORT TITLE.

This Act may be cited as the "National Institutes of Health Reform Act of 2006".

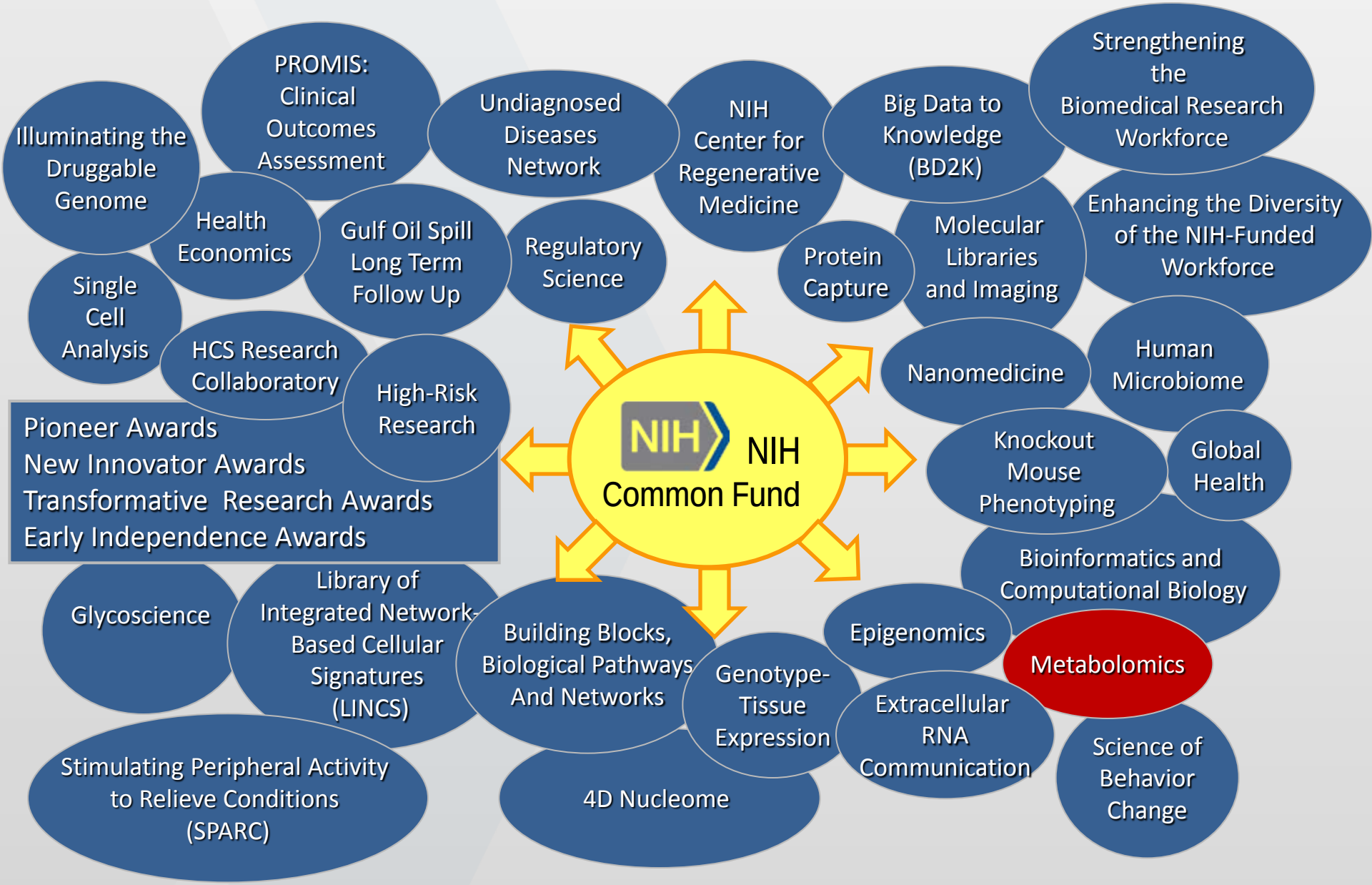
TITLE I—NIH REFORM



- **Exceptionally high impact**
- **Broadly applicable**
- **Benefit from strategic planning and coordination**

Managed by the Office of Strategic Coordination in the Office of the Director, NIH

Current Common Fund Programs



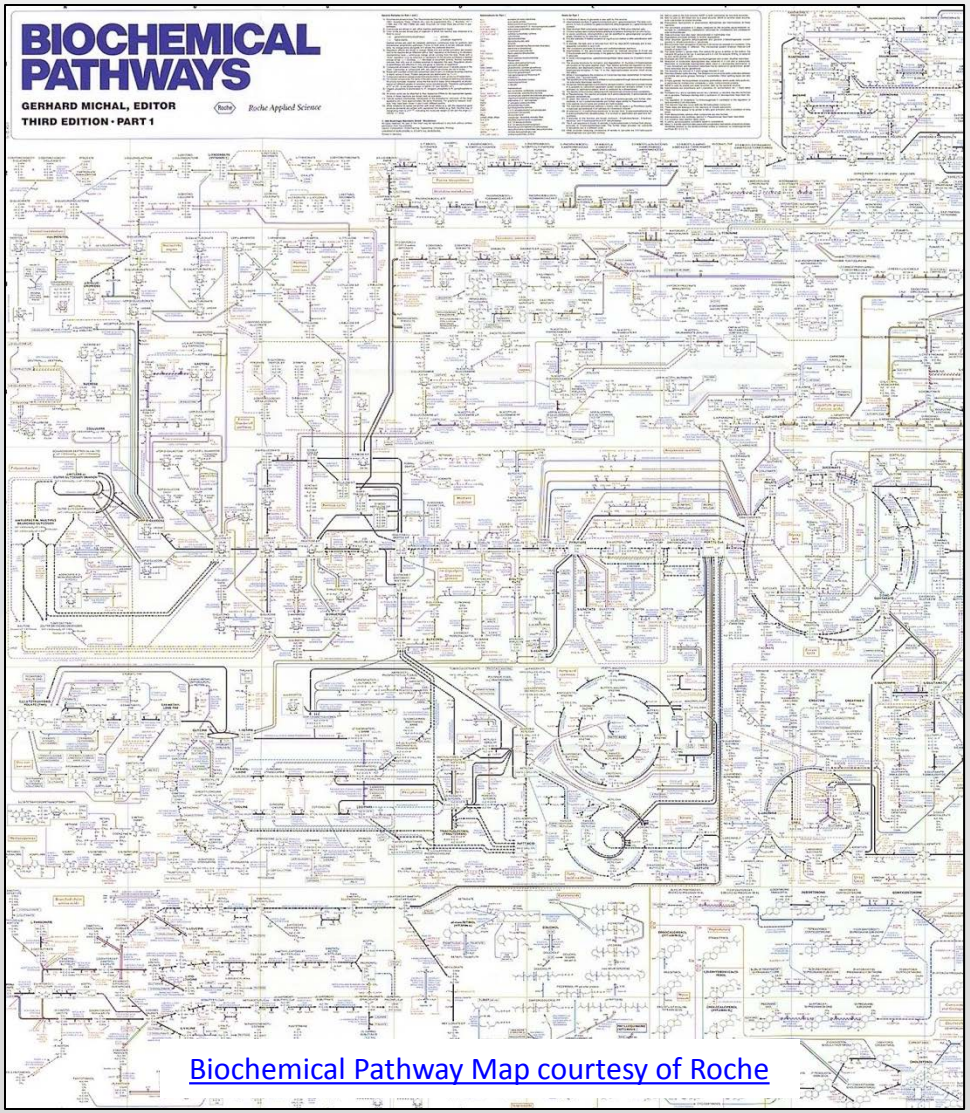
An Introduction to Metabolomics

Metabolomics: study of all the small molecules produced or consumed during the chemical reactions that sustain life.

Metabolome: sum of all metabolites at any given moment; chemical readout of the functional state of an organism.

Major Technologies: MS, NMR

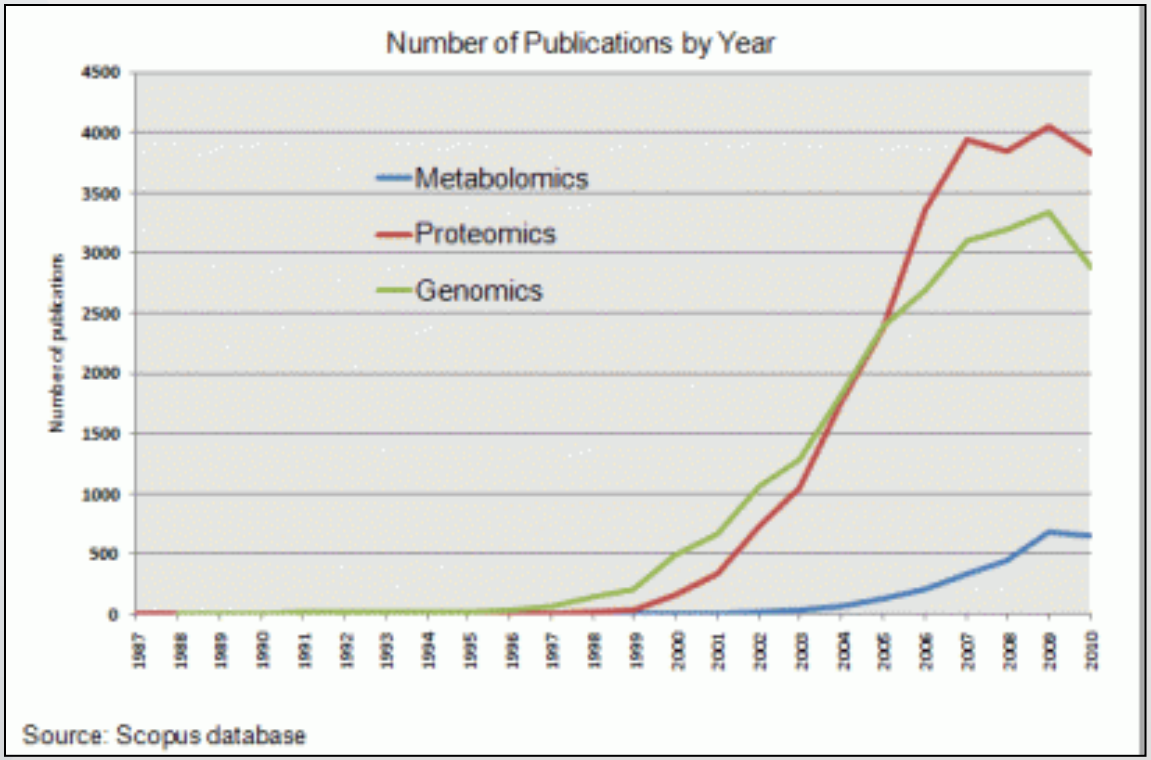
Biological samples: cells, tissues, biofluids



Biochemical Pathway Map courtesy of Roche

Metabolomics: Meeting Common Fund Criteria

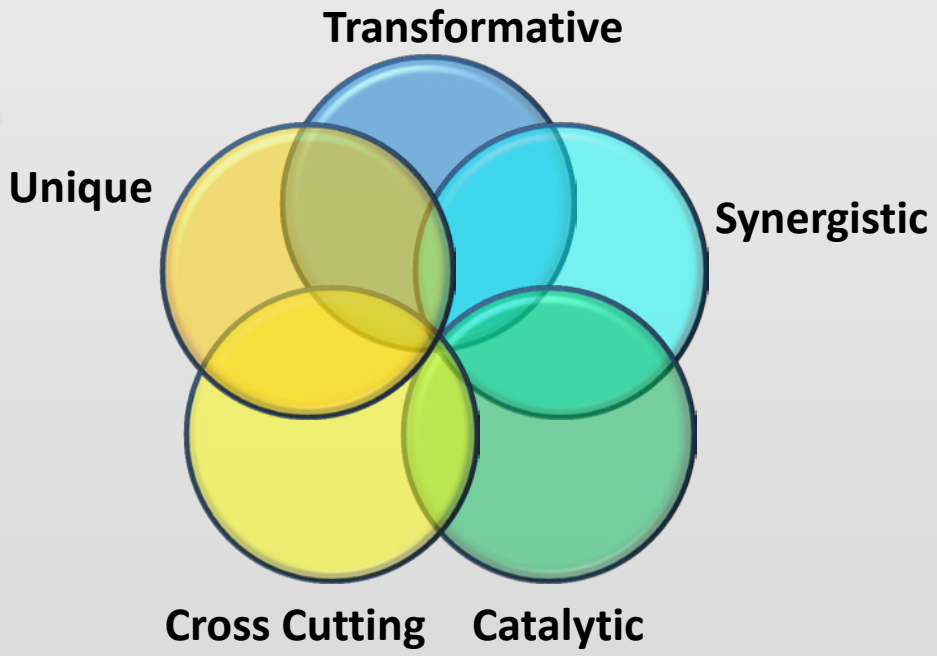
- High potential to contribute greatly to our knowledge of health and disease.
- Applicable to basic, clinical, and translational research.
- Trans-NIH relevance.
- Likely to benefit from strategic coordination.



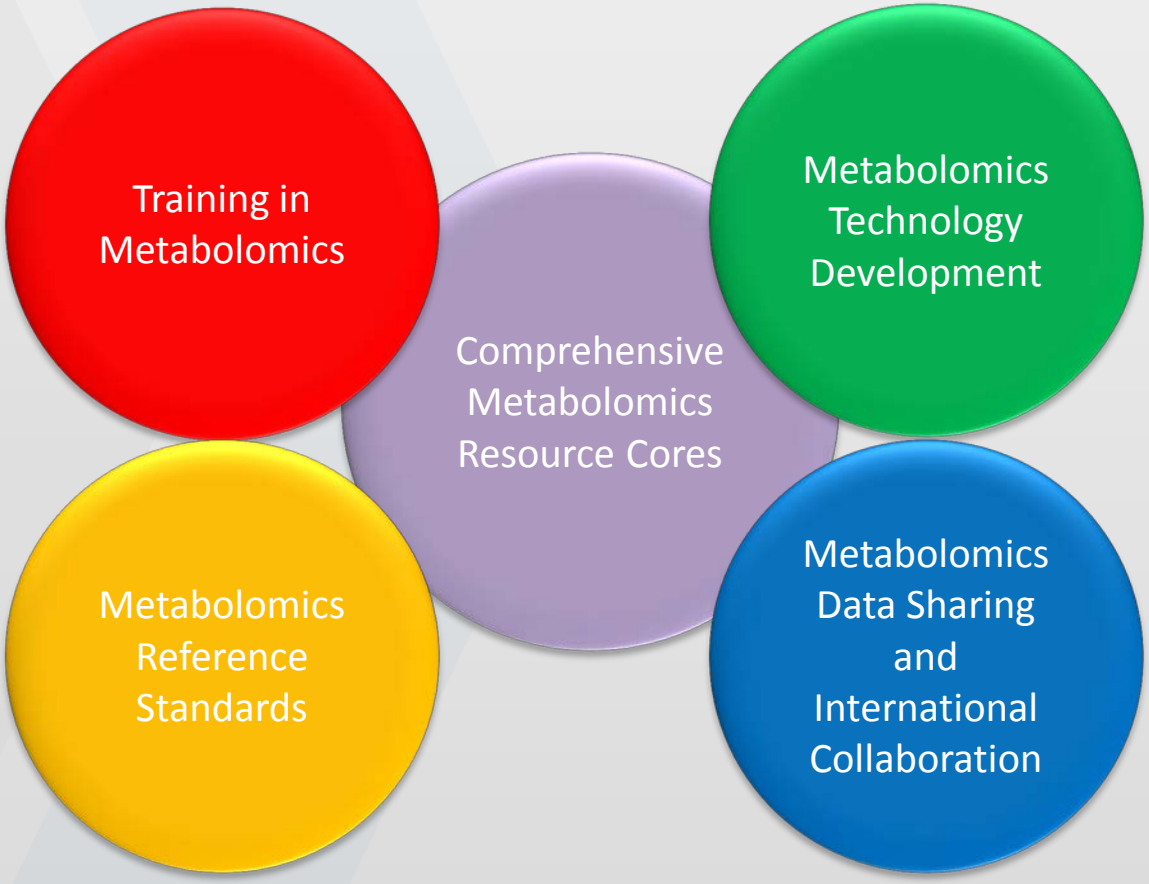
Rationale for the Metabolomics Program

The Common Fund Metabolomics Program was initiated in 2012 **to increase the national capacity in metabolomics** by developing:

- **New Tools**
- **Infrastructure**
- **Data**
- **Training**



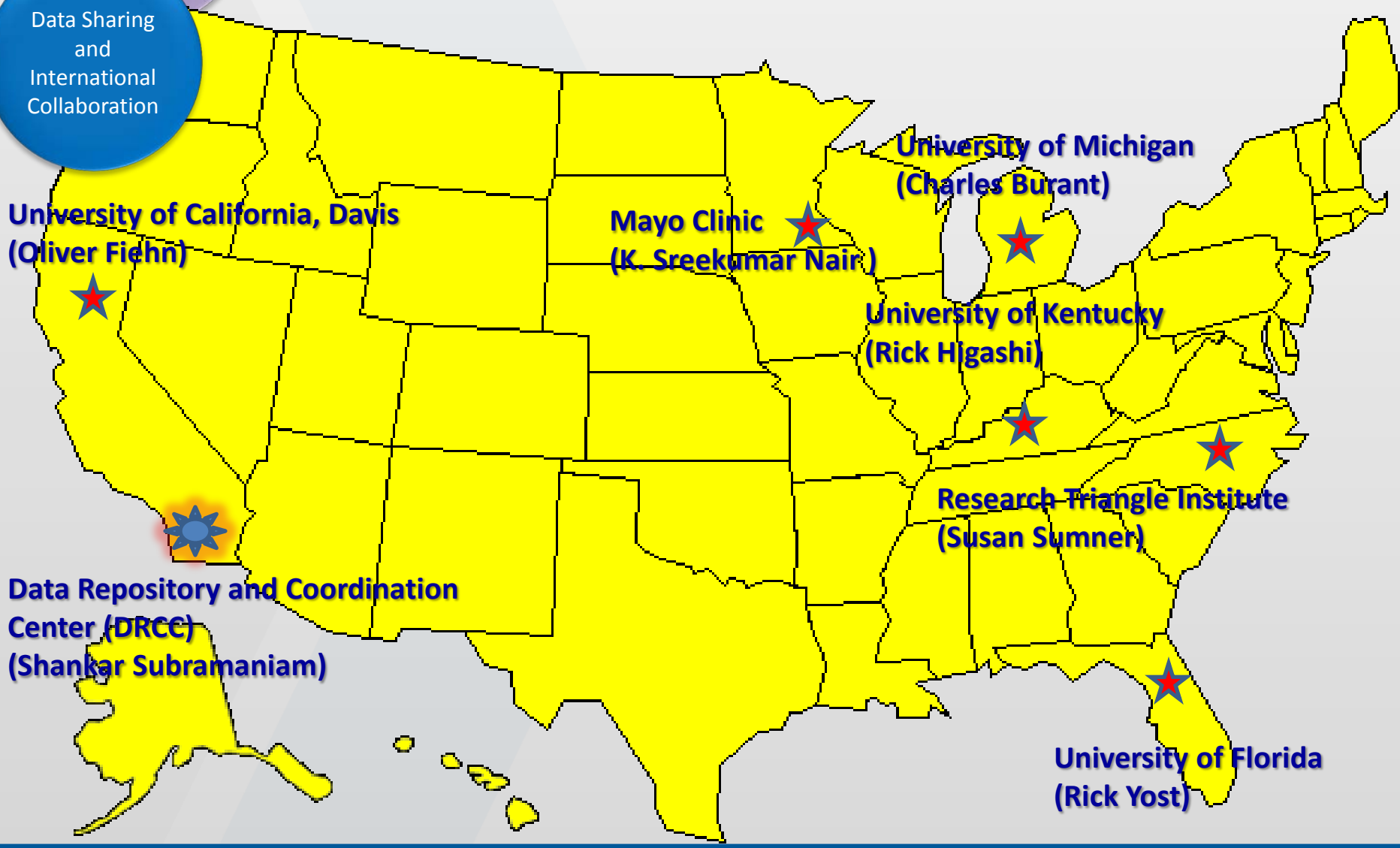
Metabolomics Program Activities



Metabolomics Resource Cores

Data Sharing and International Collaboration

Funded RCMRCs and DRCC



Metabolomics Resource Cores

Goals of the Regional Comprehensive Metabolomics Resource Cores (RCMRCs)

- **Provide metabolomics services for the research community**
  - Fee-for-Service (at cost) Support
  - Pilot and Feasibility (P&F) Program
- **Technology development**
  - Data generation and analysis
- **Training**
  - Workshops
  - Symposia
- **Work as a consortium to enhance the field**
- **Submit data to DRCC**

Data Sharing and International Collaboration

- Data repository with >200 metabolomics studies from a wide variety of organisms.
- Online browsing, searching, plotting and statistical analysis tools.
- Searchable Metabolite Database covering over 60,000 structures.
- General and study-specific experimental protocols available for download.
- Metabolite standards nomination and request.
- Metabolomics training materials and information.

http://www.metabolomicsworkbench.org

Reference Standards

Metabolite Standards Synthesis Core

- **High-quality metabolite standards** are synthesized under contracts to SRI International and RTI International.
- Provides metabolomics researchers with high quality metabolite standards that have high potential in translational research at no cost.
- Nominate a compound or request an aliquot today!

View the list of nominated compounds or nominate new compounds at:

http://www.metabolomicsworkbench.org/standards/index.html

Training

Metabolomics Training

Metabolomics Training ranges from introductory training for researchers new to the field to advanced training for metabolomics experts in emergent approaches.

- University of Alabama Annual Workshop on Metabolomics
- On-line course Metabolomics in Medicine
- RCMRC workshops and symposia
- Videos and on-line training material on Metabolomics Workbench
- Hand-on research training:
  - 10 early career awards (K01)
  - P&F Program
  - Administrative Supplement Program

http://metabolomicsinmedicine.org

Training

Administrative Supplements to Existing NIH Grants

- PIs of eligible grants from any NIH IC can apply for additional funding to support metabolomics analyses within the scope of the specific aims.
- Must form highly interactive collaboration with a metabolomics expert.
- Proposal must include training component to help increase the number of investigators trained in metabolomics.
- If PI is not new to metabolomics, must be proposing to learn a substantially different sub-field of metabolomics.
- 12 month timeframe; $100K direct cost cap

http://grants.nih.gov/grants/guide/pa-files/PA-16-005.html

Last Due Date Feb 15, 2016 ; Feb 2017 if reissued

Summary of Resources Available through Metabolomics Workbench

- **Metabolomics data** from Program consortium members and international sources.
- **Protocols**, **analytical tools**, searchable **metabolite database**
- **Access to RCMRCs**: metabolomics services, Pilot & Feasibility programs, training.
- **Training materials**: hands-on and on-line training courses, videos, information on workshops and symposia.
- **Metabolite standards** request and nomination forms

Common Fund Metabolomics Program Working Group Leadership

Working Group Co-Chairs:

Phil Smith (NIDDK), Dinah Singer (NCI)

Working Group Coordinators:

Barbara Spalholz (NCI), Arthur Castle (NIDDK)

Common Fund Program Leader: Leslie Derr (OD)

Additional Members of Core Leadership Team:

Keren Witkin (NCI), Padma Maruvada (NIDDK), David Balshaw (NIEHS), Richard Okita (NIGMS), Pothur Srinivas (NHLBI), Laurie Nadler (NIMH), Mukesh Verma (NCI), Katrina Gwinn (NINDS)

Program Website: https://commonfund.nih.gov/metabolomics/index

DIABETES RESEARCH CENTERS

2016 Institutional Diabetes Center Websites

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

Baltimore Area (JHU/UMD): http://www.hopkinsmedicine.org/drtc/index.html

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

Columbia University: http://cumc.columbia.edu/derc/

Indiana University: http://cdmd.indiana.edu/

Joslin Diabetes Center: http://www.joslin.org/diabetes-research/DRC-core-labs.html

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

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

UCSF: http://diabetes.ucsf.edu/DERC

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/derc/

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

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

Washington University in St. Louis: http://diabetesresearchcenter.dom.wustl.edu/index.htm

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

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 2017

- **RFA-DK-15-026:** Published October 6, 2015 (electronic applications; ASSIST)
- Application deadline: June 14, 2016
- Initial Review: October/November 2016
- Earliest Funding: April 1, 2017 (FY2017)
- Renewal Applications:
  - Joslin Diabetes Center
  - University of Pennsylvania
  - Vanderbilt University

NIH National Institute of Diabetes and Digestive and Kidney Diseases

Fiscal Year 2018

- **RFA**: Hope to publish by early summer 2016
- Application deadline: 1st quarter 2017
- Renewal Applications:
  - Columbia University
  - Johns Hopkins University & University of Maryland
  - University of Alabama at Birmingham
  - University of California, San Diego & UCLA
  - University of Chicago
  - University of Michigan
  - University of Washington
  - Washington University in St. Louis
  - Yale University

Next NIDDK Diabetes Research Center Directors' Meeting

- Timing: after FY2018 awards are made
- Primary Focus: Presentations from Centers—innovative aspects of Center Cores, partnerships, etc.

National Institute of
Diabetes and Digestive
and Kidney Diseases

National Institute of Diabetes and Digestive and Kidney Diseases

Submitting Electronic NIH Grant Applications: Annual Progress Reports

National Institute of Diabetes and Digestive and Kidney Diseases

Submitting Electronic Diabetes Research Center (P30) Progress Reports

- RPPR instructions updated frequently (latest update 1/25/2016)

http://grants.nih.gov/grants/rppr/rppr_instruction_guide.pdf

- Supplemental instructions: 7.6 Multi-Project RPPRs and Single-Project RPPRs with Complicated Structure

- Jim will send updated RPPR template as a guide (summer 2016)

Multi-Project RPPRs

- Multi-Project RPPRs
  - The RPPR will include all activity codes that have been coded to have multiple components.
  - Multi-project (e.g., P01, P30)
    - Projects with more than one component
- RPPR structure
  - RPPR questions at overall project level
  - RPPR questions at the component level
    - Questions not applicable to component
      - E.g., Publications, Websites, Inventions, Participants, Inclusion/Enrollment, Unobligated Balance

Public Access Policy

- NIH will delay processing Type 5s that are not compliant with the public access policy.
- Bringing papers into compliance:
  - Use My NCBI's My Bibliography
  - Process may take several weeks
- PD/PIs must collaborate with project leads and supported authors to ensure all papers are in My Bibliography and thus linked to the RPPR.
- For additional resources: see http://publicaccess.nih.gov/index.htm

Common Issues with P30 RPPRs

- Other support for key personnel not included in RPPR
- Other support exceeds 12 calendar months for key personnel
- Biographical sketch not included for new key personnel
- IACUC and/or IRB approvals not submitted
- Budget: P30 grants may not support graduate student or postdoctoral fellows
- Core Directors must have a minimum of 0.6 calendar months devoted to the Core
- Centers Directors must have a minimum of 2.4 calendar months devoted to the P30 grant (may be spread over P30 components)
- Enrollment forms for human subjects involved in P&F projects or electronic Inclusion Data Records for those P30 grants now using the Inclusion Management System

Changes to NIH Applications Instructions and Forms

- Changes are effective starting with application deadlines on May 25, 2016 and beyond.
- Significant changes webpage: http://grants.nih.gov/grants/how-to-apply-application-guide/forms-d/general/g.120-significant-changes.htm
- Updated SF424 application instructions for Multi-project applications (e.g. P30): http://grants.nih.gov/grants/how-to-apply-application-guide/forms-d/multi-project-forms-d.pdf

Examples of Some Applications Changes

- Separate attachment for “Data and Safety Monitoring Plan”
- New “Authentication of Key Biological and/or Chemical Resources” attachment
- New Vertebrate Animal section added to Cover Page:
  - Are animals euthanized? Yes/No
  - If Yes, is method consistent with AVMA guidelines? Yes/No
  - If No to AVMA guidelines, describe method/provide scientific justification
- Vertebrate Animal section now has 3 points:
  - Description of Procedures (describe animals and proposed use)
  - Justifications (e.g. choice of species; alternate models)
  - Minimization of Pain and Distress
  - Description of veterinary care is no longer required
  - Justification of the number of animals has been eliminated

Delayed-Onset Human Subjects Research

Scenario D. Delayed-Onset Human Subjects Research

If human subjects research is anticipated within the period of the award but plans for involvement of human subjects cannot be described in the application as allowed by the HHS regulations (45 CFR part 46.118), you will have designated "Yes" to Human Subjects Research on the PHS 398 face page (or you will have designated **Yes** in response to "Are Human Subjects Involved?" on the SF424 (R&R) Other Project Information Form and entered your OHRP assurance number). In the section on Protection of Human Subjects in the Research Plan (or on the PHS Fellowship Supplemental Form for Fellowship applicants), you should either include an explanation of anticipated protections for human subjects or an explanation of why protections cannot be described.

Examples of delayed-onset of human subjects research include:

- Human subjects research is dependent upon the completion of animal or other studies; or
- Human subjects research protocols to be included will be determined at a later time after award; (often defined by a FOA).

See instructions for Scenario D.

National Institute of
Diabetes and Digestive
and Kidney Diseases

National Institute of Diabetes and Digestive and Kidney Diseases

NIDDK Diabetes Research Centers Website Update

National Institute of Diabetes and Digestive and Kidney Diseases

Diabetes Research Centers

IN THE SPOTLIGHT

Rudolph L. Leibel

Dr. Leibel has worked in obesity research for over 25 years. His initial research efforts were focused on adrenergic receptor-mediated effects on lipolysis, and on the control of fatty acid re-esterification in human adipose tissue. He was among the first i.. MORE

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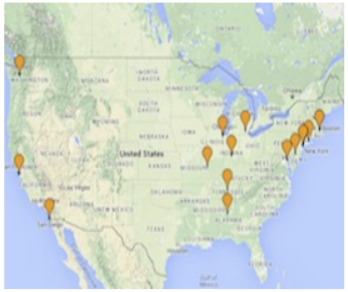
Diabetes Centers Overview



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Publication in the Spotlight

University of Alabama at Birmingham

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Diabetes , 2014

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Study in the Spotlight

Johns Hopkins University/ University of Maryland

Thomas O. Obisesan, M.D.

2013 P&F Awardee

The Role of Cardiovascular DIsease Risk in Memory and Alzheimer's diseaseRole of Lipolysis in Intermittent-Hypoxia Induced Insulin Resistance

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- Small Grants for New Investigators to Promote

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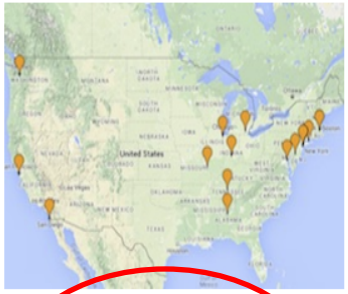

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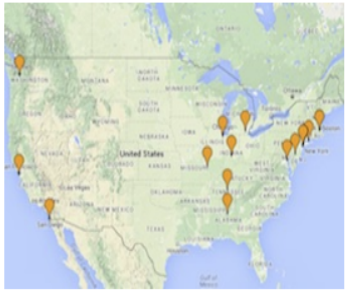

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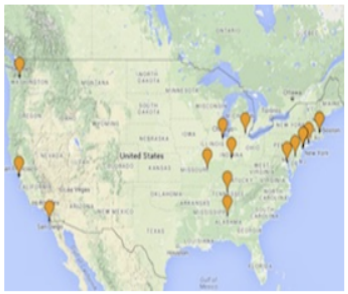

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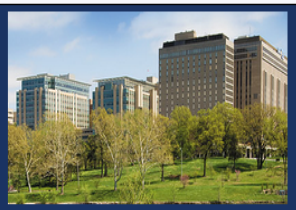
IN THE SPOTLIGHT

Kevin Yarasheski, Ph.D.

ski received his Ph.D. from Kent State University in 1986, and completed a arch fellowship in Metabolism, Endocrinology and Geriatrics at Washington of Medicine under Dennis Bier, M.D. (1986-89). He recei.. MORE

Washington University



The mission of the Washington University School of Medicine (WUMS) Diabetes Research Center (DRC) is to support and enhance research in diabetes and related metabolic diseases through expert Biomedical Research Cores, a vibrant Pilot & Feasibility Program a... MORE

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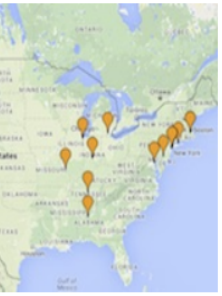

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Centers In The News

- PENN: Guenther H. Boden,

- new investigators
- established investigators new to diabetes research
- established diabetes investigators pursuing high impact/high risk projects or projects that are a significant departure from their usual work

Individual Center P&F programs may also be structured to provide support for establishing interdisciplinary collaborations and to help forge new partnerships between basic scientists and clinical researchers.

Study In the Spotlight

UCSD/UCLA
Wenxian Fu, PhD

2014 P&F Awardee

Identifying the Initiating Factors in Type 1 Diabetes by Disclosing the Crosstalk among Islet Vascular Inflammation, Immune Responses and Beta Cell Death

Partnered with UCSD CTRI and UCSD Dean's Research Award.

Boston Area
Jae Hyuang Chang, MD

2014 P&F Awardee

Role of proximal tubule injury in the pathogenesis of diabetic nephropathy

Diabetic nephropathy (DN) has been traditionally considered as a primary glomerular disease. We suggest that the kidney tubule plays a primary role in the development of DN, and that subsequent tubulointerstitial injury may lead to glomerular changes in diabetes.We will investigate if selective proximal tubule injury alone can accelerate diabetic kidney disease in a genetic model of type 1 diabetes (Akita mice). For these studies, we have crossed a mouse containing the tubule-specific Six2-Cre allele with a mouse transgenic for a Cre-inducible simian diphtheria toxin receptor (iDTR). Six2-Cre...

For more information about Pilot and Feasibility opportunities in the NIDDK Diabetes Center program, please visit the following individual center P&F websites:

Diabetes Research Centers Pilot and Feasibility	
Albert Einstein College of Medicine	University of Alabama at Birmingham
Boston Area	University of Chicago
Columbia University	University of Michigan
Indiana Diabetes Research Center	University of Pennsylvania
Johns Hopkins/University of Maryland	University of Washington
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UCSD/UCLA	Washington University
UCSF	Yale University

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Funding Opportunities

- Small Grants for New Investigators to Promote Diversity in Health-Related Research (R21)
- Diabetes Research Centers (P30)
- Clinical, Behavioral and

Diabetes Research Centers Website

- P&F reviewers in database (2016): 1,439
- Researcher In the Spotlight (Center investigators): 211
- Study in the Spotlight (P&F Awardees): 56 (2014); 113 (2016)
- We presently have ~ 300 Center personnel listed on website.
- Banner Images: Send images to Jim or Jodee Allen

Diabetes Research Centers Website

- Plan to accrue P&F awardees across the Diabetes Research Centers program, and will create a sortable (by Center) listing of P&F awardee information

- Only information posted on individual Center websites (i.e. is publically available) will be used on central website

- Information will first be used as a P&F Awardee in the Spotlight (Study in the Spotlight), and then 'archived' as part of sortable listing

National Institute of
Diabetes and Digestive
and Kidney Diseases

nature COMMUNICATIONS

ARTICLE

Received 8 Dec 2014 | Accepted 20 Feb 2015 | Published 7 Apr 2015

DOI: 10.1038/ncomms7704

Metabolic learning and memory formation by the brain influence systemic metabolic homeostasis

Yumin Zhang[1,2,*], Gang Liu[1,*], Jingqi Yan[1], Yalin Zhang[1], Bo Li[1] & Dongsheng Cai[1]

Metabolic homeostasis is regulated by the brain, but whether this regulation involves learning and memory of metabolic information remains unexplored. Here we use a calorie-based, taste-independent learning/memory paradigm to show that *Drosophila* form metabolic memories that help in balancing food choice with caloric intake; however, this metabolic learning or memory is lost under chronic high-calorie feeding. We show that loss of individual learning/memory-regulating genes causes a metabolic learning defect, leading to elevated trehalose and lipid levels. Importantly, this function of metabolic learning requires not only the mushroom body but also the hypothalamus-like pars intercerebralis, while NF-κB activation in the pars intercerebralis mimics chronic overnutrition in that it causes metabolic learning impairment and disorders. Finally, we evaluate this concept of metabolic learning/memory in mice, suggesting that the hypothalamus is involved in a form of nutritional learning and memory, which is critical for determining resistance or susceptibility to obesity. In conclusion, our data indicate that the brain, and potentially the hypothalamus, direct metabolic learning and the formation of memories, which contribute to the control of systemic metabolic homeostasis.

[1] Department of Molecular Pharmacology, Diabetes Research Center, Institute of Aging, Albert Einstein College of Medicine, Bronx, New York 10461, USA. [2] Department of Endocrinology and Metabolism, the First Hospital of Jilin University, Changchun 130021, China. * These authors contributed equally to this work. Correspondence and requests for materials should be addressed to D.C. (email: dongsheng.cai@einstein.yu.edu).

Original article

MOLECULAR METABOLISM

Cholinergic neurons in the dorsomedial hypothalamus regulate mouse brown adipose tissue metabolism

Jae Hoon Jeong [1], Dong Kun Lee [1], Clemence Blouet [2], Henry H. Ruiz [3,4], Christoph Buettner [3,4], Streamson Chua Jr. [1], Gary J. Schwartz [1], Young-Hwan Jo [1,5,*]

ABSTRACT

Objective: Brown adipose tissue (BAT) thermogenesis is critical in maintaining body temperature. The dorsomedial hypothalamus (DMH) integrates cutaneous thermosensory signals and regulates adaptive thermogenesis. Here, we study the function and synaptic connectivity of input from DMH cholinergic neurons to sympathetic premotor neurons in the raphe pallidus (Rpa).

Methods: In order to selectively manipulate DMH cholinergic neuron activity, we generated transgenic mice expressing channelrhodopsin fused to yellow fluorescent protein (YFP) in cholinergic neurons (choline acetyltransferase (ChAT)-Cre::ChR2-YFP) with the Cre-LoxP technique. In addition, we used an adeno-associated virus carrying the Cre recombinase gene to delete the floxed *Chat* gene in the DMH. Physiological studies in response to optogenetic stimulation of DMH cholinergic neurons were combined with gene expression and immunocytochemical analyses.

Results: A subset of DMH neurons are ChAT-immunopositive neurons. The activity of these neurons is elevated by warm ambient temperature. A phenotype-specific neuronal tracing shows that DMH cholinergic neurons directly project to serotonergic neurons in the Rpa. Optical stimulation of DMH cholinergic neurons decreases BAT activity, which is associated with reduced body core temperature. Furthermore, elevated DMH cholinergic neuron activity decreases the expression of BAT uncoupling protein 1 (*Ucp1*) and peroxisome proliferator-activated receptor γ coactivator 1 α (*Pgc1*α) mRNAs, markers of BAT activity. Injection of M2-selective muscarinic receptor antagonists into the 4th ventricle abolishes the effect of optical stimulation. Single cell qRT-PCR analysis of retrogradely identified BAT-projecting neurons in the Rpa shows that all M2 receptor-expressing neurons contain tryptophan hydroxylase 2. In animals lacking the *Chat* gene in the DMH, exposure to warm temperature reduces neither BAT *Ucp1* nor *Pgc1*α mRNA expression.

Conclusion: DMH cholinergic neurons directly send efferent signals to sympathetic premotor neurons in the Rpa. Elevated cholinergic input to this area reduces BAT activity through activation of M2 mAChRs on serotonergic neurons. Therefore, the direct DMH^{ACh}—$Rpa^{5\text{-}HT}$ pathway may mediate physiological heat-defense responses to elevated environmental temperature.

Keywords Acetylcholine; Muscarinic; Nicotinic; Neuronal tracing; Serotonin; Hypothalamus

1. INTRODUCTION

Brown adipose tissue (BAT) contributes to energy homeostasis by burning carbohydrates and lipids to generate heat using uncoupling protein-1 (*Ucp1*), a protein that uncouples electron transport from ATP production [1—3]. The hypothalamus is implicated in the regulation of BAT activity [4—10]. In particular, the dorsomedial hypothalamus (DMH) appears to be a key structure for BAT thermogenesis [4,6,8,11]. In fact, DMH neurons directly project to sympathetic premotor neurons in the raphe pallidus (Rpa) that innervate BAT sympathetic preganglionic neurons in the spinal intermediolateral nucleus [8,12,13]. Furthermore, optical or pharmacogenetic stimulation of DMH neurons increases BAT temperature [8,11], suggesting that excitatory synaptic inputs from the DMH to the Rpa activate BAT thermogenesis. Indeed, local infusion of the AMPA and NMDA glutamate receptor antagonists into the Rpa blocks the effects of optical stimulation and disinhibition of DMH neurons [8,14]. These prior studies suggest that glutamatergic neurons in the DMH are part of the neural circuitries that positively control BAT activity.

In addition, it has been recently described that the DMH expresses acetylcholine (ACh)-containing neurons [15]. Activation of central cholinergic receptors, including nicotinic and muscarinic ACh receptors oppositely regulates body temperature. For instance, central injection of ACh or muscarinic ACh receptor (mAChR) agonists, such as

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*Corresponding author. Department of Medicine, Albert Einstein College of Medicine of Yeshiva University, 1300 Morris Park Avenue, Bronx, NY 10461, USA. Tel.: +1 718 430 3495; fax: +1 718 430 8557. E-mail: young-hwan.jo@einstein.yu.edu (Y.-H. Jo).

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http://dx.doi.org/10.1016/j.molmet.2015.03.006

nature medicine

LETTERS

A high-throughput chemical screen reveals that harmine-mediated inhibition of DYRK1A increases human pancreatic beta cell replication

Peng Wang[1,2], Juan-Carlos Alvarez-Perez[1,2], Dan P Felsenfeld[3,4], Hongtao Liu[1], Sharmila Sivendran[3,4], Aaron Bender[1,2], Anil Kumar[1,2], Roberto Sanchez[3], Donald K Scott[1,2,5], Adolfo Garcia-Ocaña[1,2,5] & Andrew F Stewart[1,2]

Types 1 and 2 diabetes affect some 380 million people worldwide. Both ultimately result from a deficiency of functional pancreatic insulin-producing beta cells. Beta cells proliferate in humans during a brief temporal window beginning around the time of birth, with a peak percentage (~2%) engaged in the cell cycle in the first year of life[1–4]. In embryonic life and after early childhood, beta cell replication is barely detectable. Whereas beta cell expansion seems an obvious therapeutic approach to beta cell deficiency, adult human beta cells have proven recalcitrant to such efforts[1–8]. Hence, there remains an urgent need for antidiabetic therapeutic agents that can induce regeneration and expansion of adult human beta cells *in vivo* or *ex vivo*. Here, using a high-throughput small-molecule screen (HTS), we find that analogs of the small molecule harmine function as a new class of human beta cell mitogenic compounds. We also define dual-specificity tyrosine-regulated kinase-1a (DYRK1A) as the likely target of harmine and the nuclear factors of activated T cells (NFAT) family of transcription factors as likely mediators of human beta cell proliferation and differentiation. Using three different mouse and human islet *in vivo*–based models, we show that harmine is able to induce beta cell proliferation, increase islet mass and improve glycemic control. These observations suggest that harmine analogs may have unique therapeutic promise for human diabetes therapy. Enhancing the potency and beta cell specificity of these compounds are important future challenges.

The growth-mediating MYC proteins are key normal drivers of cell growth for many tissues[9–16] and lie downstream of numerous physiological, developmental and regenerative mitogenic signaling pathways[9–17]. More specifically, c-MYC is an essential driver of proliferation in Ins1 and RINm5F rat pancreatic beta cell lines and can drive human beta cell proliferation[17]. Reasoning that the *MYC* promoter might serve as a useful downstream 'sensor' for multiple diverse upstream signals leading to proliferation, we developed a luciferase-based small-molecule HTS strategy to detect molecules that directly or indirectly activate the *MYC* promoter in human cells (**Fig. 1a**, **Supplementary Fig. 1** and Online Methods).

We generated multiple stable cell lines expressing a luciferase reporter under control of the human *MYC* promoter. Among these, the human hepatocyte cell line HepG2 yielded the most robust luciferase responses and the least variability in pilot HTS screens, and we selected it for further screening using two small-molecule libraries: a library from the US Food and Drug Administration consisting of 2,300 small molecules and a 100,000-compound commercial library (see Online Methods). Of the 102,300 compounds tested, 4,500 scored >3 for median absolute deviation (MAD)[18] for luciferase activation (**Fig. 1b**). Among these, we assessed the 86 that generated the greatest normalized percentage activation (NPA >7.5%)[19] further for their ability both to induce c-MYC protein expression in HepG2 cells (**Supplementary Fig. 1**) and to induce BrdU incorporation in dispersed rat pancreatic beta cells (**Fig. 1c**). Only one compound, harmine, induced both a mild increase of c-MYC in HepG2 cells and substantial BrdU incorporation into rat beta cells. Harmine also induced BrdU incorporation and Ki-67 labeling in human beta cells, with the frequent appearance of double nuclei, suggesting recent cell division (**Fig. 1d–g**).

Harmine is a competitive inhibitor of ATP binding to the kinase pocket of DYRK1A, but it can also inhibit other DYRK family members, monoamine oxidases (MAOs) and cdc-like kinases (CLKs). We therefore surveyed additional harmine analogs[20–24] (**Fig. 2a**). Harmaline and harmane (inhibitors of MAO but not DYRK1A) did not induce proliferation; conversely, inhibitor of DYRK1A, (INDY, a chemical compound that inhibits DYRK1A but not MAO), activated proliferation in both rat and human beta cells (**Fig. 2b–d**), also yielding Ki-67$^+$ and BrdU$^+$ double nuclei (**Fig. 2d**). Harmine and INDY both also induced phosphorylation of histone H3, a third marker of cell cycle transition (**Supplementary Fig. 2**). Effective harmine and

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Cell

Discovery of a Class of Endogenous Mammalian Lipids with Anti-Diabetic and Anti-inflammatory Effects

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SUMMARY

Increased adipose tissue lipogenesis is associated with enhanced insulin sensitivity. Mice overexpressing the Glut4 glucose transporter in adipocytes have elevated lipogenesis and increased glucose tolerance despite being obese with elevated circulating fatty acids. Lipidomic analysis of adipose tissue revealed the existence of branched *f*atty *a*cid esters of *h*ydroxy *f*atty *a*cids (FAHFAs) that were elevated 16- to 18-fold in these mice. FAHFA isomers differ by the branched ester position on the hydroxy fatty acid (e.g., *p*almitic-*a*cid-9-*h*ydroxy-stearic-*a*cid, 9-PAHSA). PAHSAs are synthesized in vivo and regulated by fasting and high-fat feeding. PAHSA levels correlate highly with insulin sensitivity and are reduced in adipose tissue and serum of insulin-resistant humans. PAHSA administration in mice lowers ambient glycemia and improves glucose tolerance while stimulating GLP-1 and insulin secretion. PAHSAs also reduce adipose tissue inflammation. In adipocytes, PAHSAs signal through GPR120 to enhance insulin-stimulated glucose uptake. Thus, FAHFAs are endogenous lipids with the potential to treat type 2 diabetes.

INTRODUCTION

Obesity and type 2 diabetes (T2D) are at epidemic proportions worldwide (Hu, 2011). The major pathogenic factors underlying T2D are resistance to insulin action in peripheral tissues and dysregulated insulin secretion. The Glut4 glucose transporter is the major insulin-regulated glucose transporter and mediates glucose uptake into skeletal muscle, heart, and adipocytes in response to rising insulin after a meal (Shepherd and Kahn, 1999). In humans and rodents with obesity or T2D, Glut4 is downregulated selectively in adipose tissue (AT) and not in muscle (Shepherd and Kahn, 1999). This alters AT biology leading to systemic insulin resistance (Abel et al., 2001). Glut4 knockdown selectively in adipocytes in mice results in insulin resistance and increased T2D risk (Abel et al., 2001), whereas adipose-selective overexpression of Glut4 (AG4OX) lowers fasting glycemia and enhances glucose tolerance (Carvalho et al., 2005; Shepherd et al., 1993). These effects in AG4OX mice are mediated by glucose-dependent induction of lipogenesis in AT driven by ChREBP (Herman et al., 2012), a transcription factor that regulates both glycolysis and lipogenesis (Iizuka et al., 2004; Ma et al., 2005). ChREBP knockout in AG4OX mice completely reverses the enhanced glucose tolerance (Herman et al., 2012). Expression of ChREBP and lipogenic genes in AT is highly associated with insulin sensitivity in humans and rodents (Herman et al., 2012; Roberts et al., 2009) and increased de novo lipogenesis in AT has favorable metabolic effects including potentially increasing longevity (Bruss et al., 2010).

Elevated circulating fatty acids are generally associated with insulin resistance and glucose intolerance (Boden and Shulman, 2002). However, certain fatty acids such as dietary omega-3 fatty acids (Oh et al., 2010; Virtanen et al., 2014) and the endogenously produced palmitoleate (Cao et al., 2008) have favorable metabolic effects. Furthermore, large epidemiological studies show that an increased ratio of unsaturated to saturated fatty acids in serum triacylglycerols is associated with a reduced risk of T2D (Rhee et al., 2011; Risérus et al., 2009). Similarly, an increased ratio of monounsaturated to saturated fatty acids in the liver is associated with insulin sensitivity even with extensive hepatic steatosis (Benhamed et al., 2012). AG4OX mice have elevated circulating fatty acids and increased adiposity,

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The Dynamics of the Human Infant Gut Microbiome in Development and in Progression toward Type 1 Diabetes

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SUMMARY

Colonization of the fetal and infant gut microbiome results in dynamic changes in diversity, which can impact disease susceptibility. To examine the relationship between human gut microbiome dynamics throughout infancy and type 1 diabetes (T1D), we examined a cohort of 33 infants genetically predisposed to T1D. Modeling trajectories of microbial abundances through infancy revealed a subset of microbial relationships shared across most subjects. Although strain composition of a given species was highly variable between individuals, it was stable within individuals throughout infancy. Metabolic composition and metabolic pathway abundance remained constant across time. A marked drop in alpha-diversity was observed in T1D progressors in the time window between seroconversion and T1D diagnosis, accompanied by spikes in inflammation-favoring organisms, gene functions, and serum and stool metabolites. This work identifies trends in the development of the human infant gut microbiome along with specific alterations that precede T1D onset and distinguish T1D progressors from non-progressors.

INTRODUCTION

The initial colonization of the human gut microbiota begins in utero (Aagaard et al., 2014) and is strongly influenced by

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miR-222 Is Necessary for Exercise-Induced Cardiac Growth and Protects against Pathological Cardiac Remodeling

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SUMMARY

Exercise induces physiological cardiac growth and protects the heart against pathological remodeling. Recent work suggests exercise also enhances the heart's capacity for repair, which could be important for regenerative therapies. While microRNAs are important in certain cardiac pathologies, less is known about their functional roles in exercise-induced cardiac phenotypes. We profiled cardiac microRNA expression in two distinct models of exercise and found microRNA-222 (miR-222) was upregulated in both. Downstream miR-222 targets modulating cardiomyocyte phenotypes were identified, including HIPK1 and HMBOX1. Inhibition of miR-222 in vivo completely blocked cardiac and cardiomyocyte growth in response to exercise while reducing markers of cardiomyocyte proliferation. Importantly, mice with inducible cardiomyocyte miR-222 expression were resistant to adverse cardiac remodeling and dysfunction after ischemic injury. These studies implicate miR-222 as necessary for exercise-induced cardiomyocyte growth and proliferation in the adult mammalian heart and show that it is sufficient to protect the heart against adverse remodeling.

INTRODUCTION

Heart failure is a growing cause of morbidity and mortality throughout the world and often develops after a period of abnormal growth termed pathological hypertrophy. Loss of cardiomyocytes contributes to decreased cardiac function and heart failure. While the heart has some regenerative capacity, little is known about what regulates this ability or whether it can be effectively harnessed to mitigate these processes. Thus, understanding the pathways that promote cardiomyocyte survival and/or regeneration could have important fundamental and clinical implications.

Many pathways have been implicated in heart disease, but less is understood about what keeps the heart healthy. Clinical and experimental studies document the impact of exercise in both primary and secondary prevention of cardiovascular disease (Lim et al., 2012; Young et al., 2014). Prior work from our groups utilized a genome-wide analysis to compare transcriptional components involved in the exercise response versus those involved in pathological hypertrophy after pressure overload (Boström et al., 2010). These studies demonstrated that exercise induces a transcriptional network distinct from that seen with pathological stimuli, even at an early stage when the hearts were structurally and functionally indistinguishable. In addition, physiological growth was associated with transcriptional components linked to cell-cycle progression, and exercised hearts showed an increase in proliferation markers, specifically in cells expressing cardiomyocyte sarcomere proteins (Boström et al., 2010). These data suggested that exercise may provide physiological cues that enhance the heart's limited endogenous capacity for regeneration, similar to effects that have been documented in other organ systems, including the brain (van Praag et al., 1999, 2005; Zhang et al., 2008). These studies also implicated a transcriptional network regulated by C/EBPβ and CITED4, which appeared central to the cardiac exercise response and protected the heart against adverse remodeling (Boström et al., 2010).

MicroRNAs have been shown to regulate entire gene expression networks and play important roles in cardiovascular disease (Small and Olson, 2011). Although several studies have examined microRNAs regulated by exercise (Carè et al., 2007; DA Silva et al., 2012; Fernandes et al., 2011; Martinelli et al., 2014; Soci et al., 2011), less is known about their functional roles in this context. To identify microRNAs that are differentially regulated and functionally important in exercised

Differentiation of hypothalamic-like neurons from human pluripotent stem cells

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The hypothalamus is the central regulator of systemic energy homeostasis, and its dysfunction can result in extreme body weight alterations. Insights into the complex cellular physiology of this region are critical to the understanding of obesity pathogenesis; however, human hypothalamic cells are largely inaccessible for direct study. Here, we developed a protocol for efficient generation of hypothalamic neurons from human embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) obtained from patients with monogenetic forms of obesity. Combined early activation of sonic hedgehog signaling followed by timed NOTCH inhibition in human ESCs/iPSCs resulted in efficient conversion into hypothalamic NKX2.1$^+$ precursors. Application of a NOTCH inhibitor and brain-derived neurotrophic factor (BDNF) further directed the cells into arcuate nucleus hypothalamic-like neurons that express hypothalamic neuron markers proopiomelanocortin (POMC), neuropeptide Y (NPY), agouti-related peptide (AGRP), somatostatin, and dopamine. These hypothalamic-like neurons accounted for over 90% of differentiated cells and exhibited transcriptional profiles defined by a hypothalamic-specific gene expression signature that lacked pituitary markers. Importantly, these cells displayed hypothalamic neuron characteristics, including production and secretion of neuropeptides and increased p-AKT and p-STAT3 in response to insulin and leptin. Our results suggest that these hypothalamic-like neurons have potential for further investigation of the neurophysiology of body weight regulation and evaluation of therapeutic targets for obesity.

Introduction

The mediobasal hypothalamus is a functional integrator of homeostatic processes, including food intake, energy expenditure, neuroendocrine regulation, body temperature, and circadian rhythms (1). Constituent cell bodies with distinct physiological functions include the arcuate ventromedial (VMH), dorsal medial (DMH), and paraventricular (PVH) nuclei (2, 3). Arcuate nucleus (ARC) neurons, such as those expressing proopiomelanocortin (POMC) and neuropeptide Y (NPY)/agouti-related peptide (AGRP), can sense peripheral hormones — insulin, leptin, ghrelin, PYY — and secrete neuropeptides α melanocyte–stimulating hormone (αMSH) and NPY/AGRP to engage receptors on so-called "second order" DMH, PVH, and other neurons to regulate aspects of energy homeostasis through melanocortin 4 receptor (MC4R), neuropeptide Y receptor type 1 (NPY1R), and other receptors (3). Hypomorphic mutations of genes involved in hypothalamic leptin-melanocortin signaling, such as leptin, leptin receptor, POMC, and MC4R, result in monogenic severe obesity in humans and rodents, confirming the biological importance of these pathways(4–8). Though various neuronal cell types have been generated by directed differentiation from human pluripotent stem cells and applied for the study of neurodegenerative diseases, including Alzheimer's disease, Parkinson's disease, and ALS (9–11), there is currently no published protocol for the differentiation of human hypothalamic neurons. For the analysis of obesity-related pathophysiology, access to hypothalamic cell types would be extremely useful.

Establishing directed differentiation protocols relies upon an understanding of the details of cellular ontogenesis. The hypothalamus is a complex organ subserving roles in energy homeostasis, endocrine physiology, temperature regulation, arousal, circadian rhythms, and other functions that are mediated by specific hypothalamic cell types (12). A growing number of transcription factors (TFs) have been implicated in the differentiation and specification of hypothalamic neuronal subtypes (Supplemental Figure 1A; supplemental material available online with this article; doi:10.1172/JCI79220DS1). In the E10.5 mouse brain, Nkx2.1 is expressed in both the ventral diencephalon and telencephalon, while FoxG1 is expressed in telencephalon progenitors, but not in hypothalamic progenitors, suggesting that hypothalamic neurons likely develop from Nkx2.1$^+$FoxG1$^-$ precursors (13). RAX, VAX, and SIX3 are specifically expressed in rostral hypothalamic neuroepithelia (14, 15). Moreover, Achaete-scute–like 1 (ASCL1, also called MASH1),

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Cell Metabolism
Article

Activation of Calcium/Calmodulin-Dependent Protein Kinase II in Obesity Mediates Suppression of Hepatic Insulin Signaling

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SUMMARY

A hallmark of obesity is selective suppression of hepatic insulin signaling ("insulin resistance"), but critical gaps remain in our understanding of the molecular mechanisms. We now report a major role for hepatic CaMKII, a calcium-responsive kinase that is activated in obesity. Genetic targeting of hepatic CaMKII, its downstream mediator p38, or the p38 substrate and stabilizer MK2 enhances insulin-induced p-Akt in palmitate-treated hepatocytes and obese mouse liver, leading to metabolic improvement. The mechanism of improvement begins with induction of ATF6 and the ATF6 target $p58^{IPK}$, a chaperone that suppresses the PERK—p-eIF2α—ATF4 branch of the UPR. The result is a decrease in the ATF4 target TRB3, an inhibitor of insulin-induced p-Akt, leading to enhanced activation of Akt and its downstream metabolic mediators. These findings increase our understanding of the molecular mechanisms linking obesity to selective insulin resistance and suggest new therapeutic targets for type 2 diabetes and metabolic syndrome.

INTRODUCTION

Obesity is the leading cause of insulin resistance, metabolic syndrome, and type 2 diabetes (T2D), but therapeutic options are limited due to critical gaps in our knowledge of molecular mechanisms linking obesity with the metabolic disturbances of insulin resistance and T2D (Samuel and Shulman, 2012). A key factor in T2D is an inappropriate increase in hepatic glucose production (HGP), which results from selective hepatic insulin resistance together with impaired suppression of glucagon signaling (Lin and Accili, 2011). In addition to elevated HGP, selective insulin resistance contributes to other critical maladies associated with T2D, including cardiovascular disease, the leading cause of death in these patients (Bornfeldt and Tabas, 2011; Leavens and Birnbaum, 2011).

We recently elucidated a pathway through which glucagon stimulates HGP in fasting and in obesity, and in obesity this pathway contributes to hyperglycemia (Ozcan et al., 2012; Wang et al., 2012). The pathway is triggered downstream of the glucagon receptor by PKA-mediated activation of the endoplasmic reticulum (ER) calcium release channel, inositol 1,4,5-triphosphate receptor (IP3R). Channel opening, which is also promoted by a glucagon receptor-phospholipase C pathway that generates IP3, results in release of calcium from ER stores, which then activates the cytoplasmic calcium-sensitive kinase, calcium/calmodulin dependent-protein kinase II (CaMKII). CaMKII then activates the MAPK p38α, which phosphorylates FoxO1 in a manner that promotes FoxO1 nuclear translocation. Nuclear FoxO1 induces target genes that are rate limiting for glycogenolysis and gluconeogenesis, notably *G6pc* and *Pck1*. This CaMKII-FoxO1 pathway is complemented by the activation of the calcium-sensitive phosphatase calcineurin, which promotes CRTC2-mediated induction of the FoxO1 transcriptional partner, PGC1α (Wang et al., 2012). Moreover, recent studies have shown that calcium transport back into the ER, mediated by sarcoplasmic/endoplasmic reticulum calcium ATPase (SERCA), is dysfunctional in obesity (Fu et al., 2011; Park et al., 2010), which could contribute to both the amplitude and the duration of the pathological calcium response. Collectively, these data point to the importance of intracellular calcium metabolism and CaMKII in enhanced HGP in obesity. However, a critical remaining question in this area was whether CaMKII plays a role in the other major pathological process in obesity and T2D, namely selective insulin resistance.

Defective insulin signaling is a major feature of selective hepatic insulin resistance in obesity (Brown and Goldstein, 2008; Könner and Brüning, 2012). In normal physiology, insulin stimulates insulin autophosphorylation of the insulin receptor (IR), which promotes to Tyr-phosphorylation of insulin receptor substrates 1 and 2 (IRS-1/2). Through a series of downstream processes involving lipid mediators and protein kinases, p-IRS-1/2 leads to Ser/Thr-phosphorylation and activation of

Cell Metabolism
Article

Metabolic Inflexibility Impairs Insulin Secretion and Results In MODY-like Diabetes in Triple FoxO-Deficient Mice

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SUMMARY

Pancreatic β cell failure in type 2 diabetes is associated with functional abnormalities of insulin secretion and deficits of β cell mass. It's unclear how one begets the other. We have shown that loss of β cell mass can be ascribed to impaired FoxO1 function in different models of diabetes. Here we show that ablation of the three FoxO genes (1, 3a, and 4) in mature β cells results in early-onset, maturity-onset diabetes of the young (MODY)-like diabetes, with abnormalities of the MODY networks Hnf4α, Hnf1α, and Pdx1. FoxO-deficient β cells are metabolically inflexible, i.e., they preferentially utilize lipids rather than carbohydrates as an energy source. This results in impaired ATP generation and reduced Ca^{2+}-dependent insulin secretion. The present findings demonstrate a secretory defect caused by impaired FoxO activity that antedates dedifferentiation. We propose that defects in both pancreatic β cell function and mass arise through FoxO-dependent mechanisms during diabetes progression.

INTRODUCTION

Patients with type 2 diabetes display qualitative and quantitative defects in pancreatic β cell function and mass (Ferrannini, 2010). These deficits are critical components of the transition from compensated insulin resistance (Weyer et al., 1999)—a state that may affect up to a quarter of the population (Reaven, 1995)—to overt hyperglycemia, a condition that affects 5%–8% of the population (National Institute of Diabetes and Digestive and Kidney Diseases, 2005). Functional defects appear to antedate quantitative ones at the onset of hyperglycemia (Prentki and Madiraju, 2012; Rahier et al., 2008), but there is no clear mechanism to explain how β cell dysfunction spawns loss of β cells.

The functional impairment of β cells has both genetic (Fajans and Bell, 2011) and acquired components, with the latter being partly reversible, at least initially (Savage et al., 1979). Thus, a challenge for this field of investigation is to identify mechanisms whereby genetic, environmental, and reversible defects of β cell function can be subsumed with loss of β cell mass under a general theory of β cell failure (Accili et al., 2010).

Transient elevations of glucose or lipids activate transcription factor FoxO1 through nuclear translocation in β cells (Kawamori et al., 2006; Kitamura et al., 2005; Martinez et al., 2006), thereby preventing a chronic dedifferentiation process that appears to be a shared feature of otherwise different types of murine diabetes (Talchai et al., 2012b). In addition, we have recently shown that key tenets of this theory, i.e., dedifferentiation, loss of FOXO1, and conversion of β cells into α and δ cells, also occur in human type 2 diabetes (Cinti et al., manuscript submitted), providing further impetus to understand how to forestall or reverse this process. This model possibly reflects an integrative function of FoxO1 downstream of heterogeneous signaling pathways, but doesn't explain the functional abnormalities that precede β cell dedifferentiation. β cells express also FoxO3a and FoxO4 (Kitamura et al., 2002). The present study fills this gap in knowledge by demonstrating that, when all three FoxO isoforms are ablated, β cells develop signal abnormalities of insulin secretion resulting in MODY-like diabetes, paving the way for eventual dedifferentiation.

RESULTS

Rationale for the Generation of Triple FoxO Knockout Mice in β Cells

Genetic ablation of FoxO1 results in β cell dedifferentiation following repeated pregnancies or in aging animals, as does the acquired loss of FoxO1 observed in disparate models of murine diabetes (Talchai et al., 2012b). We first investigated whether this functional loss of FoxO in diabetic islets extends to the other two isoforms, FoxO3 and FoxO4. Indeed, in islets from frankly diabetic mice (random glucose ~450 mg/dl), all FoxOs were decreased (Figures 1A and 1B). Consistent with

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Polyamine biosynthesis is critical for growth and differentiation of the pancreas

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The pancreas, in most studied vertebrates, is a compound organ with both exocrine and endocrine functions. The exocrine compartment makes and secretes digestive enzymes, while the endocrine compartment, organized into islets of Langerhans, produces hormones that regulate blood glucose. High concentrations of polyamines, which are aliphatic amines, are reported in exocrine and endocrine cells, with insulin-producing β cells showing the highest concentrations. We utilized zebrafish as a model organism, together with pharmacological inhibition or genetic manipulation, to determine how polyamine biosynthesis functions in pancreatic organogenesis. We identified that inhibition of polyamine biosynthesis reduces exocrine pancreas and β cell mass, and that these reductions are at the level of differentiation. Moreover, we demonstrate that inhibition of ornithine decarboxylase (ODC), the rate-limiting enzyme in polyamine biosynthesis, phenocopies inhibition or knockdown of the enzyme deoxyhypusine synthase (DHS). These data identify that the pancreatic requirement for polyamine biosynthesis is largely mediated through a requirement for spermidine for the downstream posttranslational modification of eIF5A by its enzymatic activator DHS, which in turn impacts mRNA translation. Altogether, we have uncovered a role for polyamine biosynthesis in pancreatic organogenesis and identified that it may be possible to exploit polyamine biosynthesis to manipulate pancreatic cell differentiation.

The growth and development of a multicellular organism necessitates the successive differentiation of specialized cell types from pools of undifferentiated progenitor cells. This coordinated process ultimately results in the specification of tissues composed of distinct cell types that harmonize to produce functioning organs; each distinct cell type must pass through several semi-differentiated pluripotent cell populations between the zygote and mature organism. Deciphering the mechanisms that direct this cell-specific differentiation is a key biological question with important ramifications for understanding the pathogenesis or treatment of many diseases, including diabetes mellitus.

Diabetes is a syndrome that results from the destruction or dysfunction of the insulin-producing β cells in the pancreas[1]. In all organisms, β cell development begins during embryogenesis. Pancreatic progenitor cells are specified in the endoderm following the reception of secreted signals. These secreted signals then initiate a cascade of pancreas-specific transcription factors within the pancreatic progenitor cells[2]. In particular, these progenitor cells first co-express the transcription factors pancreatic and duodenal homeobox 1 (Pdx1) and pancreas transcription factor 1a (Ptf1a), and subsequently differentiate into cells composing all pancreatic lineages: exocrine, endocrine and duct cells[3–5]. However before differentiation occurs, the progenitor cells increase in number and spatially organize into an intricate epithelial tree; progenitors in the trunk domain upregulate Neurogenin3 (Neurog3) before differentiating into endocrine cells[6,7], whereas those in the tip domain express CarboxypeptidaseA (Cpa1) and

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Elevations in Circulating Methylated and Unmethylated Preproinsulin DNA in New-Onset Type 1 Diabetes

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Elevated ratios of circulating unmethylated to methylated preproinsulin (*INS*) DNA have been suggested to reflect β-cell death in type 1 diabetes (T1D). We tested the hypothesis that absolute levels (rather than ratios) of unmethylated and methylated *INS* DNA differ between subjects with new-onset T1D and control subjects and assessed longitudinal changes in these parameters. We used droplet digital PCR to measure levels of unmethylated and methylated *INS* DNA in serum from subjects at T1D onset and at 8 weeks and 1 year post-onset. Compared with control subjects, levels of both unmethylated and methylated *INS* DNA were elevated at T1D onset. At 8 weeks post-onset, methylated *INS* DNA remained elevated, but unmethylated *INS* DNA fell. At 1 year post-onset, both unmethylated and methylated *INS* DNA returned to control levels. Subjects with obesity, type 2 diabetes, and autoimmune hepatitis exhibited lower levels of unmethylated and methylated *INS* compared with subjects with T1D at onset and no differences compared with control subjects. Our study shows that elevations in both unmethylated and methylated *INS* DNA occurs in new-onset T1D and that levels of these DNA species change during T1D evolution. Our work emphasizes the need to consider absolute levels of differentially methylated DNA species as potential biomarkers of disease.

The diagnosis of type 1 diabetes (T1D) is made at a time when individuals have lost substantial β-cell mass and function (1,2). Interventions instituted at T1D diagnosis have failed to result in recovery of β-cell function, raising the possibility that earlier detection of β-cell death might provide an opportunity for preventative interventions prior to T1D onset (3). Recently, several groups have proposed the measurement of circulating unmethylated DNA encoding preproinsulin (*INS*) as a biomarker of β-cell death (4–10), since β-cells have a much higher frequency of unmethylated CpG sites compared with other cell types (6,11,12) and might release this DNA species into the circulation upon death. In these studies, unmethylated *INS* DNA was expressed as a ratio relative to methylated *INS* DNA for normalization purposes. However, because β-cells and many other cell types in the islet contain some fraction of both unmethylated and methylated *INS* (6,12,13), it remains unclear to what extent each species of *INS* might be independently informative of the underlying disease process in T1D.

Droplet digital PCR (ddPCR) uses the analysis of discrete individual PCR reactions (~20,000/sample) to identify the presence of target DNA and uses Poisson statistics to extrapolate the copy number of target DNA per sample (14). This technology enables direct quantitation of differentially methylated DNA species in serum without the need for normalization. We used ddPCR to analyze serum from individuals with new-onset T1D to test the hypothesis that absolute levels (rather than ratios) of unmethylated and methylated *INS* differ between subjects with new-onset T1D and control subjects. We also assessed longitudinal

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ARTICLE

Sirtuin 6 regulates glucose-stimulated insulin secretion in mouse pancreatic beta cells

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Abstract
Aims/hypothesis Sirtuin 6 (SIRT6) has been implicated in ageing, DNA repair and metabolism; however, its function in pancreatic beta cells is unclear. The aim of this study is to elucidate the role of SIRT6 in pancreatic beta cells.
Methods To investigate the function of SIRT6 in pancreatic beta cells, we performed *Sirt6* gene knockdown in MIN6 cells and generated pancreatic- and beta cell-specific *Sirt6* knockout mice. Islet morphology and glucose-stimulated insulin secretion (GSIS) were analysed. Glycolysis and oxygen consumption rates in SIRT6-deficient beta cells were measured. Cytosolic calcium was monitored using the Fura-2-AM fluorescent probe (Invitrogen, Grand Island, NY, USA). Mitochondria were analysed by immunoblots and electron microscopy.
Results *Sirt6* knockdown in MIN6 beta cells led to a significant decrease in GSIS. Pancreatic beta cell *Sirt6* knockout mice showed a ~50% decrease in GSIS. The knockout mouse islets had lower ATP levels compared with the wild-type controls. Mitochondrial oxygen consumption rates were significantly decreased in the SIRT6-deficient beta cells. Cytosolic calcium dynamics in response to glucose or potassium chloride were attenuated in the *Sirt6* knockout islets. Numbers of damaged mitochondria were increased and mitochondrial complex levels were decreased in the SIRT6-deficient islets.
Conclusions/interpretation These data suggest that SIRT6 is important for GSIS from pancreatic beta cells and activation of SIRT6 may be useful to improve insulin secretion in diabetes.

Keywords Beta cell · Calcium · Glucose metabolism · Insulin secretion · Mitochondria · SIRT6

Abbreviations

ADPR	ADP ribose
bMko	*Sirt6* beta cell-specific knockout
bPko	*Sirt6* pancreas-specific knockout
$[Ca^{2+}]_i$	Intracellular calcium
ECAR	Extracellular acidification rate
ES	Embryonic stem
GSIS	Glucose-stimulated insulin secretion
H3K9	Histone H3 at lysine 9
HFD	High-fat diet
ITT	Insulin tolerance test
KCl	Potassium chloride
KIC	α-Ketoisocaproate
NIDDK	National Institute of Diabetes and Digestive and Kidney Diseases
NIH	National Institutes of Health
OAADPR	*O*-Acetyl-ADP ribose
OCR	Oxygen consumption rate

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PLOS | ONE

RESEARCH ARTICLE

Insulin Receptor Signaling in the GnRH Neuron Plays a Role in the Abnormal GnRH Pulsatility of Obese Female Mice

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Abstract

Infertility associated with obesity is characterized by abnormal hormone release from reproductive tissues in the hypothalamus, pituitary, and ovary. These tissues maintain insulin sensitivity upon peripheral insulin resistance. Insulin receptor signaling may play a role in the dysregulation of gonadotropin-releasing hormone (GnRH) secretion in obesity, but the interdependence of hormone secretion in the reproductive axis and the multi-hormone and tissue dysfunction in obesity hinders investigations of putative contributing factors to the disrupted GnRH secretion. To determine the role of GnRH insulin receptor signaling in the dysregulation of GnRH secretion in obesity, we created murine models of diet-induced obesity (DIO) with and without intact insulin signaling in the GnRH neuron. Obese control female mice were infertile with higher luteinizing hormone levels and higher GnRH pulse amplitude and total pulsatile secretion compared to lean control mice. In contrast, DIO mice with a GnRH specific knockout of insulin receptor had improved fertility, luteinizing hormone levels approaching lean mice, and GnRH pulse amplitude and total secretion similar to lean mice. Pituitary responsiveness was similar between genotypes. These results suggest that in the obese state, insulin receptor signaling in GnRH neurons increases GnRH pulsatile secretion and consequent LH secretion, contributing to reproductive dysfunction.

Introduction

Obesity and conditions with hyperinsulinemia such as type 2 diabetes mellitus, metabolic syndrome, and polycystic ovary syndrome (PCOS) are often accompanied by infertility in females [1]. The role of hyperinsulinism in female reproductive dysfunction is undisputed [2]. Insulin is a co-gonadotropin with LH, causing increased steroidogenesis and altered follicular maturation in animal models [3] and women with PCOS [2]. The effect of hyperinsulinemia on central reproductive tissues such as the pituitary and hypothalamus is not well defined.

Experiments using various animal models and experimental paradigms have suggested a role of insulin signaling in central reproductive function. Central infusion of insulin to diabetic male sheep [4] or female rats [5] is associated with increased LH secretion; a similar study in

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Adipose Fatty Acid Oxidation Is Required for Thermogenesis and Potentiates Oxidative Stress-Induced Inflammation

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SUMMARY

To understand the contribution of adipose tissue fatty acid oxidation to whole-body metabolism, we generated mice with an adipose-specific knockout of carnitine palmitoyltransferase 2 ($CPT2^{A-/-}$), an obligate step in mitochondrial long-chain fatty acid oxidation. $CPT2^{A-/-}$ mice became hypothermic after an acute cold challenge, and $CPT2^{A-/-}$ brown adipose tissue (BAT) failed to upregulate thermogenic genes in response to agonist-induced stimulation. The adipose-specific loss of CPT2 resulted in diet-dependent changes in adiposity but did not result in changes in body weight on low- or high-fat diets. Additionally, $CPT2^{A-/-}$ mice had suppressed high-fat diet-induced oxidative stress and inflammation in visceral white adipose tissue (WAT); however, high-fat diet-induced glucose intolerance was not improved. These data show that fatty acid oxidation is required for cold-induced thermogenesis in BAT and high-fat diet-induced oxidative stress and inflammation in WAT.

INTRODUCTION

Ingestion of a calorically dense diet, generally high in fat content, coupled with inactivity leads to increased adiposity and eventual obesity. Obesity in turn is highly correlated with the development of type 2 diabetes, the metabolic syndrome, and cardiovascular disease, among others. The molecular mechanisms by which high-fat diets contribute to these pathologies are not well understood, but several themes have emerged. Implicated in the etiology and progression of obesity-related pathologies is oxidative stress, endoplasmic reticulum stress, and inflammation originating locally at adipose depots but acting systemically to promote insulin resistance (Glass and Olefsky, 2012; Hotamisligil, 2010; Keaney et al., 2003; Kusminski and Scherer, 2012). Reversing or preventing local adipose tissue inflammation may have beneficial systemic effects against insulin resistance. Alternatively, strategies to reverse obesity by increasing adipose energy expenditure have been suggested to improve systemic obesity-related complications (Tseng et al., 2010).

Adult mammals have at least two functionally distinct adipose lineages: unilocular white adipocytes, which function mainly to store fat, and multilocular brown adipocytes, which function mainly to burn fat for thermogenesis. Dysfunctional white adipose tissue (WAT) and brown adipose tissue (BAT) have been implicated in the pathogenesis of obesity and diabetes. BAT is densely packed with mitochondria and requires fatty acid oxidation to fuel heat generation (Ellis et al., 2010; Guerra et al., 1998; Ji et al., 2008; Schuler et al., 2005; Tolwani et al., 2005). Although the oxidation of fatty acids in WAT in the fed state is relatively low, fasting doubles the rate of white adipocyte fatty acid oxidation and is presumably a major fuel in insulin suppressed states (Wang et al., 2003). Changing macronutrient metabolism specifically in adipocytes can lead to changes in adiposity, body weight, and glucose tolerance (Abel et al., 2001; Ahmadian et al., 2011; Lodhi et al., 2012; Vernochet et al., 2012). However, the autonomous contribution of adipose fatty acid oxidation to obesity and insulin resistance remains unknown.

Mitochondrial long-chain fatty acid β-oxidation requires successive carnitine acyltransferases to translocate acyl-coenzyme As (acyl-CoAs) from the cytoplasm into the mitochondrial matrix (Wolfgang and Lane, 2006). The initial and rate-setting enzyme, CPT1, generates acylcarnitines that can traverse the mitochondrial membranes via specific transporters. CPT1 is allosterically inhibited by the rate-determining metabolite in de novo fatty acid synthesis, malonyl-CoA; therefore, the balance of fatty acid synthesis and oxidation is metabolically coordinated posttranslationally. Once inside the mitochondrial matrix, CPT2 generates acyl-CoAs from acylcarnitines to initiate the β-oxidation of long-chain fatty acids to acetyl-CoA. Fatty acids contain an abundant energy potential, making them ideal for storage during energy surplus and mobilization during energy deficits. Fatty acid oxidation efficiently generates energy but can also promote the generation of reactive oxygen species (ROS). ROS can potentiate oxidative stress and inflammation, which can impair insulin sensitivity (Houstis et al., 2006).

Although it is clear that fatty acid oxidation is a critical and fundamental metabolic endpoint in humans (Longo et al., 2006) and rodents (Ji et al., 2008; Nyman et al., 2005), it is not clear

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Article

TRANSPARENT PROCESS

THE EMBO JOURNAL

Parkin-independent mitophagy requires Drp1 and maintains the integrity of mammalian heart and brain

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Abstract

Mitochondrial dynamics and mitophagy have been linked to cardiovascular and neurodegenerative diseases. Here, we demonstrate that the mitochondrial division dynamin Drp1 and the Parkinson's disease-associated E3 ubiquitin ligase parkin synergistically maintain the integrity of mitochondrial structure and function in mouse heart and brain. Mice lacking cardiac Drp1 exhibited lethal heart defects. In Drp1KO cardiomyocytes, mitochondria increased their connectivity, accumulated ubiquitinated proteins, and decreased their respiration. In contrast to the current views of the role of parkin in ubiquitination of mitochondrial proteins, mitochondrial ubiquitination was independent of parkin in Drp1KO hearts, and simultaneous loss of Drp1 and parkin worsened cardiac defects. Drp1 and parkin also play synergistic roles in neuronal mitochondrial homeostasis and survival. Mitochondrial degradation was further decreased by combination of Drp1 and parkin deficiency, compared with their single loss. Thus, the physiological importance of parkin in mitochondrial homeostasis is revealed in the absence of mitochondrial division in mammals.

Keywords mice; mitochondria; organelle division; respiration
Subject Categories Membrane & Intracellular Transport; Metabolism
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Introduction

Mitochondria are highly abundant in the brain and heart. In these tissues, neurons and cardiomyocytes produce high levels of oxidative stress and live for a long period of time without proliferation; therefore, mitochondrial homeostasis is critical in these cells. Indeed, alterations in mitochondrial structure and function have been linked to many neurodegenerative and cardiovascular diseases (Nunnari & Suomalainen, 2012; Itoh *et al*, 2013; Ong *et al*, 2013).

Mitochondria grow and divide to control their number and size. Mitochondrial division is mediated by a dynamin-related GTPase, Drp1, and its receptors, which are located in the mitochondrial outer membrane (Okamoto & Shaw, 2005; Chang & Blackstone, 2010; Westermann, 2010; Kageyama *et al*, 2011; Tamura *et al*, 2011; Sesaki *et al*, 2013). Although Drp1 is ubiquitously expressed in many mammalian tissues, different cell types have different frequencies of mitochondrial division. In neurons, mitochondria are distributed throughout the cytoplasm, actively moving along axons and dendrites. Neuronal mitochondria also undergo frequent division and fusion and regulate their shape, size, and number. In contrast, mitochondria in cardiomyocytes are located underneath the sarcolemma, between myofibrils and around nuclei (Ong *et al*, 2013; Piquereau *et al*, 2013). Intermyofibrillar mitochondria run parallel to myofibrils to efficiently provide ATP for muscle contraction. Mitochondrial movement is limited due to this spatial organization in the cytoplasm, and mitochondrial division is estimated to only occur at an extremely low frequency in adult cardiomyocytes under physiological conditions (Beraud *et al*, 2009). In contrast, under pathological conditions such as ischemic reperfusion, mitochondria divide and induce apoptosis, promoting myocardial damage. In ischemic reperfusion, a pharmacological inhibitor of

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nature medicine

Clonal analyses and gene profiling identify genetic biomarkers of the thermogenic potential of human brown and white preadipocytes

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Targeting brown adipose tissue (BAT) content or activity has therapeutic potential for treating obesity and the metabolic syndrome by increasing energy expenditure. However, both inter- and intra-individual differences contribute to heterogeneity in human BAT and potentially to differential thermogenic capacity in human populations. Here we generated clones of brown and white preadipocytes from human neck fat and characterized their adipogenic and thermogenic differentiation. We combined an uncoupling protein 1 (UCP1) reporter system and expression profiling to define novel sets of gene signatures in human preadipocytes that could predict the thermogenic potential of the cells once they were maturated. Knocking out the positive UCP1 regulators, *PREX1* and *EDNRB*, in brown preadipocytes using CRISPR-Cas9 markedly abolished the high level of UCP1 in brown adipocytes differentiated from the preadipocytes. Finally, we were able to prospectively isolate adipose progenitors with great thermogenic potential using the cell surface marker CD29. These data provide new insights into the cellular heterogeneity in human fat and offer potential biomarkers for identifying thermogenically competent preadipocytes.

Obesity, mainly characterized by increased adiposity, has reached pandemic proportions and is a major contributor to metabolic disorders. In mammals, there are two functionally distinct types of fat: white adipose tissue (WAT), which is specialized for energy storage, and BAT, which dissipates energy for thermogenesis[1,2] via the activity of UCP1. In addition to the classical brown adipocytes, UCP1-positive 'beige' or 'brite' adipocytes can be recruited within WAT upon chronic cold or β3-adrenergic stimulation[3–6].

Because of the immense capacity of BAT to combust fuels for heat production[7,8] and owing to its presence in adult humans[9–14], increasing the amount or activity of brown or beige fat has been considered an appealing approach for the treatment or prevention of obesity and related metabolic disorders. Indeed, in rodents the activation of brown or beige fat can increase energy expenditure and is protective against diet-induced obesity[5,6,15]. In humans, BAT mass or activity is inversely correlated to body mass index and percentage of body fat[10–12], and cold exposure can elevate BAT volume and activity and increase energy expenditure, thus pointing toward the therapeutic potential of BAT in humans for the treatment of obesity and metabolic disease[16–18].

Recent data indicate that the neck, supraclavicular and spinal cord regions of adult humans contain substantial deposits of UCP1-positive adipocytes[19–22]. The presence of brown, beige, and white adipocytes, and possibly other unidentified adipose cell types, highlights the heterogeneity of adipose tissue depots, which is potentially related to their diverse functions in energy metabolism. Both inter-subject differences and various cellular compositions within a given fat tissue contribute to the heterogeneity of human BAT and affect thermogenic potential. In rodents, lineage tracing and cell sorting analyses have demonstrated that the various types of fat cells arise from discrete pools of progenitors, which express distinct molecular markers[19,23–26]. However, whether the markers identified in mouse cells can unambiguously define different types of human adipose progenitors is currently unknown.

A key impediment for these studies is the lack of human-derived brown and white fat progenitor cell models. To investigate the heterogeneous nature of the progenitor cell population in human BAT and WAT, we generated clonal cell lines from human neck fat and characterized their adipogenic differentiation and metabolic function *in vitro* and *in vivo* after transplantation into immune-deficient nude mice. Using clonal analysis and gene-expression profiling, we defined unique sets of gene signatures in human preadipocytes that could predict the thermogenic potential of these cells once they have

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Restoring Systemic GDF11 Levels Reverses Age-Related Dysfunction in Mouse Skeletal Muscle

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Parabiosis experiments indicate that impaired regeneration in aged mice is reversible by exposure to a young circulation, suggesting that young blood contains humoral "rejuvenating" factors that can restore regenerative function. Here, we demonstrate that the circulating protein growth differentiation factor 11 (GDF11) is a rejuvenating factor for skeletal muscle. Supplementation of systemic GDF11 levels, which normally decline with age, by heterochronic parabiosis or systemic delivery of recombinant protein, reversed functional impairments and restored genomic integrity in aged muscle stem cells (satellite cells). Increased GDF11 levels in aged mice also improved muscle structural and functional features and increased strength and endurance exercise capacity. These data indicate that GDF11 systemically regulates muscle aging and may be therapeutically useful for reversing age-related skeletal muscle and stem cell dysfunction.

Skeletal muscle is a highly specialized tissue composed predominantly of contractile, multinucleated fibers whose regeneration after injury depends on the activity of a specialized subset of muscle fiber–associated mononuclear stem cells called satellite cells (*1*, *2*). Satellite cells can be isolated by fluorescence-activated cell sorting based on their unique surface marker profile ($CD45^-Sca\text{-}1^-CD11b^-CXCR4^+\beta1\text{-}integrin^+$), which effectively distinguishes them from non-myogenic cells and more differentiated myoblasts within the muscle (*3*, *4*).

Aged muscle exhibits decreased satellite cell number, impaired satellite cell function, and reduced regenerative potential (*2*, *5*–*9*). To evaluate satellite cell function in aged muscle on a per cell basis, we performed clonal myogenesis assays (*5*, *9*) and found that $CD45^-Sca\text{-}1^-CD11b^-CXCR4^+\beta1\text{-}Integrin^+$ satellite cells (fig. S1) from aged mice formed fewer colonies by up to a factor of 4 compared with young cells (fig. S2A) (*5*, *9*). To investigate the molecular basis of this reduced satellite cell activity in aged muscle, we examined DNA integrity in young and aged satellite cells using single-cell gel electrophoresis assays. Freshly sorted satellite cells showed a marked increase in DNA damage with age (fig. S2, B and C), with ~60% of aged cells exhibiting severely compromised DNA integrity (red bars, fig. S2B). Likewise, nearly 60% of satellite cells sorted from aged muscle (fig. S2, D and E) or identified by Pax7 staining on isolated muscle fibers (fig. S3) showed increased immunoreactivity for the phosphorylated form of histone H2AX (pH2AX), a marker of DNA damage (*10*). In contrast, 40% of freshly isolated young satellite cells were devoid of detectable DNA damage by gel electrophoresis assay (fig. S2, B and C), and young satellite cell nuclei rarely contained more than two pH2AX foci when assayed after cell sorting (fig. S2, D and E) or on single myofibers (fig. S3). Induction of DNA damage by x-irradiation reduced the myogenic function of young satellite cells in transplantation assays (fig. S4), which suggests

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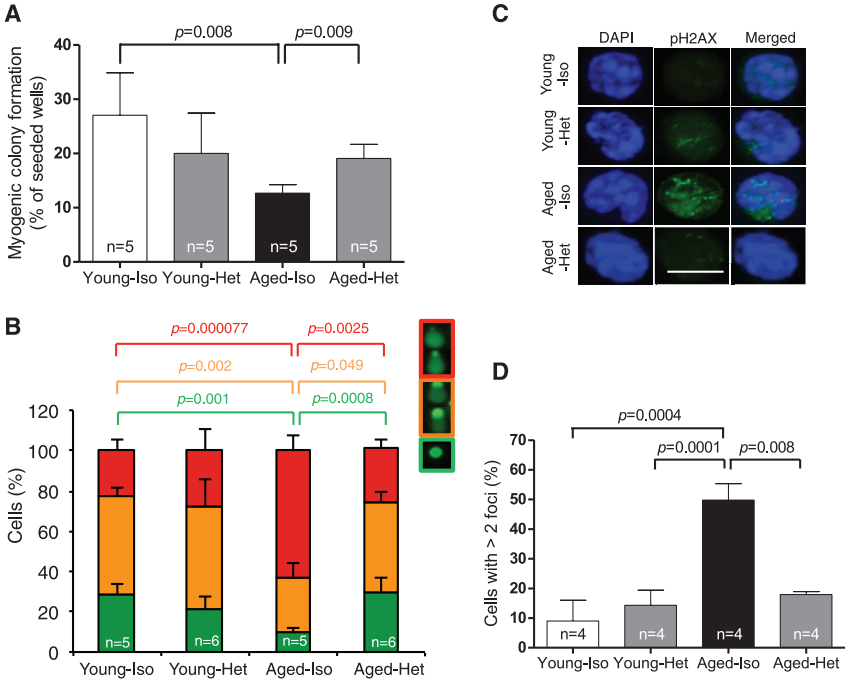

Fig. 1. Rejuvenation of muscle stem cells by heterochronic parabiosis. (**A**) Frequency of clone-sorted satellite cells from isochronic (Iso) or heterochronic (Het) mice forming colonies after 5 days in culture. All colonies showed characteristic morphology of muscle lineage cells. (**B**) DNA damage in freshly sorted satellite cells assessed by single-cell gel electrophoresis under alkaline conditions. Damage was quantified using a visual scoring metric (*25*) (key at top) and represented by color coding: no damage, green; moderate damage, orange; maximal damage, red. (**C**) Representative images (confocal *z* stacks) of freshly sorted satellite cells stained with 4′,6-diamidino-2-phenylindole (DAPI) (blue) and antibody to pH2AX (green). Data are quantified in (**D**). All graphs represent mean ± SD, with *P* values calculated by Mann-Whitney analysis. *n*, number of mice used for each analysis. Scale bar, 10 μm.

Cell Metabolism
Article

Preserved DNA Damage Checkpoint Pathway Protects against Complications in Long-Standing Type 1 Diabetes

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SUMMARY

The mechanisms underlying the development of complications in type 1 diabetes (T1D) are poorly understood. Disease modeling of induced pluripotent stem cells (iPSCs) from patients with longstanding T1D (disease duration $\geq$ 50 years) with severe (Medalist +C) or absent to mild complications (Medalist −C) revealed impaired growth, reprogramming, and differentiation in Medalist +C. Genomics and proteomics analyses suggested differential regulation of DNA damage checkpoint proteins favoring protection from cellular apoptosis in Medalist −C. In silico analyses showed altered expression patterns of DNA damage checkpoint factors among the Medalist groups to be targets of miR200, whose expression was significantly elevated in Medalist +C serum. Notably, neurons differentiated from Medalist +C iPSCs exhibited enhanced susceptibility to genotoxic stress that worsened upon miR200 overexpression. Furthermore, knockdown of miR200 in Medalist +C fibroblasts and iPSCs rescued checkpoint protein expression and reduced DNA damage. We propose miR200-regulated DNA damage checkpoint pathway as a potential therapeutic target for treating complications of diabetes.

INTRODUCTION

Type 1 diabetes (T1D) is associated with micro- and macrovascular complications (Chang-Chen et al., 2008; Forbes and Cooper, 2013; Rask-Madsen and King, 2013; Fang et al., 2004; Schalkwijk and Stehouwer, 2005), but the mechanisms underlying their development remain elusive, due in part to a lack of suitable cellular models for molecular investigation (Calcutt et al., 2009; Reddy and Natarajan, 2011; Reddy et al., 2012; Lacolley et al., 2009). Diabetic complications affecting the heart and vascular cells (cardiovascular), kidney (nephropathy), eyes (retinopathy), or nerves (neuropathy) arise as a consequence of stress-induced apoptosis, resulting in loss of the functional cellular pool and a concomitant failure of the body's inherent mechanism to compensate (Brownlee, 2001, 2005). Animal modeling of these phenotypes has met with challenges over the years, in part due to an inability to precisely mimic the human disease phenotype. To overcome such challenges, we derived induced pluripotent stem cells (iPSCs) from individuals with long-standing T1D ($\geq$50 years), termed Medalist patients (Keenan et al., 2007; Maehr et al., 2009; Park et al., 2008; Tiscornia et al., 2011), and age-matched healthy controls. The Medalists were extensively phenotyped by clinical examination and assessed for the presence of complications and classified as those with severe (Medalist +C) and those with absent to mild (Medalist −C) complications (Keenan et al., 2007, 2010). Mass spectrometry (MS)-based quantitative proteomics analyses of these iPSCs, along with microarray gene expression profiling of the patient fibroblasts used to derive the iPSCs, implicated preserved DNA damage checkpoint pathway function, due to suppressed miR200 expression as a mechanism underlying protection against diabetic complications in the Medalist −C subgroup. Consistently, we observed elevated miR200 levels in sera from Medalist +C patients. Furthermore, overexpression of miR200 in two target cell types, namely primary human neurons and primary endothelial cells, caused downregulation of ATM protein, increased pH2AX, and cellular apoptosis. Corroboratively,

Development of a Weighted Cardiometabolic Disease Staging (CMDS) System for the Prediction of Future Diabetes

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Context: Metabolic syndrome traits are important risk factors for diabetes; however, each trait has different predictive power for future diabetes. Additionally, the impact of insulin resistance on metabolic profile can differ by gender and racial group, suggesting that gender-race specific prediction algorithms for diabetes may be warranted.

Objective: To develop a quantitative scoring system based on weighting of risk components in the cardiometabolic disease staging (CMDS) system for the prediction of future diabetes.

Design, Setting, and Participants: We derived the CMDS score in 2857 participants with valid follow-up information on incident diabetes from the Coronary Artery Risk Development in Young Adults study and validated it in 6425 older participants from the Atherosclerosis Risk in Communities study. We assigned a simple integer value for each CMDS risk factor component.

Main Outcome Measures: Incident diabetes.

Results: Fasting glucose, 2-hour glucose, waist circumference, and blood pressure components contributed similarly for the prediction of future diabetes (CMDS scores, 23, 21, 26, and 20, respectively). The area under the receiver operating characteristic curve was 0.7158 for the CMDS scoring system, whereas it was 0.7053 for the Framingham diabetes score. The CMDS components performed differently for prediction of future diabetes in Black and White men and women. The components with the highest predictive power for diabetes were waist circumference in Black men, 2-hour glucose in Black women, and fasting glucose in both White men and White women.

Conclusions: The weighted CMDS score has high model discrimination power for diabetes and can be used clinically to identify patients for weight loss therapy based on differential risk for future diabetes. (***J Clin Endocrinol Metab*** **100: 3871–3877, 2015)**

Obesity is associated with elevated risk for diabetes and cardiovascular diseases (1). Recent approval of new weight loss medications (2, 3) has enabled a pharmaceutical approach for obesity therapy. Although an average weight loss of approximately 10% will often not suffice to meet the cosmetic goals of patients or even bring many patients below the body mass index (BMI) threshold for obesity, it is sufficient to exert powerful benefits regarding weight-related complications (4, 5). However, with nearly 70% of US adults being overweight or obese (6), and due to concerns of safety and cost, it is impracticable to treat all overweight and obese subjects with medical or surgical therapy. The patients who will benefit most from treatment with medications or surgery have obesity-related comorbidities that can be categorized into two general classes: insulin resis-

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Abbreviations: AUC, area under the ROC curve; BMI, body mass index; CARDIA, Coronary Artery Risk Development in Young Adults; CMDS, cardiometabolic disease staging; HDL, high-density lipoprotein; OGTT, oral glucose tolerance test; ROC, receiver operating characteristic; T2DM, type 2 diabetes mellitus.

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Analysis of the Human Proteome in Subcutaneous and Visceral Fat Depots in Diabetic and Non-diabetic Patients with Morbid Obesity

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Abstract

No longer regarded as simply a storage depot, fat is a dynamic organ acting locally and systemically to modulate energy homeostasis, glucose sensitivity, insulin resistance, and inflammatory pathways. Here, mass spectrometry was used to survey the proteome of patient matched subcutaneous fat and visceral fat in 20 diabetic vs 22 nondiabetic patients with morbid obesity. A similar number of proteins (~600) were identified in each tissue type. When stratified by diabetic status, 19 and 41 proteins were found to be differentially abundant in subcutaneous fat and omentum, respectively. These proteins represent pathways known to be involved in metabolism. Five of these proteins were differentially abundant in both fat depots: moesin, 78 kDa glucose-regulated protein, protein cordon-bleu, zinc finger protein 611, and cytochrome c oxidase subunit 6B1. Three proteins, decorin, cytochrome c oxidase subunit 6B1, and 78 kDa glucose-regulated protein, were further tested for validation by western blot analysis. Investigation of the proteins reported here is expected to expand on the current knowledge of adipose tissue driven biochemistry in diabetes and obesity, with the ultimate goal of identifying clinical targets for the development of novel therapeutic interventions in the treatment of type 2 diabetes mellitus. To our knowledge, this study is the first to survey the global proteome derived from each subcutaneous

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Conflict of Interest

The authors have declared no conflict of interest.

Peer Coaches to Improve Diabetes Outcomes in Rural Alabama: A Cluster Randomized Trial

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ABSTRACT

PURPOSE It is unclear whether peer coaching is effective in minority populations living with diabetes in hard-to-reach, under-resourced areas such as the rural South. We examined the effect of an innovative peer-coaching intervention plus brief education vs brief education alone on diabetes outcomes.

METHODS This was a community-engaged, cluster-randomized, controlled trial with primary care practices and their surrounding communities serving as clusters. The trial enrolled 424 participants, with 360 completing baseline and follow-up data collection (84.9% retention). The primary outcomes were change in glycated hemoglobin (HbA_{1c}), systolic blood pressure (BP), low density lipoprotein cholesterol (LDL-C), body mass index (BMI), and quality of life, with diabetes distress and patient activation as secondary outcomes. Peer coaches were trained for 2 days in community settings; the training emphasized motivational interviewing skills, diabetes basics, and goal setting. All participants received a 1-hour diabetes education class and a personalized diabetes report card at baseline. Intervention arm participants were also paired with peer coaches; the protocol called for telephone interactions weekly for the first 8 weeks, then monthly for a total of 10 months.

RESULTS Due to real-world constraints, follow-up was protracted, and intervention effects varied over time. The analysis that included the 68% of participants followed up by 15 months showed only a significant increase in patient activation in the intervention group. The analysis that included all participants who eventually completed follow-up revealed that intervention arm participants had significant differences in changes in systolic BP ($P = .047$), BMI ($P = .02$), quality of life ($P = .003$), diabetes distress ($P = .004$), and patient activation ($P = .03$), but not in HbA_{1c} ($P = .14$) or LDL-C ($P = .97$).

CONCLUSION Telephone-delivered peer coaching holds promise to improve health for individuals with diabetes living in under-resourced areas.

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Conflicts of interest: authors report none.

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INTRODUCTION

The Southeast has both the highest prevalence of diabetes and the highest stroke and heart disease mortality in the United States.[1] Risks of poor outcomes can be reduced by controlling blood glucose, blood pressure (BP), cholesterol levels and body weight, but accomplishing this is difficult. In rural areas like the Alabama Black Belt,[2] barriers to achieving risk factor control are particularly daunting: one-third of area residents live below the federal poverty line (compared with 15% nationally)[3,4] and there are fewer than half as many primary care physicians per 10,000 population as the US average.[3,5,6] Low educational attainment is common[7]; the area's residents are predominantly African Americans, and mistrust of the health care system is widespread.[8] Low-cost programs that can overcome these barriers are urgently needed.

Peer-support interventions to promote self care are particularly attractive for such under-resourced regions. Peer coaches are typically lay people who receive minimal training. They live in the same communities

Article

Cell

Environment Drives Selection and Function of Enhancers Controlling Tissue-Specific Macrophage Identities

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SUMMARY

Macrophages reside in essentially all tissues of the body and play key roles in innate and adaptive immune responses. Distinct populations of tissue macrophages also acquire context-specific functions that are important for normal tissue homeostasis. To investigate mechanisms responsible for tissue-specific functions, we analyzed the transcriptomes and enhancer landscapes of brain microglia and resident macrophages of the peritoneal cavity. In addition, we exploited natural genetic variation as a genome-wide "mutagenesis" strategy to identify DNA recognition motifs for transcription factors that promote common or subset-specific binding of the macrophage lineage-determining factor PU.1. We find that distinct tissue environments drive divergent programs of gene expression by differentially activating a common enhancer repertoire and by inducing the expression of divergent secondary transcription factors that collaborate with PU.1 to establish tissue-specific enhancers. These findings provide insights into molecular mechanisms by which tissue environment influences macrophage phenotypes that are likely to be broadly applicable to other cell types.

INTRODUCTION

Macrophages are phagocytic cells of the innate immune system that populate every organ, making key contributions to their development, functions, and protection against infections and injuries (Geissmann et al., 2010; Gordon et al., 2014; Wynn et al., 2013). Accordingly, each population of tissue macrophages must adapt to its surrounding environment and engage in tissue-specific functions to be effective auxiliary cells. In support of this, recent mRNA profiling studies revealed significant differences between distinct populations of resident tissue macrophages (Gautier et al., 2012; Okabe and Medzhitov, 2014). Thus, in spite of common elements shared across all subtypes of tissue macrophages, including dependency on the transcription factor PU.1 and signaling downstream of the CSF1 receptor for ontology and survival (Schulz et al., 2012; Wynn et al., 2013), each subset of tissue macrophage possesses its own unique gene expression profile that presumably allows it to function in synergy with the tissue in which it resides.

Accumulating evidence suggests that signaling factors derived from tissue environments play key roles in promoting the ontology and phenotype of the residing macrophage populations. For example, absence of TGF-β1 signaling in the mouse brain impairs the development of the microglia population (Butovsky et al., 2014; Makwana et al., 2007). In the peritoneum, omentum-derived retinoic acid (RA) promotes expression of Gata6 in a subpopulation of local macrophages (Okabe and Medzhitov, 2014). Interestingly, Gata6 expression is exclusive to this particular tissue macrophage population, and decreasing or eliminating its expression interferes with their functions and survival (Gautier et al., 2012, 2014; Okabe and Medzhitov, 2014; Rosas et al., 2014).

Precisely how these and other signals act on macrophages at the genomic level to promote specialized phenotypes and unique transcriptional signatures remains unknown. However, strong evidence suggests that enhancers, which are fundamental determinants of gene expression, may play a key role in this context (Andersson et al., 2014; Levine, 2010; Shlyueva et al., 2014). Enhancers, in comparison to promoters, exhibit significant enrichment for combinations of DNA recognition motifs that correspond to binding sites for lineage-determining transcription factors (LDTFs), which are required for the development of distinct cell types. Different patterns of LDTF expression drive the selection of cell-specific repertoires of enhancers that are considered to be central to the establishment of cell identity and regulatory potential.

Studies of primary macrophages and B cells indicated that PU.1 acts as an essential LDTF that contributes to the selection

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Low-frequency and rare exome chip variants associate with fasting glucose and type 2 diabetes susceptibility

Jennifer Wessel, Audrey Y. Chu, Sara M. Willems, Shuai Wang *et al.*#

Fasting glucose and insulin are intermediate traits for type 2 diabetes. Here we explore the role of coding variation on these traits by analysis of variants on the HumanExome BeadChip in 60,564 non-diabetic individuals and in 16,491 T2D cases and 81,877 controls. We identify a novel association of a low-frequency nonsynonymous SNV in *GLP1R* (A316T; rs10305492; MAF = 1.4%) with lower FG ($\beta = -0.09 \pm 0.01$ mmol l^{-1}, $P = 3.4 \times 10^{-12}$), T2D risk (OR[95%CI] = 0.86[0.76–0.96], $P = 0.010$), early insulin secretion ($\beta = -0.07 \pm 0.035$ pmol$_{insulin}$ mmol$^{-1}_{glucose}$, $P = 0.048$), but higher 2-h glucose ($\beta = 0.16 \pm 0.05$ mmol l^{-1}, $P = 4.3 \times 10^{-4}$). We identify a gene-based association with FG at *G6PC2* ($p_{SKAT} = 6.8 \times 10^{-6}$) driven by four rare protein-coding SNVs (H177Y, Y207S, R283X and S324P). We identify rs651007 (MAF = 20%) in the first intron of *ABO* at the putative promoter of an antisense lncRNA, associating with higher FG ($\beta = 0.02 \pm 0.004$ mmol l^{-1}, $P = 1.3 \times 10^{-8}$). Our approach identifies novel coding variant associations and extends the allelic spectrum of variation underlying diabetes-related quantitative traits and T2D susceptibility.

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nature medicine

A Gpr120-selective agonist improves insulin resistance and chronic inflammation in obese mice

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It is well known that the ω–3 fatty acids (ω–3-FAs; also known as *n*–3 fatty acids) can exert potent anti-inflammatory effects[1–4]. Commonly consumed as fish products, dietary supplements and pharmaceuticals, ω–3-FAs have a number of health benefits ascribed to them, including reduced plasma triglyceride levels, amelioration of atherosclerosis and increased insulin sensitivity[5–7]. We reported that Gpr120 is the functional receptor for these fatty acids and that ω–3-FAs produce robust anti-inflammatory, insulin-sensitizing effects, both *in vivo* and *in vitro*, in a Gpr120-dependent manner[8]. Indeed, genetic variants that predispose to obesity and diabetes have been described in the gene encoding GPR120 in humans (*FFAR4*)[9]. However, the amount of fish oils that would have to be consumed to sustain chronic agonism of Gpr120 is too high to be practical, and, thus, a high-affinity small-molecule Gpr120 agonist would be of potential clinical benefit. Accordingly, Gpr120 is a widely studied drug discovery target within the pharmaceutical industry. Gpr40 is another lipid-sensing G protein–coupled receptor[10], and it has been difficult to identify compounds with a high degree of selectivity for Gpr120 over Gpr40 (ref. 11). Here we report that a selective high-affinity, orally available, small-molecule Gpr120 agonist (cpdA) exerts potent anti-inflammatory effects on macrophages *in vitro* and in obese mice *in vivo*. Gpr120 agonist treatment of high-fat diet–fed obese mice causes improved glucose tolerance, decreased hyperinsulinemia, increased insulin sensitivity and decreased hepatic steatosis. This suggests that Gpr120 agonists could become new insulin-sensitizing drugs for the treatment of type 2 diabetes and other human insulin-resistant states in the future.

Gpr120 and Gpr40 are both lipid-sensing G protein–coupled receptors (GPCRs)[10,12], but although there is limited homology between these two polyunsaturated fatty acid (PUFA) receptors, identification of ligands that are highly selective for Gpr120 over Gpr40 has been challenging[11,13–15]. We have generated a small-molecule Gpr120 agonist, compound A (cpdA) (**Fig. 1a**), and have examined its selectivity for Gpr120 compared to Gpr40 using a Ca^{2+} FLIPR (fluorometric imaging plate reader) assay (**Fig. 1b**). We found that cpdA was fully selective for Gpr120 (log of the half-maximum effective concentration (EC_{50}) = −7.62 ± 0.11 M) with negligible activity toward Gpr40 (**Fig. 1b**). Gpr120 couples to Gαq/Gα11-initiated signal transduction pathways, and we therefore assessed the activity of cpdA in an inositol-1,4,5-triphosphate (IP_3) production assay, employing HEK 293 cells that stably express human or mouse Gpr120. The Gpr120 agonist produced concentration-dependent increases in IP_3 production from both human and mouse Gpr120–expressing cells (**Fig. 1c**).

In addition to promoting signaling via Gαq/11, Gpr120 also directly couples to β-arrestin-2 (refs. 8,14). Therefore, we examined the potency of cpdA in a β-arrestin-2 recruitment assay (**Fig. 1d**). We found that cpdA led to a concentration-dependent response to recruit β-arrestin-2 in both human and mouse Gpr120–expressing cells, with EC_{50}s of ~0.35 μM (**Fig. 1d**). As Gpr120 is a Gαq/11-coupled receptor, it stimulates both protein kinase C and mitogen-activated protein kinase, and both of these biological effects can be detected in a serum response element (SRE)-driven reporter system[8]. We transiently transfected HEK 293 cells with a plasmid construct encoding mouse Gpr120 and another plasmid encoding an SRE-luciferase promoter-reporter (SRE-luc). We treated the Gpr120:SRE-luc reporter cells with either docosahexaenoic acid (DHA) or cpdA. Gpr120 stimulation by cpdA was ~50-fold more sensitive than that achieved by DHA (**Fig. 1e**). We used DHA and cpdA at 100 μM and 10 μM, respectively, in all subsequent studies to achieve maximal effects.

In our previous studies[8], we showed that Gpr120 stimulation mediated anti-inflammatory responses in macrophages. To link these observations to cpdA, we evaluated the effect of DHA and cpdA on nuclear factor-κB (NF-κB) response element–driven reporter gene activity in wild-type (WT) and Gpr120-knockout primary mouse macrophages.

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A Comparative Analysis of the Safety, Efficacy, and Cost of Islet Versus Pancreas Transplantation in Nonuremic Patients With Type 1 Diabetes

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Few current studies compare the outcomes of islet transplantation alone (ITA) and pancreas transplantation alone (PTA) for type 1 diabetes (T1D). We examined these two beta cell replacement therapies in nonuremic patients with T1D with respect to safety, graft function and cost. Sequential patients received PTA (n = 15) or ITA (n = 10) at our institution. Assessments of graft function included duration of insulin independence; glycemic control, as measured by hemoglobin A1c; and elimination of severe hypoglycemia. Cost analysis included all normalized costs associated with transplantation and inpatient management. ITA patients received one (n = 6) or two (n = 4) islet transplants. Mean duration of insulin independence in this group was 35 mo; 90% were independent at 1 year, and 70% were independent at 3 years. Mean duration of insulin independence in PTA was 55 mo; 93% were insulin independent at 1 year, and 64% were independent at 3 years. Glycemic control was comparable in all patients with functioning grafts, as were overall costs ($138 872 for ITA, $134 748 for PTA). We conclude that with advances in islet isolation and posttransplant management, ITA can produce outcomes similar to PTA and represents a clinically viable option to achieve long-term insulin independence in selected patients with T1D.

Abbreviations: BELA, belatacept; CAD, coronary artery disease; CMV, cytomegalovirus; Cr, creatinine; EFA, efalizumab; eGFR, estimated GFR; HbA1c, hemoglobin A1c; IEQ, islet equivalents; IAK, islet after kidney; ITA, islet transplant alone; IV, intravenously; MMF, mycophenolate mofetil; MMTT, mixed meal tolerance test; PAK, pancreas after kidney; PTA, pancreas transplant alone; PTLD, posttransplant lymphoproliferative disorder; PVD, peripheral vascular disease; SD, standard deviation; SIK, simultaneous islet and kidney; SPK, simultaneous pancreas and kidney; T1D, type 1 diabetes; Tx, transplant; UCSF, University of California San Francisco

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Introduction

The most physiological method of achieving normoglycemia without the associated risk of hypoglycemia in patients with type 1 diabetes (T1D) is currently to restore islet function by vascularized pancreas transplantation or transplantation of isolated pancreatic islets. Although combined solid organ pancreas and kidney transplantation in uremic patients with diabetes is a complex procedure, advances in immunosuppressive regimens and surgical technique have made it increasingly successful, particularly because these patients enjoy the benefits of independence from dialysis (1,2). In contrast, solitary pancreas transplantation in nonuremic patients has received limited acceptance, primarily because of the associated surgical complications, the high risk of rejection and the nephrotoxic side effects of current immunosuppressive regimens in a patient population that is already at risk for renal dysfunction (1,3,4). This perception continues despite advances in immunosuppression and patient management that have improved 5-year graft survival rates in this patient population to >50% (2,5,6).

Islet transplantation offers a promising minimally invasive approach to restore insulin independence in patients with T1D without the surgical complications associated with whole-organ transplantation. Success rates for islet transplant alone (ITA) have traditionally been much lower than for pancreas transplant alone (PTA); however, this is changing with improvements in islet processing and immunosuppressive management. Consequently, islet transplantation is increasingly being considered as a realistic beta cell replacement option for patients with T1D who are not candidates for whole-organ transplant (7–13). Despite these innovations, there have not been any recent comparisons of these two therapies with respect to graft and patient outcomes. In the current study, we addressed

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Activated Type 2 Innate Lymphoid Cells Regulate Beige Fat Biogenesis

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SUMMARY

Type 2 innate lymphoid cells (ILC2s), an innate source of the type 2 cytokines interleukin (IL)-5 and -13, participate in the maintenance of tissue homeostasis. Although type 2 immunity is critically important for mediating metabolic adaptations to environmental cold, the functions of ILC2s in beige or brown fat development are poorly defined. We report here that activation of ILC2s by IL-33 is sufficient to promote the growth of functional beige fat in thermoneutral mice. Mechanistically, ILC2 activation results in the proliferation of bipotential adipocyte precursors (APs) and their subsequent commitment to the beige fat lineage. Loss- and gain-of-function studies reveal that ILC2- and eosinophil-derived type 2 cytokines stimulate signaling via the IL-4Rα in PDGFRα$^+$ APs to promote beige fat biogenesis. Together, our results highlight a critical role for ILC2s and type 2 cytokines in the regulation of adipocyte precursor numbers and fate, and as a consequence, adipose tissue homeostasis.

INTRODUCTION

White adipose tissue (WAT) is a highly dynamic organ that responds to nutrient and environmental stress (Berry et al., 2014; Rosen and Spiegelman, 2006; Rosen and Spiegelman, 2014; Zeve et al., 2009). When mammals are in positive energy balance, WAT expands by hyperplasia and hypertrophy to store excess nutrients. In contrast, prolonged cold stress induces catabolic programs in WAT depots, in particular in the subcutaneous WAT (scWAT) of mice, to support thermogenesis (Harms and Seale, 2013; Wu et al., 2013). In this case, adrenergic stimulation of scWAT promotes tissue "browning" via induction of beige adipocytes that express the uncoupling protein 1 (UCP1). This de novo recruitment of beige adipocytes alleviates cold stress to restore thermal homeostasis (Nedergaard and Cannon, 2014). Despite progress in this field, the physiologic signals that regulate adipocyte precursor proliferation and their subsequent commitment to the beige adipocyte lineage remain poorly understood.

Fate mapping studies have led to the identification of progenitor or precursor cell populations that give rise to brown and beige adipocytes in adult mice. These studies have revealed that interscapular brown adipocytes arise from a mesodermal progenitor that transiently expresses the myogenic transcription factors Myf5 and Pax7 (Lepper and Fan, 2010; Seale et al., 2008). In contrast, beige adipocytes, which are found in WAT depots of mice, primarily arise from Myf5$^-$ PDGFRα$^+$ precursor cells (Sanchez-Gurmaches et al., 2012; Seale et al., 2008). Fate mapping studies by the Granneman laboratory have elegantly demonstrated that pharmacologic activation of the β3-adrenergic receptor stimulates the proliferation of PDGFRα$^+$ precursor cells, which subsequently differentiate into beige adipocytes (Lee et al., 2012). Interestingly, these PDGFRα$^+$ precursor cells can also give rise to white adipocytes in the setting of dietary obesity (Berry and Rodeheffer, 2013; Hudak et al., 2014; Lee et al., 2012; Wang et al., 2014), suggesting that environmental signals likely dictate the commitment of PDGFRα$^+$ precursor cells to the beige or white adipocyte lineage.

Exposure of adult animals to environmental cold stimulates the growth of thermogenic beige fat via activation of adrenergic signaling pathways (Harms and Seale, 2013; Wu et al., 2013). In contrast to interscapular BAT, we recently reported that the scWAT relies on a hematopoietic circuit consisting of eosinophils and alternatively activated macrophages for the maintenance of its adrenergic tone. In response to environmental cold, we found that eosinophil-derived interleukin (IL)-4 induces the expression tyrosine hydroxylase (TH), the rate-limiting enzyme in the synthesis of catecholamines, in alternatively activated macrophages (Nguyen et al., 2011; Qiu et al., 2014). Accordingly, genetic deletion of *Il4ra* or *Th* in myeloid cells significantly impaired the development of thermogenic beige fat in mice (Qiu et al., 2014). The observation that other browning factors, such as meteorin-like (METRNL) also utilize this pathway for their thermic effects (Rao et al., 2014), suggests that type 2 innate immunity might be integrally linked with the development of beige adipose tissue.

ILC2s, which are present in lymphoid and nonlymphoid tissues (Moro et al., 2010; Neill et al., 2010; Price et al., 2010),

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Cell

Sensory Detection of Food Rapidly Modulates Arcuate Feeding Circuits

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SUMMARY

Hunger is controlled by specialized neural circuits that translate homeostatic needs into motivated behaviors. These circuits are under chronic control by circulating signals of nutritional state, but their rapid dynamics on the timescale of behavior remain unknown. Here, we report optical recording of the natural activity of two key cell types that control food intake, AgRP and POMC neurons, in awake behaving mice. We find unexpectedly that the sensory detection of food is sufficient to rapidly reverse the activation state of these neurons induced by energy deficit. This rapid regulation is cell-type specific, modulated by food palatability and nutritional state, and occurs before any food is consumed. These data reveal that AgRP and POMC neurons receive real-time information about the availability of food in the external world, suggesting a primary role for these neurons in controlling appetitive behaviors such as foraging that promote the discovery of food.

INTRODUCTION

Food intake is controlled by evolutionarily hard-wired neural circuits that contain specialized neural cell types. Two cell types in the arcuate nucleus (ARC) of the hypothalamus are known to be particularly important for the control of feeding. These neurons are identified by expression of the neuropeptides Agouti-related Protein (AgRP) and Proopiomelanocortin (POMC) and have opposing functions. AgRP neurons are activated by energy deficit (Hahn et al., 1998) and promote food seeking and consumption. Optogenetic or chemogenetic activation of AgRP neurons induces voracious eating in sated mice (Aponte et al., 2011; Krashes et al., 2011), whereas inhibition or ablation of AgRP neurons results in aphagia (Gropp et al., 2005; Krashes et al., 2011; Luquet et al., 2005). These effects of AgRP neurons are mediated by release of GABA as well as two neuropeptides, AgRP and NPY, that stimulate food intake when delivered into the brain (Clark et al., 1985; Fan et al., 1997; Ollmann et al., 1997; Tong et al., 2008). POMC neurons by contrast are activated by energy surfeit and their activity inhibits food intake and promotes weight loss. These two cell types interact in part through a common set of downstream neural targets that express melanocortin receptors, which are activated by POMC and inhibited by AgRP (Fan et al., 1997; Ollmann et al., 1997; Seeley et al., 1997). Thus, AgRP and POMC neurons are two intermingled, interacting neural cell types that have opposing roles in the control of feeding.

Despite intense investigation of these cells over the past 20 years, their activity dynamics during behavior remain unknown. This knowledge gap reflects the difficulty of recording cell-type-specific neural activity within heterogeneous deep brain structures such as the hypothalamus. As a result, our current understanding of the regulation of AgRP and POMC neurons is based on a combination of approaches that include in vitro electrophysiology, *c-fos* staining, pharmacology, and genetic manipulations. These pioneering studies have revealed a dominant role for circulating hormones and nutrients in the control of these cells (Williams and Elmquist, 2012). AgRP and POMC neurons are modulated by hormones such as ghrelin and leptin (Cowley et al., 2001, 2003; Nakazato et al., 2001; Pinto et al., 2004) as well as circulating nutrients (Blouet and Schwartz, 2010) in part via their metabolic effects on mitochondrial dynamics (Dietrich et al., 2013; Schneeberger et al., 2013). Together, these findings have led to a generally accepted model in which AgRP and POMC neurons function as interoceptors that monitor the concentration of hormones and nutrients in the blood and then gradually adjust their activity in parallel with changes in nutritional state. This model provides a compelling explanation for how nutritional changes can be translated into counterregulatory responses but leaves unanswered the question of whether these neurons are also subject to rapid regulation on the timescale of behavior.

AgRP and POMC neurons also receive abundant synaptic input which provides the potential for more rapid modulation. However, the function of this afferent input is not well understood. Fasting increases excitatory tone onto AgRP neurons (Liu et al., 2012; Yang et al., 2011), and one source of such excitatory input is neurons in the paraventricular hypothalamus (PVH) (Krashes et al., 2014). AgRP neurons also receive inhibitory input from the dorsomedial hypothalamus (DMH) among other sources (Krashes et al., 2014). POMC neurons by contrast receive inhibitory input from cells in the ARC, including neighboring AgRP neurons, as well as excitatory input from the ventromedial hypothalamus (VMH) and other regions (Cowley et al., 2001; Krashes et al., 2014; Pinto et al., 2004; Sternson et al., 2005; Vong et al., 2011). As these circuit connections have only recently been described, their regulation and function are not yet clear. An important open question regards the nature of the information that these presynaptic cells communicate to their AgRP and POMC targets.

In the present study, we have used an optical approach to record the natural activity of AgRP and POMC neurons in awake

Original Investigation

Association of a Low-Frequency Variant in *HNF1A* With Type 2 Diabetes in a Latino Population

The SIGMA Type 2 Diabetes Consortium

IMPORTANCE Latino populations have one of the highest prevalences of type 2 diabetes worldwide.

OBJECTIVES To investigate the association between rare protein-coding genetic variants and prevalence of type 2 diabetes in a large Latino population and to explore potential molecular and physiological mechanisms for the observed relationships.

DESIGN, SETTING, AND PARTICIPANTS Whole-exome sequencing was performed on DNA samples from 3756 Mexican and US Latino individuals (1794 with type 2 diabetes and 1962 without diabetes) recruited from 1993 to 2013. One variant was further tested for allele frequency and association with type 2 diabetes in large multiethnic data sets of 14 276 participants and characterized in experimental assays.

MAIN OUTCOME AND MEASURES Prevalence of type 2 diabetes. Secondary outcomes included age of onset, body mass index, and effect on protein function.

RESULTS A single rare missense variant (c.1522G>A [p.E508K]) was associated with type 2 diabetes prevalence (odds ratio [OR], 5.48; 95% CI, 2.83-10.61; $P = 4.4 \times 10^{-7}$) in hepatocyte nuclear factor 1-α (*HNF1A*), the gene responsible for maturity onset diabetes of the young type 3 (MODY3). This variant was observed in 0.36% of participants without type 2 diabetes and 2.1% of participants with it. In multiethnic replication data sets, the p.E508K variant was seen only in Latino patients (n = 1443 with type 2 diabetes and 1673 without it) and was associated with type 2 diabetes (OR, 4.16; 95% CI, 1.75-9.92; $P = .0013$). In experimental assays, HNF-1A protein encoding the p.E508K mutant demonstrated reduced transactivation activity of its target promoter compared with a wild-type protein. In our data, carriers and noncarriers of the p.E508K mutation with type 2 diabetes had no significant differences in compared clinical characteristics, including age at onset. The mean (SD) age for carriers was 45.3 years (11.2) vs 47.5 years (11.5) for noncarriers ($P = .49$) and the mean (SD) BMI for carriers was 28.2 (5.5) vs 29.3 (5.3) for noncarriers ($P = .19$).

CONCLUSIONS AND RELEVANCE Using whole-exome sequencing, we identified a single low-frequency variant in the MODY3-causing gene *HNF1A* that is associated with type 2 diabetes in Latino populations and may affect protein function. This finding may have implications for screening and therapeutic modification in this population, but additional studies are required.

Supplemental content at jama.com

The Authors and other collaborators of the SIGMA Type 2 Diabetes Consortium are listed at the end of this article.

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ARTICLE

Age at the time of sulfonylurea initiation influences treatment outcomes in *KCNJ11*-related neonatal diabetes

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Abstract
Aims/hypothesis Individuals with heterozygous activating mutations of the *KCNJ11* gene encoding a subunit of the ATP-sensitive potassium channel (KATP) can usually be treated with oral sulfonylurea (SU) pills in lieu of insulin injections. The aim of this study was to test our hypothesis that younger age at the time of initiation of SU therapy is correlated with lower required doses of SU therapy, shorter transition time and decreased likelihood of requiring additional diabetes medications.
Methods We performed a retrospective cohort study using data on 58 individuals with neonatal diabetes due to *KCNJ11* mutations identified through the University of Chicago Monogenic Diabetes Registry (http://monogenicdiabetes.uchicago.edu/registry). We assessed the influence of age at initiation of SU therapy on treatment outcomes.
Results HbA_{1c} fell from an average of 8.5% (69 mmol/mol) before transition to 6.2% (44 mmol/mol) after SU therapy ($p<0.001$). Age of initiation of SU correlated with the dose (mg kg^{-1} day^{-1}) of SU required at follow-up ($r=0.80$, $p<0.001$). Similar associations were observed across mutation subtypes. Ten participants required additional glucose-lowering medications and all had initiated SU at age 13 years or older. No serious adverse events were reported.
Conclusions/interpretation Earlier age at initiation of SU treatment is associated with improved response to SU therapy. Declining sensitivity to SU may be due to loss of beta cell mass over time in those treated with insulin. Our data support the need for early genetic diagnosis and appropriate personalised treatment in all cases of neonatal diabetes.

Keywords Dosing · Glibenclamide · Glyburide · HbA_{1c} · K_{ATP} channel · *KCNJ11* · Monogenic diabetes · Neonatal diabetes · Pharmacotherapy · Sulfonylurea

Abbreviations

K_{ATP} channel	ATP-sensitive potassium channel
SU	Sulfonylurea

Introduction

Activating heterozygous mutations in *KCNJ11* and *ABCC8* genes encoding the two subunits of the ATP-sensitive potassium (K_{ATP}) channel are the most common causes of diabetes in the first months of life [1–3]. *KCNJ11* mutations most commonly cause permanent neonatal diabetes. Identifying individuals with heterozygous activating mutations of this gene is of significant clinical value as oral sulfonylurea (SU) treatment can routinely replace insulin therapy with improvement of glycaemic control [1, 4–8]. *KCNJ11*-related neonatal diabetes may be distinguished from other monogenic or

The list of members of the United States Neonatal Diabetes Working Group is shown in the Acknowledgements.

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Sulfonylurea Treatment Before Genetic Testing in Neonatal Diabetes: Pros and Cons

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Context: Diabetes in neonates nearly always has a monogenic etiology. Earlier sulfonylurea therapy can improve glycemic control and potential neurodevelopmental outcomes in children with *KCNJ11* or *ABCC8* mutations, the most common gene causes.

Objective: Assess the risks and benefits of initiating sulfonylurea therapy before genetic testing results become available.

Design, Setting, and Patients: Observational retrospective study of subjects with neonatal diabetes within the University of Chicago Monogenic Diabetes Registry.

Main Outcome Measures: Response to sulfonylurea (determined by whether insulin could be discontinued) and treatment side effects in those treated empirically.

Results: A total of 154 subjects were diagnosed with diabetes before 6 months of age. A genetic diagnosis had been determined in 118 (77%), with 73 (47%) having a mutation in *KCNJ11* or *ABCC8*. The median time from clinical diagnosis to genetic diagnosis was 10.4 weeks (range, 1.6 to 58.2 wk). In nine probands, an empiric sulfonylurea trial was initiated within 28 days of diabetes diagnosis. A genetic cause was subsequently found in eight cases, and insulin was discontinued within 14 days of sulfonylurea initiation in all of these cases.

Conclusions: Sulfonylurea therapy appears to be safe and often successful in neonatal diabetes patients before genetic testing results are available; however, larger numbers of cases must be studied. Given the potential beneficial effect on neurodevelopmental outcome, glycemic control, and the current barriers to expeditious acquisition of genetic testing, an empiric inpatient trial of sulfonylurea can be considered. However, obtaining a genetic diagnosis remains imperative to inform long-term management and prognosis. **(*J Clin Endocrinol Metab* 99: E2709–E2714, 2014)**

Persistent hyperglycemia occurring at a very early age has long been termed neonatal diabetes mellitus (NDM), although it is often diagnosed after the first few weeks of life. Infants diagnosed within the first 6 months of life are especially likely to have an underlying monogenic cause, but a small fraction of those diagnosed at later ages may also be found to have similar single gene mutations (1). Studies of European populations provide inci-

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Abbreviations: DEND, developmental delay, epilepsy, and neonatal diabetes; KATP, ATP-sensitive potassium; NDM, neonatal diabetes mellitus; 6q24-TND, 6q24-related transient neonatal diabetes.

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Cell Metabolism
Article

Maximal Oxidative Capacity during Exercise Is Associated with Skeletal Muscle Fuel Selection and Dynamic Changes in Mitochondrial Protein Acetylation

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SUMMARY

Maximal exercise-associated oxidative capacity is strongly correlated with health and longevity in humans. Rats selectively bred for high running capacity (HCR) have improved metabolic health and are longer-lived than their low-capacity counterparts (LCR). Using metabolomic and proteomic profiling, we show that HCR efficiently oxidize fatty acids (FAs) and branched-chain amino acids (BCAAs), sparing glycogen and reducing accumulation of short- and medium-chain acylcarnitines. HCR mitochondria have reduced acetylation of mitochondrial proteins within oxidative pathways at rest, and there is rapid protein deacetylation with exercise, which is greater in HCR than LCR. Fluxomic analysis of valine degradation with exercise demonstrates a functional role of differential protein acetylation in HCR and LCR. Our data suggest that efficient FA and BCAA utilization contribute to high intrinsic exercise capacity and the health and longevity benefits associated with enhanced fitness.

INTRODUCTION

Exercise capacity and cardiovascular fitness are highly predictive of metabolic health, including lower fat mass, higher insulin sensitivity, lower blood pressure, and, importantly, age-adjusted mortality (Blair et al., 1996; Church et al., 2004; Dvorak et al., 2000; Kodama et al., 2009). The mechanisms underlying these associations are not fully understood. One important link between exercise capacity and overall metabolic health is the fuel selection for energy production. Higher exercise capacity is associated with increased fatty acid (FA) oxidation during exercise (Hall et al., 2010; Morris et al., 2013; Nordby et al., 2006; Venables et al., 2005), while poor metabolic health is associated with high basal use of carbohydrates and impaired fuel switching during the fast-fed transition (Kelley and Mandarino, 2000). The glucose-FA cycle described by Randle et al. (1963) states that fat availability will drive fat oxidation and reciprocally lead to decreased glucose oxidation; however, this theory cannot explain instances when fat availability is high but carbohydrates are preferentially oxidized, as is the case during high-intensity exercise and insulin resistance (Kelley and Mandarino, 2000; Mittendorfer and Klein, 2001; Sidossis et al., 1997).

Recent advances in metabolomics and proteomics allow the quantification of tens to thousands of metabolites or peptides in a single biological sample. Integrating these techniques can provide insight into the changes in nutrient utilization under different physiological conditions. In these studies, we employed a combination of metabolomics and proteomics to investigate fuel selection in rats selectively bred for high and low intrinsic running capacity (HCR and LCR). The HCR-LCR rat model was derived from a heterogeneous founder population (N:NIH) with breeder selection based solely on intrinsic (untrained) treadmill running capacity (Koch and Britton, 2001). In this model, as in humans, exercise capacity is a heritable trait (Fagard et al., 1991; Ren et al., 2013), and like humans who differ in running capacity, HCR and LCR diverge in susceptibility to metabolic and related disease traits (Koch et al., 2011; Naples et al., 2010; Noland et al., 2007; Novak et al., 2010; Wisløff et al., 2005). Compared to LCR, HCR animals diverge more strongly in running capacity from the founder stocks and show a 2.4-fold increased running capacity over the highest capacity observed in inbred lines (Ren et al., 2013). HCR animals weigh significantly less than LCR animals throughout their lifespan, despite similar food consumption, and there is evidence of increased capacity of substrate oxidation (Rivas et al., 2011). A recent study (Gavini et al., 2014) showed that HCR and LCR have similar resting

nature neuroscience

Leptin-inhibited PBN neurons enhance responses to hypoglycemia in negative energy balance

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Hypoglycemia initiates the counter-regulatory response (CRR), in which the sympathetic nervous system, glucagon and glucocorticoids restore glucose to appropriate concentrations. During starvation, low leptin levels restrain energy utilization, enhancing long-term survival. To ensure short-term survival during hypoglycemia in fasted animals, the CRR must overcome this energy-sparing program and nutrient depletion. Here we identify in mice a previously unrecognized role for leptin and a population of leptin-regulated neurons that modulate the CRR to meet these challenges. Hypoglycemia activates neurons of the parabrachial nucleus (PBN) that coexpress leptin receptor (LepRb) and cholecystokinin (CCK) (PBN LepRb^{CCK} neurons), which project to the ventromedial hypothalamic nucleus. Leptin inhibits these cells, and *Cck^{cre}*-mediated ablation of LepRb enhances the CRR. Inhibition of PBN LepRb cells blunts the CRR, whereas their activation mimics the CRR in a CCK-dependent manner. PBN LepRb^{CCK} neurons are a crucial component of the CRR system and may be a therapeutic target in hypoglycemia.

Hypoglycemia and glucoprivation (which mimics low glucose availability by interfering with cellular glucose metabolism) activate a neurohormonal CRR that stimulates the hypothalamic-pituitary-adrenal axis and the sympathetic nervous system (SNS) to promote glucose release into the bloodstream[1,2]. The SNS also acts on pancreatic islets to promote glucagon release and suppress insulin secretion[3]. The CRR serves to restore normoglycemia and protect the brain and body from damage due to hypoglycemia. An appropriately robust CRR is crucial to prevent cognitive impairment, unconsciousness or even death when blood glucose levels are too low. This response is especially crucial to counteract insulin-induced hypoglycemia (IIH) in patients with diabetes, for whom the risk of hypoglycemia (especially during the night and other periods of fasting) is the most serious limitation to achieving tight glycemic control[2,4]. Defining the neural systems that mediate and modulate the CRR to hypoglycemia will reveal mechanisms that may be potential targets for the prevention and therapy of this life-threatening complication of insulin therapy.

Diminished nutritional reserves present a particularly severe challenge to mounting an appropriate CRR to hypoglycemia; not only does fasting predispose to hypoglycemia[5] and deplete stores of glycogen and gluconeogenic substrates, but negative energy balance also initiates a neuroendocrine starvation response, decreasing overall SNS tone and initiating energy-sparing changes in endocrine function[6]. Teleologically, it would thus make sense to deploy a more robust CRR in the face of depleted energy stores, although such a system has not been described previously.

The energy-conserving response to starvation results in large part from decreased circulating concentrations of the adipose-derived hormone leptin, which is produced in proportion to fat stores[6–9]. In general, low leptin levels signal the insufficiency of energy reserves to decrease energy utilization and promote hunger, along with other adaptations to cope with decreased energy availability, including alterations in anxiety, motivation, locomotor activity, glucose homeostasis and a wide range of other behavioral and physiologic parameters[10–14]. Here we test the hypothesis that low leptin levels also enhance the CRR to permit an appropriately robust response to hypoglycemia in the context of decreased nutritional reserves.

RESULTS

Electrical and structural properties of PBN LepRb neurons

Most effects of leptin are mediated by LepRb in the brain, especially the hypothalamus and brainstem, where most LepRb neurons reside[15–17]. Commensurate with the diverse processes controlled by leptin, various subtypes of LepRb neurons each contribute to distinct aspects of energy balance and metabolism[13,18–20]. Hypothalamic LepRb neurons in aggregate mediate most of leptin's action on food intake and energy expenditure[21–23]. Although ablation of LepRb in the nucleus tractus solitarius (NTS) has revealed that NTS LepRb cells participate in the control of satiety[24–28], roles for leptin and LepRb in most brainstem

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CellPress

Cell Metabolism
Short Article

Bone Marrow Adipose Tissue Is an Endocrine Organ that Contributes to Increased Circulating Adiponectin during Caloric Restriction

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SUMMARY

The adipocyte-derived hormone adiponectin promotes metabolic and cardiovascular health. Circulating adiponectin increases in lean states such as caloric restriction (CR), but the reasons for this paradox remain unclear. Unlike white adipose tissue (WAT), bone marrow adipose tissue (MAT) increases during CR, and both MAT and serum adiponectin increase in many other clinical conditions. Thus, we investigated whether MAT contributes to circulating adiponectin. We find that adiponectin secretion is greater from MAT than WAT. Notably, specific inhibition of MAT formation in mice results in decreased circulating adiponectin during CR despite unaltered adiponectin expression in WAT. Inhibiting MAT formation also alters skeletal muscle adaptation to CR, suggesting that MAT exerts systemic effects. Finally, we reveal that both MAT and serum adiponectin increase during cancer therapy in humans. These observations identify MAT as an endocrine organ that contributes significantly to increased serum adiponectin during CR and perhaps in other adverse states.

INTRODUCTION

White adipose tissue (WAT) is a major endocrine organ that exerts diverse systemic effects. One of the most extensively studied adipocyte-secreted factors is the hormone adiponectin, which promotes insulin sensitivity, fat oxidation, antiatherogenic, and anticancer effects (Ye and Scherer, 2013). Serum adiponectin is also a well-established biomarker for insulin resistance and cardiovascular disease; indeed, circulating adiponectin is low in obese, insulin-resistant individuals and other adverse metabolic states (Ye and Scherer, 2013). Conversely, serum adiponectin increases in lean, insulin-sensitive states such as with calorie restriction (CR) in animals and anorexia nervosa (AN) in humans (Combs et al., 2003; Dolezalova et al., 2007; Pannacciulli et al., 2003). Reduced circulating adiponectin in obesity most likely derives from decreased adiponectin expression and secretion, which may result from mitochondrial dysfunction or aberrantly increased inflammation, hypoxia, or endoplasmic reticulum stress (Ye and Scherer, 2013). Far less is known about why serum adiponectin increases in lean states. Although some studies report increased adiponectin expression in WAT during extensive CR (Qiao et al., 2011), most studies in mice and humans find that prolonged CR or extensive weight loss increases serum adiponectin without affecting adiponectin expression or secretion from WAT (Behre et al., 2007; Combs et al., 2003; Kovacova et al., 2009; Wang et al., 2006). Indeed, adiponectin expression in WAT decreases in human subjects with AN (Dolezalova et al., 2007). Adiponectin clearance is also unaltered during CR (Qiao et al., 2011). Thus, in lean states such as CR or AN, the paradoxical increase in serum adiponectin can occur without greater expression or secretion from WAT or decreased adiponectin clearance.

Our knowledge of adiponectin derives from extensive study of WAT biology over the past generation. In comparison, metabolic research has largely neglected another adipose depot, bone

Cell

Genetic Variation Determines PPARγ Function and Anti-diabetic Drug Response In Vivo

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SUMMARY

SNPs affecting disease risk often reside in non-coding genomic regions. Here, we show that SNPs are highly enriched at mouse strain-selective adipose tissue binding sites for PPARγ, a nuclear receptor for anti-diabetic drugs. Many such SNPs alter binding motifs for PPARγ or cooperating factors and functionally regulate nearby genes whose expression is strain selective and imbalanced in heterozygous F1 mice. Moreover, genetically determined binding of PPARγ accounts for mouse strain-specific transcriptional effects of TZD drugs, providing proof of concept for personalized medicine related to nuclear receptor genomic occupancy. In human fat, motif-altering SNPs cause differential PPARγ binding, provide a molecular mechanism for some expression quantitative trait loci, and are risk factors for dysmetabolic traits in genome-wide association studies. One PPARγ motif-altering SNP is associated with HDL levels and other metabolic syndrome parameters. Thus, natural genetic variation in PPARγ genomic occupancy determines individual disease risk and drug response.

INTRODUCTION

A major unanswered question is how most genetic variation causes phenotypic differences, as only a small fraction of single-nucleotide polymorphisms (SNPs) affect protein sequence (Shastry, 2002). Current genome-wide association studies (GWAS) reveal a large gap between known causal genes and the observed heritability of common diseases and treatment outcomes (Sadee et al., 2014). Another limitation of GWAS is that each locus nominates a large group of SNPs in linkage disequilibrium, such that causal and neutral variants cannot easily be distinguished. Non-coding SNPs in regulatory regions may affect transcription factor (TF) binding and gene expression, thus contributing to complex phenotypes like disease association and response to drugs (Edwards et al., 2013).

There are examples of regulatory variants causing Mendelian syndromes (De Gobbi et al., 2006; Smemo et al., 2012), but such SNPs may be more likely to associate with complex non-Mendelian diseases in GWAS (Sakabe et al., 2012). Overall, putative causal GWAS SNPs cluster more in promoters and enhancers than in exons (Andersson et al., 2014), and a recent effort to computationally identify causal GWAS SNPs for autoimmune diseases found that ~90% were non-coding, with ~60% in distal immune cell enhancers (Farh et al., 2015). A few specific examples have emerged. The causal SNP for an LDL cholesterol and myocardial infarction locus is a regulatory variant altering hepatic *SORT1* expression (Musunuru et al., 2010). Regulatory SNPs in distant enhancers for *MYC* result in associations with multiple cancers (Sur et al., 2013), and an intronic enhancer SNP in *TCF7L2* may mediate type 2 diabetes (T2D) risk (Gaulton et al., 2010). For the *PPARG* T2D locus, the causal SNP was thought to be a coding Pro12Ala polymorphism, yet recent evidence has implicated a tightly linked regulatory SNP (Claussnitzer et al., 2014).

PPARγ provides an excellent system to study effects of regulatory variation on TF binding, gene expression, drug response, and phenotype. PPARγ is a nuclear receptor TF required for adipocyte development (Wang et al., 2013) that activates many adipocyte genes. PPARγ is genetically implicated in metabolic disease, both through the common SNP associated with T2D (Altshuler et al., 2000) and also through rare ligand binding domain mutations, causing an autosomal dominant syndrome of lipodystrophic insulin resistance (Barroso et al., 1999). Since variants affecting the PPARγ TF itself have these consequences, then genetic variation in key PPARγ genomic binding sites may similarly have metabolic effects.

PPARγ is also the target of anti-diabetic thiazolidinedione (TZD) drugs, which have a unique and powerful insulin-sensitizing effect, yet clinical use has declined due to concerns over side effects and adverse events (Soccio et al., 2014). Individuals differ in drug response, and ~20%–30% of diabetic patients fail to respond to TZDs (Sears et al., 2009). Most pharmacogenomic studies focus on coding or non-coding variants affecting the

TALE-mediated epigenetic suppression of *CDKN2A* increases replication in human fibroblasts

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Current strategies to alter disease-associated epigenetic modifications target ubiquitously expressed epigenetic regulators. This approach does not allow specific genes to be controlled in specific cell types; therefore, tools to selectively target epigenetic modifications in the desired cell type and strategies to more efficiently correct aberrant gene expression in disease are needed. Here, we have developed a method for directing DNA methylation to specific gene loci by conjugating catalytic domains of DNA methyltransferases (DNMTs) to engineered transcription activator–like effectors (TALEs). We demonstrated that these TALE-DNMTs direct DNA methylation specifically to the targeted gene locus in human cells. Further, we determined that minimizing direct nucleotide sequence repeats within the TALE moiety permits efficient lentivirus transduction, allowing easy targeting of primary cell types. Finally, we demonstrated that directed DNA methylation with a TALE-DNMT targeting the *CDKN2A* locus, which encodes the cyclin-dependent kinase inhibitor p16, decreased *CDKN2A* expression and increased replication of primary human fibroblasts, as intended. Moreover, overexpression of p16 in these cells reversed the proliferative phenotype, demonstrating the specificity of our epigenetic targeting. Together, our results demonstrate that TALE-DNMTs can selectively target specific genes and suggest that this strategy has potential application for the development of locus-specific epigenetic therapeutics.

Introduction

Epigenetic modifications are a major determinant of gene expression programs, and inappropriate changes in these modifications can lead to a wide spectrum of diseases. Cancer is perhaps the most widely recognized disease area associated with aberrant epigenetic programs, and more recently, epigenetic changes have been implicated in neurological, metabolic, and cardiovascular diseases (1). These modifications are known to be reversible, making them attractive drug targets. To date, clinicians have relied exclusively on general inhibitors of globally expressed epigenetic regulators, which are responsible for maintaining integrity of the entire epigenome (2). Thus, unintended effects of such epigenetic inhibitors may be particularly pervasive and deleterious. Therefore, there is a need for novel tools for producing specific epigenetic changes in the laboratory to enable novel therapeutic strategies.

DNA methylation has emerged as an important mechanism governing cellular reprogramming processes such as cell differentiation, cellular senescence, and disease. In mammalian cells, DNA methylation is most abundant on cytosine residues in the context of cytosine-guanine dinucleotides, or CpGs, and, when occurring at enhancers and promoters, is frequently associated with gene repression (3). DNA methylation patterns are established by the de novo DNA methyltransferases DNMT3a and DNMT3b and propagated across cell divisions by the maintenance DNA methyltransferase DNMT1 (4, 5).

In an experimental or therapeutic setting, targeted de novo DNA methylation may be accomplished by tethering of the catalytic domain of a DNA methyltransferase (DNMT) to DNA-binding proteins designed to bind specific gene loci, thereby affecting gene expression. Siddique and colleagues have pioneered this strategy by fusing DNMT catalytic subunits to an artificial zinc finger protein targeting the promoter of vascular endothelial cell growth factor A (VEGF-A) in a human cancer cell line, SOKV3 (6). However, challenges in designing artificial zinc fingers have limited the widespread use of this technology (7). Transcription activator–like effectors (TALEs) are a newer technology that is extremely modular, easy to assemble, and therefore a more efficient choice for targeted epigenome editing.

TALEs are DNA-binding proteins endogenous to bacterial plant pathogens, including the genus *Xanthomonas*. This class of proteins binds to regulatory regions in the host genome to modulate gene expression and promote bacteria survival. The central DNA binding domain of TALE proteins consists of a series of approximately 34–amino acid repeats, or monomers, which are polymorphic only at positions 12 and 13. These polymorphic residues, termed the repeat-variable-di-residue (RVD), determine DNA binding specificity, as each amino acid pair preferentially binds to 1 of the 4 nucleotides (8). Consequently, by assembly of monomers in a particular order, TALEs can be engineered to bind specific DNA sequences.

Customized TALEs have been used to modulate transcription through conjugation to activator domains, such as VP64, and repressor domains, such as the mSin interaction domain (SID) (9, 10). The potential for implementing TALEs to direct targeted epigenetic modifications has become increasingly recognized, as

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Restoration of Glucose Counterregulation by Islet Transplantation in Long-standing Type 1 Diabetes

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Patients with long-standing type 1 diabetes (T1D) may exhibit defective glucose counterregulation and impaired hypoglycemia symptom recognition that substantially increase their risk for experiencing severe hypoglycemia. The purpose of this study was to determine whether intrahepatic islet transplantation improves endogenous glucose production (EGP) in response to hypoglycemia in T1D patients experiencing severe hypoglycemia. We studied longitudinally subjects (n = 12) with ~30 years, disease duration before and 6 months after intrahepatic islet transplantation using stepped hyperinsulinemic-hypoglycemic and paired hyperinsulinemic-euglycemic clamps with infusion of 6,6-$^2H_2$-glucose and compared the results with those from a nondiabetic control group (n = 8). After islet transplantation, HbA_{1c} was normalized, and time spent while hypoglycemic (<70 mg/dL) was nearly abolished as indicated by continuous glucose monitoring. In response to insulin-induced hypoglycemia, C-peptide (absent before transplant) was appropriately suppressed, glucagon secretion was recovered, and epinephrine secretion was improved after transplantation. Corresponding to these hormonal changes, the EGP response to insulin-induced hypoglycemia, which was previously absent, was normalized after transplantation, with a similar effect seen for autonomic symptoms. Because the ability to increase EGP is ultimately required to circumvent the development of hypoglycemia, these results provide evidence that intrahepatic islet transplantation can restore glucose counterregulation in long-standing T1D and support its consideration as treatment for patients with hypoglycemia unawareness experiencing severe hypoglycemia.

Defective glucose counterregulation develops in long-standing type 1 diabetes (T1D) due to progressive impairments in defense mechanisms against a falling plasma glucose concentration in the setting of therapeutic hyperinsulinemia (1). This includes loss of inhibition in endogenous insulin secretion with associated loss of activation in glucagon secretion, which together normally increase endogenous (primarily hepatic) glucose production (EGP); impairment in sympathoadrenal epinephrine secretion, which contributes to EGP; and symptom generation, which leads to a syndrome of hypoglycemia unawareness also known as hypoglycemia-associated autonomic failure (HAAF) (2). Hypoglycemia unawareness in T1D is associated with a 20-fold increased risk for experiencing severe hypoglycemia (3), which itself contributes importantly to increased morbidity (4) and mortality (5).

In cross-sectional studies of long-standing T1D, after intrahepatic islet transplantation, insulin-induced hypoglycemia is associated with normal inhibition of endogenous insulin secretion and either defective or partially restored glucagon secretion, epinephrine secretion, and symptom responses (6–8). We sought to determine whether the recovery of islet responses to hypoglycemia after transplantation together with avoidance of hypoglycemia afforded by functioning islet grafts would reverse HAAF and restore the EGP

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Impact of Islet Autoimmunity on the Progressive β-Cell Functional Decline in Type 2 Diabetes

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OBJECTIVE

Cross-sectional studies have suggested that islet autoimmunity may be more prevalent in type 2 diabetes (T2D) than previously appreciated and may contribute to the progressive decline in β-cell function. In this study, we longitudinally evaluated the effect of islet autoimmune development on the progressive β-cell dysfunction in T2D patients.

RESEARCH DESIGN AND METHODS

Twenty-three T2D patients negative for islet autoantibodies (GAD antibody and insulinoma-associated protein 2) and islet-specific T cells were evaluated prospectively for up to 36 months. We investigated the percentage of patients who developed islet autoantibodies (Ab+) and/or islet-reactive T cells (T+) and the effect of the islet autoimmunity on fasting and glucagon-stimulated C-peptide responses. We defined positive islet autoimmunity as Ab+ and/or T+ for at least two study visits.

RESULTS

Of the 23 patients, 6 (26%) remained negative for islet autoimmunity (Ab−T−), 14 (61%) developed Ab+ and/or T+, and 3 (13%) were unclassifiable because they developed islet autoimmunity at only one study visit. Islet Ab+ was observed to be less stable than islet-specific T-cell responses. Development of islet autoimmunity was significantly associated with a more rapid decline in fasting ($P < 0.0001$) and glucagon-stimulated ($P < 0.05$) C-peptide responses.

CONCLUSIONS

These pilot data suggest that the development of islet autoimmunity in T2D is associated with a significantly more rapid β-cell functional decline.

Historically, type 2 diabetes (T2D) has not been considered to be immune mediated. However, many notable discoveries in recent years have provided evidence to support the concept of immune system involvement in T2D pathophysiology (1–5). Immune cells have been identified in the pancreases of phenotypic T2D patients (3–5). Moreover, treatment with interleukin-1 receptor agonist improves β-cell function in T2D patients (6–8). These studies suggest that β-cell damage/destruction mediated by the immune system may be a component of T2D pathophysiology.

Although the β-cell damage and destruction in autoimmune diabetes is most likely T-cell mediated (T), immune markers of autoimmune diabetes have primarily centered on the presence of circulating autoantibodies (Abs) to various islet antigens (9–15). Abs commonly positive in type 1 diabetes (T1D), especially GAD

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Article

Metabolic Dysfunction Drives a Mechanistically Distinct Proinflammatory Phenotype in Adipose Tissue Macrophages

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SUMMARY

Adipose tissue macrophage (ATM)-driven inflammation plays a key role in insulin resistance; however, factors activating ATMs are poorly understood. Using a proteomics approach, we show that markers of classical activation are absent on ATMs from obese humans but are readily detectable on airway macrophages of patients with cystic fibrosis, a disease associated with chronic bacterial infection. Moreover, treating macrophages with glucose, insulin, and palmitate—conditions characteristic of the metabolic syndrome—produces a "metabolically activated" phenotype distinct from classical activation. Markers of metabolic activation are expressed by proinflammatory ATMs in obese humans/mice and are positively correlated with adiposity. Metabolic activation is driven by independent proinflammatory and anti-inflammatory pathways, which regulate balance between cytokine production and lipid metabolism. We identify PPARγ and p62/SQSTM1 as two key proteins that promote lipid metabolism and limit inflammation in metabolically activated macrophages. Collectively, our data provide important mechanistic insights into pathways that drive the metabolic-disease-specific phenotype of macrophages.

INTRODUCTION

Macrophages accumulate in adipose tissue of obese mice and humans (Weisberg et al., 2003; Xu et al., 2003) and are key contributors to inflammation and obesity-induced insulin resistance (Chawla et al., 2011; Lumeng and Saltiel, 2011; Olefsky and Glass, 2010; Wellen and Hotamisligil, 2005). The evidence implicating adipose tissue macrophage (ATM) inflammation in potentiating insulin resistance is substantial. Indeed, ablation of proinflammatory ($CD11c^+$) ATMs using a diphtheria toxin system led to rapid improvements in insulin sensitivity and glucose tolerance, associated with marked decreases in local and systemic inflammation in obese mice (Patsouris et al., 2008). Moreover, targeting pathways that mediate inflammation in the macrophage revealed significant roles for TLR4, JNK, and IKKβ in potentiating ATM inflammation and insulin resistance in mice (Arkan et al., 2005; Han et al., 2013; Saberi et al., 2009). Anti-inflammatory effects may also help explain the insulin-sensitizing action of thiazolidinediones (TZDs). Indeed, myeloid-specific deletion of PPARγ, the molecular target of TZDs, exacerbated macrophage inflammation and insulin resistance (Odegaard et al., 2007).

Macrophages are heterogeneous, and based on patterns of gene expression and function, they have been classified as classically (M1) or alternatively (M2) activated (Gordon and Taylor, 2005). The M1 phenotype is promoted by Th1 mediators such as LPS and IFNγ and is characterized by the overproduction of proinflammatory cytokines. In contrast, Th2 mediators (e.g., IL-4) drive the M2 phenotype, which activates expression of immunosuppressive factors and peroxisome proliferator-activated receptor gamma (PPARγ) that promote tissue remodeling and helps resolve inflammation (Odegaard et al., 2007). It has been proposed that during weight gain macrophages undergo a "phenotypic switch" from an anti-inflammatory M2 phenotype to a proinflammatory M1 state, a conversion that has been linked to the emergence of systemic insulin resistance (Lumeng et al., 2007).

Although it is clear that ATM activation is involved in regulating insulin sensitivity, the mechanisms that underlie transition to the

STEM CELLS

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Neural Stem Cells in the Adult Subventricular Zone Oxidize Fatty Acids to Produce Energy and Support Neurogenic Activity

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Key Words. Neural stem cell • Progenitor • Neurogenesis • Fatty acid oxidation • Proliferation • Differentiation

ABSTRACT

Neural activity is tightly coupled to energy consumption, particularly sugars such as glucose. However, we find that, unlike mature neurons and astrocytes, neural stem/progenitor cells (NSPCs) do not require glucose to sustain aerobic respiration. NSPCs within the adult subventricular zone (SVZ) express enzymes required for fatty acid oxidation and show sustained increases in oxygen consumption upon treatment with a polyunsaturated fatty acid. NSPCs also demonstrate sustained decreases in oxygen consumption upon treatment with etomoxir, an inhibitor of fatty acid oxidation. In addition, etomoxir decreases the proliferation of SVZ NSPCs without affecting cellular survival. Finally, higher levels of neurogenesis can be achieved in aged mice by ectopically expressing proliferator-activated receptor gamma coactivator 1 alpha (PGC1α), a factor that increases cellular aerobic capacity by promoting mitochondrial biogenesis and metabolic gene transcription. Regulation of metabolic fuel availability could prove a powerful tool in promoting or limiting cellular proliferation in the central nervous system. STEM CELLS *2015;33:2306—2319*

SIGNIFICANCE STATEMENT

Most cell types in the adult brain rely on sugars such as glucose to produce energy. In this study, we find that neural stem cells in the adult brain use fatty acids to power respiratory activity and cell division. The identification of metabolic substrates required by the adult neural stem cell is imperative to understanding the process of regeneration. Regulation of metabolic fuel availability may even prove a powerful tool in promoting or limiting cellular proliferation in the central nervous system.

INTRODUCTION

Neural stem/progenitor cells (NSPCs), which retain the capacity to produce new neurons and glia in the adult mammalian brain, reside in the subventricular zone (SVZ) of the lateral ventricle and the subgranular zone of hippocampal dentate gyrus (DG) [1, 2]. While newly born cells in the inner molecular cell layer of DG migrate locally, cells from SVZ migrate long distances along the rostral migratory stream to populate the olfactory bulb (OB) with new GABAergic interneurons including dopaminergic neurons [3]. The control of neurogenesis in the adult brain is notoriously complex, and depends upon factors in both the extracellular environment and limitations within the cellular machinery [4]. As NSPCs reside in a "neurogenic niche" fortified by blood vessels and astrocytes [5], these cells have direct access to contents in the bloodstream, including substrates to fuel energy metabolism. In this study, we investigated the substrates required by adult mammalian neural stem cells to maintain their metabolic and neurogenic activity.

Catabolism, the production of energy in the form of ATP, is necessary to sustain cellular activity. The adult brain primarily depends upon the oxidation of carbohydrates such as glucose to fuel energy production. Although the brain comprises about 2% of body weight, this organ accounts for approximately 25% of organismal glucose consumption and 20% of oxygen consumption. Neuronal activity is indeed tightly coupled to glucose uptake [6]. However, intriguingly, neurons and astrocytes have recently been shown to compartmentalize metabolic processes [7, 8]. Glutamate

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Restoring Insulin Secretion (RISE): Design of Studies of β-Cell Preservation in Prediabetes and Early Type 2 Diabetes Across the Life Span

*The RISE Consortium**

OBJECTIVE

The Restoring Insulin Secretion (RISE) Consortium is testing interventions designed to preserve or improve β-cell function in prediabetes or early type 2 diabetes.

RESEARCH DESIGN AND METHODS

β-Cell function is measured using hyperglycemic clamps and oral glucose tolerance tests (OGTTs). The adult medication protocol randomizes participants to 12 months of placebo, metformin alone, liraglutide plus metformin, or insulin (3 months) followed by metformin (9 months). The pediatric medication protocol randomizes participants to metformin or insulin followed by metformin. The adult surgical protocol randomizes participants to gastric banding or metformin (24 months). Adult medication protocol inclusion criteria include fasting plasma glucose 95–125 mg/dL (5.3–6.9 mmol/L), OGTT 2-h glucose ≥140 mg/dL (≥7.8 mmol/L), HbA_{1c} 5.8–7.0% (40–53 mmol/mol), and BMI 25–40 kg/m^2. Adult surgical protocol criteria are similar, except for fasting plasma glucose ≥90 mg/dL (≥5.0 mmol/L), BMI 30–40 kg/m^2, HbA_{1c} <7.0% (<53 mmol/mol), and diabetes duration <12 months. Pediatric inclusion criteria include fasting plasma glucose ≥90 mg/dL (≥5.0 mmol/L), 2-h glucose ≥140 mg/dL (≥7.8 mmol/L), HbA_{1c} ≤8.0% (≤64 mmol/mol), BMI >85th percentile and ≤50 kg/m^2, 10–19 years of age, and diabetes <6 months.

RESULTS

Primary outcomes are clamp-derived glucose-stimulated C-peptide secretion and maximal C-peptide response to arginine during hyperglycemia. Measurements are made at baseline, after 12 months on treatment, and 3 months after treatment withdrawal (medication protocols) or 24 months postintervention (surgery protocol). OGTT-derived measures are also obtained at these time points.

CONCLUSIONS

RISE is determining whether medication or surgical intervention strategies can mitigate progressive β-cell dysfunction in adults and youth with prediabetes or early type 2 diabetes.

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Insulin Delivery Into the Peripheral Circulation: A Key Contributor to Hypoglycemia in Type 1 Diabetes

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Hypoglycemia limits optimal glycemic control in type 1 diabetes mellitus (T1DM), making novel strategies to mitigate it desirable. We hypothesized that portal (Po) vein insulin delivery would lessen hypoglycemia. In the conscious dog, insulin was infused into the hepatic Po vein or a peripheral (Pe) vein at a rate four times of basal. In protocol 1, a full counterregulatory response was allowed, whereas in protocol 2, glucagon was fixed at basal, mimicking the diminished α-cell response to hypoglycemia seen in T1DM. In protocol 1, glucose fell faster with Pe insulin than with Po insulin, reaching 56 ± 3 vs. 70 ± 6 mg/dL ($P = 0.04$) at 60 min. The change in area under the curve (ΔAUC) for glucagon was similar between Pe and Po, but the peak occurred earlier in Pe. The ΔAUC for epinephrine was greater with Pe than with Po (67 ± 17 vs. 36 ± 14 ng/mL/180 min). In protocol 2, glucose also fell more rapidly than in protocol 1 and fell faster in Pe than in Po, reaching 41 ± 3 vs. 67 ± 2 mg/dL ($P < 0.01$) by 60 min. Without a rise in glucagon, the epinephrine responses were much larger (ΔAUC of 204 ± 22 for Pe vs. 96 ± 29 ng/mL/180 min for Po). In summary, Pe insulin delivery exacerbates hypoglycemia, particularly in the presence of a diminished glucagon response. Po vein insulin delivery, or strategies that mimic it (i.e., liver-preferential insulin analogs), should therefore lessen hypoglycemia.

Hypoglycemia is a key barrier to optimal glycemic control in the management of type 1 diabetes mellitus (T1DM). Previous research has established the importance of aggressive control of hyperglycemia to mitigate microvascular (1–4) and possibly macrovascular (5–9) complications, but a principle limitation of this approach is increased hypoglycemia and its potential for devastating neurologic consequences (6,10–15). The homeostatic response to hypoglycemia is compromised in patients with T1DM for several reasons, including *1*) the circulating insulin concentration does not fall in response to decreasing glucose concentrations, *2*) the glucagon response is deficient (16–19), and *3*) patients with antecedent hypoglycemia are predisposed to subsequent hypoglycemia because the sympathoadrenal response is diminished (18,20). Collectively, the presence of these abnormalities contributes to defective counterregulation and hypoglycemic unawareness.

Current therapy in T1DM is further limited by the necessity of injecting insulin into subcutaneous tissue, which delivers insulin into the peripheral (Pe) circulation, rather than the hepatic portal (Po) circulation. This approach results in a reversal of the normal insulin distribution, with higher insulin concentrations in the Pe circulation and lower insulin levels in the hepatic Po blood. A therapeutic balance must therefore be achieved, such that the excess of insulin in the Pe circulation and its effect on glucose uptake offsets the deficit of insulin at the liver and its effect on glucose production. Because Pe overinsulinization shifts the primary site of insulin action away from the liver and toward skeletal muscle, a conceivable result is a predisposition to hypoglycemia. Skeletal muscle has an inherently slower response time than the liver to fluxes in insulin, glucose, and counterregulatory factors (21–25). Further, skeletal muscle provides a larger "glucose sink" than the liver because it comprises a higher percentage of total body mass than the liver (26,27) and takes up glucose at all glycemic levels, as opposed

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LETTER

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G-protein-independent coupling of MC4R to Kir7.1 in hypothalamic neurons

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The regulated release of anorexigenic α-melanocyte stimulating hormone (α-MSH) and orexigenic Agouti-related protein (AgRP) from discrete hypothalamic arcuate neurons onto common target sites in the central nervous system has a fundamental role in the regulation of energy homeostasis. Both peptides bind with high affinity to the melanocortin-4 receptor (MC4R); existing data show that α-MSH is an agonist that couples the receptor to the $G\alpha_s$ signalling pathway[1], while AgRP binds competitively to block α-MSH binding[2] and blocks the constitutive activity mediated by the ligand-mimetic amino-terminal domain of the receptor[3]. Here we show that, in mice, regulation of firing activity of neurons from the paraventricular nucleus of the hypothalamus (PVN) by α-MSH and AgRP can be mediated independently of $G\alpha_s$ signalling by ligand-induced coupling of MC4R to closure of inwardly rectifying potassium channel, Kir7.1. Furthermore, AgRP is a biased agonist that hyperpolarizes neurons by binding to MC4R and opening Kir7.1, independently of its inhibition of α-MSH binding. Consequently, Kir7.1 signalling appears to be central to melanocortin-mediated regulation of energy homeostasis within the PVN. Coupling of MC4R to Kir7.1 may explain unusual aspects of the control of energy homeostasis by melanocortin signalling, including the gene dosage effect of MC4R[4] and the sustained effects of AgRP on food intake[5].

To better understand the diametrically opposed regulation of food intake by α-MSH and AgRP, we sought to identify mechanism(s) by which these peptides control firing activity of MC4R neurons in the paraventricular nucleus of the hypothalamus (PVN), a brain nucleus in which MC4R is known to control food intake[6]. Using electrophysiology with murine hypothalamic slice preparations in which MC4R PVN neurons are labelled with green fluorescent protein (GFP), α-MSH increases the frequency of action potential firing in PVN MC4R neurons recorded from loose patches (Fig. 1a), depolarizing these cells on average by ~8 mV through action on postsynaptic MC4R (Fig. 1b)[7]. α-MSH had no effect on neighbouring non-GFP-labelled neurons (Fig. 1c). AgRP hyperpolarized PVN MC4R neurons (Fig. 1d), inhibiting their firing activity.

We next examined if α-MSH depolarized neurons through activation of the $G\alpha_s$–adenylyl cyclase-cAMP-PKA pathway. PKAi (20 μM intrapipette), a peptide inhibitor of PKA, failed to abolish the α-MSH-induced increase in firing frequency in PVN neurons during whole cell recording, (not shown) or to block α-MSH-induced depolarization of membrane potential (Fig. 1e). Inhibition of adenylyl cyclase with SQ22536 (25 μM) similarly failed to block α-MSH-induced depolarization of membrane potential (Fig. 1f). Finally, we examined whether blocking G protein signalling can inhibit MC4R-mediated depolarization by loading cells with the inhibitory GDP analogue, GDPβS. To verify that GDPβS blocked G-protein function, we examined effects of activation of D1 dopamine receptor, known to depolarize neurons via activation of $G\alpha_s$. Activation of D1 dopamine receptor by the D1 agonist SKF83822 (5 μM) depolarized PVN neurons (Fig. 1g). GDPβS (5 mM) blocked D1-mediated depolarization, but failed to block α-MSH-induced depolarization of PVN MC4R neurons (Fig. 1g). Several other inhibitors of components of G protein signalling were also ineffective in blocking neuronal firing

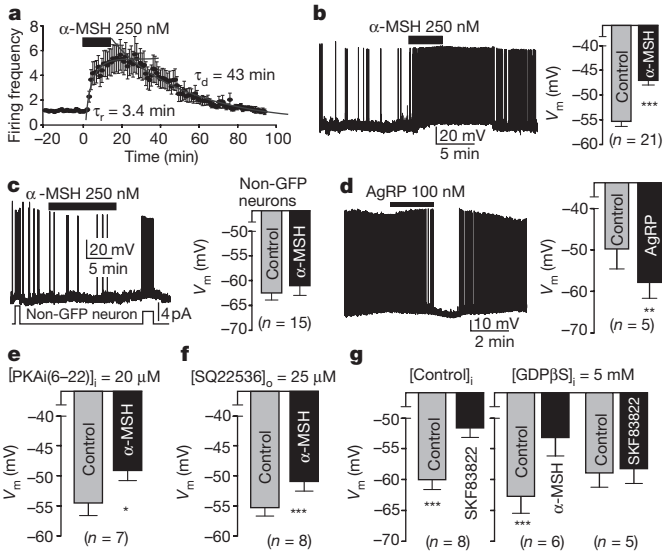

Figure 1 | Depolarization of hypothalamic PVN MC4R neurons by α-MSH is G-protein independent. **a**, Normalized mean amplitude (±s.e.m.) and time-course of α-MSH action on firing frequencies of PVN MC4R neurons ($n = 14$) recorded in loose-patch configuration using hypothalamic slice preparations from MC4R–GFP mice before and after the addition of 250 nM α-MSH and washout. **b**, A representative depolarizing response of a PVN MC4R neuron recorded in current clamp to bath application of 250 nM α-MSH. The bar graph represents mean ± s.e.m. (***$P < 0.001$). V_m, membrane potential in millivolts (mV). **c**, A representative response of a non-GFP-expressing PVN neuron recorded in current clamp to bath application of 250 nM α-MSH. Application of brief current pulses (lower trace) caused depolarization and burst firing (upper trace), while α-MSH failed to depolarize this neuron. The bar graph represents mean ± s.e.m. ($P > 0.1$). **d**, A representative hyperpolarizing response of a PVN MC4R neuron recorded in current clamp to bath application of 100 nM AgRP. The bar graph represents mean ± s.e.m. (**$P < 0.01$). **e**, Intracellular PKA inhibitor, PKAi (6–22 amide) at 20 μM, fails to block the α-MSH-induced depolarization of membrane potential in PVN MC4R neurons. **f**, Inhibition of adenylyl cyclase by SQ22536 fails to block depolarizing effects of α-MSH. **g**, Application of 5 μM SKF83822, a selective D1 receptor agonist, causes depolarization of PVN neurons (left panel). Intrapipette addition of 5 mM GDPβS, a blocker of G-protein signalling (right panel), fails to block the α-MSH-induced depolarization in PVN MC4R neurons, but blocks the depolarization and firing activity induced by SKF83822. Data in panels **e**, **g** show mean ± s.e.m., *$P < 0.05$, ***$P < 0.001$, paired *t*-test. In all electrophysiological studies, each *n* represents an independent neuron and slice, and no more than two slices were used per animal.

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Novel Stable Isotope Analyses Demonstrate Significant Rates of Glucose Cycling in Mouse Pancreatic Islets

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A polymorphism located in the *G6PC2* gene, which encodes an islet-specific glucose-6-phosphatase catalytic subunit, is the most important common determinant of variations in fasting blood glucose (FBG) levels in humans. Studies of *G6pc2* knockout (KO) mice suggest that G6pc2 represents a negative regulator of basal glucose-stimulated insulin secretion (GSIS) that acts by hydrolyzing glucose-6-phosphate (G6P), thereby reducing glycolytic flux. However, this conclusion conflicts with the very low estimates for the rate of glucose cycling in pancreatic islets, as assessed using radioisotopes. We have reassessed the rate of glucose cycling in pancreatic islets using a novel stable isotope method. The data show much higher levels of glucose cycling than previously reported. In 5 mmol/L glucose, islets from C57BL/6J chow-fed mice cycled ∼16% of net glucose uptake. The cycling rate was further increased at 11 mmol/L glucose. Similar cycling rates were observed using islets from high fat–fed mice. Importantly, glucose cycling was abolished in *G6pc2* KO mouse islets, confirming that G6pc2 opposes the action of the glucose sensor glucokinase by hydrolyzing G6P. The demonstration of high rates of glucose cycling in pancreatic islets explains why *G6pc2* deletion enhances GSIS and why variants in *G6PC2* affect FBG in humans.

Glucose-6-phosphatase catalyzes the hydrolysis of glucose-6-phosphate (G6P) to glucose and inorganic phosphate (1–5). It exists as a multicomponent system located in the endoplasmic reticulum (ER) and is composed of several integral membrane proteins, namely, a catalytic subunit (G6PC), a glucose transporter, and a G6P/inorganic phosphate antiporter (1–5). Three G6PC isoforms have been identified, which are designated G6PC, G6PC2, and G6PC3 (5). Each isoform is encoded by a separate gene with a distinct pattern of tissue-specific expression (5). *G6PC* is predominantly expressed in the liver, where it catalyzes the final step in gluconeogenesis and glycogenolysis (1–5). *G6PC3*, also known as *UGRP* and *G6Pase-β*, is ubiquitously expressed (6,7). Mutations that reduce G6PC3 activity result in neutropenia; however, the physiological function of G6PC3 is unclear (8,9). *G6PC2*, also known as *IGRP* (10,11), is selectively expressed in pancreatic islet β-cells (12). G6PC2 is a major autoantigen in both mouse (13–15) and human (16,17) type 1 diabetes.

Historically, the question as to whether glucose-6-phosphatase activity is present in islets has been controversial, though it is now generally agreed that activity is detectable, but at a lower level than that found in the liver (2,10,12,18,19). While a majority of studies agree that glucose-6-phosphatase activity exists in pancreatic islets, the issue as to whether the level of activity is enough to affect glucose-stimulated insulin secretion (GSIS), and therefore whether the activity is of biological significance, is currently unresolved. Sweet et al. (19) concluded that, while glucose-6-phosphatase activity is present in rat islets, the level of activity is not enough to result in sufficient G6P hydrolysis so as to affect GSIS. However, two caveats have subsequently arisen with respect to this conclusion. First, in contrast to all other vertebrate species examined (see http://genome.ucsc.edu/), *G6PC2* is a pseudogene in rats (11). Second, in various rat models associated with impaired glucose tolerance, *G6PC* expression is induced such that G6P hydrolysis would be elevated (20–22).

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A calcium-dependent protease as a potential therapeutic target for Wolfram syndrome

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Wolfram syndrome is a genetic disorder characterized by diabetes and neurodegeneration and considered as an endoplasmic reticulum (ER) disease. Despite the underlying importance of ER dysfunction in Wolfram syndrome and the identification of two causative genes, Wolfram syndrome 1 (*WFS1*) and Wolfram syndrome 2 (*WFS2*), a molecular mechanism linking the ER to death of neurons and β cells has not been elucidated. Here we implicate calpain 2 in the mechanism of cell death in Wolfram syndrome. Calpain 2 is negatively regulated by WFS2, and elevated activation of calpain 2 by WFS2-knockdown correlates with cell death. Calpain activation is also induced by high cytosolic calcium mediated by the loss of function of WFS1. Calpain hyperactivation is observed in the WFS1 knockout mouse as well as in neural progenitor cells derived from induced pluripotent stem (iPS) cells of Wolfram syndrome patients. A small-scale small-molecule screen targeting ER calcium homeostasis reveals that dantrolene can prevent cell death in neural progenitor cells derived from Wolfram syndrome iPS cells. Our results demonstrate that calpain and the pathway leading its activation provides potential therapeutic targets for Wolfram syndrome and other ER diseases.

Wolfram syndrome | endoplasmic reticulum | diabetes | neurodegeneration | treatment

The endoplasmic reticulum (ER) takes center stage for protein production, redox regulation, calcium homeostasis, and cell death (1, 2). It follows that genetic or acquired ER dysfunction can trigger a variety of common diseases, including neurodegenerative diseases, metabolic disorders, and inflammatory bowel disease (3, 4). Breakdown in ER function is also associated with genetic disorders such as Wolfram syndrome (5–8). It is challenging to determine the exact effects of ER dysfunction on the fate of affected cells in common diseases with polygenic and multifactorial etiologies. In contrast, we reasoned that it should be possible to define the role of ER dysfunction in mechanistically homogenous patient populations, especially in rare diseases with a monogenic basis, such as Wolfram syndrome (9).

Wolfram syndrome (OMIM 222300) is a rare autosomal recessive disorder characterized by juvenile-onset diabetes mellitus and bilateral optic atrophy (7). Insulin-dependent diabetes usually occurs as the initial manifestation during the first decade of life, whereas the diagnosis of Wolfram syndrome is invariably later, with onset of symptoms in the second and ensuing decades (7, 10, 11). Two causative genes for this genetic disorder have been identified and named Wolfram syndrome 1 (*WFS1*) and Wolfram syndrome 2 (*WFS2*) (12, 13). It has been shown that multiple mutations in the *WFS1* gene, as well as a specific mutation in the *WFS2* gene, lead to β cell death and neurodegeneration through ER and mitochondrial dysfunction (5, 6, 14–16). *WFS1* gene variants are also associated with a risk of type 2 diabetes (17). Moreover, a specific *WFS1* variant can cause autosomal dominant diabetes (18), raising the possibility that this rare disorder is relevant to common molecular mechanisms altered in diabetes and other human chronic diseases in which ER dysfunction is involved.

Despite the underlying importance of ER malfunction in Wolfram syndrome, and the identification of *WFS1* and *WFS2* genes, a molecular mechanism linking the ER to death of neurons and β cells has not been elucidated. Here we show that the calpain protease provides a mechanistic link between the ER and death of neurons and β cells in Wolfram syndrome.

Results

The causative genes for Wolfram syndrome, *WFS1* and *WFS2*, encode transmembrane proteins localized to the ER (5, 12, 13). Mutations in the *WFS1* or *WFS2* have been shown to induce neuronal and β cell death. To determine the cell death pathways emanating from the ER, we sought proteins associated with Wolfram syndrome causative gene products. HEK293 cells were transfected with a GST-tagged WFS2 expression plasmid. The GST-WFS2 protein was purified along with associated proteins on a glutathione affinity resin. These proteins were separated by

Significance

Wolfram syndrome is an autosomal recessive disorder characterized by juvenile diabetes and neurodegeneration, and is considered a prototype of human endoplasmic reticulum (ER) disease. Wolfram syndrome is caused by loss of function mutations of Wolfram syndrome 1 or Wolfram syndrome 2 genes, which encode transmembrane proteins localized to the ER. Despite its rarity, Wolfram syndrome represents the best human disease model currently available to identify drugs and biomarkers associated with ER health. Furthermore, this syndrome is ideal for studying the mechanisms of ER stress-mediated death of neurons and β cells. Here we report that the pathway leading to calpain activation offers potential drug targets for Wolfram syndrome and substrates for calpain might serve as biomarkers for this syndrome.

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Ketogenesis prevents diet-induced fatty liver injury and hyperglycemia

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Nonalcoholic fatty liver disease (NAFLD) spectrum disorders affect approximately 1 billion individuals worldwide. However, the drivers of progressive steatohepatitis remain incompletely defined. Ketogenesis can dispose of much of the fat that enters the liver, and dysfunction in this pathway could promote the development of NAFLD. Here, we evaluated mice lacking mitochondrial 3-hydroxymethylglutaryl CoA synthase (HMGCS2) to determine the role of ketogenesis in preventing diet-induced steatohepatitis. Antisense oligonucleotide–induced loss of HMGCS2 in chow-fed adult mice caused mild hyperglycemia, increased hepatic gluconeogenesis from pyruvate, and augmented production of hundreds of hepatic metabolites, a suite of which indicated activation of the de novo lipogenesis pathway. High-fat diet feeding of mice with insufficient ketogenesis resulted in extensive hepatocyte injury and inflammation, decreased glycemia, deranged hepatic TCA cycle intermediate concentrations, and impaired hepatic gluconeogenesis due to sequestration of free coenzyme A (CoASH). Supplementation of the CoASH precursors pantothenic acid and cysteine normalized TCA intermediates and gluconeogenesis in the livers of ketogenesis-insufficient animals. Together, these findings indicate that ketogenesis is a critical regulator of hepatic acyl-CoA metabolism, glucose metabolism, and TCA cycle function in the absorptive state and suggest that ketogenesis may modulate fatty liver disease.

Introduction

Nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH) are now the most common causes of liver disease in Western countries (1). NAFLD-induced liver failure is one of the most common reasons for liver transplantation. NAFLD increases the risk of developing type 2 diabetes, worsens glycemic control, and contributes to the pathogenesis of cardiovascular disease and chronic kidney disease (2–4). The pathogenic mechanisms of NAFLD and NASH are incompletely understood but are thought to involve abnormalities of hepatocyte metabolism, hepatocyte autophagy and endoplasmic reticulum stress, hepatic immune cell function, adipose tissue inflammation, and systemic inflammatory mediators (2, 4–6). Perturbations of carbohydrate, lipid, and amino acid metabolism occur in and contribute to obesity, diabetes, and NAFLD in humans and in model organisms (reviewed in refs. 7–11). While hepatocyte abnormalities in cytoplasmic lipid metabolism are commonly observed in NAFLD (12), the role of mitochondrial metabolism, which governs the oxidative and terminal "disposal" of fats, in NAFLD pathogenesis is less clear. Nonetheless, most investigators agree that abnormalities of mitochondrial metabolism occur in and contribute to NAFLD (reviewed in refs. 13–15).

Ketogenesis can dispose of as much as two-thirds of the fat entering the liver (16). Thus, dysregulation of ketone body metabolism could potentially contribute to NAFLD pathogenesis. Hepatic ketogenesis is activated in states of high fatty acid and diminished carbohydrate availability and/or when circulating insulin concentrations are very low (17–20). Within hepatic mitochondria, ketogenic reactions condense β-oxidation–derived acetyl-CoA into the ketone bodies acetoacetate (AcAc) and β hydroxybutyrate (βOHB). In doing so, ketogenesis disposes of acetyl-CoA generated in excess of the liver's own energy needs and simultaneously recycles 2 moles of free coenzyme A (CoASH) per mole of ketone produced (reviewed in refs. 17, 19, 20). Robust ketogenesis is limited to hepatocytes due to relatively restricted expression of the fate-committing ketogenic enzyme mitochondrial 3-hydroxymethylglutaryl-CoA synthase (HMGCS2) under normal conditions (21). In contrast, oxidative disposal of ketone bodies is nearly ubiquitous, as all cells except hepatocytes express the fate-committing enzyme of ketone body oxidation succinyl-CoA:3-oxoacid CoA transferase (SCOT) (22, 23). Within the mitochondria of extrahepatic tissues, βOHB is oxidized to AcAc, which is then directed toward the TCA cycle for terminal oxidation via a reaction uniquely catalyzed by SCOT (17). Despite its high capacity as a disposal pathway for hepatic fatty acids, ketone body metabolism has been overlooked as a potential therapeutic target in NAFLD.

Through incompletely defined mechanisms, obesity-associated hyperinsulinemia suppresses ketogenesis, creating a state of relative ketogenic insufficiency and leading to hypoketonemia in obese animal models and humans when compared with lean

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Global Biochemical Profiling Identifies β-Hydroxypyruvate as a Potential Mediator of Type 2 Diabetes in Mice and Humans

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Glucose-dependent insulinotropic polypeptide (GIP) and GLP-1 are incretins secreted by respective K and L enteroendocrine cells after eating and amplify glucose-stimulated insulin secretion (GSIS). This amplification has been termed the "incretin response." To determine the role(s) of K cells for the incretin response and type 2 diabetes mellitus (T2DM), diphtheria toxin–expressing (DT) mice that specifically lack GIP-producing cells were backcrossed five to eight times onto the diabetogenic NONcNZO10/Ltj background. As in humans with T2DM, DT mice lacked an incretin response, although GLP-1 release was maintained. With high-fat (HF) feeding, DT mice remained lean but developed T2DM, whereas wild-type mice developed obesity but not diabetes. Metabolomics identified biochemicals reflecting impaired glucose handling, insulin resistance, and diabetes complications in prediabetic DT/HF mice. β-Hydroxypyruvate and benzoate levels were increased and decreased, respectively, suggesting β-hydroxypyruvate production from D-serine. In vitro, β-hydroxypyruvate altered excitatory properties of myenteric neurons and reduced islet insulin content but not GSIS. β-Hydroxypyruvate–to–D-serine ratios were lower in humans with impaired glucose tolerance compared with normal glucose tolerance and T2DM. Earlier human studies unmasked a neural relay that amplifies GIP-mediated insulin secretion in a pattern reciprocal to β-hydroxypyruvate–to–D-serine ratios in all groups. Thus, K cells may maintain long-term function of neurons and β-cells by regulating β-hydroxypyruvate levels.

Glucose-dependent insulinotropic polypeptide (GIP) and GLP-1 are incretins produced predominantly by enteroendocrine K and L cells, respectively, located in the proximal (K cells) and distal (L cells) gut (1,2). Both are released into the circulation immediately after eating in response to nutrients in the gut lumen but not blood (1,2). Orally derived glucose elicits a greater insulin secretory response compared with isoglycemic levels of intravenous glucose. This enhancement of glucose-stimulated insulin secretion (GSIS), or "incretin response," is thought to be mediated by incretins released from the gut acting on β-cells in the pancreas. Neurotransmitters and neuropeptides also regulate GSIS (3).

Abnormalities in the pancreatic β-cell and insulin secretion play a central role in the pathogenesis of type 2 diabetes mellitus (T2DM) (4). The incretin response, but not GIP or GLP-1 release, is blunted in humans with T2DM (5–7). Nevertheless, exogenously infused GLP-1 remains active in T2DM and forms the rationale for incretin-based pharmacotherapies for T2DM (8). Although effects of exogenous GIP on insulin secretion are reportedly blunted in T2DM (9–11), we recently showed that the magnitude of the augmentation in the insulin secretory response to exogenously infused GIP, but not glucose, is retained in humans with T2DM (12). Thus, the endogenous incretin response may not be mediated by direct action of GIP or GLP-1 on β-cells.

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METABOLIC DISEASE

Controlled-release mitochondrial protonophore reverses diabetes and steatohepatitis in rats

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Nonalcoholic fatty liver disease (NAFLD) is a major factor in the pathogenesis of type 2 diabetes (T2D) and nonalcoholic steatohepatitis (NASH). The mitochondrial protonophore 2,4 dinitrophenol (DNP) has beneficial effects on NAFLD, insulin resistance, and obesity in preclinical models but is too toxic for clinical use. We developed a controlled-release oral formulation of DNP, called CRMP (controlled-release mitochondrial protonophore), that produces mild hepatic mitochondrial uncoupling. In rat models, CRMP reduced hypertriglyceridemia, insulin resistance, hepatic steatosis, and diabetes. It also normalized plasma transaminase concentrations, ameliorated liver fibrosis, and improved hepatic protein synthetic function in a methionine/choline–deficient rat model of NASH. Chronic treatment with CRMP was not associated with any systemic toxicity. These data offer proof of concept that mild hepatic mitochondrial uncoupling may be a safe and effective therapy for the related epidemics of metabolic syndrome, T2D, and NASH.

Nonalcoholic fatty liver disease (NAFLD) affects 15 to 30% of the world's population (*1*) and is a key predisposing factor for nonalcoholic steatohepatitis (NASH), cirrhosis, and hepatocellular carcinoma. The role of hepatic steatosis in the pathogenesis of NASH and liver fibrosis remains undefined, and thus far, no therapeutic agents improve liver histology or hepatic protein synthetic function in animal models of NASH. In addition, NAFLD is strongly associated with hepatic insulin resistance and type 2 diabetes (T2D); however, efforts to ameliorate NAFLD or diabetes with pharmacologic agents have met with limited success.

The mitochondrial protonophore 2,4-dinitrophenol (DNP) has been investigated since the early 20th century for its ability to promote weight loss; however, after numerous reports of deaths in individuals taking DNP, production of the drug ceased in the United States in the late 1930s. Nevertheless, given its ability to promote insulin sensitivity in the rat (*2*), we investigated whether DNP could be pharmacologically manipulated to improve its safety margin. In a previous study (*3*), we showed that promoting subtle increases in hepatic mitochondrial uncoupling with a liver-targeted derivative of DNP ameliorates NAFLD and T2D in the rat. Although liver-targeted DNP was well tolerated, we hypothesized that we could further improve the safety and efficacy of DNP by developing a version of the drug with lower peak plasma concentrations and sustained-release pharmacokinetics.

To test this hypothesis, we first examined whether a 5-day continuous, low-dose intragastric infusion of DNP to achieve sustained plasma DNP concentrations in the 1 to 5 μM range would lead to reductions in hepatic steatosis and improve whole-body insulin sensitivity in high-fat–fed rats. This intragastric infusion of DNP resulted in steady-state plasma and liver DNP concentrations of ~3 and ~1 μM, respectively (fig. S1A). Nevertheless, these very low concentrations of DNP resulted in lower fasting plasma glucose and insulin concentrations as well as 80% reductions in plasma, liver, and skeletal muscle triacylglycerol (TAG) content (fig. S1, B to F).

Given the encouraging results of the intragastric infusion studies, we synthesized an orally available, controlled-release formulation of DNP, which is described in the supplemental materials, materials and methods. This formulation, called CRMP (controlled-release mitochondrial protonophore), was fed to rats in a small amount of peanut butter. In contrast to DNP, which caused a dose-dependent increase in body temperature at doses above 25 mg/kg, CRMP had a negligible effect on temperature at doses less than 100 mg/kg (fig. S2, A and B). To compare the safety and efficacy of CRMP and DNP, we performed 5-day parallel group dosing studies in high-fat–fed rats and found that the minimum effective dose of CRMP to decrease liver TAG was 0.5 mg/kg, whereas that of DNP was 5 mg/kg (fig. S2, C and D). In concert with this, the median lethal dose (LD_{50}) of CRMP was more than 10-fold higher than that of DNP (fig. S2E). No changes to alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN), or creatinine were observed with any of the doses of CRMP below 125 mg/kg, whereas DNP treatment at doses above 0.5 mg/kg raised AST concentrations (fig. S3, A to H). Thus, the 5-day no observed adverse effect level (NOAEL) of CRMP was 100 mg/kg, as compared with 0.5 mg/kg for DNP.

We next examined whether the improved safety of CRMP might be related to differences in pharmacokinetic properties (fig. S4, A to F). Peak plasma DNP concentrations at each toxic dose of DNP were significantly higher than equimolar doses of CRMP, whereas the area under the curve of DNP concentration was higher after treatment with CRMP, likely accounting for CRMP's improved efficacy and reduced toxicity (fig. S4, E and F). Detailed pharmacologic data can be found in the supplementary materials (fig. S5, A to H).

To further evaluate the safety margins of CRMP as compared with DNP, we treated rats for 6 weeks with oral DNP or CRMP. Six weeks of CRMP treatment at 1 mg/kg was well tolerated and did not result in any alterations in behavior, food intake, body weight, body temperature, liver or kidney histology, or induction of neuropathy (fig. S6, A to I). In addition, no toxic effects were seen with doses up to 100 mg/kg CRMP, whereas increases in AST were seen at 1 mg/kg DNP treatment (fig. S6, D to G). Thus, the 6-week NOAEL for CRMP is at least 100-fold greater for CRMP (more than 100 mg/kg) than for DNP (less than 1 mg/kg). Taken together, our data indicate that the toxicity of a DNP derivative is predicted by the maximum concentration of DNP (fig. S4F), whereas its efficacy is predicted by the area under the curve of plasma DNP concentrations (fig. S4E).

To examine the impact of CRMP on rates of hepatic mitochondrial glucose and fat oxidation, we assessed these rates using a combined liquid chromatography–mass spectrometry (MS)/MS–nuclear magnetic resonance method (*4*). We observed a 60% increase in rates of hepatic mitochondrial tricarboxylic acid cycle flux (V_{TCA}) flux in CRMP-treated rats, which could be attributed to a 65% increase in rates of fat oxidation (Fig. 1A). In contrast, there were no differences in fat oxidation relative to V_{TCA} in kidney, brain, heart, or skeletal muscle, indicating that the uncoupling effect of CRMP is confined to the liver (fig. S7A). To examine whether uncoupling with CRMP reduces tissue lipid content and improves insulin sensitivity, we treated a high-fat–fed rat model of NAFLD and insulin resistance with daily CRMP (1 mg/kg) or vehicle for 5 days. Despite identical body weight and fat content at the time of study, CRMP-treated rats exhibited 30 to 40% reductions in fasting plasma glucose, fatty acid, and triglyceride concentrations; a 30% increase in high-density lipoprotein concentration; and a 50% reduction in plasma insulin concentration, without any difference in hepatic gluconeogenic protein expression (Fig. 1, B to D, and fig. S7, B to H).

Rats treated with CRMP manifested improved glucose tolerance, with lower plasma glucose and insulin concentrations throughout an intraperitoneal glucose tolerance test (Fig. 1, E and F, and fig. S7, I and J). To evaluate the effect of CRMP on whole-body insulin sensitivity, we performed hyperinsulinemic-euglycemic clamps with

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Current State of Type 1 Diabetes Treatment in the U.S.: Updated Data From the T1D Exchange Clinic Registry

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To examine the overall state of metabolic control and current use of advanced diabetes technologies in the U.S., we report recent data collected on individuals with type 1 diabetes participating in the T1D Exchange clinic registry. Data from 16,061 participants updated between 1 September 2013 and 1 December 2014 were compared with registry enrollment data collected from 1 September 2010 to 1 August 2012. Mean hemoglobin A_{1c} (HbA_{1c}) was assessed by year of age from <4 to >75 years. The overall average HbA_{1c} was 8.2% (66 mmol/mol) at enrollment and 8.4% (68 mmol/mol) at the most recent update. During childhood, mean HbA_{1c} decreased from 8.3% (67 mmol/mol) in 2–4-year-olds to 8.1% (65 mmol/mol) at 7 years of age, followed by an increase to 9.2% (77 mmol/mol) in 19-year-olds. Subsequently, mean HbA_{1c} values decline gradually until ~30 years of age, plateauing at 7.5–7.8% (58–62 mmol/mol) beyond age 30 until a modest drop in HbA_{1c} below 7.5% (58 mmol/mol) in those 65 years of age. Severe hypoglycemia (SH) and diabetic ketoacidosis (DKA) remain all too common complications of treatment, especially in older (SH) and younger patients (DKA). Insulin pump use increased slightly from enrollment (58–62%), and use of continuous glucose monitoring (CGM) did not change (7%). Although the T1D Exchange registry findings are not population based and could be biased, it is clear that there remains considerable room for improving outcomes of treatment of type 1 diabetes across all age-groups. Barriers to more effective use of current treatments need to be addressed and new therapies are needed to achieve optimal metabolic control in people with type 1 diabetes.

Results of the Diabetes Control and Complications Trial (DCCT) and the Epidemiology of Diabetes Interventions and Complications (EDIC) follow-up study of the DCCT cohort have demonstrated that most people with type 1 diabetes should be treated intensively to achieve hemoglobin A_{1c} (HbA_{1c}) levels as close to normal as possible and as early in the course of the disease as possible to prevent and delay the late micro- and macrovascular complications of the disease (1). Most recently, the DCCT/EDIC study group reported that all-cause mortality also was reduced over 30 years of follow-up during DCCT/EDIC in the original DCCT intensive treatment group compared with the original conventional treatment group (2). Consequently, the American Diabetes Association (ADA) treatment guidelines indicate that adults with type 1 diabetes should aim at target HbA_{1c} levels <7.0% (53 mmol/mol) unless there is a reason, such as recurrent severe hypoglycemia (SH), to set a higher target, whereas the target is set slightly higher in children and adolescents at <7.5% (58 mmol/mol) by both the ADA and the International Society for Pediatric and Adolescent Diabetes (ISPAD) (3,4).

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INTRACELLULAR TRANSPORT

PI4P/phosphatidylserine countertransport at ORP5- and ORP8-mediated ER–plasma membrane contacts

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Lipid transfer between cell membrane bilayers at contacts between the endoplasmic reticulum (ER) and other membranes help to maintain membrane lipid homeostasis. We found that two similar ER integral membrane proteins, oxysterol-binding protein (OSBP)–related protein 5 (ORP5) and ORP8, tethered the ER to the plasma membrane (PM) via the interaction of their pleckstrin homology domains with phosphatidylinositol 4-phosphate (PI4P) in this membrane. Their OSBP-related domains (ORDs) harbored either PI4P or phosphatidylserine (PS) and exchanged these lipids between bilayers. Gain- and loss-of-function experiments showed that ORP5 and ORP8 could mediate PI4P/PS countertransport between the ER and the PM, thus delivering PI4P to the ER-localized PI4P phosphatase Sac1 for degradation and PS from the ER to the PM. This exchange helps to control plasma membrane PI4P levels and selectively enrich PS in the PM.

Membrane lipids can be exchanged between bilayers at contact sites between the endoplasmic reticulum (ER) and other membranes (*1*–*7*). One class of molecules mediating these contacts are oxysterol-binding proteins (OSBP) and the closely related OSBP-related proteins (ORPs) (Osh proteins in yeast), which harbor lipids in a hydrophobic cavity of their OSBP-related domain (ORD) (fig. S1) (*3*, *7*–*13*). Members of this protein family (more than 10 in mammals) have been thought to function selectively as sterol sensors or transport proteins (*12*, *13*), but recent studies show that they can also harbor different lipids (*3*, *7*, *9*–*11*). OSBP and Osh4/Kes1function in a lipid countertransport between the Golgi complex and membranes of the ER by delivering cholesterol to the Golgi in exchange for phosphatidylinositol 4-phosphate (PI4P), which is degraded by the Sac1 phosphatase in the ER (*9*, *14*). Whether other ORPs also function in lipid countertransport reactions to help maintain membrane heterogeneity, such as a selective concentration of phosphatidylserine (PS) in the plasma membrane (PM) (*15*), is unclear. We focused on two very similar mammalian ORPs, ORP5 and ORP8, which are anchored to the ER membrane, where they reside via a hydrophobic tail sequence (*12*, *16*, *17*). Their ORDs are the mammalian ORDs most closely related to the ORDs of Osh6 and Osh7, which transport PS to the PM in yeast, although ORP5/8 and Osh6/7 are otherwise different in domain organization (fig. S2A) (*3*, *8*, *11*, *12*).

Green fluorescent protein (GFP)–ORP5 and GFP-fusions of the two splice variants of ORP8 (*17*), which differ by the inclusion (ORP8L) or exclusion (ORP8S) of an N-terminal 42 amino acids sequence (fig. S5), were independently expressed in HeLa cells and analyzed by means of confocal microscopy. ORP5 predominantly accumulated in small patches at the cell periphery in a pattern reminiscent of ER-PM contacts (*18*, *19*): a row of peripheral dots in mid-cell optical sections and tightly apposed patches in optical sections of the flat base of the cell (Fig. 1A). More numerous and longer ER-PM contacts were detected as a result of excess ORP5 expression (Fig. 1, C to E). In contrast, GFP-ORP8L had a broad reticular distribution throughout the cell (Fig. 1A) that overlapped with that of the ER marker Sec61β (fig. S3), with only a faint puncta at the cell periphery. GFP-ORP8S had a somewhat intermediate localization pattern (Fig. 1A). Coexpressed GFP-ORP5 and mCherry-ORP8L partially colocalized at the cell cortex (Fig. 1F), and ORP8L coimmunoprecipitated with ORP5 (Fig. 1G), indicating that ORP5 may help mediate ORP8 recruitment to ER-PM contacts via heteromerization.

Because both ORP5 and ORP8 contain a PH domain, we next investigated whether their tethering function depended on phosphoinositides in the PM. The cortical pool of GFP-ORP5 and GFP-ORP8S, and even the very weak cortical accumulation of GFP-ORP8L, increased upon overexpression of phosphatidylinositol 4-kinase IIIα (PI4KIIIα) and its associated factors [the enzyme complex responsible for PI4P synthesis in the PM (*20*–*23*)] (Fig. 1, A and B). In cells treated with the PI4KIIIα inhibitor A1 (*25*), both ORP5 and ORP8S dissociated from the PM and dispersed throughout the ER (Fig. 1H and movies S1 and S2). This redistribution correlated with the dissociation from the PM of near-infrared fluorescent protein (iRFP)–P4M, a PI4P reporter (*24*), but not of $PH_{PLC\delta}$, a phosphatidylinositol 4,5-bisphosphate [$PI(4,5)P_2$] reporter (movie S3) (*25*). Thus, PI4P is required for the binding of ORP5 and ORP8 to the PM.

ORP5 and ORP8L constructs lacking their PH domains were localized throughout the ER even upon coexpression of PI4KIIIα (Fig. 1, I and J, and fig. S4). Constructs lacking the transmembrane region or comprising the PH domain and upstream N-terminal sequences mimicked the properties of the full-length proteins (cortical localization of ORP5 constructs and strong dependence on PI4KIIIα overexpression for the cortical accumulation of ORP8L constructs) (Fig. 1, I and J). Both PH domain-only constructs were similarly targeted to the PM and more prominently upon PI4KIIIα overexpression (Fig. 1, I and J). Thus, differences in the PM recruitment of ORP5 and ORP8L are dictated by their N-terminal amino acid sequences that differ substantially in the two proteins and confer an overall more negative charge to ORP8L. Most of this negative charge is accounted for by the ORP8L-specific extension (fig. S5), which explains the greater accumulation of ORP8S at ER-PM contacts. The ORD is dispensable for cortical localization (fig. S4).

The PI4P-dependent cortical accumulation of ORP5 was supported by studies of tamoxifen-inducible PI4KIIIα-conditional knockout embryonic fibroblasts (MEFs) (*20*). In sham-treated MEFs, GFP-ORP5 was concentrated at ER-PM contacts, but in knockout (tamoxifen-treated) cells, in which global PI4P levels are 70% lower than in controls with dramatic loss of PM PI4P (*20*), GFP-ORP5 was dispersed throughout the ER (Fig. 2A). ORP5 and ORP8 expression was up-regulated in knockout MEFs, pointing to a functional link between ORP5/ORP8 function and PI4KIIIα (Fig. 2B). This phenotype, as well as the loss of cortical localization of ORP5, was rescued by expression of PI4KIIIα in the knockout MEFs (Fig. 2, A and B). PI4KIIIα knockout MEFs also had lower PS levels (about 50% reduction) (Fig. 2C and fig. S6) but no major changes in levels of phosphatidylethanolamine (PE) and phosphatidylcholine (PC), the precursors of PS (Fig. 2C) (*15*, *26*).

If ORP5 and ORP8 operate in a countertransport mechanism, they should contain more than one lipid in their ORD domains, which are the most similar to each other in their portion defining the lipid-harboring cavity (fig. S2A). Because only the ORD of ORP8 (amino acids 370 to 809) could be purified in sufficient yield (Fig. 3A), we focused on this domain. Mass spectrometry comparison between the denatured ORD [apo form, molecular weight (MW) 53,865 daltons] and the native ORD (mixture of native apo and holo forms) revealed two main ORD-lipid complexes: MW 54,654 daltons (apo form + 789 daltons) and MW 54,808 daltons (apo form + 943 daltons) (Fig. 3B). These mass increments correspond

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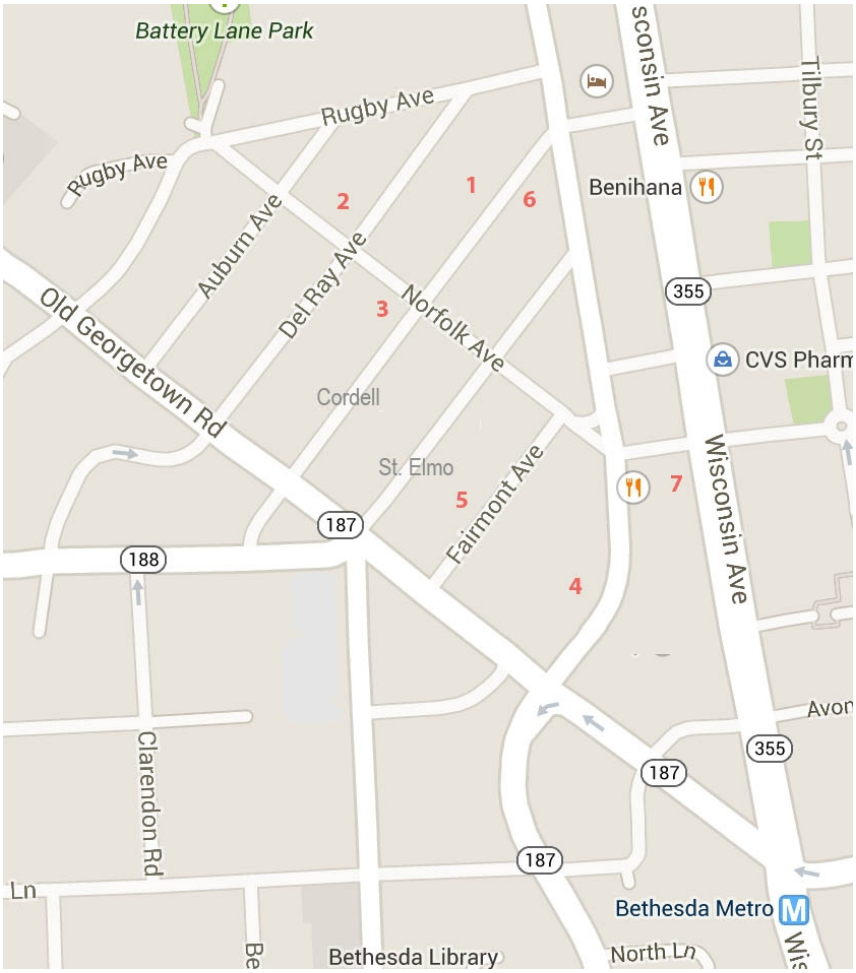
Bethesda Restaurant Districts

There are two Bethesda Restaurant districts in walking distance from the Bethesda Metro. A free circulator bus connects the two areas.

Bethesda Row or Bethesda West

The more trendy one is south, Bethesda Row or Bethesda West.
Walk about 3 blocks south of the Bethesda Metro station (at the Hyatt) along Wisconsin and 2 blocks west to reach the Bethesda restaurant district known as "Bethesda Row". The numbers indicate a few of the restaurants in that area that are described below.



All restaurants along this street have both sidewalk dining and indoor dining

1. American Tap Room – casual, burgers, sandwiches, beer, loud
2. The Mussel Bar – good selection of Belgian and other ales on tap, mussels, flatbread. Trendy
3. *Raku – Asian fusion, one of our favorites
4. Tandoori Nights – recently replaced the prior Indian restaurant that was good. We haven't eaten at this new restaurant yet.
5. *Jaleo's – Tapas, bistro very lively atmosphere, loud, a favorite. Trendy

6. Mon Ami Gabi – steak frites, bistro. Sometimes musicians in the bar

7. Deluxe café – modern American food, lamb sandwiches. Popular happy hour in the bar.
8. Mama Lucia's – pasta, Italian, relatively inexpensive.
9. Lebanese Taverna – middle eastern food, big tables for groups, moderate prices, but the food is always a little disappointing to me
10. *Redwood – new American food, big restaurant with many large tables indoors and outdoors. Not too loud. Wine specials, music certain days, entrees in $25-30 range. Good for group dinners.
11. Many less expensive restaurants are on this block
    a. Nando peri-peri (rotisserie chicken; counter ordering, bring to table)
    b. Sweetgreens (salads; counter ordering)
    c. 5 guys (burgers; counter ordering)
    d. Cava – (gyros, falafal; counter ordering)
    e. Georgetown cupcake
    f. Tara Thai (table service). Moderate cost, good food.

Outside the "row"

12. *Food Wine and Company -Modern american bistro, raw bar, charcuterie. $20-30 entrées. A current favorite
13. Ruth's Chris steakhouse (expensive)
14. *Pines of Rome (inexpensive, family style Italian, no reservations taken, very old Bethesda restaurant, not trendy but a favorite of ours)
15. Cesco Osteria (italian, expensive, reservations recommended). We haven't eaten here since it moved and went upscale. Reputation for good food.
16. Morton's the steak (expensive)
17. The very large arrow points to the Bethesda Crab House about 1 block further down Bethesda Ave. Whole crabs on newspaper with mallets on outdoor picnic tables - a Maryland tradition, Chesapeake Bay blue crabs. The menu is very limited: crabs, shrimp, beer and occasionally soda crackers.

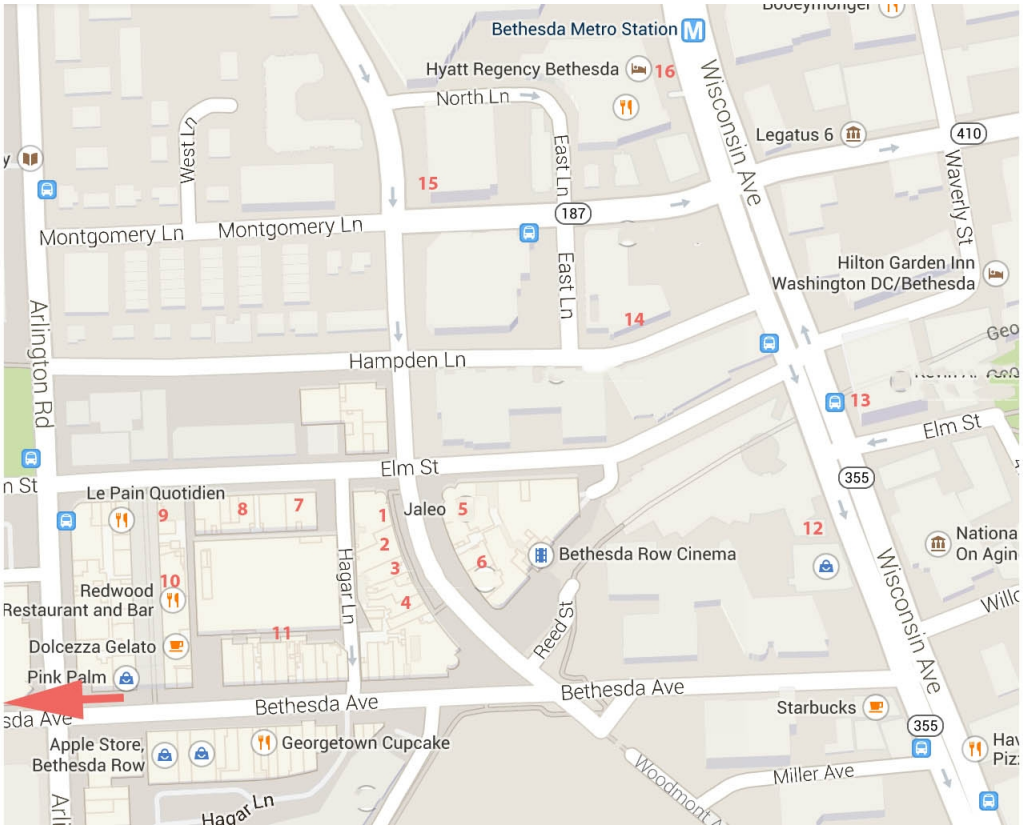
A few other favorites, not marked on the map:

- Rock Bottom—Pub, beer brewed on site, pub food, loud, long tables. 7900 Norfolk Ave, Bethesda, MD 20814 (301) 652-1311
- Tastee Diner—Classic greasy spoon diner. Not particularly good but authentic. 30+ years 7731 Woodmont Ave, Bethesda, MD 20814 (301) 652-3970

Cordell Triangle

The other restaurant district is Cordell Triangle, north of the Bethesda Metro. To reach this area, walk 2-3 blocks north on Old Georgetown Road, and turn right on any of the little streets. Turn left on Norfolk to reach the "epicenter" at Cordell and Norfolk. There are about 30 restaurants within a few blocks that run the gamut of price (burger, pizza, burritos, Chinese at the low end), some good mid-range restaurants (Italian, Indian, cajun, thai, a brewpub) and a couple of foodie type-restaurants (Blacks, Grapeseed, Bistro Provence). Here are a few:

1. *Grapeseed – dishes are created to complement wines. Changing seasonal menu. Moderately expensive. (a favorite, on Cordell Ave)
2. Steamers – another crab house with picnic tables and mallets, somewhat classier and less authentic than the Bethesda crab house, and more extensive menu.
3. Roof – a new restaurant in this area on an open roof. American food – haven't eaten here yet but menu looks good.
4. *Black's bar and grill – One of Jeff Black's restaurants (local famous chef) raw bar, good seafood, good happy hour deal. Moderately expensive
5. Bisto Provence – Yannick Cam's restaurant (famous chef) French, reservations essential, very expensive. Good reputation but we have not eaten here…
6. Harp and Fiddle – Irish pub/sports bar. Outdoor area for smoking.
7. *Tako grill – Japanese sushi and robataki, moderate prices. A favorite



A few other favorites, not marked on the map:

- Chef Tony's is on St. Elmo between Old G'town and Norfolk– seafood
- Aji-Nippon—Small pleasant "neighborhood" Japanese restaurant. Booths for 4, a few larger tables. 6937 Arlington Rd, Bethesda, MD 20814 (301) 654-0213
- Persimmon—American, very good, "classy", must have reservations. 7003 Wisconsin Ave, Chevy Chase, MD 20815 (301) 654-9860